

THE EFFECTS OF PROGESTINS ON ANIMAL MODELS OF  
COCAINE BINGEING AND RELAPSE

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## **Dedication**

Dedicated to my great grandmother Melinda Roever,  
the most exceptional person I have had the privilege of knowing.

You are my inspiration.

## **Abstract**

Female gonadal hormones influence responses to stimulant drugs, and estrogen (EST) facilitates while progesterone (PROG) attenuates these responses. The PROG metabolite allopregnanolone (ALLO) also suppresses responses to cocaine, and it may be responsible for PROG's attenuating effects. The research included in this thesis is concerned with the influence of PROG and its metabolite ALLO on animal models of escalation or bingeing of cocaine of intake (Experiments 1 and 2) and reinstatement of extinguished drug seeking or relapse (Experiments 3, 4, and 5). Experiment 1 focused on the influence of PROG on the escalation of cocaine self-administration that occurs with long (6 h) daily access in ovariectomized (OVX) and gonadally intact female rats treated with either EST or VEH. Following the escalation procedure, treatment groups were compared on a progressive ratio (PR) schedule for several doses of cocaine. This research was extended to the PROG metabolite ALLO in Experiment 2a. Gonadally intact female rats were treated with ALLO or VEH and allowed to earn either i.v. cocaine infusions or sucrose deliveries under a long-access (LgA) escalation procedure. In Experiment 2b sucrose was used to determine the specificity of ALLO's effects on the escalation of cocaine self-administration, and in Experiment 2c control conditions were used to examine the effect of ALLO on locomotor activity and operant behavior reinforced by food. In Experiment 3 the effects of PROG on the reinstatement of cocaine-seeking behavior were examined in OVX and intact female rats that received EST or VEH treatment. In Experiment 4 the mechanism of PROG's effects was further studied by using finasteride, a 5-alpha reductase inhibitor that prevents the metabolism of

PROG into ALLO. Finasteride (FIN) was administered concurrently with PROG to determine whether it could block the attenuating effects of PROG on cocaine-primed reinstatement, thus implicating ALLO in PROG's actions. Allopregnanolone was also tested on cocaine-primed reinstatement in male and female rats to examine possible sex-specific treatment effects. In the final experiment (Experiment 5), the effects of ALLO were examined on stress-induced reinstatement by injecting rats with yohimbine (YOH) to generate a stressful condition. Results from Experiment 1 indicated that EST facilitated while PROG attenuated the escalation of cocaine self-administration during LgA, but neither hormone affected cocaine-maintained responding under a PR schedule. Experiment 2a confirmed that ALLO also attenuated the escalation of cocaine self-administration, and 2b indicated that ALLO did not affect the escalation of intake of a nondrug reward (i.e., sucrose). Experiment 2c revealed that ALLO also had no effect on locomotor activity or operant food-reinforced behavior. Experiments 3 and 4 demonstrated that PROG and ALLO also decreased the reinstatement of cocaine-seeking behavior precipitated by i.p. injections of cocaine. Results from Experiment 4 further indicated that the attenuation of cocaine seeking by PROG may be attributed to its conversion into ALLO, as blocking this conversion with FIN prevented PROG's attenuating effects. In addition, the effects of ALLO were specific to females and did not extend to males. Finally, in Experiment 5, female rats exhibited greater reinstatement of cocaine seeking following a stressful stimulus (YOH) than males, and the attenuating effects of ALLO on this behavior were specific to females. Taken together, these results demonstrated a role for PROG and ALLO in suppressing the bingeing and relapse

associated with cocaine abuse. These effects were specific to cocaine seeking in females (vs. males) and did not extend to behavior maintained by a nondrug rewards (i.e., sucrose or food) or locomotor activity.



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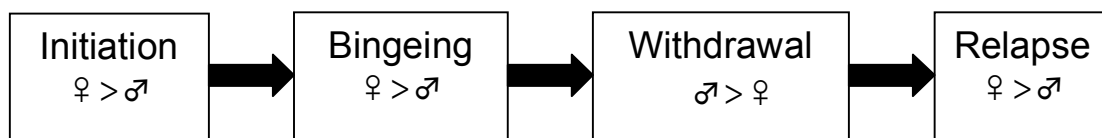
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## **Introduction**

Cocaine addiction typically begins with infrequent recreational use that rapidly progresses into compulsive drug taking that is characterized by high rates of relapse (O'Brien 2001). Next to alcohol, cocaine is the drug most commonly associated with emergency room visits (Substance Abuse and Mental Health Services Administration, SAMHSA 2008). As a result, cocaine addiction poses a serious health problem. Currently, rates of drug abuse are lower in women than men (SAMHSA 2008); however, this may be attributed to a difference in opportunity rather than vulnerability (Van Etten and Anthony 2001; Van Etten et al. 1999). Regardless, sex differences in drug abuse are narrowing (Carroll et al. 2004), and results with adolescents indicate that drug abuse rates, especially with cocaine use, are higher in adolescent girls than boys (Chen and Kandel 2002; SAMHSA 2008).

Clinical and preclinical research also indicates that females, compared to males, exhibit greater vulnerability toward drug abuse at stages of the addiction process that mark transitions in drug use. These stages are depicted in Figure 1 and include drug initiation, bingeing, withdrawal, and relapse, and they are modeled in animals using acquisition, escalation, withdrawal/extinction, and reinstatement procedures, respectively (Carroll et al. 2009a). Clinical reports indicate that women are more likely to initiate drug use at an earlier age (Chen and Kandel 2002), are more vulnerable to binge-like patterns of drug intake (Becker and Hu 2008; Brady and Randall 1999; Lynch et al. 2002; Mann et al. 2005; Randall et al. 1999), report greater difficulty in quitting (Becker and Hu 2008; Carpenter et al. 2006; Lynch et al. 2002), exhibit greater drug craving (Robbins

et al. 1999), are more sensitive to stress-induced craving (Fox and Sinha 2009), are more likely to relapse (Ignjatova and Raleva 2009), and they take more drug following relapse (Gallop et al. 2007). Animal models of drug abuse add further support to enhanced female vulnerability across these stages of the addiction process, and they indicate that female rats acquire drug self-administration at a faster rate, exhibit greater binge-like patterns of drug intake, and are more vulnerable to relapse of drug-seeking behavior (for reviews see Carroll and Anker 2009; Carroll et al. 2009a; Lynch et al. 2002; Roth et al. 2004). Thus, women may be more vulnerable to several aspects of drug abuse, possibly due to an underlying biological predisposition. As a consequence, it is important to not only identify biological vulnerability factors that could contribute to the onset and progression of drug addiction in women but to consider possible treatments as well.



**Figure 1** The drug abuse process, and relative vulnerability of females ( ♀ ) and males ( ♂ ).

A growing number of findings suggest that the biological basis for increased drug abuse vulnerability in women may be attributed to female gonadal hormones (for reviews see Carroll and Anker 2009; Becker and Hu 2008; Carroll et al. 2004; Festa and Quinones-Jenab 2004; Lynch et al. 2002; Quinones-Jenab et al. 2001; Terner and de Wit 2006). More specifically, enhanced drug seeking and subjective effects in women (Evans 2007; Terner and de Wit 2006) are associated with higher levels of endogenous estrogen

(EST). Preclinical work has further confirmed this and has demonstrated that endogenous or exogenous (e.g., estradiol benzoate, 17-beta-estradiol) EST facilitates the acquisition, escalation, and reinstatement of cocaine-seeking behavior in female rats (for a review see Carroll and Anker 2009). Progesterone (PROG) has received less attention than EST, but recent findings indicate that PROG and its metabolite allopregnanolone (ALLO), collectively termed progestins (see Frye 2007), have the opposite effect on cocaine-induced positive subjective measures in women (Evan 2007, Fox and Sinha 2009) and on the acquisition of cocaine self-administration in female rats (Jackson et al. 2006). Despite these findings, little is known about the effects of PROG and ALLO on stages of the addiction process associated with increased vulnerability in women such as drug bingeing and relapse after an abstinence period. The focus of the experiments in this thesis is on the effects of PROG and ALLO on the escalation and reinstatement of cocaine-seeking behavior in female rats. The findings from these studies are preceded by an overview of factors implicating PROG and ALLO in the attenuation of cocaine seeking.

### ***Biosynthesis of progestins***

In the female mammalian body, the primary source of PROG and EST is secretion by the ovaries (Frye 2007). Allopregnanolone is also secreted by the ovaries as well as the adrenal glands, and it is manufactured in glial cells (Mellon 2004; Kabbadj et al. 1993; Mellon 2004). However, the primary source of ALLO and pregnanolone is through metabolism of peripheral sources of PROG (Frye 2007). The conversion of

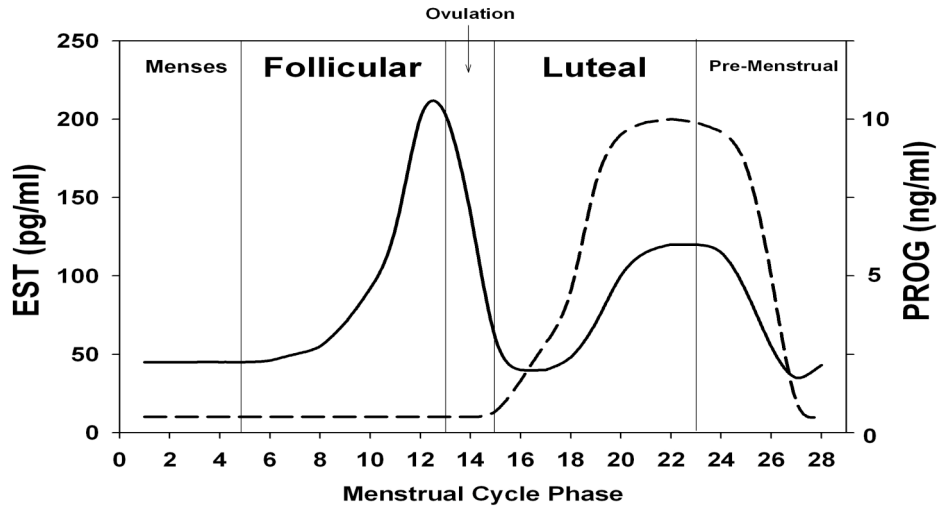


PROG into its metabolites occurs in a series of metabolic steps. Pregnenolone is converted to PROG which is then reduced to 5-alpha-dihydroprogesterone (DHP) and 5-beta-DHP by 5-alpha- and -beta-reductase, respectively. Allopregnanolone and pregnanolone are subsequently synthesized from the 5-alpha and 5-beta forms of DHP by 3-alpha-hydroxysteroid oxidoreductase (Melon et al. 2004). For clarification, pregnenolone with an e is the precursor to PROG and pregnanolone with an a is a metabolite of PROG.

### ***Progestins and cocaine-induced effects: clinical research***

Two methods are primarily used in clinical research to investigate the effects of female hormones on cocaine-related behaviors. The first compares cocaine-elicited responses across different phases of the female hormonal cycle, while the second involves direct manipulation of hormone levels by their systemic administration. An abundance of clinical research has employed the former method and has focused on menstrual cycle interactions with stimulant-elicited subjective and physiological responses. The schematic in Figure 2 shows that the 28-day human menstrual cycle is composed of two main phases: follicular and luteal, each characterized by differing levels of the female hormones, EST and PROG. During the follicular phase (~14 days) PROG levels remain low, and EST levels increase until they peak just prior to ovulation. Ovulation occurs right after the follicular phase and is characterized by declining levels of EST, low levels of PROG, and a surge of luteinizing hormone and follicle-stimulating hormone (not shown). After ovulation, during the luteal phase (~ 7 days), EST levels

continue to decline and remain relatively low while PROG levels continue to increase (Carter 1994). The luteal phase also marks the period of largest increases of the PROG metabolites ALLO and pregnanolone (Bixo et al. 1997).



**Figure 2** Schematic of plasma concentrations of EST (solid line) and PROG (dashed line) across the menstrual cycle

### *Progestins across the human menstrual cycle*

In clinical research, natural fluctuations of PROG during the menstrual cycle correspond to differences in the physiological and subjective effects of some stimulant drugs (for reviews see Evans 2007; Terner and de Wit 2006). For example, cardiovascular and/or positive subjective responses to cocaine (Evans and Foltin 2006; Evans et al. 2002; Sofuoglu et al. 1999) and amphetamine (Justice and de Wit 1999, 2000; White et al. 2002), but not nicotine (for review see Terner and de Wit 2006), were reduced during the luteal phase of the menstrual cycle (when PROG levels are high) compared to the follicular phase (However, see Collins et al. 2007; Lukas et al. 1996; Mendelson et al. 1999). These results have also been extended to measures of stress

reactivity and craving elicited by cocaine-related stimuli in cocaine-dependent women (Sinha et al. 2007). In this study, participants were separated into three groups that had high, medium, or low levels of circulating PROG, corresponding to their midluteal, early luteal, and follicular phases of their menstrual cycles, respectively. Groups were then exposed to imagery related to a personally stressful event, or to cocaine-related cues, and compared on measures of cocaine craving, subjective anxiety, and diastolic and systolic blood pressure. Following the drug-cue exposure, women with high levels of PROG showed lower diastolic and systolic blood pressure responses and scored lower on self-reported measures of anxiety and drug craving than women with low levels of PROG. Heightened plasma PROG levels were also associated with less severe stress-induced craving for cocaine (Sinha et al. 2007). The finding with stress-induced craving is especially important given that clinical and preclinical reports implicate stress as a primary factor in drug bingeing and relapse (Covington et al. 2005; Sinha 2008), and it is associated with heightened drug abuse vulnerability in women (Fox and Sinha 2009). Taken together, these results indicate that the phase of the menstrual cycle in which PROG levels are at the highest was associated with low positive affective responses to cocaine and diminished stress-induced craving in women.

### *Systemic progesterone administration*

The influence of systemic PROG administration on the subjective and physiological effects of cocaine has been examined in several clinical studies (for review see Evans 2007; Evans and Foltin 2009). In these experiments, PROG was administered

in the micronized (extended release) form at a concentration that produces a physiological level comparable to the midluteal phase of the menstrual cycle. Results from a study conducted by Sofuoglu et al. (2002) indicated that women treated with PROG showed a decrease in the positive subjective effects of smoked cocaine compared with placebo-treated controls (Sofuoglu et al. 2002). This study was subsequently extended to examine PROG's effects on i.v. cocaine self-administration in female and male participants (Sofuoglu et al. 2004), but PROG failed to alter i.v. cocaine self-administration in either group in this study. This lack of a sex difference may have been due to a ceiling effect, as both groups self-administered their total allotment of cocaine infusions. However, physiological measures such as cocaine-induced increases in diastolic blood pressure and subjective ratings of "feeling high" and "feeling the effects of the last administered dose" were diminished in PROG-treated vs. placebo-treated participants (Sofuoglu et al. 2004).

Similar results were also found in a study conducted by Evans and Foltin (2006). However, the inhibitory effects of PROG in this study were variable and dependent on the cocaine dose as well as the sex of the participant. For example, PROG attenuated cocaine-induced increases in heart rate in women across all of the cocaine doses, but this effect was dose-specific in males. Subjective ratings of "feeling high," "overall drug quality," and "willingness to pay for an experimenter-administered dose of cocaine" were also decreased following PROG treatment in females, while PROG had little or no effect on these measures in male participants (Evans and Foltin 2006). The lack of an effect in males is further supported by research in which PROG failed to decrease outpatient

cocaine use in male cocaine users (this study did not examine female cocaine users) (Sofuoglu et al. 2007). Overall, results from clinical studies indicated that endogenous and systemically administered PROG decreased cocaine-induced subjective and physiological ratings, and this effect was generally specific to women. Few clinical studies have examined the influence of PROG on motivational aspects of cocaine abuse (cf. Sofuoglu et al. 2004), and none have examined the influence of PROG's metabolites (ALLO or pregnanolone) on these measures. This would be of interest in future research given that levels of these metabolites show significant increases during the luteal phase of the menstrual cycle when PROG levels are high (Bixo et al. 1997) or following systemic PROG injections (Soderpalm et al. 2004). Table 1 summarizes results across the menstrual cycle and PROG's effects on amphetamine- and cocaine-induced subjective measures in women.

**Table 1** Summary of the effects of endogenous and exogenously administered PROG in women on subjective responses to amphetamine and cocaine in clinical research

| Drug        | Independent Variable                                     | Dependent Measure                          | Findings <sup>a</sup> | Reference                                    |
|-------------|--|--|-----------------------|--|
| Amphetamine | Menstrual cycle state                                    | Feeling high                               | L < F                 | Justice and de Wit 1999                      |
|             |  | Want more drug                             | L < F                 | Justice and de Wit 2000<br>White et al. 2002 |
| Cocaine     | Menstrual cycle state                                    | Subjective measures                        | No phase differences  | Lukas et al. 1996<br>Mendelson et al. 1999   |
|             |  | Feel high, want more drug                  | L < F                 | Sofuoglu et al. 1999                         |
|             |  | Positive effect, want more drug            | L < F                 | Evans et al. 2002                            |
|             |  | Good drug effect                           | L < F                 | Evans and Foltin 2006*                       |
|             |  | Craving following cues                     | L < F                 | Sinha et al. 2007                            |
|             |  | Subjective measures                        | No phase differences  | Collins et al. 2007                          |
|             | Systemic PROG administration during the follicular phase | Feel the effect of last dose               | F+P < F               | Sofuoglu et al. 2002                         |
|             |  | Feel the effect of last dose, feeling high | F+P < F               | Sofuoglu et al. 2004                         |
|             |  | Good drug effect, drug quality             | F+P < F               | Evans and Foltin 2006*                       |

<sup>a</sup> L: luteal phase; F: follicular phase; P: PROG

\*no effects in males

### ***Progestins and cocaine-induced effects: preclinical research***

Studies using animal models have extended clinical findings with PROG by modeling critical phases of the drug abuse process that occur in humans. There is typically close agreement between results from clinical and preclinical studies in the screening, prevention, and treatment of drug abuse; thus, preclinical models of drug self-administration have translational value for the treatment of many aspects of this disorder (Carroll et al. 2009a). In animal research, the self-administration paradigm is considered a valid model of human drug addiction (for a review see Panlilio and Goldberg 2007). Self-administration allows the animal to regulate intake and offers a controlled

longitudinal approach to the study of different hormonal conditions during critical phases of the drug abuse process (e.g., initiation, escalation, and relapse) (Carroll et al. 2009a). The following section highlights findings from nonhuman primate and rodent preclinical work examining the effects of endogenous and systemically-administered PROG on the acquisition, maintenance, extinction, and reinstatement phases of the drug abuse process in addition to PROG's effects on other addiction-related behaviors. Allopregnanolone's effects on cocaine overdose will also be discussed.

#### *Cocaine-seeking behavior across the nonhuman primate menstrual cycle*

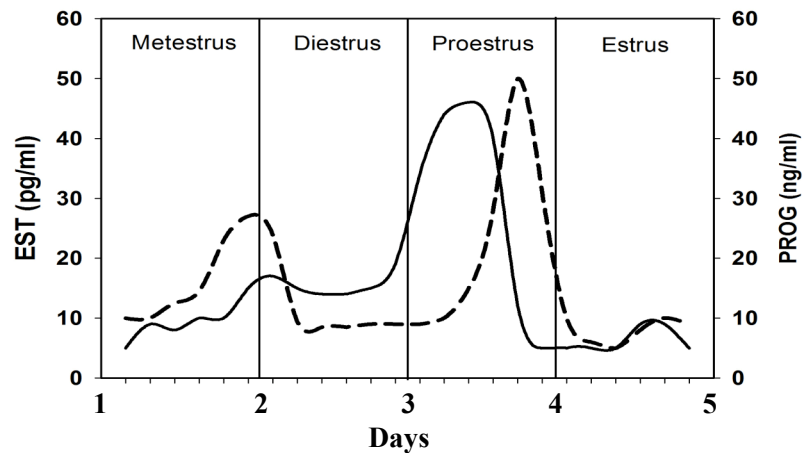
In preclinical research, the use of monkeys provides an invaluable model for the human menstrual cycle due to the similarity in its duration and hormonal changes. Similar to clinical findings (for review see Evans 2007; Evans and Foltin 2009), research with nonhuman primates indicates hormone cycle-dependent alterations of stimulant-related behaviors. For example, during the maintenance of cocaine self-administration under a progressive-ratio (PR) schedule, cynomolgus female monkeys reached significantly lower breakpoints (i.e., decreased motivation) for a low dose of cocaine (0.0032 mg/kg/i.v. injection) during the luteal phase of the menstrual cycle, when PROG levels are high compared with the follicular phase when PROG levels are low (Mello et al. 2007). However, these results contrast with a study of oral phencyclidine (PCP) self-administration in rhesus monkeys under a fixed-ratio (FR) schedule in which responding significantly increased during the luteal phase (Newman et al. 2006). These inconsistent results may be related to differences in the pharmacological class of the self-administered

substance, doses used, route of administration (i.v. vs. oral), or schedule of reinforcement (PR vs. FR), respectively.

### *Cocaine-seeking behavior across the rodent estrous cycle*

While the rodent estrous cycle is much shorter than the primate menstrual cycle (approximately 6 days vs. 28 days), changes in cocaine self-administration have been monitored across estrous phases. The rodent cycle consists of four distinct phases (metestrus, diestrus, proestrus, and estrus), each characterized by varying levels of EST and PROG (Figure 3). During early metestrus, EST and PROG levels remain relatively low until late metestrus when PROG levels rise until they decrease again during early diestrus. Estrogen levels slowly increase during both metestrus and diestrus until mid proestrus when they increase dramatically and peak. Shortly thereafter, PROG levels reach their highest level in mid proestrus. Both hormones reach their lowest levels during the estrus phase of the female rat cycle (Brown-Grant et al. 1970; Feltenstein and See 2006; Naftolin et al. 1972; Roth et al. 2002; Shaikh 1971). Despite challenges associated with rapid estrous phase transitions, preclinical research supports clinical and nonhuman primate work suggesting a role for endogenously circulating PROG in the attenuation of cocaine-related responses.





**Figure 3** Schematic of plasma concentrations of EST (solid line) and PROG (dashed line) across the rodent estrous cycle

#### Maintenance

Similar to findings with nonhuman primates (Mello et al. 2007), research with rodents indicates hormone cycle effects in the maintenance of cocaine self-administration under a PR schedule. Breakpoints under a PR schedule were higher (increased motivation) for i.v. cocaine in female rats (Hecht et al. 1999; Roberts et al. 1989), and the rats consumed more drug (Feltenstein and See 2007; Lynch et al. 2000; Lynch 2008) during the estrus phase, when both EST and PROG levels were at their lowest, than at all other phases of the estrous cycle. These results suggested that phases of the female hormone cycle associated with lower PROG levels corresponded with higher motivation to self-administer cocaine.

#### Extinction/reinstatement

The extinction and reinstatement (relapse) phases of drug abuse have been modeled in rats, and they are considered to be representative and predictive of abstinence

and relapse in humans. Female rats in the estrus phase (low PROG) were more resistant to extinction of lever responding that was previously reinforced with i.v. cocaine (Feltenstein and See 2007; Kerstetter et al. 2008). During reinstatement, females in estrus also exhibited higher levels of cocaine-primed responding on a lever previously associated with cocaine delivery compared to rats in other phases of the estrous cycle (Feltenstein and See 2007; Kerstetter et al. 2008; Kippin et al. 2005). Moreover, plasma PROG levels in free-cycling female rats were negatively correlated with cocaine seeking during extinction and cocaine-primed reinstatement (Feltenstein and See 2007). These results did not extend to cue-induced reinstatement, as female rats responded similarly following a cue previously paired with cocaine self-administration during all phases of the estrous cycle (Fuchs et al. 2005). Others have also demonstrated differential treatment effects for drug- and cue-primed reinstatement, such that a treatment effective in blocking cue-induced reinstatement of cocaine seeking had negligible effects on drug-induced reinstatement (Filip and Frankowaska 2007; Torregrossa and Kalivas 2008). These discrepancies may be explained by different neurobiological mechanisms underlying each form of reinstatement (Di Chiara 2002; Torregrossa and Kalivas 2008).

### *Systemic progestin administration*

A method that allows a controlled and systematic approach involves direct control of hormone levels through systemic injection. In preclinical research, this generally involves depleting hormone levels by ovariectomy (OVX), waiting for the hormones to dissipate (e.g., 5 days), and then administering the hormone or metabolite of interest.

The following sections describe recent findings from preclinical research using surgical methods and/or pharmacological manipulations to examine progestin effects on the acquisition of cocaine self-administration and cocaine overdose.

### Acquisition

In a study conducted by Jackson et al. 2006, OVX female rats treated with vehicle (VEH), EST, or EST+PROG were compared during the acquisition of cocaine self-administration. Results indicated that EST treatment facilitated the acquisition of cocaine self-administration in OVX female rats (but not castrated male rats) relative to VEH-treated controls, as previously reported (Lynch et al. 2001), and PROG attenuated this EST-induced facilitation. Cocaine intake was also reduced in female rats treated with EST+PROG relative to OVX rats treated with EST alone (Jackson et al. 2006). These results suggested an important role for PROG in limiting cocaine-seeking behavior; however, other critical phases of drug abuse have yet to be examined.

### Reinstatement

Systemic administration of PROG has also been implicated in the attenuation of cocaine-induced reinstatement of drug seeking in female rats. In one study, PROG administration during the estrus phase of freely-cycling female rats decreased reinstatement responding following a cocaine-priming injection relative to VEH-treated rats (Feltenstein et al. 2009). The suppressant effects of PROG on cocaine seeking did

not extend to other phases of the estrous cycle suggesting a hormone cycle-dependent treatment effect.

## Overdose

Excessive or binge-like patterns of cocaine intake can result in aversive consequences such as overdose and seizures. Kaminski et al. (2003) examined the protective effect of PROG's metabolites ALLO and pregnanolone against cocaine-kindled seizures in mice. While they were not using a self-administration model, results from this study indicated that both treatments attenuated the occurrence of cocaine-precipitated seizures after acute high-dose cocaine treatment. Allopregnanolone also prevented the development of kindling, reflected by the number of mice exhibiting seizures following repeated cocaine treatment (Kaminski et al. 2003; also see Leskiewicz et al. 2003). These results further emphasize the potential value of progestins in not only preventing the initiation of cocaine use but for protection from the toxic consequences of cocaine administration. Table 2 summarizes findings from preclinical work on the effects of the hormone cycle and progestin administration on the acquisition, maintenance, and reinstatement of cocaine-seeking behavior in addition to drug overdose.

**Table 2** Summary of the effects of endogenous and exogenously-administered progestins on behavioral responses to cocaine across phases of the drug abuse process

| Independent Variable           | Dependent Measure            | Finding <sup>a</sup>                           | Reference  |
|--------------------------------|------------------------------|--|--|
| Menstrual cycle state (Monkey) | Maintenance (PR)             | BPs: L < F                                     | Mello et al. 2007  |
| Estrous cycle state            | Maintenance (PR)             | BPs: E < all other phases                      | Roberts et al. 1989<br>Hecht et al. 1999                                 |
|                                | Maintenance (FR)             | Intake: E < all other phases                   | Lynch et al. 2000<br>Feltenstein and See 2007<br>Lynch 2008              |
|                                | Extinction                   | Resistance to extinction: E < all other phases | Feltenstein and See 2007<br>Kerstetter et al. 2008                       |
|                                | Cue-induced Reinstatement    | no phase differences                           | Fuchs et al. 2005  |
|                                | Cocaine-primed reinstatement | E < all other phases                           | Kippin et al. 2005<br>Feltenstein and See 2007<br>Kerstetter et al. 2008 |
| Systemic PROG administration   | Acquisition                  | Days to criteria: OVX+EB+P < OVX+EB            | Jackson et al. 2006  |
|                                | Cocaine-primed Reinstatement | P < V  | Feltenstein et al. 2009  |
| Systemic ALLO administration   | Overdose                     | Seizures and death: A & Preg < V               | Kaminski et al. 2003<br>Leskiewicz et al. 2003                           |

<sup>a</sup>L: luteal phase; F: follicular phase; E: estrus phase; EB: EST; P: PROG; V: vehicle; A: ALLO; Preg: pregnanolone

*Effects of progestins on cocaine-induced behaviors related to drug seeking*

Progesterone and ALLO have been implicated in other behavioral responses to cocaine, such as cocaine-induced conditioned place preference (CPP), locomotor activity, and cocaine discrimination (see Table 3). These behaviors are thought to measure different aspects of drugs of abuse associated with their conditioned rewarding effects

(CPP), behavioral-activating effects (locomotor activity), and their discriminative-stimulus properties (drug discrimination).

Similar to its effects on cocaine self-administration (Jackson et al. 2006), PROG attenuated cocaine-induced CPP in female rats (Russo et al. 2003, 2008) and male mice (Romieu et al. 2003) at doses that did not affect ambulatory or rearing behaviors. However, despite the relatively consistent findings concerning PROG's influence on other cocaine-related behaviors, the effects of PROG on cocaine-induced locomotor activity have been mixed. Progesterone increased (Niyomchai et al. 2008; Perrotti et al. 2001; Quinones-Jenab et al. 2000b; Sircar and Kim 1999), decreased (Niyomchai et al. 2008; Sell et al. 2000; Yang et al. 2007), or had no effect (Perrotti et al. 2001; Quinones-Jenab et al. 2000b; Sircar and Kim 1999; Yang et al. 2007) on locomotor activity following cocaine administration. Discrepancies in these results may be explained by the timing, dose, and/or route of administration of PROG and the presence or absence of EST. Thus, results from locomotor activity studies failed to support previous work showing an attenuating effect of progestins on the conditioned rewarding effects of cocaine.

Drugs of abuse serve as powerful discriminative stimuli, and the translational value of the drug discrimination paradigm is high, as drug discrimination in rodents has closely agreed with self-reported subjective effects in humans (Dykstra et al. 1997; Kamien et al. 1993; Schuster and Johanson 1988). Pharmacological agents that block drug discrimination may also decrease the drug's reinforcing effects; thus, drug discrimination has been useful for screening possible treatments of drug abuse (Solinas et

al. 2006). There has been only one study examining the effects of a progestin on the discrimination of cocaine. Quinton et al. (2006) trained rats to discriminate between saline or two doses of cocaine (5.6 or 10 mg/kg) for food presentation under an operant schedule. Pregnanolone failed to substitute for cocaine in the task, but when it was administered prior to cocaine, the discriminative stimulus effects of the latter were attenuated. One explanation of this finding may be related to state-dependent learning and a stimulus-generalization decrement. For example, stimulant discrimination is dependent on behavioral and neurochemical responses to stress (Kamien and Woolverton 1989; Johanson and Barret 1993; Spealman 1995; Mantsch and Goeders 1998), while the anxiolytic pregnanolone exerts a mitigating effect on stress-related responses (Bitran et al. 1991). Thus, discrimination may have been attenuated when tested in a “nonstressed” state because it was learned in a cocaine-induced “stressed” state.

**Table 3** Summary of the effects of progestins on other behaviors elicited by cocaine

| Independent Variable         | Dependent Measure                  | Decreased Response <sup>a</sup>                          | Reference  |
|------------------------------|------------------------------------|--|--|
| Systemic PROG administration | Cocaine-induced CPP                | P < V  | Russo et al. 2003<br>Romieu et al. 2003<br>Russo et al. 2008                                   |
|                              | Cocaine-induced locomotor activity | No effect  | Sircar and Kim 1999<br>Quinones-Jenab et al. 2000b<br>Perrotti et al. 2001<br>Yang et al. 2007 |
|                              |                                    | P (24 hr after EB)+EB > V,<br>P (<24 hr after EB)+EB < V | Perrotti et al. 2003   |
|                              |                                    | P (500µg)+EB(50µg) > V,<br>P(500µg)+EB(10µg) < V         | Niyomachi et al. 2008  |
|                              |                                    | EB+P > V   | Yang et al. 2007   |
|                              |                                    | P < V  | Sell et al. 2000   |
| Systemic pregnanolone        | Cocaine discrimination             | Blocked by Preg  | Quinton et al. 2006  |

<sup>a</sup> P: PROG; V: vehicle; EB: EST; Preg: pregnanolone

Despite growing evidence implicating PROG in the attenuation of addiction-related responses, little is known of its effects on phases of the drug abuse process involved in heightened female vulnerability such as drug bingeing (Becker and Hu 2008; Brady and Randall 1999; Lynch et al. 2002; Mann et al. 2005; Randall et al. 1999) and relapse (Feltenstein et al. 2009; Gallop et al. 2007). Furthermore, the effects of PROG's metabolite ALLO during these phases have yet to be examined. This is especially important given that ALLO has been shown to attenuate cocaine-elicited responses (Kaminski et al. 2003). In addition, no studies have compared males and females while examining the effects of PROG on cocaine reinforcement. Thus, the primary objective of this thesis work was to investigate the influence of PROG and its metabolite, ALLO, on animal models of bingeing and relapse. The goal of Experiment 1 was to investigate the influence of PROG administration on the escalation of cocaine self-administration. These results were subsequently extended to ALLO in Experiment 2a. Allopregnanolone's effects on the escalation of sucrose self-administration (Experiment 2b), locomotor activity, and food-reinforced behavior (Experiment 2c) were also examined to determine the specificity of ALLO's effects on cocaine seeking. Experiment 3 further extended the results of Experiment 1 to the reinstatement phase, while in Experiment 4 the mechanism of PROG's attenuation of cocaine-primed reinstatement was investigated by pharmacologically blocking the metabolism of a systemically-injected dose of PROG with FIN, a 5-alpha reductase inhibitor. It was hypothesized that FIN would block the attenuation of PROG on cocaine-primed reinstatement. An additional aim of Experiment 4 was to determine sex-specific treatment effects of ALLO



on reinstatement. In Experiment 5 male and female rats were compared on stress-induced reinstatement of cocaine seeking generated by an injection of a stress-inducing agent, yohimbine (YOH), following ALLO treatment.

## **General Methods**

### ***Subjects***

A total of 233 sexually mature (90-120 days old) Wistar rats were used in these experiments. Female rats weighed 225-300 g, and male rats weighed 350-400 g (Harlan Sprague-Dawley, Madison, WI) at the beginning of the procedures. Rats were allowed at least 3 days to acclimate upon arrival in the laboratory before experimental testing or i.v. cannulation. During this time they were pair-housed in plastic cages where they had free access to food (Purina Laboratory Chow, Purina Mills, Minneapolis, MN) and water. Following i.v. cannulation, rats were placed in operant-conditioning chambers to recover for 3 days, and they remained there for the duration of the study. While in the chambers, they continued to have free access to water, and they were fed 16 g (females) or 20 g (males) of ground food (Purina Laboratory Chow) at the end of experimental sessions to maintain consistency in food intake across subjects. These amounts allowed rats to gain weight slowly during the experiments. All holding rooms were maintained at 24°C and at 40-50% humidity under a light/dark cycle (12/12 h with room lights on at 6:00 am). The experimental protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol number 0708A15141), and experiments were conducted in accordance with the Principles of Laboratory Animal Care (National

Research Council, 2003). Laboratory facilities used in these studies were accredited by the American Association for the Accreditation of Laboratory Animal Care.

## *Apparatus*

### *Cocaine self-administration*

Operant chambers were hexagonal in shape with alternating stainless steel and Plexiglas walls. Each chamber contained slots for insertion of stainless steel wall panels, lights, and operant fixtures. Chambers were also fitted with a food receptacle and a panel that allowed placement of a drinking spout into the chamber. Each chamber was enclosed in a sound-attenuating wooden cabinet that had a fan for ventilation.

Two operant levers were positioned 2.5 cm above the wire mesh floor on opposite sides of the chamber, and stimulus lights were situated above each lever. In addition, boxes were illuminated with a house light (4.76 W) located in the upper right corner of each chamber. An infusion pump (RHSYOCKC, Fluid Metering, Oyster Bay, NY) used to administer response-contingent cocaine during experimental sessions was positioned outside of the sound-attenuating wooden box. A length of plastic tubing (1.52 mm o.d., 0.51 mm i.d., Fisher Scientific, Springfield, NJ) extended from the infusion pump to a small plastic swivel (050-0022, Alice King Chatham, Hawthorne, CA) located at an opening at the top of the operant chamber. The swivel attached to a metal spring-covered tether (C313CS, Plastics One, Roanoke, VA) that extended into the operant conditioning chamber where it was secured to a metal cannula guide (C3236, Plastics One, Roanoke, VA) embedded in the rat's infusion harness. The tether was connected to the rat's

infusion harness the day after catheterization, and it remained attached throughout the duration of the study.

### *Sucrose self-administration*

Chambers for the sucrose self-administration study (Experiment 2b) were identical to the cocaine self-administration chambers described above with the exception that two automated liquid-delivery devices (lick-o-meters), activated following a lick contact, were inserted in place of the levers. Stimulus lights were located above the lick-o-meters and were activated for 5 sec following a lick on the spout below. A lick on the active spout delivered a single 0.07 ml delivery of 10% sucrose or water depending on the experimental condition.

### *Locomotor activity*

To measure locomotor activity in Experiment 2c, a circular track (inner diameter 46 cm; outer diameter 71 cm; height 25 cm) previously described by Carroll et al. (2007) was used. Four infrared sensors positioned 5 cm above the wire-mesh floor were mounted on the outer wall of the track at 0°, 90°, 180° and 270°. A VersaMax programmable logic controller (IC200UDR001, GE Fanuc Automation, Charlottesville, VA) and VersaPro software (GE Fanuc Automation, Charlottesville, VA) were used to record sensor beam breaks during experimental sessions.

### *Operant responding for food under a delay-discounting task*

The same octagonal operant conditioning chambers were used to conduct a food-reinforced, delay-discounting task as a nondrug control condition in Experiment 2c. They contained two levers (Coulbourn Instruments, Allentown, NJ) located approximately 2.5 cm above the wire mesh floor, and one set of three multi-colored stimulus lights was located above each lever. A house light (4.76 W) was fixed at the top of the operant chamber, and a food hopper (Coulbourn Instruments, Allentown, NJ) dispensed grain-based 45-mg food pellets (PJA1-0045, Research Diets Inc., New Brunswick, NJ) into a food trough that entered the testing apparatus. A drinking bottle was also accessible from the chamber. Data collection and experimental programming were controlled by MED-PC software (Med Associates, St. Albans, VT) on PCs.

### ***Drugs***

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC), dissolved in a sterile 0.9% saline solution at a concentration of 1.6 mg cocaine HCl/ ml saline, and refrigerated. To extend catheter patency, the anticoagulant heparin (1 ml heparin/200 ml of saline; 190 USP units of heparin/kg) was added to the cocaine solution. The flow rate of each infusion was 0.025 ml/sec, and the duration of pump activation (1 sec per 100 g body weight) was adjusted weekly following catheter patency checks to reflect changes in weight and maintain a constant cocaine dose.

Estrogen, ALLO, PROG, Dimethyl sulfoxide (DMSO), and peanut oil were purchased from Sigma-Aldrich (St. Louis, MO); finasteride (FIN) was purchased from Steraloids (Newport, RI); and yohimbine (YOH) was purchased from the University of Minnesota Boynton Health Service Pharmacy. Estrogen (0.5mg/ml), ALLO (20 mg/ml), and PROG (0.625 mg/ml) were dissolved in peanut oil, and FIN was dissolved in DMSO and promptly refrigerated until use. Peanut oil was used to compare results from previous studies using the same VEH. All injections were administered s.c. in the dorsal-caudal region with the exception of YOH which was administered i.p. The EST dose was chosen based on previous studies indicating that administration of this dose to OVX rats increased the acquisition and reinstatement of cocaine-seeking behavior in female rats (Lynch et al. 2001; Larson et al. 2005). Progesterone was administered at the 0.5 mg/kg dose, as this produced PROG levels within the physiological range and effective in blocking cocaine-seeking behavior (Jackson et al. 2006). A 15 mg/kg dose of ALLO was used, as it produced relatively stable brain concentrations of ALLO 2 h following a s.c. injection (Lancel et al. 1997), and it failed to disrupt motor behavior required to lick from an automated drinking spout (Experiment 2b). An additional dose of ALLO was tested (30 mg/kg) to explore possible dose response interactions with cocaine-primed reinstatement in Experiment 3. This dose (30 mg/kg) was selected as it did not interfere with general locomotor activity or food maintained responding under an operant task (Experiment 2c). A 25 mg/kg FIN injection was given based on previous findings that this dose reliably prevented increases in cortical concentrations of ALLO following a large dose of PROG (5 mg/kg) (Dazzi et al. 2002). The YOH dose of 2.5 mg/kg had

been shown to reliably reinstate cocaine seeking using a similar reinstatement procedure (Feltenstein and See 2006).

## ***Procedures***

### *Surgery*

Rats were surgically implanted with an indwelling catheter in the right jugular vein following the procedure outlined by Carroll and Boe (1982). Briefly, rats were anesthetized with a combination of ketamine (60 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and administered doxapram (5 mg/kg, i.p.) and atropine (0.4 mg/ml, 0.15 ml, s.c.) to facilitate respiration. An incision was made lateral to the trachea, the right jugular was exposed, and a small incision was made perpendicular to the vein. The beveled end of a polyurethane catheter (MRE-040, Braintree Scientific Inc., Braintree, MA, USA) was inserted and then secured to the vein with silk sutures. The free end of the catheter was guided subcutaneously to the midscapular region of the neck where it exited via a small incision and attached to a metal cannula (C3236, PlasticsOne, Roanoke, VA, USA) that was embedded in the infusion harness.

For the OVX and sham (SH) surgeries (Experiments 1 and 3), rats were first given bilateral incisions at the dorsal-lateral region of the abdomen, the underlying muscle wall was separated using a blunt cut method, and the ovaries were localized from within the abdominal cavity. The ovaries were then externalized and removed for OVX, or returned to the abdominal cavity for SH surgeries. After the OVX or SH surgery, rats were implanted with indwelling catheters to allow for i.v. cocaine self-administration.

Following the surgical procedure, rats were allowed a 3-day recovery period during which antibiotic (gentamicin) and analgesic (buprenorphine) medications were administered. Every 7 days at 3:00 pm body weights were recorded, and catheter patency was checked by injecting a 0.1-ml solution containing ketamine (60 mg/kg), midazolam (3 mg/kg), and saline (KMS). If a loss of the righting reflex was not manifest upon a KMS catheter patency check, a second catheter was implanted in the left jugular vein following the methods described above, and the experiment resumed in 3 days.

#### *Confirmation of estrous phase*

Samples of the vaginal mucosa were taken at 3:00 pm in Experiments 1 and 3. In Experiment 1 samples were taken each day following recovery from surgery. In Experiment 3 samples were collected during the first 3 to 5 days before the experiment began, and they were also taken daily during the treatment phase to confirm hormone or VEH treatment. Cells were collected from the rat via a saline-dampened cotton applicator, placed on microscope slides, stained with methylene blue, and promptly coverslipped. Information related to the prototypic cytologic morphology corresponding to experimental groups was referenced from Montes and Luque (1998), and the hormonal conditions were defined as follows: the OVX-EST rats were characterized by a predominance of cornified epithelial cells; the OVX-V group displayed increased numbers of leukocytes; the OVX-EST+PROG and SH-PROG groups showed a predominance of nucleated epithelial cells; and the SH-V group showed daily changes in cytology corresponding to the diestrus, estrus, proestrus, and metestrus phases.

### *Cocaine self-administration training*

Following recovery from surgery, rats (Experiments 1-5) were trained to self-administer 0.4 mg/kg/infusion i.v. cocaine during daily sessions under a fixed-ratio 1 (FR 1) schedule of reinforcement. Self-administration sessions began daily at 9:00 am with the illumination of the house light and ended at 11:00 am with its termination. During each session, a response on the active lever resulted in a single 0.4 mg/kg cocaine infusion (0.025 ml/100 g body wt) and the simultaneous illumination of three multi-colored stimulus lights located directly above the lever. Responses on the other (inactive) lever produced only the illumination of the stimulus lights for the duration of an infusion, but they did not activate the infusion pump. Responses on the inactive lever were considered a measure of activity, and responses on both levers were recorded using Med-PC IV software (Med Associates, St Albans, VT., USA) during each session. During self-administration training, sessions began with three experimenter-administered cocaine infusions each separated by 2 sec, and levers were subsequently baited with approximately 0.5 g of food. The criteria for the acquisition of cocaine self-administration consisted of no steadily increasing or decreasing trend in responses, an average of  $\geq 30$  infusions over a 3-day period, and a minimum active/inactive lever response ratio of 2:1. Once rats met these criteria, priming injections and food placement on levers ended, and the subsequent experimental condition commenced.

### *Escalation of cocaine self-administration*

Following the acquisition of cocaine self-administration, rats in Experiments 1



and 2a were allowed to self-administer cocaine (0.4 mg/kg/inf, i.v.) during three short access (ShA) sessions (2 h/day, 9:00-11:00 am) under a FR 1, 20-sec timeout schedule of reinforcement. Rats were then given long access (LgA) (6 h, 9:00 am-3:00 pm) to cocaine self-administration (0.4 mg/kg/inf, i.v.) for 21-days under a FR 1, 20-sec timeout schedule of reinforcement. Self-administration under the 3-day ShA condition was retested after the LgA phase in order to determine if escalation led to changes in ShA cocaine intake.

#### *Maintenance, extinction, and reinstatement of cocaine-seeking behavior*

Once rats achieved acquisition criteria, in Experiments 3, 4, and 5 they were allowed to maintain cocaine intake during 2-h sessions (9:00-11:00 am) for 14 days (maintenance phase) under the same experimental conditions described for the training procedure. After this maintenance period, rats began the extinction condition, and cocaine solutions were replaced with saline. They were then allowed to extinguish cocaine-maintained responding over a 21-day period (2 h/day, 9:00-11:00 am). During this time, all experimental conditions, with the exception of response-contingent i.v. saline, were identical to those during maintenance.

Following the extinction of cocaine-maintained responding, stimulus lights, house lights, and syringe pumps were deactivated 3 days prior to reinstatement testing and remained off throughout the rest of the study (de Vries et al. 1998). This was done as previous studies have shown that the presentation of a compound stimulus (cocaine and auditory/visual cues) increased reinstatement responding (relative to exposure to a single

stimuli) even after responding for one of the stimuli (e.g., cue light) had been extinguished (See et al. 1999; Beardsley et al. 2005). Responding on the previously active lever was counted but had no consequence. During this 3-day period, pretreatment with hormones or VEH began in order to allow rats to acclimate to the treatment procedure and achieve stable hormone levels. Rats from Experiment 3 received EST, PROG, EST+PROG, or VEH, while rats from Experiment 4 received ALLO, PROG, FIN+PROG or VEH. Injections occurred 30 min (8:30 am) prior to the sessions and continued daily throughout reinstatement testing. Subjects from Experiment 5 did not receive pretreatment with hormones or VEH until the reinstatement procedure began.

## **Experiment 1: The role of progesterone in the escalation of cocaine self-administration in female rats**

### ***Background***

The transition from steady to dysregulated drug consumption characterizes the escalation phase of the drug abuse process (Ahmed and Koob 1998, 1999; Lynch et al. 2000). In humans, escalation represents out-of-control drug bingeing (Kalivas and Volkow 2005) that is linked to overdose and death (Kalivas and Volkow 2005). Escalation also occurs in animals with several drugs (Ahmed and Koob 1998, 1999, 2005; Kitamura et al. 2006), and it is typically achieved by increasing the animal's access to the drug (Fitch and Roberts 1993; Lynch and Carroll 2001; Lynch et al. 2000; Morgan and Roberts 2004). Following a change to extended access, drug intake increases over time, and binge and rest patterns replace steady-state patterns of intake.

Studies using differential-access paradigms (LgA vs. ShA) indicate that escalation of cocaine self-administration in rats (Roth and Carroll 2004) and PCP in rhesus monkeys (Carroll et al. 2005) was greater for females than males during LgA. In two studies using a 24 h/day discrete trial procedure, it was found that females self-administered more cocaine and had less ability to titrate and/or control their cocaine intake compared to males (Lynch and Taylor 2004, 2005), and administration of EST enhanced drug intake in OVX female rats relative to VEH-treated controls (Lynch and Taylor 2005). In another study using dose self-selection methodology during 5-h daily sessions, females were less able to regulate and/or control their drug intake compared to males, and females showed greater dysregulation during the estrus phase than other phases of the estrous cycle (Lynch et al. 2000). These findings implicate female gonadal hormones in the regulation and escalation of cocaine self-administration under extended-access conditions. However, despite evidence implicating EST in the facilitation of cocaine bingeing, little is known of the effect of PROG.

The goal of Experiment 1 was to examine the effects of EST and PROG on the escalation of cocaine self-administration in female rats given LgA to cocaine. Using the escalation procedure outlined in the General Methods section, five groups of female rats were compared: 1) SH-operated rats treated with VEH (SH-VEH), 2) SH treated with PROG (SH-PROG), 3) ovariectomized rats treated with VEH (OVX-VEH), 4) OVX rats treated with estradiol benzoate (OVX-EST), and 5) OVX treated with EST and PROG (OVX-EST+PROG). They were subsequently tested under a PR schedule of reinforcement to determine their relative motivation for cocaine-seeking as a function of

hormonal status. It was hypothesized that PROG would suppress cocaine self-administration under the LgA and PR conditions in the PROG-treated (SH-PROG and OVX-EST+PROG) and OVX-VEH groups relative to the SH-VEH and OVX-EST groups. It was also hypothesized that OVX-EST rats would show enhanced cocaine self-administration under these conditions relative to OVX-VEH rats.

## ***Methods***

### *Subjects*

A total of 58 experimentally naïve adult (>60 days) female Wistar rats (Harlan Sprague-Dawley, Madison, WI) were used in this study. Prior to surgery, rats were randomly assigned to one of five experimental groups: 1) SH-VEH (n=13), 2) SH-PROG (N=9) 3) OVX-VEH (n=13), 4) OVX-EST (n=13), and 5) OVX-EST+PROG (N =10).

### *Procedure*

Rats were tested on cocaine self-administration under ShA and LgA conditions using the escalation procedure described in the General Methods. In order to further assess the motivation for cocaine self-administration after the LgA phase, they were given 3-h (9:00 am-12:00 pm) daily access to cocaine self-administration under a PR-schedule of reinforcement following the second ShA condition. To generate dose-effect functions, the total infusions per session were assessed at four different cocaine doses presented in nonsystematic order: 0.2, 0.4, 0.8, and 1.6 mg/kg. Each dose was tested

until stable rates of responding were achieved (daily infusions within 20% of the mean for 3 days, with no increasing or decreasing trends).

### *Data analysis*

All statistical analyses were conducted using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD) and MacAnova (Gary W. Oehlert and Christopher Bingham, University of Minnesota, MN). The number of cocaine infusions self-administered during LgA was averaged into 7 blocks of 3 days each and subsequently analyzed using a two-way ANOVA (group and block) with block as a repeated measure. Comparisons of the number of infusions self-administered during ShA sessions either before or after LgA were analyzed by a two-way repeated measures ANOVA (group and phase), and the number of infusions achieved during PR testing was also analyzed with a two-way repeated measures ANOVA (group and dose) with dose as a repeated measure. Post-hoc comparisons of these data were made using Fischer's LSD protected t-tests. Results were considered significant if  $p < 0.05$ .

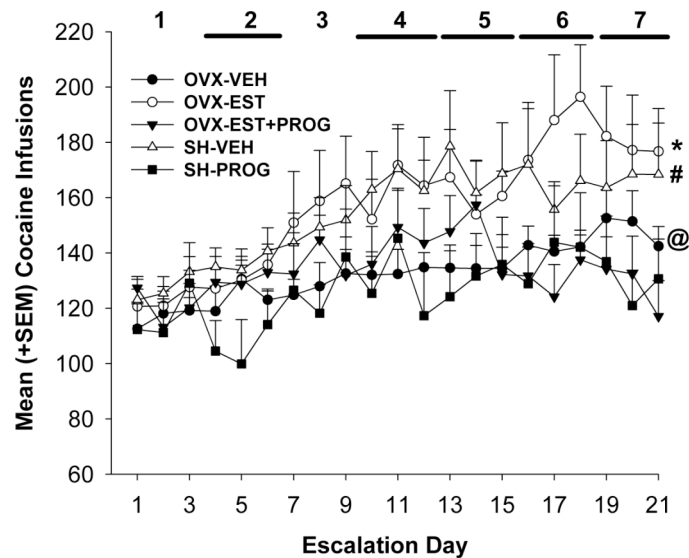
## ***Results***

### *Training*

During training when rats self-administered cocaine (0.4 mg/kg) in daily 2-h ShA sessions the mean ( $\pm$  SEM) number of days to reach training criteria was 10.9 ( $\pm$  2.1), 25 ( $\pm$  6), 9.4 ( $\pm$  1.5), 17.6 ( $\pm$  4.3), and 14.1 ( $\pm$  2) for the SH-VEH, SH-PROG, OVX-VEH, OVX-EST, and OVX-EST+PROG groups, respectively.

### *LgA Phase*

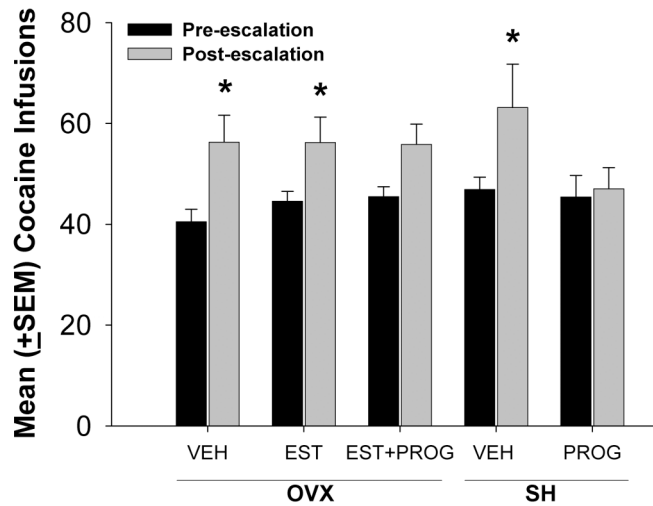
Figure 4 illustrates the mean ( $\pm$  SEM) number of cocaine infusions (0.4 mg/kg) self-administered under the FR 1 schedule during each day of LgA (6 h/day) to cocaine self-administration. There was a significant main effect of group ( $F_{4,300} = 2.748$ ,  $p < 0.05$ ) and block ( $F_{6,300} = 16.667$ ,  $p < 0.01$ ) on the number of cocaine infusions self-administered. Post-hoc comparisons indicated that compared to the first 3-day block OVX-EST rats had elevated (escalated) cocaine self-administration during blocks 3-7 ( $p < 0.05$ ), and SH-VEH showed escalated cocaine self-administration during blocks 4-7 ( $p < 0.05$ ). Cocaine self-administration also increased over time in the OVX-VEH group: block 1 was less than blocks 5, 6, and 7. There were no block differences in the PROG-treated groups (SH-PROG and OVX-EST+PROG). Group comparisons of cocaine self-administration within individual blocks indicated that in block 2, the SH-PROG group was significantly lower ( $p < 0.05$ ) than the OVX-VEH, OVX-EST, OVX-EST+PROG, and SH-VEH groups. In blocks 4 and 5 the SH-PROG group was significantly lower ( $p < 0.05$ ) than the OVX-EST and SH-VEH groups. In block 6, the OVX-VEH, OVX-EST+PROG, and SH-PROG groups were significantly lower than OVX-EST group and in block 7 the SH-PROG group was significantly lower ( $p < 0.05$ ) than the SH-VEH and OVX-EST groups, while the OVX-EST+PROG was lower than the OVX-EST group. Thus, administration of EST alone increased cocaine intake, while exogenous or administered PROG (SH-PROG, OVX-EST+PROG) resulted in reduced escalation of cocaine self-administration.



**Figure 4** Data represent the mean ( $\pm$  SEM) cocaine infusions self-administered each day of the LgA phase. Horizontal lines indicate the 3-day intervals during which there were significant group differences in drug deliveries ( $p < 0.05$ ). \* =  $p < 0.05$  block 1 < blocks 3-7; block 2 < blocks 5-7 in the OVX-EST group and # =  $p < 0.05$  block 1 < blocks 4-7 in the SH-VEH group. Reprinted with permission from: Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007) Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Exp Clin Psychopharmacol* 15: 461-71 (Figure 1, pg 466)

#### Comparison of cocaine ShA self-administration Pre- vs. Post-LgA

Figure 5 illustrates the mean ( $\pm$  SEM) number of cocaine infusions self-administered under ShA (2 h/day) conditions as assessed either before (black bars) or after (gray bars) exposure to LgA conditions. When examining individual rat data, there was a significant pre- vs. post-LgA effect on cocaine infusions ( $F_{1,47} = 14.2792$ ,  $p < 0.01$ ), and post-hoc comparisons indicated that all groups significantly increased their cocaine infusions after the LgA condition ( $p < 0.05$ ), except the PROG-treated groups (SH-PROG, and OVX-EST+PROG). There were no significant differences between groups in cocaine self-administration during either the pre-LgA or post-LgA phases, and there was no group X phase interaction.

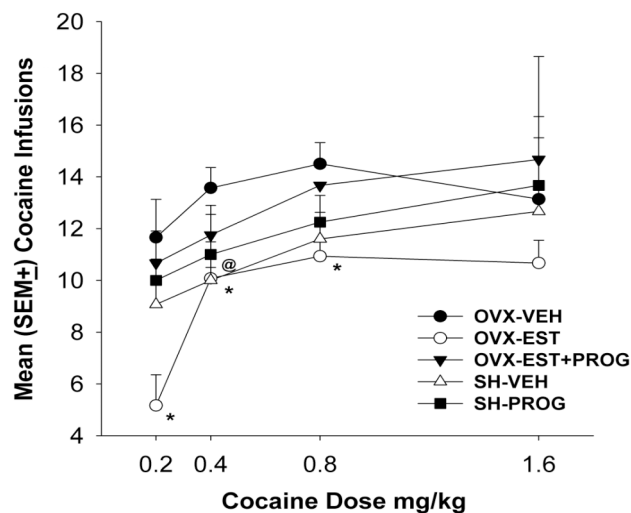


**Figure 5** Mean ( $\pm$  SEM) cocaine infusions self-administered during ShA (2-h) sessions either before (black bars) or after (gray bars) the LgA phase \* =  $p < 0.05$  post LgA vs. pre LgA phase, within group. Reprinted with permission from: Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007) Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Exp Clin Psychopharmacol* 15: 461-71 (Figure 2, pg 467)

### Post-LgA PR

Figure 6 depicts the mean ( $\pm$  SEM) number of infusions obtained under a PR schedule of reinforcement during 3-h access sessions for the four doses of cocaine. The maximum number of cocaine infusions obtained varied as a function of cocaine dose ( $F_{3,89} = 8.2757$ ,  $p < 0.0001$ ), and responding peaked at the 0.8 mg/kg dose in all groups. The number of cocaine infusions obtained under the PR schedule also differed between groups ( $F_{4,89} = 5.4970$ ,  $p = 0.001$ ); however, there was no group X dose interaction. Post-hoc between-group comparisons at each individual cocaine dose revealed only a few group differences. OVX-EST rats earned fewer infusions compared to OVX-VEH rats at the 0.2, 0.4, and 0.8 mg/kg doses ( $p < 0.05$ ). Similarly, SH-VEH rats earned fewer cocaine infusions compared to OVX-VEH rats at the 0.4 mg/kg dose ( $p < 0.05$ ).





**Figure 6** Mean ( $\pm$  SEM) cocaine infusions obtained under a PR schedule (3 h/day), assessed after the LgA phase. \* =  $p < 0.05$  OVX-EST compared to OVX-VEH group, and @ =  $p < 0.05$  SH-PROG compared to OVX-VEH group. Reprinted with permission from: Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007) Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Exp Clin Psychopharmacol* 15: 461-71 (Figure 3, pg 468)

### Vaginal Cytology

Examination of vaginal cytology in the five groups confirmed that rats' hormonal status was consistent with their treatment group; however, cycles became irregular following prolonged exposure to cocaine under the extended-access condition. There were no differences in cocaine intake across the estrus cycle.

### Discussion

The objective of the present study was to compare the effects of PROG and EST on the escalation of cocaine self-administration in female rats during LgA to cocaine. Prior to LgA, all groups exhibited similar levels of ShA cocaine intake, a finding that is consistent with previous studies (Caine et al. 2004; Larson et al. 2005; Lynch and Carroll

2000; Roth and Carroll 2004). However, when access was subsequently extended to 6 h/day (LgA), groups SH-VEH, OVX-EST, and OVX-VEH escalated cocaine intake, while the PROG-treated groups (SH-PROG, OVX-EST+PROG) did not show a significant escalation. Furthermore, OVX-EST rats escalated more rapidly and self-administered more cocaine during LgA than OVX-VEH. Similar to results reported for the acquisition of cocaine self-administration (Jackson et al. 2006), exogenously-administered PROG inhibited the escalation of cocaine intake, while EST potentiated this process. These findings suggest that PROG's inhibitory effects were due to the presence of EST (Jackson et al. 2006; Peris et al. 1991; Quinones-Jenab et al. 2000b; Romieu et al. 2003), as PROG reduced the facilitating effects of EST to levels that were comparable to the OVX-VEH rats.

During PR testing, OVX-VEH rats earned more cocaine infusions than SH-PROG and OVX-EST rats, an effect that was more pronounced at lower cocaine doses (0.2 and 0.8 mg/kg). These results were unexpected, as it has been reported in previous studies that female rats earned more infusions of cocaine than male under PR schedules (Roberts et al. 1989; Roth and Carroll 2004). However, this finding is consistent with other work that compared responding on a PR schedule before and after LgA under a discrete trials procedure in OVX female rats treated with EST or VEH (Lynch and Taylor 2005). These results also indicated that i.v. cocaine intake under a PR schedule was decreased in OVX-VEH rats compared to pre-LgA baselines, and EST administration reversed this post LgA decrement in PR cocaine intake (Lynch and Taylor 2005).

Overall, the results of Experiment 1 suggest that gonadal hormones, EST and PROG, have opposite effects on the escalation of cocaine self-administration in female rats. Thus, hormonal status may contribute to both the susceptibility toward (EST) and protection against (PROG) drug bingeing, a stage of the drug abuse process to which women are especially vulnerable.

### **Experiment 2a: The role of allopregnanolone in the escalation of cocaine self-administration in female rats**

#### ***Background***

The effects of PROG on the escalation of cocaine self-administration may be related to its metabolite ALLO. As previously mentioned, ALLO plasma levels increase following systemic PROG injection (Soderpalm et al. 2004), and ALLO has been shown to suppress behaviors elicited by drugs of abuse (Kaminski et al. 2003; Reddy and Kulkarni 1997a). Therefore, ALLO may contribute to PROG-induced decreases in the escalation of cocaine self-administration. The goal of Experiment 2a was to extend the results with PROG in Experiment 1 and investigate a role for ALLO in suppressing the escalation of cocaine self-administration in female rats.

#### ***Methods***

##### ***Subjects***

A total of 9 ALLO-treated female Wistar rats were compared to 10 VEH-treated rats from Experiment 1. Rats received jugular catheterization surgery as described in the

General Methods section. Vehicle or ALLO was administered 30 min before the start of each session (8:30 am) beginning 3 days after surgery and throughout the duration of the experiment.

### *Procedure*

Rats were trained and allowed to self-administer cocaine under the training and escalation procedures described in the General Methods section. Administration of ALLO and all other details of the experiment were the same as Experiment 1.

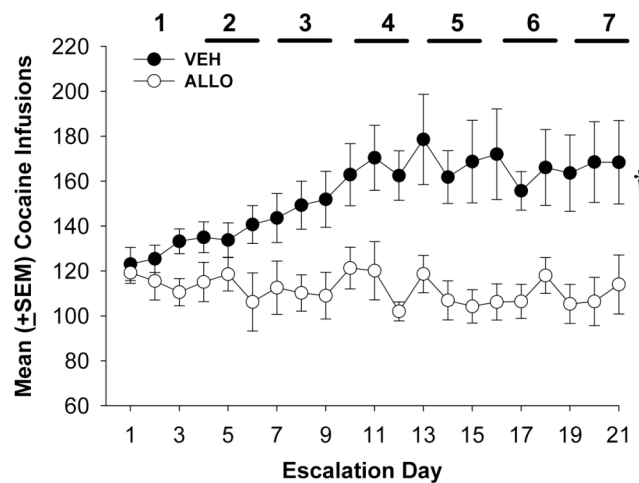
### *Data Analysis*

Active and inactive lever responses and cocaine infusions during LgA were averaged into 7 blocks of 3 days each and analyzed using a two-factor repeated measures ANOVA (group X Block). Comparison of the number of cocaine infusions during ShA sessions before and after LgA were analyzed using a two-factor repeated measures ANOVA (group X phase). Post-hoc comparisons were conducted using Fisher's LSD protected t-tests, and all statistical analyses were conducted using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD) and MacAnova (Gary W. Oehlert and Christopher Bingham, University of Minnesota, MN).

### *Results*

Figure 7 illustrates the mean ( $\pm$  SEM) number of cocaine infusions (0.4 mg/kg) self-administered under the FR 1 schedule during each day of LgA (6 h/day) to cocaine

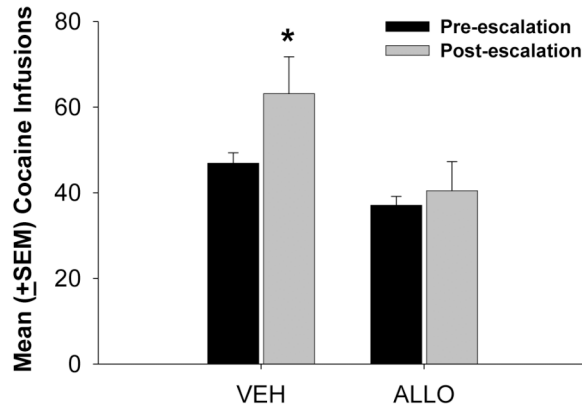
self-administration. There was a significant main effect of group ( $F_{1,132} = 12.367$ ,  $p < 0.01$ ) and block ( $F_{6,132} = 2.495$ ,  $p < 0.05$ ) and a significant group X block interaction ( $F_{6,132} = 3.782$ ,  $p < 0.01$ ) on the number of cocaine infusions self-administered during LgA. Post-hoc comparisons indicated that, compared to the first 3-day block, VEH-treated rats had increased (escalated) cocaine self-administration during blocks 2-7 ( $p < 0.05$ ), while ALLO-treated rats maintained stable cocaine intake throughout the escalation procedure. Group comparisons revealed that the VEH group self-administered significantly more cocaine than the ALLO group in blocks 2-7. Thus, ALLO blocked the escalation of cocaine self-administration and attenuated cocaine intake.



**Figure 7** Data represent the mean ( $\pm$ SEM) cocaine infusions self-administered by female rats each day of the LgA phase. Horizontal lines indicate 3-day intervals during which there were significant group differences ( $p < 0.05$ ). † =  $p < 0.05$  block 1 < blocks 3-7 in the VEH group

Figure 8 illustrates the mean ( $\pm$  SEM) number of cocaine infusions self-administered under ShA (2 h/day) conditions, assessed either before (black bars) or after (gray bars) exposure to LgA conditions. There was a significant effect of group on

cocaine infusions ( $F_{1,37} = 8.053$ ,  $p < 0.05$ ), but there was not a main effect for the pre- or post-ShA phases nor a significant group X phase interaction.



**Figure 8** Mean ( $\pm$ SEM) cocaine infusions self-administered by female rats during ShA sessions before (black bars) and after (gray bars) the LgA phase \* =  $p < 0.05$  post LgA vs. pre LgA within group.

## **Experiment 2b: The role of allopregnanolone in the escalation of sucrose self-administration in female rats**

### ***Background***

To control for the specificity of ALLO's effects on the escalation of cocaine intake, a group self-administering orally delivered sucrose was compared to the cocaine group. Previous studies have shown that escalation occurs with behavior reinforced by nondrug rewards such as sucrose (Colantuoni et al. 2001, 2002) and wheel running (Larson and Carroll 2005). Furthermore, clinical (Edler et al. 2007; Gladis and Walsh 1987; Price et al. 1987) and preclinical (Leibowitz et al. 2007; Klump et al. 2008) research implicates ALLO's precursor PROG in the modulation of binge-like patterns of food intake. Thus, Experiment 2b examined the effects of ALLO on the escalation of responding for sucrose.

## ***Methods***

### *Subjects*

Fourteen female Wistar rats were used in Experiment 2b (VEH, n=6; ALLO, n=8). Rats underwent a SH jugular catheterization surgery (see General Methods) to match conditions with the cocaine group, but they were not implanted with a catheter. Following surgery, they were fitted with an infusion harness and tether and allowed to recover for a 3-day period before being tested on sucrose intake.

### *Procedure*

Following the stabilization of water intake after surgery ( $\geq 20$  ml water consumed for 3 consecutive days) rats were trained to drink through the lick-o-meter devices described in the General Methods section. The morning after rats achieved stable water intake, water bottles were removed from the operant chambers, the 500-ml reservoir was filled with tap water, and they subsequently had 24-h access (8:00 am to 8:00 am the next day) to water contingent on contact with the active spout. During this time, a contact on the inactive spout was recorded and produced the same experimental consequence as a contact on the active spout with the exception that it did not deliver water. The next day the water bottle was returned to the chamber. If a rat obtained 100 or more deliveries during the 24-h training session, the water in the 500-ml reservoir was replaced with a 10% sucrose solution, the session length was changed to a 2-h session (9:00 am to 11:00 am), and the rat began treatment with VEH or ALLO (s.c. at 8:30 am). If the rat did not achieve the acquisition criteria during the 24-h training session, the water bottle was

returned for at least 3 days until water intake again stabilized, and the 24-h training session resumed. If a rat did not achieve acquisition after three training sessions, it was excluded from the study. Following acquisition, rats self-administered sucrose during three 2-h ShA sessions (9:00 am to 11:00 am) and then session length was extended to 6 h (LgA) (9:00 am to 3:00 pm) for 21 days. Sucrose intake under the ShA condition (3 days) was then reassessed following LgA. A 10% sucrose solution was used in this experiment, as it has previously been shown to reliably produce escalation of sucrose intake under extended access conditions (Rada et al. 2005).

### *Data Analysis*

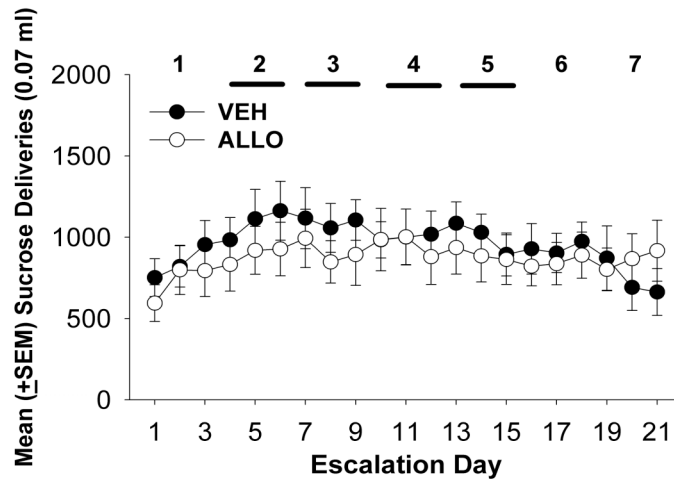
The same statistical procedures and software used in Experiment 2b for analyzing sucrose deliveries were used to analyze cocaine deliveries during the ShA and LgA conditions in Experiment 2a.

### *Results*

Figure 9 depicts mean sucrose deliveries for ALLO- and VEH-treated rats across the 21 days of extended access. Analysis of deliveries indicated a significant main effect of block ( $F_{6,90} = 2.566$ ,  $p < 0.05$ ) but no main effect of group nor a significant group X block interaction. Given the lack of a main interaction, the two groups were combined and reanalyzed across days. The results indicated a significant main effect ( $F_{6,90} = 2.743$ ,  $p < 0.05$ ), and subsequent post-hoc analyses showed that rats significantly increased

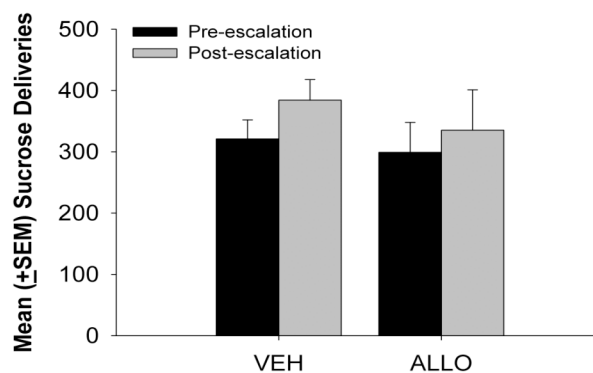


sucrose intake during session blocks 2, 3, 4, and 5 compared to block 1 ( $p < 0.05$ ), regardless of treatment group.



**Figure 9** Data represent mean ( $\pm$ SEM) sucrose deliveries self-administered by female rats under the 21-day LgA condition. There were no significant differences under this condition. Horizontal lines indicate significant difference between the 1<sup>st</sup> block of days and blocks 2-5.

Comparison of mean sucrose deliveries earned during three ShA sessions before and after LgA for the ALLO and VEH groups is shown in Figure 10. Data analysis revealed no significant main effects or interactions.



**Figure 10** Mean ( $\pm$ SEM) sucrose deliveries self-administered by female rats during ShA sessions.

## **Experiment 2c: Controls for the sedative effects of allopregnanolone**

### ***Background***

Results from humans (van Broekhoven et al. 2007; Timby et al. 2006) and rodents (Korneyev and Costa 1996; Lancel et al. 1997) indicated that ALLO results in sedation when administered in large doses. Thus, in order to determine whether ALLO's effect on cocaine escalation was due to ataxia, ALLO was tested for its influence on locomotor activity in a locomotor track similar to that described by Carroll et al. (2007) and food-maintained responding under an operant conditioning delay-discounting task similar to that previously described by Perry et al. (2005).

### ***Methods***

#### ***Subjects***

Fourteen experimentally naïve female Wistar rats (7 VEH treated and 7 ALLO treated) were used for a locomotor control condition, and 9 naïve females were used for a delay discounting (food-maintained) control experiment.

#### ***Procedure***

##### **Locomotor activity**

Prior to activity testing in the locomotor tracks (General methods), rats received 4 days of ALLO (n=7) or VEH (n=7) pretreatment at 8:30 am. Locomotor activity was then assessed in daily 45-min sessions beginning at 9:00 am for 5 days, while rats also continued to receive ALLO or VEH daily.

## Delay discounting for food

When not in the test apparatus, rats were individually housed in plastic home cages. During experimental sessions, chambers identical to the self-administration chambers were used with the exception that the food receptacle was replaced with a 45-mg pellet feeder that was secured to a pellet-delivery trough (Coulbourn Instruments, Allentown, NJ). During the sessions, two levers were available, and a response on one lever produced a small-immediate reward (one 45-mg pellet), while a response on the other lever produced a large-delayed reward (three 45-mg pellets). Experimental sessions consisted of 15, 4-trial blocks. Trials one and two were a forced-choice trial on one lever (associated with immediate small reward) followed by a forced-choice trial on the opposite lever (associated with large delayed reward), respectively. Trials three and four were free-choice trials.

The initial delay of the large reward was set at 6 sec, and thereafter the delay decreased by 1 sec for each response on the small-immediate reward lever and increased by 1 sec after each response on the large-delayed reward lever. The adjusting delay determined on the fourth trial of each block was used as the delay in the forced-choice trials in the next block of trials, and the final adjusting delay of the session was used for the initial adjusting delay for the subsequent session. A mean adjusted delay (MAD) was calculated for each session by averaging the delays during 30 free-choice trial.

Once rats reached stability on the delay-discounting task, they were administered s.c. injections of VEH 30 min before session. Stability on the delay discounting task was defined as 4 consecutive days with 60 trials and a difference in MAD values of no more

than 5 sec with no increasing or decreasing trend. They were maintained an additional 5 days on the delay discounting procedure in order to establish pretreatment baseline measures of food intake, trials, and MAD values. Subsequently, rats were treated with 30 mg/kg ALLO 30 min prior to the session for 9 daily sessions to determine ALLO's effect on this sensitive operant baseline. A 9-day ALLO treatment regimen, with injections occurring 30 min prior to behavioral testing, was selected for the locomotor and delay discounting conditions, as it was the same treatment schedule employed in reinstatement experiments (e.g., Experiment 4).

### *Data analysis*

Beam breaks (locomotor control experiment only), trials, food pellets, and MAD (delay discounting) values served as the primary dependent measures, and they were compared using a within-subject, repeated-measures ANOVAs with day as the repeated factor.

### ***Results***

Table 4 shows the mean ( $\pm$  SEM) values for locomotor activity and food maintained responding under the delay-discounting schedule. There were no significant group differences in the total number of beam breaks during locomotor testing; however, there was an effect of day on locomotor testing (not shown), and beam breaks decreased similarly over subsequent testing days in both groups ( $F_{4, 42} = 4.48, p < 0.01$ ). Following ALLO administration, the number of trials completed, pellets earned, trials, and MAD

values obtained during delay-discounting sessions did not significantly differ in the VEH and ALLO treated groups/conditions. These results suggest that the ALLO doses used in this study did not alter locomotor activity or responding under an operant schedule maintained by food.

**Table 4** ALLO does not alter locomotor activity or food-maintained responding in female (F) rats

| Injection | Locomotor   | Food-reinforced behavior |                |                           |
|-----------|-------------|--------------------------|----------------|---------------------------|
|           | Beam breaks | Trials completed         | Pellets earned | Mean adjusted delay (sec) |
| F-VEH     | 33.8 ± 6.4  | 56.9 ± 0.9               | 113.3 ± 1.7    | 12.7 ± 0.8                |
| F-ALLO    | 26.0 ± 5.4  | 55.8 ± 0.7               | 111.4 ± 1.5    | 15.1 ± 0.4                |

### *Discussion*

The results of Experiment 2a extended the findings of Experiment 1 and showed that ALLO, like its precursor PROG, attenuated the escalation of cocaine self-administration in female rats. The effects of ALLO on reinforcement under an extended-access condition were specific to cocaine, as ALLO failed to interfere with the escalation of sucrose intake in Experiment 2b. These results indicated that ALLO reliably and specifically blocked the escalation of cocaine self-administration in female rats. The sucrose study (Experiment 2b) also showed that ALLO did not interfere with motor behavior required to lick from an automated drinking spout.

Palatable substances and drugs of abuse share several common features (for a review see Avena et al. 2008; Carroll et al. 2008), and this was demonstrated in the present study. Similar to cocaine, extended access to a 10% sucrose solution resulted in

an escalation of intake over a 21-day period. This result was consistent with previous reports demonstrating escalated sucrose intake under similar extended-access procedures (Avena 2007; Avena et al. 2006; 2009). For example, in one study, rats given 12-h access to a 10% sucrose solution exhibited binge-like patterns of sucrose intake (Rada et al. 2005) that resembled the escalated patterns typically seen in rats with extended access to i.v. cocaine. However, similar to its effects on ShA (1 h) sucrose intake in a previous study (Janak and Gill 2003), ALLO did not suppress sucrose intake under an extended-access condition in the present study, demonstrating the specificity of ALLO's attenuating effects to the escalation of cocaine self-administration.

Allopregnanolone (at the highest dose tested during reinstatement) also had no effect on general locomotor activity or performance under an operant-conditioning schedule for food reinforcement in females (Experiment 2c). Therefore, it is unlikely that ALLO reduced cocaine-seeking by nonspecific behavioral suppressant effects due to sedation. In fact, previous data indicated that ALLO brain levels following a 15 mg/kg injection were far below the levels needed to produce a loss of a righting reflex [64.5 pmol/g (Lancel et al. 1997) vs. 3000 pmol/g (Korneyev and Costa 1996 in Lancel et al. 1997)] in rats. Results from the control conditions also agree with previous findings showing that ALLO did not decrease locomotor activity (Beauchamp et al. 2000; Ford et al. 2007) or responding maintained by saccharin (Sinnott et al. 2002) or sucrose (Experiment 2b; Janak and Gill 2003).

In summary, the results of Experiment 2 extended those of Experiment 1 and demonstrated that the PROG metabolite, ALLO, blocked the escalation of cocaine self-

administration. Sucrose, like cocaine, also produced escalation of intake over the 21-day extended-access condition, but it was unaffected by pretreatment with ALLO, and ALLO did not produce a general disruption in locomotor or operant behavior. These results suggest the action of ALLO on escalation of cocaine intake is specific to the drug's reinforcing effects and does not extend to nondrug reinforcers. However, the effects of ALLO on the escalation of other drugs of abuse such as alcohol and heroin remains to be examined.

### **Experiment 3: The effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats**

#### ***Background***

In addition to a heightened propensity toward drug bingeing, reports indicate that women are also more vulnerable to drug abuse relapse (Ignjatova and Raleva 2009). Preclinical work supports clinical findings and indicates that females exhibit greater cocaine-induced reinstatement of cocaine seeking than males (Lynch and Carroll 2000), and reinstatement differs during different phases of the hormone cycle (Carroll and Anker 2009; Feltenstein and See 2007; Kerstetter et al. 2008; Kippin et al. 2005). Furthermore, administration of EST in OVX female rats potentiates cocaine-induced reinstatement relative to OVX female rats treated with VEH (Larson et al. 2005). Thus, both clinical and preclinical research indicate that there are sex differences in relapse to drug-seeking behavior, and initial preclinical work suggests that EST contributes to this sex difference.

However, few studies have examined the contribution of PROG in the reinstatement of cocaine-seeking behavior.

The goal of the present study was to extend the results from Experiment 1 to another phase of the drug abuse process, reinstatement, that is considered a model of relapse and a major hallmark of addiction. The effects of PROG on the reinstatement of cocaine seeking in female rats was compared under different hormonal conditions, and it was hypothesized that the administration of PROG in intact (i.e., SH) and OVX-EST female rats would decrease reinstatement of cocaine seeking (i.e., pressing on the previously active lever).

## ***Methods***

### *Subjects*

Forty-nine female Wistar rats, weighing between 250-350 g at the start of the experiment, were assigned to 5 groups (OVX-EST, n = 10; OVX-EST+PROG, n = 7; OVX-VEH, n = 9; SH-PROG, n = 10; and SH-VEH, n = 13. Ovariectomized animals in the EST and VEH groups and the SH-VEH group were part of previously published research (Larson et al. 2005), and additional animals were added for the OVX-EST+PROG and SH-PROG groups in the present study.

### *Procedure*

Procedural details for the training, maintenance, extinction, pre-reinstatement, and reinstatement conditions are outlined in the General Methods section.



### *Data Analysis*

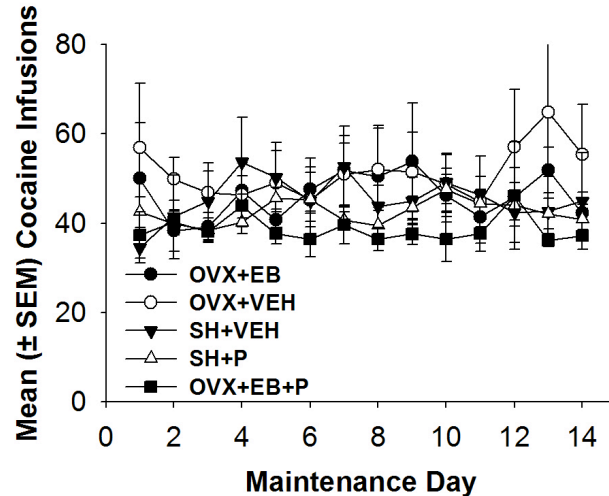
Infusions during maintenance and extinction and responses during reinstatement served as the primary dependent measures in this study. The numbers of infusions during the maintenance and extinction periods were compared using two-factor ANOVA with group as the between-subjects factor and day as the repeated factor. Active and inactive lever responses during reinstatement were compared using separate two-factor ANOVA with group as the between-subjects factor and dose (i.e., saline or cocaine) as the repeated factor. After a significant main effect, post-hoc tests were conducted using Fisher's LSD protected t-tests. Results were considered significant if  $p < 0.05$ .

### ***Results***

There were no group differences in the number of days to acquire cocaine self-administration. The mean ( $\pm$  SEM) number of days to reach the acquisition criteria was 14.4 ( $\pm$  3.4) days in the SH-VEH group, 8.9 ( $\pm$  2.1) in the SH-PROG group, 17.0 ( $\pm$  4.1) in the OVX-VEH group, 16.2 ( $\pm$  3.7) in the OVX-EST group, and 22.0 ( $\pm$  9.6) in the OVX-EST+PROG group. It should be stressed that during acquisition, maintenance, and extinction groups were not treated with either VEH or hormone, and treatment did not occur until the pre-reinstatement phase, 3 days prior to the reinstatement testing.

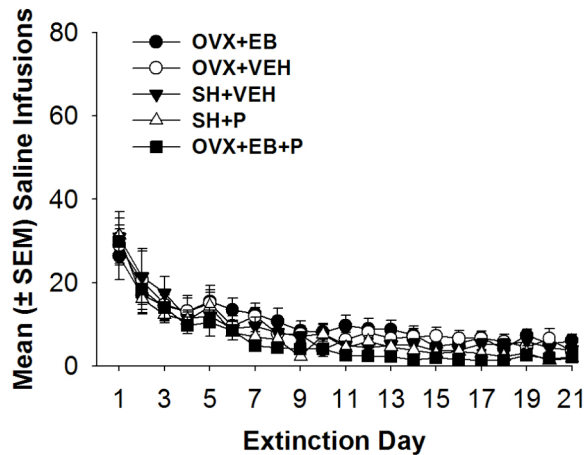
Figure 11 shows that there were also no significant differences in cocaine self-administration between groups or across the 14 days of the maintenance phase. Thus, all groups self-administered similar amounts of cocaine during maintenance, and the number of cocaine infusions remained relatively stable across the 14 days. For all groups, active

lever responding was significantly greater than inactive lever responding during the maintenance period ( $F_{1, 97} = 87.07, p < 0.0001$ ).



**Figure 11** Mean cocaine (0.4 mg/kg) infusions ( $\pm$ SEM) self-administered during 14 consecutive 2-h sessions by 5 groups of female rats. During this time, groups did not receive VEH or hormone treatment. Reprinted with permission from: Anker JJ, Larson EB, Gliddon LA, Carroll ME (2007) Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Exp Clin Psychopharmacol* 15: 472-80 (Figure 2, pg 476)

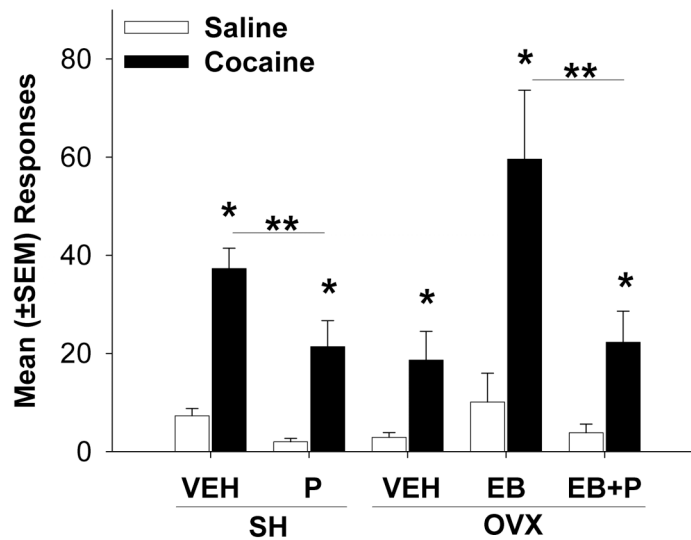
Figure 12 indicates that during extinction there were also no group differences in the number of saline infusions self-administered; however, there was a significant day effect, as the number of infusions of saline self-administered decreased in all rats across the 21-day extinction period ( $F_{20, 1028} = 32.18, p < 0.0001$ ). There was also no significant group X day interaction, nor were there group differences in the number of responses on the inactive lever across the extinction period. On the last 3 days before reinstatement, when either hormone or VEH treatment began, conditioned stimuli were eliminated. This did not produce any changes in responding, and lever responding remained at low levels (data not shown).



**Figure 12** Mean saline infusions ( $\pm$ SEM) self-administered during 21 consecutive 2-h extinction sessions by 5 groups of female rats. As with the maintenance phase, groups were not treated with either VEH, EST, or PROG at this time. Reprinted with permission from: Anker JJ, Larson EB, Gliddon LA, Carroll ME (2007) *Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. Exp Clin Psychopharmacol 15: 472-80 (Figure 2, pg 477)*

Figure 13 illustrates responding on the previously active lever during the reinstatement period when a saline (S) or cocaine (C) i.p. priming injection was given before the start of the session. A priming injection of saline had no significant effect on the number of responses made on the previously active lever relative to the inactive lever. In addition, a priming injection of cocaine (10 mg/kg) significantly increased the number of active lever responses relative to those made after a saline priming injection ( $F_{1, 97} = 67.780, p < 0.01$ ). Post-hoc comparisons indicated significant cocaine-induced reinstatement in all groups ( $p < 0.05$ ). Comparisons also indicated significant group differences in the amount of active lever responding ( $F_{4, 97} = 4.511, p = 0.05$ ) and a significant group X priming injection interaction ( $F_{4, 97} = 3.69, p = 0.01$ ). Post-hoc comparisons revealed no group differences in active lever responding after the saline priming injection. In contrast, group differences in active lever responding indicated that

cocaine-induced reinstatement responding in the SH-PROG rats was reduced compared to SH-VEH ( $p < 0.05$ ) and OVX-EST ( $p < 0.01$ ) rats. Similarly, the OVX-EST+PROG group had lower cocaine-induced reinstatement of active lever responding compared to the OVX-EST ( $p < 0.01$ ) and SH-VEH ( $p < 0.01$ ) groups. Cocaine-induced reinstatement responding in the PROG-treated groups (SH-PROG, OVX-EST+PROG) was not significantly different than the OVX-VEH group.



**Figure 13** Black bars represent the mean ( $\pm$ SEM) responses on the cocaine-paired lever and white bars refer to responses on the same lever after the saline priming injections. All groups responded more on the cocaine-paired lever after a 10 mg/kg cocaine i.p. than after a saline i.p. (\* =  $p < 0.05$ ). After the cocaine priming injection the SH-VEH group responded more than the SH-PROG group (\*\* =  $p < 0.01$ ), and the OVX-EST group responded more than the OVX-EST+PROG group (\*\* =  $p < 0.01$ ). Reprinted with permission from: Anker JJ, Larson EB, Gliddon LA, Carroll ME (2007) Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Exp Clin Psychopharmacol* 15: 472-80 (Figure 3, pg 477)

Comparisons of inactive lever responding during reinstatement revealed that cocaine produced increases in general locomotor activity, as indicated by elevated inactive-lever responding after the cocaine (vs. saline) priming injection ( $F_{1,95} = 17.81$ ,  $p < 0.01$ ). Analysis of the active- vs. inactive-lever responding during reinstatement

showed that active responding was higher following cocaine priming injection ( $F_{1,97} = 27.157$   $p < 0.01$ ) than after the saline priming injection. This suggests that cocaine-induced reinstatement of active-lever responding was specific to an increased motivation for cocaine-seeking and not due to general increases in activity.

### ***Discussion***

Similar to the results for the escalation of cocaine self-administration in Experiment 1, PROG treatment attenuated cocaine-induced reinstatement of cocaine-seeking behavior in OVX females treated with EST, while EST treatment alone facilitated reinstatement responding in OVX rats. These effects were not attributable to group differences in behavioral history or recent drug consumption because all groups self-administered cocaine at comparable levels during the maintenance phase, and they showed equivalent responding during the extinction phase that immediately preceded reinstatement. Findings from the present study support previous work by Feltenstein and See (2007) who reported that high plasma PROG levels during the proestrus phase were inversely related to high cocaine seeking during extinction and cocaine-primed reinstatement.

As with previous studies (Jackson et al. 2006; Peris et al. 1991; Quinones-Jenab et al. 2000b; Romieu et al. 2003) and Experiment 1, PROG's inhibitory effects may have been dependent on the presence of EST. For example, Jackson et al. (2006) found that PROG administration in OVX-EST rats decreased the facilitating effects of EST on the acquisition of cocaine self-administration to a level that was similar to that of OVX-VEH

rats. In the present study, PROG treatment in the OVX-EST group decreased the magnitude of cocaine-induced reinstatement to levels found in the OVX-VEH group. However, EST-dependent effects of PROG were not supported by findings from Feltenstein et al. (2009). In this study, attenuation of cocaine-primed reinstatement by systemic PROG was specific to the estrus phase of the hormone cycle, when EST levels are relatively low. Determining if PROG would further reduce drug-seeking behavior in OVX rats that had not received EST would clarify PROG's dependency on EST.

In summary, the present study extended results from Experiment 1 and showed that PROG inhibited cocaine-induced reinstatement in intact female rats. Similar to its effects on escalation, the enhancement of cocaine-induced reinstatement by EST in OVX female rats was attenuated by the administration of PROG, suggesting that PROG counteracted the effects of EST.

#### **Experiment 4: The influence of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats**

##### ***Background***

Experiment 3 demonstrated that PROG attenuated cocaine seeking in an animal model of relapse. However, as mentioned previously (Experiment 2), PROG may act through its metabolite ALLO to produce this effect. The purpose of the present study was to extend initial findings of PROG's inhibitory effects on the reinstatement of cocaine-seeking behavior (Experiment 3) to ALLO. The mechanism of ALLO's effect was further studied by using FIN, a 5-alpha reductase inhibitor that prevents the

metabolism of PROG into ALLO. It was hypothesized that FIN, when administered concurrently with PROG, would block the attenuating effects of PROG on cocaine-primed reinstatement, thus implicating ALLO in PROG's actions. Another goal was to compare the effects of ALLO in male and female rats, as previous findings indicate the presence of sex differences (females>males) in receptivity to behavioral (Cosgrove et al. 2002) and pharmacological (Campbell et al. 2002) interventions for reducing cocaine self-administration in rats.

## ***Methods***

### *Subjects*

Seventy-five sexually mature Wistar rats were used in the present study. Fifty-seven gonadally-intact female (between the ages of 90-120 days and weighing 250-300 g) and 18 male (between the ages of 90-120 days and weighing 350-400 g) rats were used for the self-administration experiment and were assigned (prior to experimentation) to 2 male (M) and 5 female (F) groups that received either ALLO, PROG, PROG+FIN, or VEH treatment: F-VEH, n = 10; F-ALLO (15 mg/kg), n = 11; F-ALLO (30 mg/kg), n = 11; F-PROG, n = 12; F-PROG+FIN, n = 13; M-VEH, n = 9; and M-ALLO (30 mg/kg), n = 9. Rats were assigned to their respective treatment group at the beginning of self-administration training, but treatment did not occur until the last 9 days of the experiment.

## *Procedure*

The self-administration training, maintenance, extinction, pre-reinstatement, and reinstatement phases of Experiment 4 are described in the General Methods section.

## *Data analysis*

Responses and infusions during maintenance and extinction and responses during reinstatement served as the primary dependent measures in this study. Responses and infusions during maintenance and extinction were compared using two-factor mixed ANOVAs with group as the between-subjects factor and day as the repeated factor. Active and inactive lever responses during reinstatement were compared using separate two-factor mixed ANOVA with group as the between-subjects factor and dose (i.e., saline or cocaine) as the repeated factor. Post-hoc tests were conducted using Fisher's LSD protected t-tests. Results were considered significant if  $p < 0.05$ . Statistical analyses were conducted using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD) and MacAnova (Gary W. Oehlert and Christopher Bingham, University of Minnesota, MN).

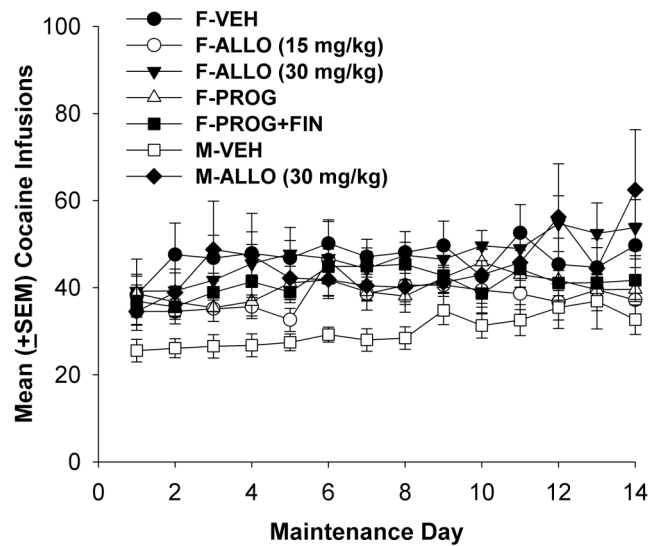
## ***Results***

The mean ( $\pm$  SEM) number of days to reach acquisition criteria differed between treatment groups ( $F_{6,73} = 4.08$ ,  $p < 0.01$ ). Females from all groups took significantly fewer days ( $p < 0.05$ ) to initiate cocaine self-administration than males.

Figure 14 shows the number of infusions self-administered across the 14 days of the maintenance phase in seven 2-day blocks for the 7 groups. Results from the ANOVA



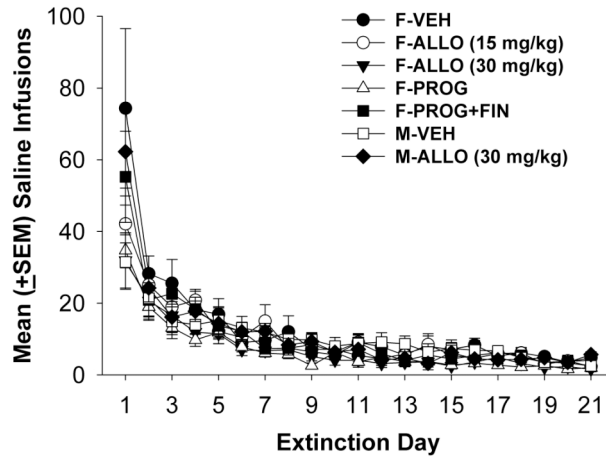
indicated a significant main effect for group ( $F_{6, 524} = 3.81, p < 0.05$ ) and session block ( $F_{6, 524} = 5.75, p < 0.01$ ). Similar analysis of active-lever responding revealed a significant main effect of session block ( $F_{6, 524} = 5.51, p < 0.01$ ). Although the number of infusions differed, the number of active-lever responses during the maintenance phase was comparable among groups. Responses on the inactive lever were negligible (not shown), and there were no significant group differences in this measure.



**Figure 14** Mean cocaine (0.4 mg/kg/infusion) infusions ( $\pm$ SEM) self-administered during 14 consecutive daily 2-h sessions by 5 groups of rats. During this time groups did not receive VEH, PROG, FIN, or ALLO treatment. Reprinted with permission from: Anker JJ, Holtz NA, Zlebnik N, Carroll ME (2009) Effects of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats. *Psychopharmacology (Berl)* 203: 63-72 (Figure 1, pg 67)

Responding on the active lever steadily decreased following the removal of cocaine (Figure 15). Responses and infusions were averaged across seven 3-day blocks and compared between groups. During extinction there was a significant session effect, as the number of responses ( $F_{6, 524} = 90.24, p < 0.0001$ ) and saline infusions ( $F_{6, 524} = 115.95, p < 0.0001$ ) decreased in all rats across the 21-day extinction period. No group

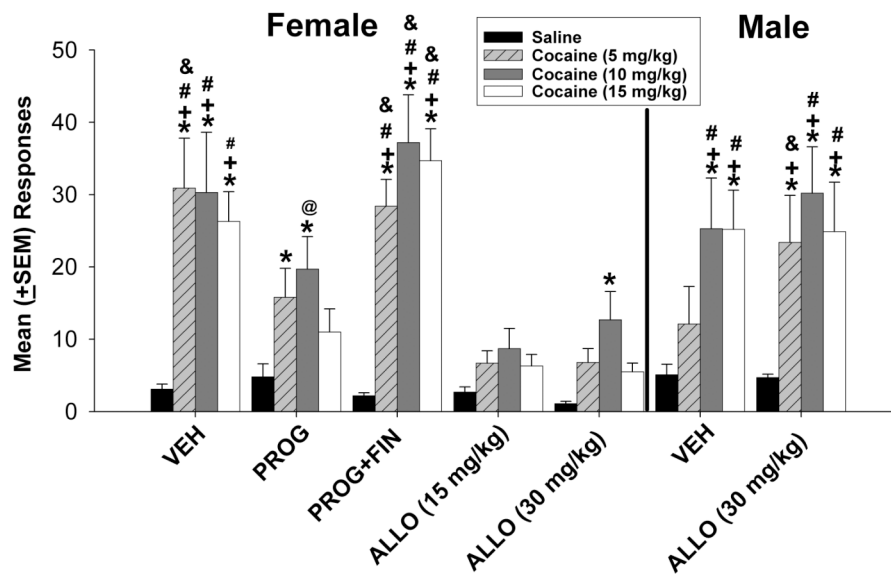
differences were present in inactive-lever responses across the extinction period or during the last 3 days before reinstatement.



**Figure 15** Mean saline infusions ( $\pm$ SEM) self-administered during 21 consecutive 2-h daily extinction sessions by the 7 groups of rats. During this time groups did not receive VEH, PROG, FIN, or ALLO treatment. Reprinted with permission from: Anker JJ, Holtz NA, Zlebnik N, Carroll ME (2009) Effects of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats. *Psychopharmacology (Berl)* 203: 63-72 (Figure 2, pg 67)

Figure 16 illustrates responding on the previously active lever during the reinstatement period when i.p. saline- or cocaine-priming injections were administered 1 min before each daily session. Responses following the saline i.p. injection were grouped and included as a mean of the three saline treatment days. There was a significant main effect of the saline- and cocaine-priming doses ( $F_{3,299} = 46.12, p < 0.0001$ ) and group ( $F_{6,299} = 7.69, p < 0.0001$ ) on reinstatement and a priming injection X group interaction ( $F_{18,299} = 2.82, p < 0.01$ ). Priming injections of 5, 10, and 15 mg/kg cocaine resulted in significantly higher responding than saline-priming injections in F-VEH, F-PROG+FIN, and M-ALLO (30 mg/kg) groups ( $p < 0.05$ ). Similarly, M-VEH rats responded significantly more after 10 and 15 mg/kg (but not 5 mg/kg) cocaine (vs. saline) on the

previously active lever ( $p < 0.05$ ). In contrast, responding after cocaine-priming injections (5, 10, and 15 mg/kg) did not significantly differ from responding following saline injections in the F-ALLO (15 mg/kg) group, while F-ALLO (30 mg/kg) rats responded significantly more following saline only after 10 mg/kg cocaine ( $p < 0.05$ ). In the F-PROG group, responding was significantly higher than saline only following the 5 and 10 mg/kg cocaine-priming injections ( $p < 0.05$ ).



**Figure 16** Mean ( $\pm$ SEM) number of responses on the previously-active lever are presented for all groups. Black bars represent the responses after a saline-priming injection, grey striped bars correspond to responses after 5 mg/kg cocaine-priming injections, dark grey bars are 10 mg/kg cocaine prime groups, and white bars are 15 mg/kg. \* =  $p < 0.05$  vs. saline; † =  $p < 0.05$  vs. F-ALLO (15 and 30 mg/kg); # =  $p < 0.05$  vs. F-PROG; & =  $p < 0.05$  vs. M-VEH; @ =  $p < 0.05$  F-ALLO (15 mg/kg). Reprinted with permission from: Anker JJ, Holtz NA, Zlebnik N, Carroll ME (2009) Effects of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats. *Psychopharmacology (Berl)* 203: 63-72 (Figure 3, pg 68)

Across all of the cocaine priming doses, female rats treated with ALLO (15 and 30 mg/kg) or PROG responded significantly less than female rats treated with VEH or FIN+PROG ( $p < 0.05$ ). This indicated an attenuating effect of PROG and its metabolite

on drug-primed reinstatement. Concurrent FIN and PROG treatment in female rats produced levels of responding similar to female rats treated with VEH alone suggesting the attenuating effects of PROG were blocked by treatment with FIN. Additionally, following a 10 mg/kg cocaine-priming injection, rats treated with 15 mg/kg ALLO responded significantly less than rats treated with PROG ( $p < 0.05$ ). Both male groups (VEH and ALLO treated) responded significantly more than the ALLO- (15 and 30 mg/kg) treated females ( $p < 0.05$ ) across all cocaine doses, and they responded more than PROG-treated females following the 10 mg/kg (M-ALLO group) and 15 mg/kg (M-VEH and M-ALLO groups) doses ( $p < 0.05$ ).

### ***Discussion***

During reinstatement testing, ALLO- (15 and 30 mg/kg) vs. VEH-treated rats decreased responding on the previously active lever across all priming doses of cocaine (5, 10, and 15 mg/kg) in female, but not male groups, suggesting that the effect of ALLO was specific to females at the doses tested. Progesterone also blocked reinstatement, as previously shown in Experiment 3, and this effect was reversed by FIN. Thus, the suppressant effects of PROG on cocaine seeking in female rats (Experiment 1 and 3; Jackson et al. 2006) may in part be attributed to the PROG metabolite, ALLO. These results lend support to the hypothesis that PROG may act through ALLO to modulate cocaine-induced effects. Allopregnanolone's attenuation of cocaine-primed reinstatement in female rats was not attributed to sedation as rats receiving a similar

dosing regimen in Experiment 2c failed to show disruption in locomotor activity or food-maintained responding.

That ALLO significantly decreased cocaine-seeking behavior in females but not in males is consistent with results from a clinical study examining the influence of PROG on the attenuation of positive-subjective ratings of cocaine in cocaine users (Evans and Foltin 2006). One possible explanation for the sex differences of ALLO's and PROG's effects may be related to the presence or absence of EST. For example, in women, PROG treatment attenuated the subjective effects of cocaine during the follicular phase of the menstrual cycle, when EST levels are high (Evans and Foltin 2006). Also, the administration of PROG in EST-treated ovariectomized rats decreased stimulant-induced behaviors relative to ovariectomized rats treated with EST alone (Jackson et al. 2006; Larson et al. 2005; Quinones-Jenab et al. 2000b). Administering PROG alone had negligible effects on stimulant-induced behavior in ovariectomized female rats (Perrotti et al. 2001; Quinones-Jenab et al. 2000b; Sell et al. 2000; Sircar and Kim 1999). A limitation of the present study was that the estrous phase and hormone levels were not controlled or monitored; thus, we could not examine EST/ALLO interactions on cocaine-seeking.

In summary, ALLO and PROG decreased cocaine-primed reinstatement of cocaine seeking, and the effect of ALLO was specific to female (but not male) rats. Additionally, FIN reversed PROG-induced decreases in reinstatement by blocking PROG's conversion into ALLO. Thus, ALLO may account for the suppression of reinstatement responding previously obtained by administering PROG in Experiment 3.

The results with ALLO could not be explained by a general reduction in activity or motivation to respond in a food-maintained operant conditioning task (see Experiment 2c).

### **Experiment 5: The role of allopregnanolone in stress-induced cocaine seeking following a yohimbine-priming injection**

#### ***Background***

Experiment 4 showed that ALLO reduced cocaine-primed reinstatement as was demonstrated for both PROG and ALLO with escalation (Experiments 1 and 2, respectively). A goal of the present study was to extend these results to stress (YOH)-induced reinstatement of cocaine-seeking behavior. Previous work indicates that progesterone was involved in modulating craving elicited by stressful stimuli in women. In a study conducted by Sinha et al. (2007), women reported less stress-induced cocaine craving when they had high to moderate, compared with low levels of circulating PROG. The anxiolytic effects of PROG may be attributed to its rapid metabolism into ALLO, a more potent anxiolytic (Russell et al. 2008). However, little is known regarding how ALLO affects stress as it relates to cocaine addiction. It is also not known whether this effect applies to males. Female and male rats were trained to lever press for i.v. infusions of cocaine. They were then allowed to extinguish lever pressing, and subsequently they were tested using a within-subject reinstatement procedure using priming injections of YOH, ALLO+YOH, or saline.

## **Methods**

### *Subjects*

Eleven female and 8 male 90-day-old Wistar rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 250-300 g and 350-400 g, respectively, served as subjects in the present study.

### *Procedure*

The training, maintenance, and extinction conditions were identical to those described in the General Methods section. However, a within-subject reinstatement procedure was used in this experiment. Following the extinction day 21, the stimulus lights, house light, and pump were unplugged for 3 days before the reinstatement condition. During reinstatement, rats received a daily i.p. injection of saline (S) or YOH (Y) immediately before the beginning of the session (9:00 am) for six days according to the following sequence: S, Y, S, A+Y, S, Y. Thirty min before (8:30 am) the second YOH injection that was given on Day 3, a 15 mg/kg s.c. injection of ALLO (A) was administered.

### *Data analysis*

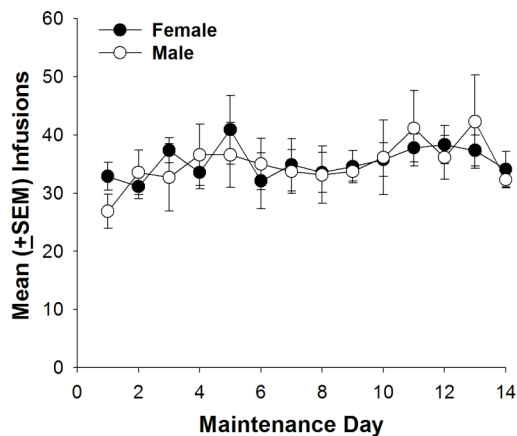
Responses and infusions were averaged across 7-day blocks for the maintenance and extinction conditions. Within-group comparisons of active vs. inactive lever responses during the maintenance and extinction phases in female and male groups were analyzed using separate two-factor repeated-measures ANOVA with block as the repeated measure and lever as the fixed factor. Between-group comparisons of active-

lever responses and infusions during maintenance and extinction were analyzed using two-factor repeated measures ANOVA. For reinstatement, responses following S vs. YOH priming injections were compared in female and male groups using separate single-factor repeated measures ANOVA. Between-group comparisons of responses following priming injections were analyzed using two-factor repeated-measures ANOVA. After a significant main effect, post-hoc tests were conducted using Fisher's LSD protected t-tests. Statistical analyses were conducted using GB Stat (Dynamic Microsystems, Inc., Silver Spring, MD).

## Results

### *Maintenance*

Females ( $F_{1,43} = 99.84, p < 0.01$ ) and males ( $F_{1,31} = 27.31, p < 0.01$ ) responded significantly more on the active- vs. the inactive-lever during maintenance. Between-group comparisons of active and inactive lever responses and infusions (Figure 17) during the maintenance phase did not lead to significant sex differences.

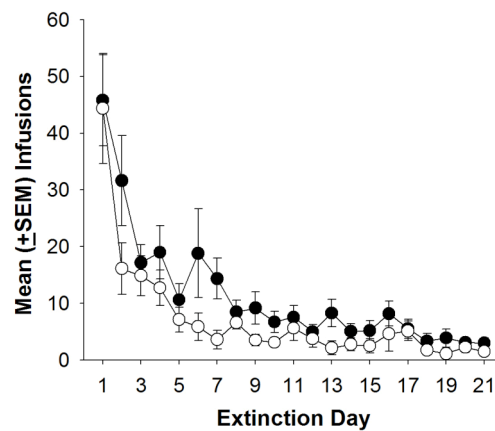


**Figure 17** Mean (+ SEM) cocaine (0.4 mg/kg) infusions earned during maintenance.



### *Extinction*

During the extinction phase females ( $F_{1,65} = 23.90, p < 0.01$ ) and males ( $F_{1,47} = 21.40, p < 0.01$ ) responded on the active lever significantly more than the inactive lever, and responding on both levers decreased over 21 days for both females ( $F_{2,65} = 22.47, p < 0.01$ ) and males ( $F_{2,41} = 6.38, p < 0.01$ ). Females made significantly more active-lever responses than males during the extinction phase ( $F_{1,56} = 4.64, p < 0.05$ ); however, there were no group differences in saline infusions during extinction (Figure 18).

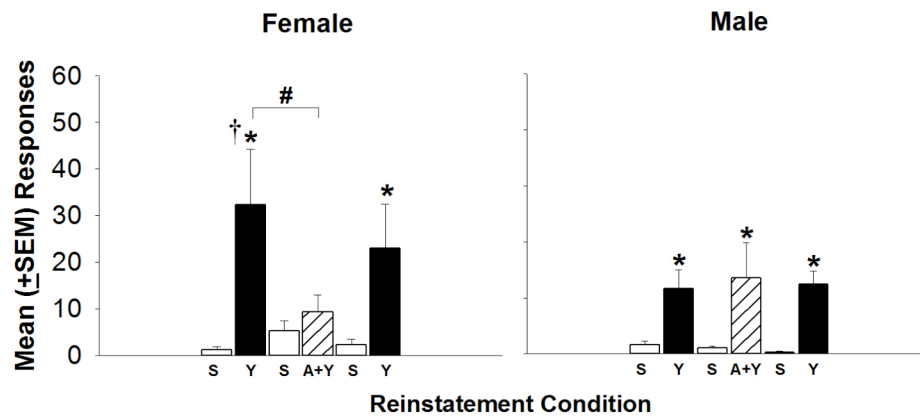


**Figure 18** Mean (+ SEM) saline infusions earned during extinction.

### *Reinstatement*

Figure 19 shows the mean responses on the previously drug- paired lever (active lever). Responding varied depending on priming injections in males ( $F_{5,65} = 9.87, p < 0.01$ ) and females ( $F_{5,65} = 5.05, p < 0.01$ ). Females significantly increased responding on the previously drug-paired lever when YOH (vs. S) was administered alone ( $p < 0.05$ ); however, when the YOH injection was preceded by a s.c. injection of ALLO (A+Y), subsequent responding did not differ from responding following S-priming injections,

and responding was significantly lower than when YOH was administered alone ( $p < 0.05$ ). Thus, YOH facilitated reinstatement responding in female and male rats, and administration of ALLO attenuated this facilitation in females but not males. In contrast to the results with females, responding on the A+Y day was significantly greater than responding on S days ( $p < 0.01$ ) for males. This suggests YOH increased reinstatement responding in male rats, but unlike the results with females, it was unaltered by ALLO. Comparison of active vs. inactive responding indicated that females, but not males, responded significantly more on the active lever when YOH was administered alone ( $p < 0.05$ ) (not shown). However, no differences were observed in responding on the two levers on the A+Y day. Between-group comparisons indicated that females, compared to males, responded significantly more following the first YOH injection ( $p < 0.01$ ).



**Figure 19** Mean ( $\pm$  SEM) responses on the previously drug-paired lever following saline (S) or YOH (Y) priming injections during the reinstatement procedure. Asterisks indicate significantly greater responding following YOH compared to S priming injections ( $p < 0.05$ ). # = symbol indicates a significant difference in YOH compared to responding following A+Y in the female group ( $p < 0.05$ ), and † = a significant sex difference (females > males) in responding following the first YOH injection ( $p < 0.05$ ).

## ***Discussion***

There were no sex differences during the maintenance of cocaine self-administration; however, females were more resistant to extinction of lever pressing following the removal of cocaine. This result supports previous work showing heightened extinction responding in female compared to male rats (Kerstetter et al. 2008; Kippin et al. 2005; Lynch and Carroll 2000; Lynch et al. 2005; Perry et al. 2008), and it supports clinical data that women may have greater difficulty in abstaining from cocaine. Indeed, female cocaine addicts exhibited greater levels of cocaine craving than males (Elman et al. 2001; Kosten and Zhang 2008).

While several preclinical studies have reported YOH-induced cocaine seeking in males (Bongiovanni and See 2008; Brown et al. 2009; Dzung Le et al. 2009; Feltenstein and See 2006; Kupferschmidt et al. 2009), this was the first to extend these results to females. In the present study, female rats were more sensitive than males to the potentiating effects of YOH on the reinstatement of cocaine seeking. This finding supports recent clinical work showing that female cocaine addicts have greater sensitivity to stress during withdrawal from cocaine than males (Fox and Sinha 2009), and it suggests that women may be more prone to stress-induced relapse to cocaine abuse.

An additional finding was that ALLO attenuated the effects of YOH on reinstatement responding in females but not males, suggesting that ALLO's effects on cocaine-seeking behavior may be anxiolytic and sex-specific. These results agree with previous findings of greater effectiveness of pharmacological (Carroll et al. 2001; Campbell et al. 2002; Cosgrove and Carroll 2004; Sershen et al. 1998) and behavioral

(Carroll et al. 2000; Cosgrove and Carroll 2003, 2004; Cosgrove et al. 2002) treatments in females than males. In Experiment 4, ALLO attenuated cocaine-primed reinstatement of cocaine-seeking behavior in female rats across several priming doses of cocaine, but it had no effect on males. Similarly, in humans, ALLO's precursor, PROG, attenuated cocaine-induced positive subjective effects in women (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al. 2004) but not in men (Evans and Foltin 2006). Taken together, these results suggest the presence of sex-specific treatment effects of PROG and ALLO on cocaine seeking.

## **General Discussion**

The overall goal of this thesis was to examine the influence of PROG and its metabolite, ALLO, on animal models of cocaine bingeing and relapse, stages of the drug abuse process associated with increased vulnerability in women. Experiment 1 demonstrated that EST and PROG produced opposite effects on the escalation of cocaine self-administration, an enhancement of self-administration by EST and a reduction by PROG. Experiment 2 extended these results with PROG to its metabolite ALLO and demonstrated that ALLO reliably and selectively blocked the escalation of cocaine self-administration without disrupting the escalation of a nondrug reward (i.e., sucrose) or reducing locomotor behavior or food-rewarded operant behavior. Similar to the results with escalation in Experiment 1 and 2, Experiment 3 demonstrated that EST and PROG had facilitating and suppressant effects, respectively, on the reinstatement of cocaine-seeking behavior in female rats. Experiment 4 supplied further evidence that PROG's

attenuating effects on cocaine seeking were mediated by its metabolite ALLO. In this experiment, the suppressant effects of PROG on cocaine-primed reinstatement were blocked by the coadministration of FIN, a pharmacological agent that prevents PROG from metabolizing into ALLO. Finally, in Experiment 5, females compared to males reinstated more following the stressful stimulus YOH, and ALLO selectively blocked this response in female but not male rats. Together, results from these experiments indicated that PROG decreased cocaine seeking during critical transition phases of the drug abuse process, escalation and reinstatement. This effect may be attributed to the conversion of PROG into ALLO, and ALLO's attenuation of these responses were specific to cocaine seeking in females and not males.

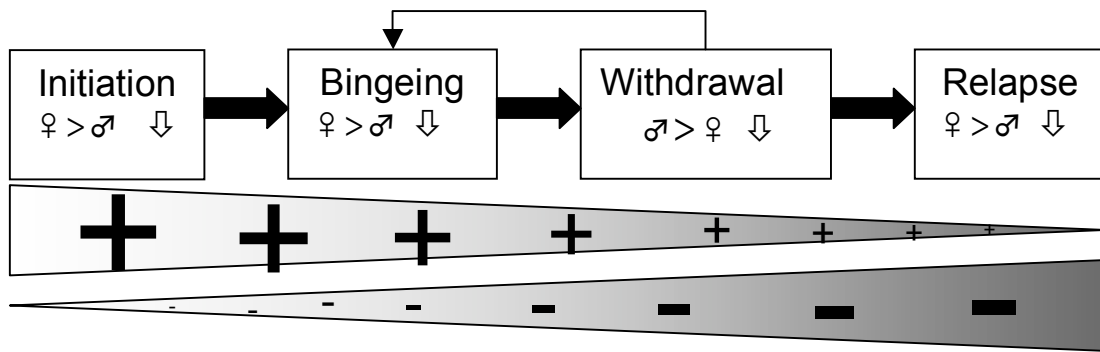
### ***Behavioral specificity of allopregnanolone***

An issue that arises with drug abuse medications is that they incur unwanted side effects such as decreases in general activity and attenuation of rewarding behavior such as food intake. Results from previous studies indicate that ALLO has sedative-like effects (Korneyev and Costa 1996; Lancel et al. 1997), and this may have contributed to suppressed cocaine seeking in the present studies (Experiments 2a, 4, and 5). However, an additional noteworthy finding of the present studies was that ALLO doses that selectively blocked the escalation (Experiment 2a) and reinstatement (Experiment 4) of cocaine seeking in female rats failed to disrupt sucrose-rewarded escalation (Experiment 2b) and general locomotor activity and operant responding maintained by food (Experiments 2c). Together, these results suggest that ALLO selectively blocked the

escalation and reinstatement of cocaine seeking without disrupting activity and responding for nondrug rewards.

***Suppressant effects of progesterone and allopregnanolone across phases of the drug abuse process***

It is important to emphasize that hormones can influence both the positive and negative reinforcing effects of drugs of abuse that are present throughout the stages of addiction. As previously mentioned, these stages include drug initiation, bingeing, withdrawal, and relapse (Carroll et al. 2009a; Koob and Volkow 2009). As suggested by Koob and Volkow (2009), and as outlined in Figure 20, drug taking is initially regulated by positive reinforcement through interaction with the mesolimbic dopamine system. However, over time and with repeated binge and withdrawal cycles, this behavior is increasingly regulated by negative reinforcement through heightened withdrawal, craving, and increased sensitivity to stress that may further enhance vulnerability to drug bingeing and/or relapse.



**Figure 20** Schematic of drug abuse process. + represents positively reinforced drug taking mediated by activation of the mesolimbic dopamine pathway; - represents negatively-reinforced drug taking mediated by activation of the HPA axis; > indicates relative vulnerability of females (♀) and males (♂); ↓ indicates phases during which PROG or ALLO decrease drug-related effects in women and female rats in animal models.

Work from the present studies and others implicates the progestins, PROG and ALLO, in neurobiological mechanisms associated with the positive and negative effects of drugs of abuse. The following section discusses the interaction of EST, PROG, and ALLO with neurobiological correlates of the positive and negative reinforcing effects of drug abuse during the stages shown in Figure 20.

### *Initiation (Acquisition)*

Positive reinforcement influences the initiation of drug abuse; it is involved in motivational aspects of drug addiction across all phases of the disorder (Figure 20) (Koob and Volkow 2009), and it is guided by activation of the mesolimbic dopamine pathway. The initiation of cocaine self-administration may be primarily under the control of the positive reinforcing effects of cocaine, as withdrawal that promotes negative reinforcement has not yet occurred. Jackson et al. (2006) demonstrated that OVX female

rats treated with EST exhibited an increase in the rate of cocaine acquisition relative to OVX rats treated with VEH or EST+PROG, suggesting that EST facilitates and PROG reduces the effects of cocaine at this early stage.

Several mechanisms could account for the attenuating effects of PROG on cocaine's positive reinforcing effects. One hypothesis concerns the modulation of PROG on EST-induced enhancement of cocaine-stimulated dopamine release. Dopamine influx in the nucleus accumbens plays a pivotal role in cocaine-reinforced responding in rats (Anderson et al. 2006; Anderson et al. 2003; Schmidt et al. 2006). Systemic administration of EST increases stimulant-induced dopamine transmission in the striatum and nucleus accumbens (Becker 1990a,b; Disshon and Dluzen 1999; Thompson and Moss 1997) and contributes to the enhanced behavioral response of female rats to cocaine (Febo et al. 2005; Zhou et al. 2002). In contrast, PROG attenuates EST enhancement of amphetamine-stimulated dopamine release in striatal tissue (Dluzen and Ramirez 1987). According to this hypothesis, PROG acts to inhibit EST-facilitated increases in stimulant-induced dopamine release within major reward systems of the brain that would, in turn, decrease the positive-reinforcing effects of cocaine.

### *Bingeing (Escalation)*

As individuals increase drug intake they may experience increased aversive effects (Figure 20). Animal research demonstrates irregularities in corticosterone levels (a neurobiological marker of stress) resulting from prolonged cocaine intake (Mantsch et al. 2007). Other preclinical findings indicate that administration of corticotropin



releasing factor (CRF) antagonists attenuate the escalation of cocaine self-administration in rats (Koob 2008). These results implicate heightened hypothalamic-pituitary-adrenal axis (HPA) activation in animal models of cocaine bingeing. Thus, PROG and/or ALLO may interact with the HPA axis to attenuate the escalation of cocaine self-administration. Indeed, PROG and/or ALLO decrease CRF levels, thus blunting HPA activation (Brunton and Russell 2008; Guo et al. 1995). This evidence may explain why ALLO and PROG blocked the escalation of cocaine self-administration in Experiments 1 and 2 and stress-induced reinstatement in Experiment 5.

### *Withdrawal*

Withdrawal from several drugs of abuse (e.g., cocaine, nicotine, alcohol, cannabinoids, opiates) produces anxiogenic-like behavior in animals (Basso et al. 1999; George et al. 2007; Knapp et al. 2004; Overstreet et al. 2004; Rodriguez de Fonseca et al. 1997; Sarnyai et al. 1995; Stinus et al. 2005); it enhances HPA activation by increasing neurobiological substrates of stress such as extracellular CRF in both humans (Sinha et al. 2003; Stewart 2003) and animals (George et al. 2007; Richter and Weiss 1999; Rodriguez de Fonseca et al. 1997; Weiss et al. 2001); and it enhances measures of drug seeking in animals (Harris and Aston-Jones 2003; Hutcheson et al. 2001; Shaham et al. 1996; Valdez et al. 2002) and craving in humans (Piasecki et al. 2003; Stine et al. 2002). Furthermore, withdrawal severity, as measured by increases in intracranial self-stimulation thresholds, was significantly correlated with escalation of cocaine self-

administration in rats (Ahmed et al. 2002), suggesting that withdrawal may increase drug bingeing (see Figure 20).

Interestingly, PROG and/or ALLO alleviate anxiogenic-like behaviors (Bitran et al. 1993, 1995; Brot et al. 1997; Laconi et al. 2001) and normalize overactive HPA activity by attenuating the release of CRF following exposure to stress (Drugan et al. 1993; Frye et al. 2006; Owens et al. 1992; Patchev et al. 1994; Purdy et al. 1991). Both are also implicated in suppressing stress-related measures of cocaine craving in humans (Sinha et al. 2007) and they attenuate stress-induced reinstatement of cocaine seeking in female rats as demonstrated in Experiment 5. Progesterone and ALLO also blocked the escalation of cocaine self-administration in Experiments 1 and 2. Thus, PROG and/or ALLO seem to attenuate cocaine seeking by interfering with the negative reinforcing and aversive effects of drug abuse that are related to bingeing and stress-induced relapse.

Progesterone and ALLO are present to a greater extent in blood and brain tissue in female rats compared to male rats (Quinones-Jenab et al. 2008), and this may partially explain why females show less withdrawal effects than males from several drugs such as alcohol (Alele and Devaud 2007; Cicero et al. 2002; Devaud and Chadda 2001; Gatch and Lal 2001; Varlinskaya and Spear 2004; Woodstock-Striley et al. 2004), pentobarbital (Suzuki et al. 1985), methaqualone (Suzuki et al. 1988), and PCP (Carroll et al. 2009b). Together, these results indicate a strong role for progestins in the treatment of the aversive aspects of drugs of abuse in women such as symptoms associated with drug withdrawal and overdose (See Figure 20).

The lessening of aversive and withdrawal-related responses by PROG and/or ALLO extends to other drugs as well, and this may explain sex differences in the expression of drug withdrawal effects. Studies examining drug withdrawal indicate that treatment with PROG's precursor (pregnenolone), its metabolite (ALLO), or PROG blocked the development of tolerance and withdrawal symptoms following termination of benzodiazepine (Reddy and Kulkarni 1997a) and morphine administration (Reddy and Kulkarni 1997b) in mice. In addition, similar to its suppressant effects on cocaine-induced seizures (Kaminski et al. 2003; Leskiewicz et al. 2003), systemic (Luntz-Leybmann et al. 1990) or intrahippocampal (Martin-Garcia and Pallares 2005) administration of ALLO resulted in an attenuation of seizures precipitated by large doses of nicotine, purportedly through its mediation of the GABAergic neurotransmitter system (Frye 1995; Frye and Scalise 2000). This suggests that progestins may act as compensatory mechanisms in the regulation of negative aspects of drug abuse. For example, findings from animal and human research indicate that ALLO levels dramatically increase during withdrawal from several drug of abuse such as cocaine (Chin et al. 2002; Festa and Quinones-Jenab 2004; Fox et al. 2008; Grobin et al. 2005; Walker et al. 2001), nicotine (Concas et al. 2006; Porcu et al. 2003), alcohol (Morrow et al. 2001), tetrahyrocannabinol (Grobin et al. 2005), and morphine (Concas et al. 2006; Grobin et al. 2005).

### *Relapse (Reinstatement)*

Drug relapse marks the final stage of addiction that is depicted in Figure 20 (Koob and Le Moal 2008), and the propensity for relapse may be directly related to the severity of the previous withdrawal phase (Koob and Volkow 2009). For example, several studies indicate that activation of the HPA axis via administration of CRF reliably reinstates cocaine-seeking behavior (Brown et al. 2009; Erb et al. 2001; Shaham et al. 2000; Stewart 2003), while administration of CRF antagonists block both stress- and drug-primed reinstatement of stimulant seeking (Marinelli et al. 2007; Moffett and Goeders 2007; Wang et al. 2005). In the present series of experiments, we demonstrated that PROG (Experiment 3) and/or its metabolite ALLO (Experiments 4 and 5) decreased cocaine seeking on two different reinstatement measures: drug- (Experiments 3 and 4) and stress-induced (Experiment 5) reinstatement. Others have shown that plasma PROG and ALLO levels were increased following systemic injection of cocaine (Chin et al. 2002; Festa and Quinones-Jenab 2004; Grobin et al. 2005; Quinones-Jenab et al. 2000a, 2008; Walker et al. 2001) or following the presentation of a stressful stimulus (Brunton and Russel 2008; Guo et al. 1995). As previously mentioned, progestins decrease HPA activation (Drugan et al. 1993; Frye et al. 2006; Owens et al. 1992; Patchev et al. 1994; Purdy et al. 1991). Thus, progestins may act as homeostatic regulators that normalize heightened HPA function following cocaine administration, and they may protect against stress responses leading to cocaine craving and relapse.

*Opposite effects of estrogen (vs. progesterone) on the HPA axis*

In contrast to PROG and ALLO, it was noted that EST facilitated the escalation and reinstatement of cocaine-seeking behavior in Experiments 1 and 3, respectively. This corroborates several other reports showing a facilitating effect of EST on responses to cocaine. Interestingly, the effects of EST on HPA activation and HPA-mediated behaviors are opposite to those of PROG and its metabolite, ALLO. Estrogen potentiated the release of CRF (Patchev et al. 1995; Swanson and Simmons 1989) and increased adrenocorticotrophic hormone (ACTH) and corticosterone (Burgess and Handa 1992), which led to increased HPA activity (Dallman et al. 2004). These effects also extended to cocaine-induced HPA activation. In a study by Niyomchai and colleagues (2005), EST increased cocaine-induced corticosterone levels relative to VEH-treated controls. Behavioral studies provided further support for the opposing effects of PROG and EST. For example, EST facilitated fear-potentiated startle in OVX female rats relative to OVX rats treated with VEH, while the administration of PROG attenuated this facilitation (Hiroi and Neumaier 2006; Toufexis et al. 2004). Further work is needed to examine the interaction between EST and progestins in relation to their effects on stress-related drug abuse. One question for further research is whether PROG or ALLO can block EST's potentiating effect on cocaine-induced corticosterone release and whether this effect is temporally related to cocaine seeking.

## *Sex differences in response to progestins*

### *Cocaine seeking*

Another important finding from the present studies was the presence of sex differences in cocaine seeking. Females showed more cocaine-seeking behavior following a low priming dose of cocaine (5 mg/kg) (Experiment 4) or an injection of the pharmacological stressor YOH (Experiment 5) than males; thus, sex differences were extended to two different forms of reinstatement. These results support the growing body of literature on sex differences in drug abuse that suggest that females may have increased vulnerability to several addiction-related measures (see Introduction and Figure 20). Importantly, they suggest that females are more vulnerable than males to the interaction between drug seeking and reactivity to stress (Experiment 5). The sex differences that are consistently found also highlight the importance of continued research on females, a group that has historically been underrepresented in drug abuse research but is increasing their drug use at a faster rates than males in younger populations.

### *Treatment effects*

An additional finding of the present series of studies was the presence of sex-specific treatment effects of ALLO on cocaine-seeking behavior. For example, ALLO attenuated cocaine- (Experiment 4) and stress- (Experiment 5) induced reinstatement of cocaine seeking in female but not male rats. Several other studies have demonstrated sex-specific treatment effects on responses to drugs of abuse. In fact, preclinical

evidence suggest that females are affected more by pharmacological (Carroll et al. 2001; Campbell et al. 2002; Cosgrove and Carroll 2004; Sershen et al. 1998) and behavioral (Carroll et al. 2000; Cosgrove and Carroll 2003, 2004; Cosgrove et al. 2002) treatments than males.

Further work is needed to characterize the mechanisms underlying sex differences in drug abuse treatment. One area of inquiry concerns the contribution of female gonadal hormones to treatment receptivity. This would be of interest when designing treatment regimens tailored to the sex of the patient. For example, fluctuation of hormone levels may influence treatment outcomes by engendering increased vulnerability to, or protection against, addictive behaviors. The findings presented in the present studies highlight the importance of sex-specific treatment strategies that take into account the unique interactions between female gonadal hormones and drug abuse disorders. The feasibility of this strategy has been demonstrated in previous clinical research. For example, women who quit smoking during the follicular phase of the menstrual cycle were more likely to abstain from smoking compared to women who quit during luteal phase (Franklin et al. 2004; Carpenter et al. 2008). These studies indicate that scheduling smoking quit dates around periods of the menstrual cycle that mark periods of increased vulnerability to relapse may be beneficial to treatment outcome. Similar methods could be implemented in the treatment of other addictions that are influenced by menstrual cycle phase such as cocaine and amphetamine dependence.

In summary, results from this thesis demonstrated that PROG and its metabolite, ALLO, attenuated behavior during the bingeing and relapse phases that are associated

with heightened vulnerability to drug abuse in women. These findings provide further support for the influence of female gonadal hormones and their metabolites in drug addiction. An additional implication is that women may respond differently to drugs of abuse than men, and this may largely be attributed to fluctuations of EST and PROG and/or ALLO during their menstrual cycle. Thus, further research is needed to determine if menstrual cycle phase should be a factor when designing treatment interventions. It was also noted that PROG may act through ALLO to decrease addiction-related responses. This finding is especially pertinent if PROG were to be pursued as a possible treatment in cocaine addiction. An additional and noteworthy result was that ALLO blocked stress-induced relapse. This is an important finding given that women cocaine addicts show greater vulnerability to stress-induced craving (Fox and Sinha 2009). In conclusion, the results from this thesis highlight the complexity of hormonal influences on drug-seeking behavior, and they demonstrate a role for hormones in the susceptibility toward (EST) and protection against (PROG and ALLO) drug abuse during critical phases of the drug abuse process.



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