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Effects of Cocaine on Sexual Behavior and Sexual Inhibition in the Male Rat.

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ABSTRACT

Effects of Cocaine on Sexual Behavior and Sexual Inhibition in the Male Rat.

Michael Benibgui

The effects of cocaine on male rat sexual behavior and sexual inhibition were studied. The first study addressed acute and long-term effects of cocaine (5, 10, 20, and 40 mg/kg, i.p.) on sexual responsiveness using a paradigm where cocaine was administered intermittently prior to sexual behavior and was followed by tests during withdrawal and drug challenge. The subsequent studies attempted to use the models of sexual inhibition described above to determine whether acute administration of cocaine could disrupt first-order and second-order conditioned inhibition of sexual behavior. Results indicated that cocaine administration lead to decreased ejaculation latencies compounded with fewer intromissions prior to ejaculation. The effects of cocaine (20 and 40 mg/kg, i.p.) on sexual inhibition were subsequently tested using first order and second order conditioned sexual inhibition paradigms. Cocaine did not release male copulatory behavior from inhibition, at any of the doses tested, on the inhibitory test with non-receptive females. In contrast, in an experiment in which males were conditioned to inhibit their sexual responses in the presence of a neutral odor (almond) paired with female non-receptivity, cocaine (20 mg/kg, i.p.) was shown to block the learned sexual inhibition against females bearing the odor in a copulatory preference test with two receptive females, as measured by the choice of first mount. Paradoxically, the same dose of cocaine (20 mg/kg, i.p.) also resulted in a preference to repeatedly mount the female bearing the odor associated with non-receptivity, as indicated by repeated attempts to mount. The present findings were interpreted in light of proposed links between cocaine use and sexual risk-taking reported in the human literature.

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Introduction

Society places strong constraints on the expression of sexual behavior, and most individuals have a tendency to inhibit their sexual behavior in situations deemed too risky or inappropriate. These inhibitions on sexual expression tend to be transmitted by the culture and internalized by the individual. In addition to sexual constraints, sexual inhibition can be defined as an inhibition, either voluntary or involuntary, of forward-directed responding to sexual incentives. It has been postulated (Bancroft, 1999) that individuals with a high propensity for sexual inhibition may be vulnerable to sexual dysfunction, whereas those with a low propensity may have an increased likelihood of engaging in risky sexual behavior. Sexual inhibitions may also come as the result of worries, distractions, or thought processes that interfere with sexual arousal and sexual behavior. Most psychogenic sexual dysfunctions are described by the DSM-IV (American Psychiatric Association, 1994) as an inhibited response that can occur at any or all levels of the sexual response cycle: desire, arousal/excitement, orgasm or resolution. Risky sexual behavior is defined in terms of the threat that the behavior entails, such as sexually transmitted diseases, unwanted pregnancy, damage to one's primary sexual relationship and social repercussions.

Bancroft (2000) recently proposed a multidimensional model of sexual risk-taking that emphasizes individual differences. In this model, cultural norms and attitudes, personal beliefs, and knowledge about the risk all influence the appraisal of the level of risk inherent to the situation. *Risk Appraisal* is followed by a *Risk Management* stage where a decision is made on whether the risk should be taken or avoided. The decision is based on a trade-off between costs and benefits, and is influenced by such factors as

factors as sexual arousal, mood, and sense of control. One of the assumptions of this model is that in a state of sexual arousal, normal "rational" decision-making is impaired. Thus, an individual who recognizes that a particular form of activity is risky, and should be avoided when not sexually aroused, feels less concerned about the risks involved when in an aroused state. The model also assumes that in the highly aroused state, the need to experience sexual stimulation and orgasmic release overrides the need to avoid the risks. In order to account for the fact that risky sexual situations are often avoided, even under the influence of high sexual arousal, the model relies on the proposed existence of two controlling systems in the brain: a Sexual Inhibition System and a Sexual Excitation System. These two systems are theorized to control sexual arousal and genital and behavioral responses in the face of a perceived threat. The author concedes that the model is reductionistic and has never been tested, but maintains that it does integrate both biological and socio-cultural factors. Whereas social and cultural factors have been examined at length with respect to high-risk sexual behavior, biological factors have been relatively unexplored.

Drugs of Abuse and Risky Sexual Behavior

Of the many types of threats discussed in the context of risky sexual behavior, none has received more attention than the threat of sexually transmitted diseases such as HIV. Particular attention has been placed on the role of drugs of abuse in sexual risk-taking. Many drugs of abuse, such as alcohol, cocaine, amphetamines, or heroin, are considered "prosexual" and are often used in sexual situations where they are believed to increase sexual desire and arousal, or to enhance the intensity of sexual stimulation during intercourse (Kall, 1992; Miller, 1993; Abel, 1984). Some of the effects are direct,

such as the facilitation of erection, and an increased sensory awareness that can amplify sexual stimulation and the intensity of orgasm. Other prosexual effects of these drugs are indirect and may stem from a general cognitive disinhibition that prompts individuals to engage in unsafe or promiscuous sexual activity or in "problem" sexual behavior (sexual assault, rape) without regard to its consequences. Also important are effects that are brought about by *drug expectancies*, a cultural belief in the powers of drugs and alcohol to disinhibit sexual activity and thus provide an "excuse" for otherwise unacceptable behavior (Critchlow-Leigh, 1986; Leigh, 1983).

Steele & Josephs (1990) proposed an *alcohol myopia model* for sexual risk taking that focused on alcohol as a powerful psychoactive drug that could affect the drinker's ability to evaluate sexual risk. Their theory is based on evidence that alcohol impairs attention, cognition, and information processing. They hypothesized that impaired attentional capacity, and cognitive resources such as the inability to process and extract meaning from incoming information may lead the intoxicated individual to focus on immediate and salient cues without processing more peripheral ones. Impaired information processing may therefore contribute to risky sexual behavior by focusing the drinker's attention on immediate positive consequences (e.g., physical pleasure, intimacy, sexual stimulation) and reducing the ability to effectively process potential negative consequences (e.g., possibility of contracting a sexually transmitted disease). More abstract or distal cues, such as suspicion that the sexual partner could be HIV infected, or the recollection of messages promoting condom use within public service announcements, are less salient and require additional cognitive resources to process. Thus, when intoxicated, the ability to consider these distal inhibitory cues decreases and

protective behavior is less likely to be enacted. Furthermore, given the results of a meta-analysis (Steele and Josephs, 1990) of studies on alcohol's effect upon social behavior their model specifies that alcohol disinhibits behavior that is under response conflict; that is, behavior for which there are strong instigating and inhibitory cues. These external factors (e.g., an attractive date and a conducive setting) might instigate sexual overtures but internal factors (e.g., sexual guilt, or fear of acquiring a STD) might serve to inhibit sexual behavior. They proposed that a person is more likely to exhibit "socially excessive" behavior when intoxicated if the behavior is one that ordinarily presents such a conflict. By decreasing the person's ability to process information, alcohol eliminates the conflict the person would experience related to the behavior. Many other drugs of abuse also interfere with cognitive processing, and attention (Julien, 1995). However, it is unclear whether the model proposed by Steele & Josephs extends to the effects of stimulant drugs such as amphetamine or cocaine, or to other central nervous system depressants such as heroin or morphine. If drinking or drug use leads to risky sexual activity, understanding the dynamic of this relationship can inform research, as well as preventative and educational efforts to control the spread of HIV and other sexually transmitted diseases.

To date, most explanations of the link between substance use and sexual risk-taking have focused on the nearly ubiquitous belief that engaging in sexual acts while intoxicated directly increases the likelihood of the activity being unsafe (Critchlow, 1986). Public education campaigns have been based on this message (e.g.: "Get high, get stupid, get AIDS"; National Institute on Drug abuse, 1994), and in many HIV-risk-

reduction programs, substance use prior to sexual behavior is discussed as a risk behavior and is assessed as an outcome.

At present, much of our knowledge about the effects of drugs of abuse on sexual behavior comes from clinical anecdotes; there are very few experimental data in humans or in animals regarding the short- or long-term effects of commonly abused drugs such as alcohol, amphetamine or cocaine on sexual function. Even less experimental data exist concerning the disinhibitory effects of these drugs. Current research into this area has focused mainly on the association between drug use, sexual behavior, and HIV transmission. A cultural belief in the powers of alcohol or drugs to disinhibit sexual activity has been demonstrated (Critchlow, 1986; Reinarman & Leigh, 1987), and literature has emerged over the past two decades to suggest that individuals who are heavy drug/alcohol users or who combine alcohol or drugs with sexual activity are more likely to engage in high risk sexual activity. However, the findings from this literature have been inconsistent, with some studies finding a statistical relationship between drug or alcohol use during sexual activity and the likelihood of participating in high risk sexual activity among gay men (Stall et al., 1986; McCusker et al., 1990; Leigh 1990), and other studies not finding any evidence of this relationship within the same population (Bolton et al., 1992; Weatherburn et al., 1993). Several studies have also established a link between general patterns of drug/alcohol use and risky sexual behaviors such as having multiple sex partners, unprotected sex (non-use of condoms), or trading sex for drugs (Woody et al, 1999; Hudgins et al., 1995; Ruiz et al., 1995; Castilla et al., 1999; Inciardi 1995; Wislar & Fendrich, 2000; Lowry et al., 1994, Chaisson et al., 1989, Iguchi and Bux, 1997). In virtually all of these studies, cocaine and alcohol were the main drugs that

were associated with a greater likelihood of engaging in sexual behavior that posed a greater risk for HIV transmission and infection.

There are several limitations in the studies mentioned above. First, the measurements used in many of these studies were aggregate measures of proximity of substance use and risky sexual behavior. For instance, a typical self report item would ask how often the individual has used alcohol/drugs in conjunction with sex and how many times the person has engaged in a variety of sexual activities. But because these are two general frequency measures, they do not provide information on the relationship between drugs and sexual behavior for a specific encounter. For instance, the times that an individual was using drugs may not be the same times that the person performed risky sexual activities. Furthermore, these types of studies do not allow examination of whether individuals' condom use changes as a function of substance use; instead, analyses are necessarily between subjects. Also, most studies rely on the report of two or three sexual events and this may not be representative of the participant's sexual behavior under the influence of alcohol/drugs.

A potentially powerful method of investigating the substance use/risky sex link is to use *multiple-event studies* in which researchers have asked participants about the details of all their recent sexual encounters. This allows for within-subjects comparisons of sexual events involving substance use to sexual events without substance use. Of the five such studies conducted to date, three have inquired solely about alcohol use, and two about alcohol and other drug use (Harvey and Beckman, 1986; Leigh, 1993; Weatherburn et al., 1993; Crosby et al., 1996; Fortenberry et al., 1997). The studies used either diary based self-reports or semi-structured retrospective interviews. Surprisingly, in *none* of the

multiple-event studies conducted to date has compelling evidence for an association between alcohol and sexual-risk behavior been found, indicating that there is either a very weak or no event-level association between alcohol/drugs and risky sexual behavior. Rather, the data indicated that people who tend to use condoms with a new or casual partner when they are sober also use them when drinking or under the influence of other drugs. However, this conclusion must be tempered by the fact that only five studies of this type have been conducted and that all of them have limitations that prohibit broad generalization of their results. Notably, of the two multiple-event study that looked at other drug use and alcohol, neither investigated the effect of alcohol use separately from other substances. Most importantly, the fact that participants were asked to self-monitor their behavior by completing diaries may have sensitized them to the potential hazards of combining substance use with sexual behavior or may have lead them to infer the hypotheses of the study. This heightened sensitization could have lead to changes in participants' responses during the course of the studies and may not have revealed accurate patterns of behavior. An attempt to overcome this limitation was made by Weatherburn et al., (1993) and Crosby et al., (1996) by using retrospective interview techniques. Both of these studies were conducted solely with gay/bisexual men and thus their results can potentially be generalized to that population. Weatherburn's team assessed the link between alcohol use and condom use during anal sex, while Crosby et al. divided participants into two groups based on condom use during anal sex while under the influence of alcohol or other drugs. Both studies found no significant alcohol/drug related differences in condom use.

An important caveat to keep in mind when attempting to draw conclusions from the results of all the aforementioned studies is that they are first and foremost correlational in nature. Several other variables may be mediating the relationship between substance use and unsafe sex, including dispositional or personality characteristics or attitudes and beliefs such as sensation-seeking, risk-taking, impulsivity, or environmental and situational characteristics. People who are likely to use alcohol and drugs more heavily may also be more likely to engage in sexual risk behavior because of a constellation of personality traits and beliefs rather than because of a causal relationship between substance use and sexual risk behavior. Kalichman et al., (1996) found that sensation-seeking is related to sexual HIV-risk behavior and substance use, and Caspi, et al., (1995) found that impulsivity predicted several health-risk behaviors, including sexual risk behavior and alcohol and drug dependence, in adolescents. In one study (Leigh, 1990), the relationship between drug use and risky sex disappeared when the overall frequency of sexual activity was controlled for. However, a notable exception to this overall finding was in gay men, whose risky behavior was strongly and independently related to the use of cocaine or other drugs in conjunction with sex. This divergent finding was explained in terms of the perceived threat of AIDS in both groups. Heterosexuals reported a very low level of concern about getting AIDS in comparison to homosexual men. Because disinhibition presumes that there is a pre-existing inhibition against the behavior, it was argued that a strong threat-induced inhibition of unsafe sex did not yet exist in heterosexuals.

Although the effect of other personality and dispositional variables has been examined, inquiries into the underlying pharmacological effect of drugs of abuse upon

sexual risk behavior have been virtually ignored. As mentioned, most studies that have been used to detect relationships between concurrent substance use and high-risk sexual activity have used non-experimental designs. As is the case with most non-experimental designs, the most important limitation is that causality cannot be established due to the probable presence of confounding variables. Furthermore, the types of studies conducted thus far have not examined whether individual's safer sex practices (e.g., condom use) changes as a function of substance use or abuse. As a rule, causal relationships are best inferred through the use of randomized experimental designs. However, due to obvious ethical concerns, it would be impossible to implement such a design with human subjects in this line of research. Animal models of human sexual behavior, on the other hand, lend themselves well to these types of experimental manipulations, and could provide insights and information not easily obtained from studies on people (Barfield 1993, Pfaus, 1996). More importantly, experimental manipulations in animals allow for the examination of pharmacological drug effects independently of the cultural beliefs and expectancy effects associated with the drug in question.

Rodent Models of Human Sexual Behavior

The rat is the most widely used animal for studies of sexual behavior. Certain tissues and neuroendocrine systems in the rat are quite similar to that of humans. Additionally, they display copulatory behaviors throughout their lives and will mate in virtually any type of testing chamber (Beach, 1938). They also display behavioral plasticity, altering their sexual behavior to meet the demands of different contexts and experimental manipulations. Male rat copulatory behaviors can be divided into appetitive, pre-copulatory and consummatory phases (Pfaus, 1999). Appetitive behaviors are often

used to infer the level of "sex drive." Anticipatory and preparatory behaviors are engaged in within the appetitive phase in anticipation of the presentation of the sexual incentive. Anticipatory behaviors such as locomotion, investigation, and grooming are used as measures of sexual excitement. Preparatory measures are operationally defined and serve to bring the animal closer to the sexual incentive and depend on the conditions of the testing environment. They can include bar presses, running a maze, crossing electrified grids, and conditioned place or partner preference. Consummatory measures, on the other hand, consist of species-specific behaviors that occur when the animal is in direct contact with the sexual incentive. They include pursuit, mounting, intromission, and ejaculation.

A novel testing environment has been developed that permits the quantification of both appetitive and consummatory sexual behaviors in male rats (Mendelson and Pfaus, 1989). This bilevel chamber consists of two levels connected by two sets of ramps, on either side. Female rats can pace their sexual contact by running from level to level, which forces the males to chase them. Various parameters can be calculated from behavioral observations of rats in uni- or bi-level chambers (van Furth & van Ree, 1996). General psychomotor activity, grooming, investigation and preparatory level changes, or instrumental responses, such as bar presses, preceding the introduction of the female are interpreted as sexual excitement by the trained observer. The number of level changes that the male rat engages in, as he "searches" for a female before she in introduced, is often used as a measure of sexual "anticipation" by researchers. Sexual arousal (sympathetic activation and erection), while not easily observable within a copulatory session in the bi-level chamber, can be inferred from latencies to mount and intromit, as

well as the timing of the post-ejaculatory refractory period. Mounts, intromissions and ejaculation frequencies are considered consummatory measures. The relative number of mounts and intromissions is referred to as "hit rate" or copulatory efficiency. The number of intromissions necessary to achieve ejaculation is considered to be an index of ejaculatory threshold, while the ejaculation latency is considered an index of copulatory performance. Thus these chambers, along with the measures derived from them, provide a sophisticated and sensitive tool for the analysis of appetitive and consummatory sexual behaviors in rats that can be used to examine the facilitative or inhibitory effects of different pharmacological or experimental treatments in ways that model human sexual function and dysfunction.

Nonetheless, there are several conceptual issues that need to be addressed when relating rat to human sexual behavior. First, human and rat copulatory behaviors differ in many respects. The human sexual response cycle consists of increasing levels of sexual excitement and arousal, plateau (during sexual stimulation), orgasm, and resolution (Masters and Johnson, 1966), and there are few differences between men and women in the response. Humans do not require vaginal intromission in order for orgasm to occur. Furthermore, with the exception of pelvic thrusting, the actual copulatory behaviors that humans engage in vary widely between cultures and from one individual to the next. In contrast, copulatory behaviors in the rat are highly sexually dimorphic and stereotyped, and involve short, intermittent bouts of vaginal intromissions followed by ejaculation. Males exposed to one female will copulate to ejaculation several times until a level of sexual exhaustion is reached. Once reached, males will not copulate with the same or a

different female for approximately 4 days – the average length of a female's estrous cycle.

In contrast to consummatory behavior, physiological measures of sexual arousal such as penile erection and ejaculation, show a high degree of similarity between rats and humans. Thus, male rats are appropriate models to use for the study of physiological sexual arousal in human males. Penile reflexes and the effects of pharmacological treatments can be measured and compared in both species. In fact, with the exception of Viagra ™ , drugs used to treat erectile dysfunction and premature ejaculation are virtually always tested first in the rat (Pfaus, 1996).

Another conceptual issue that needs to be addressed when comparing human and rat sexual behavior is that of sexual motivation or "sex drive." Sexual motivation in humans can be inferred from the speed of genital blood flow, the latency to experience orgasm, as well as the frequency of sexual behavior and sexual fantasizing within a given timeframe. Penile blood flow, ejaculation latencies, and frequency of copulation can all be measured in the rat as well. Furthermore, rat sexual motivation can be quantified, as described above, through various behavioral observations and operant preparatory responses in experimental testing environments.

Monoamines, Drugs of Abuse, and Male Sexual Behavior

Cocaine is an indirect acting dopamine (DA), norepinephrine (NE), and serotonin (5-HT) agonist. It potentiates the synaptic activity of these monoamines by blocking their reuptake by pre-synaptic transporters located on monoaminergic terminals, thus prolonging the stimulation of DA, NE and 5-HT receptors. The specific action of cocaine on the DA transporter is generally acknowledged to account for most of the reinforcing

properties of cocaine (Ritz et al., 1987). This is, in part, due to the observation that in drug discrimination or self-administration paradigms, specific DA reuptake inhibitors or DA receptor agonists can partially or fully substitute for cocaine, whereas DA antagonists block the effects of cocaine (Woods et al., 1987; Spealman, et al., 1991; Woolverton & Kleven, 1988). This relationship was not found with specific serotonin or noradrenaline reuptake inhibitors. Additionally, it was found that the abuse liability of drugs with varied affinities for DA, NE and 5-HT transporters correlates most with their affinity for the DA transporter. Finally, lesions of the mesolimbic DA system by 6-OHDA infusions decrease rates of cocaine self-administration (Roberts & Koob, 1982). Behaviorally, cocaine stimulates locomotor activity, and at high doses elicits stereotypy. These behaviors are also thought to be regulated largely by its dopaminergic effects (Delfs et al., 1990).

Cocaine also exerts powerful effects on sexual behavior. However, as will be described below, the results of studies on the effects of cocaine have not been consistent. Therefore, all three monoamines (dopamine, serotonin, and noradrenaline) should be considered when discussing the sexual effects of cocaine on behavior. Ultimately, one would expect that the net effect would depend on the activation of the various monoaminergic systems within discreet brain regions involved in sexual behavior, so that certain behaviors/responses would be facilitated while others would be inhibited.

Dopaminergic activation has been shown to potentiate arousal, copulatory activity and penile reflexes in the presence of incentive stimuli. Dopamine agonists, such as apomorphine or L-dopa generally lead to increased erections, decreased mount latencies, decreased ejaculation latencies, and reduce the number of intromissions required to

achieve ejaculation, while increasing mount, and ejaculation frequency (as reviewed in Wilson, 1994). In contrast, dopamine antagonists such as haloperidol and pimozide, reduce the number of anticipatory level changes, decrease erections, increase mount and intromission latencies, decrease the total number of ejaculations, reduce the number of intromissions required for ejaculation, and increase the post-ejaculatory interval (Ahlenius and Larson, 1984; Pfaus and Phillips, 1991; McIntosh and Barfield, 1984). In humans, dopamine agonists such as amphetamine and L-dopa have also been shown to increase sexual desire and arousal while DA antagonists commonly disrupt sexual arousal, and orgasm (Crenshaw and Goldberg, 1996).

In contrast to dopamine, stimulation of serotonin transmission impairs sexual behavior. Fluoxetine and other selective-serotonin-reuptake inhibitors have been widely reported to induce anorgasmia, delayed ejaculation, and reduce sexual desire in humans (Crenshaw and Goldberg 1996). Studies in animals have been consistent with the human clinical literature. Acute fluoxetine has been shown to increase ejaculation latencies in male rats (Yells et al., 1994) whereas long-term fluoxetine reduces the ability of male rats to ejaculate at all (Cantor et al., 1999). Reduction of serotonergic activity, on the other hand, stimulates sexual activity. PCPA, a drug that selectively depletes brain serotonin, has profound effects on the sexual behavior of rats. A number of studies have shown that PCPA leads to hypersexual behavior characterized by increased heterosexual copulation and same-sex mounting (Sheard, 1969) and reduced ejaculation latency (Larsson and Ahlenius, 1986). Subsequent studies showed that this effect is consistent only in rats that are sexually sluggish or recently castrated (Wilson, 1994). Selectively impairing serotonin transmission through agonists of the 5HT-1A receptor (8-OH-DPAT), an

autoreceptor which inhibits serotonin transmission, also results in a marked decrease in ejaculation latency (Ahlenius and Larsson, 1989; Hillegart, 1991). Noradrenaline activation facilitates sexual behavior and increases sexual arousal. Yohimbine, an alpha-2 antagonist (the alpha-2 autoreceptor normally inhibits NA release), leads to increased arousal and erection in humans (Crenshaw and Goldberg 1996) and facilitates copulation in rats by increasing the number of mounts, intromissions and ejaculations and decreasing the latency to initiate copulation (Clark et al., 1984). Agonists at the alpha-2 receptor have the opposite effect (Clark et al., 1985). Propranolol, a beta-adrenergic receptor blocker, increases ejaculatory threshold as well as the number of mounts without intromission preceding ejaculation but decreases copulatory efficiency (Smith et al., 1995). Overall, the three monoamines affected by cocaine can modify sexual behavior when targeted independently. Given that dopamine and serotonin exert opposite effects on sexual behavior, the net effect of cocaine on the aforementioned systems is not easily predictable.

Short and Long-term effects of cocaine on human sexual behavior

Much of the current information on cocaine use and sexual function is derived from a series of interviews conducted over 20 years ago (Gay & Sheppard, 1973; Gay et al., 1977; Gay et al., 1982). Based upon interviews with long-time drug users, cocaine was consistently chosen as the drug of choice for sexual enhancement effects. Cocaine was preferred due to a perceived tendency to increase sexual desire, prolong intercourse, produce more intense orgasms, enhance sensuality and increase sexual assertiveness. Subsequent research, however, found that long-term use of cocaine was associated with loss of sexual desire, inhibited orgasm, and erectile dysfunctions in some male users

(Siegel, 1982; Washton & Gold, 1984; Macdonald et al., 1988). Interestingly, the most negative effects were reported by subjects who regularly injected the drug, most of whom reported markedly diminished desire and arousal (Macdonald et al., 1988). Thus, acute use of cocaine seems to enhance sexual desire or arousal, whereas chronic use leads to sexual dysfunctions. The deterioration of sexual desire, arousal, and performance would undoubtedly have a deleterious effect on the maintenance of intimacy within a relationship. It has been suggested that the elimination of supportive and intimate relationships is a key factor in the narrowing of an addict's motivation. Eventually, drug use becomes the sole source of pleasure, and maintenance of the drug habit becomes the primary motivating force.

Effects of Cocaine on Animal Sexual Behavior

Only a handful of studies have been published on the acute and chronic effects of cocaine on sexual behavior in animals. Leavitt (1969) found that acute low doses of cocaine (10 mg/kg, i.p.) lead to shorter inter-intromission intervals, and shorter ejaculation latencies, whereas the reverse was true for a higher dose of cocaine (30 mg/kg, i.p.). The higher dose also increased the number of mounts relative to the low dose and saline controls. In another study, Abel et al. (1989) administered cocaine (0, 15, or 30 mg/kg, s.c.) daily to male rats for 72 days, after which sexual and neuroendocrine functions were examined. They found that chronic long-term administration of cocaine decreased body weight, increased locomotor activity, and increased the proportion of non-viable sperm, but did not alter sexual behavior. However, they did not test sexual behavior following every single administration of cocaine. Rather, sexual behavior was tested only once, at the very end of the experiment. A recent study by Ferrari and Giuliani

(1997) examined the acute and subchronic effects of cocaine. Acute administration of 15 mg/kg, i.p., reduced the number of intromissions prior to ejaculation, whereas subchronic administration (14 consecutive days) also reduced ejaculation latency. Both acute and subchronic cocaine facilitated penile reflexes (7.5, 15, 30mg/kg, i.p.), and all effects could be reversed by administration of the D2 dopamine receptor antagonist eticlopride (0.025 or 0.05 mg/kg, i.p.)

Two studies have examined the effects of cocaine on sexual behavior in non-human primates. Pomerantz et al., (1994) found that administration of a high dose (800 µg/kg, i.v.) of cocaine to male rhesus monkeys decreased the number of male monkeys that copulated and achieved ejaculation, and increased mount latencies and ejaculation latencies in those that did copulate. A lower dose (200 µg/kg, i.v.) did not affect sexual behavior significantly. Another study examined the effects of acute cocaine administration (0.01-1.0 mg/kg, i.v.) on sexual behavior in male stumptail macaques (Linnankoski et al., 1995). Their results indicated that cocaine dose dependently suppressed ejaculation, an effect that was reversed by the administration of the D2-receptor antagonist, haloperidol (0.003-0.01 mg/kg, i.v.).

Overall, the results of the aforementioned studies are inconsistent. The only reproducible effect has been an apparent decrease in ejaculation latency at low doses and an increase in ejaculation latency at higher doses. However, the apparent inconsistencies may be partly explained by the differences in species tested (primates vs. rodents) and the level of sexual experience of the subjects. Some studies used males that had achieved ejaculations with females over two or more sessions (Ferrari & Giuliani, 1997), whereas others only required a single ejaculation on their first test (Abel et al., 1989). Prior sexual

experience is known to markedly decrease ejaculation latencies in several species, including the rodent (Pfaus & Wilkins, 1995). Other differences include the doses used and the time limit to the behavioral test as well as the novelty of the environment where the copulatory test was performed. The level of sexual experience and novelty of the copulatory environment have been shown to markedly affect sexual behavior as well as modulate drug effects in other species such as the rat. Pfaus and Wilkins (1995) showed that naloxone and preexposure to the testing environment facilitated copulation in sexually naïve rats but that these two manipulations did not affect the copulatory performance of sexually experienced rats. Furthermore, the two studies that purported to examine chronic effects of cocaine upon sexual behavior did not perform repeated testing under the effect of the drug, over time (Ferrari and Giuliani, 1997; Abel et al., 1989). Rather, a single test of sexual behavior was performed following several days of cocaine administration. In essence, the so-called "chronic" effects reported in these articles, may be more accurately described as acute effects following daily administration.

Due to the discrepancies in the studies conducted to date, it is difficult to determine whether the effects that have been observed in animals are consistent with the pro-sexual properties of acute cocaine administration that have been suggested by anecdotal reports in the human literature. It is possible that the behavioral tests used so far have not been adequate to investigate drug-induced modifications in sexual behavior. In human social settings, pro-sexual drugs such as cocaine are used to increase confidence and arousal, and to decrease inhibitions. Drug effects can be considered prosexual when they directly facilitate sexual arousal (e.g. erection) or copulation. Drugs that interfere with the perception of tactile stimulation or inhibit or delay orgasm can also

be considered prosexual depending on individual differences in ejaculation latencies. However, drugs that cause a general cognitive disinhibition, thus facilitating the subjects' approach behaviors towards a sexual incentive, or eliminating the internal conflict caused by societal and psychological inhibitions surrounding the sexual act, can be deemed indirectly prosexual, given an appropriate eliciting context. Whereas sexual inhibitions are evident in human contexts, their existence in animals has only recently been examined. Furthermore, only a few studies have attempted to incorporate sexual inhibitions within drug testing paradigms.

Inhibition of Sexual Behavior in Animals

In animals, inhibition of sexual behavior can serve many adaptive purposes. Bancroft (1999) considered a number of conditions in which there is an adaptive needs to inhibit sexual behavior. In the first case, an animal's sexual behavior may be inhibited if the behavior carries with it some threat or danger. For example, in many species, sexual responses by low-dominance males may be met with aggression from high-dominance males. Sexual behavior may also reduce vigilance to cues in the environment, leading to an increased vulnerability to predators and other threats. In this case, an inhibition of sexual responses would allow the animal to focus on the appropriate avoidance and defensive responses. Sexual inhibition can also be adaptive at the group level: reproductive behavior has been shown to decrease in the face of population overcrowding (Christian et al., 1970). Fertility is also dependent on sexual inhibition in a number of ways. Sexual arousal is restricted following ejaculation to avoid excessive sexual activity that would result in lowering the sperm store and interfere with fertilization of the ovum (Bancroft, 1999). Another threat to fertility and survival is sexual transmission of disease.

Recently, Lockhart et al. (1996) documented the distribution of sexually transmitted diseases in the animal kingdom and suggested that the presence of such diseases may influence mating patterns. The presence of sexual inhibition to avoid such infectious risks in animals has only been speculated.

Another powerful factor that influences mating behavior is the threat of aggression from females that are not sexually receptive. Male rats in the wild, for example, are almost never observed to attempt copulation with sexually non-receptive females. "Presumably, such males learn to inhibit their sexual advances toward inappropriate stimuli during adolescence, when they attempt to mount females not in heat (who will fight them off), and thus learn to associate certain olfactory and pheromonal cues, behavioral responses, and ultrasonic vocalizations exclusively with estrus" (Pfaus, 1996, p. 197). This is in marked contrast to most laboratory settings, in which male rats will attempt to copulate with non-receptive females placed into chambers in which the males have copulated previously with receptive females (Pfaus et al., 1989). Male rats in the laboratory hardly ever learn to distinguish sexually receptive from non-receptive females.

Experimental Induction of Sexual Inhibition

To date, researchers have been relatively unsuccessful at conditioning animals to suppress their sexual responses. Beach et al. (1956) attempted to inhibit sexual responses in male rats by associating electrical shock with the process of mounting the female. Repeated administration of high levels of shock resulted in an inhibition of mounting responses, whereas low levels of shock resulted in a paradoxical increase in incomplete mounts but otherwise had no effect on sexual behavior. Although punishment with

electrical shock has been shown to suppress a variety of appetitive and consummatory behaviors (Mackintosh, 1974), such punishment has not been reported to induce sexual inhibition reliably in rats (Beach & Fowler, 1959; Beach et al., 1956; Hayward, 1957; Zimbardo, 1958). In fact, shock, pain (tail pinch), or neutral stimuli paired with them, can stimulate mounting in sexually sluggish males (Barfield & Sachs, 1968; Caggiula, 1972; Crowley et al., 1973), or reduce the number of intromissions required for ejaculation, along with the inter-intromission interval, in sexually active males (Beach & Fowler 1959; Sachs & Barfield, 1976). Thus, those later studies have shown that shock has an arousing or excitatory effect on sexual behavior, and is therefore not suitable as a means of inducing sexual inhibition. Other methods have attempted to employ aversive conditioning techniques using lithium chloride. Rats injected with LiCl following copulation displayed longer intromission latencies than controls (Peters, 1983). However, this technique, while inducing some delays in initiation of copulation, does not abolish copulatory behaviors altogether.

One effective method of abolishing copulatory behaviors is the "sexual satiation' or 'sexual exhaustion' of male rats. Unrestricted exposure of male rats to receptive females results in repeated ejaculations until a state of sexual satiation is reached – assumed to be a specific inhibition of sexual responsiveness. The average number of ejaculations required to establish this state is seven, and complete recovery takes approximately 15 days (Beach & Jordan, 1956). Several studies have used this method to investigate the effect of various drugs on sexual satiation. The $5HT_{1A}$ agonist 8-OH-DPAT, the alpha-2 adrenoreceptor antagonist, yohimbine, and the opioid antagonists naloxone and naltrexone, all partially restored mounting behavior in the sexually sated rat

(Rodriguez-Manzo & Fernando-Guasti, 1994). Apomorphine, a dopamine agonist, also restored sexual responsivity in the sated rat, as measured as an increase in mounts, intromissions and ejaculations (Mas et al., 1995). Recently Belozertseva (2000) examined the effects of cocaine on sexual exhaustion and found that it induced a dose dependent increase in the number of intromissions and ejaculations, evidence that cocaine can increase the motivation to copulate when copulation is under inhibitory control.

Thus, the sexual satiation model can be used to examine the disinhibitory effects of various drugs. Sexual satiation is assumed to be due to habituation although this has been questioned (Tiefer, 1969). It is also not likely to be a cognitive sexual inhibition. Rather, sexual satiation may be described as a complete and general fatigue, which among other things produces a lack of excitability in the presence of sexual incentives, as shown recently by Fiorino, Coury and Phillips (1997). In that study, males copulated to sexual exhaustion and dopamine release in the nucleus accumbens was monitored by in vivo microdyalisis/HPLC. Dopamine release diminished with each ejaculatory series, eventually reaching baseline as sexual exhaustion approached. Males were then presented with a new receptive female. Dopamine levels increased slightly in males that attempted to copulate, but not in males that did not attempt copulation. Additionally, because sexual satiation is an involuntary response to repeated copulations, this model would be inappropriate for use in experimental manipulations of *learned* inhibitory responses.

As mentioned previously, male rats in the wild learn to not attempt copulation with non-receptive females. Pfaus et al. (1989) made use of this observation and were able to train male rats to differentiate between sexually receptive and non-receptive females by pairing them with receptive and non-receptive females on alternating test

trials. They gradually learned to suppress their copulatory behavior during tests with non-receptive females while maintaining their baseline rates of copulatory behavior during intervening tests with receptive females. Within 5 trials, complete inhibition of mounting behavior towards non-receptive females was observed. As in the wild, aggressive and antagonistic behavior of non-receptive females towards males that attempted to mount them was presumably sufficient to train males to attend to estrous odors as indices of sexual receptivity. In this case, learned inhibition and not an unlearned lack of excitation (as in the sexual exhaustion model) was evident, because males were observed to progressively diminish their mounting behavior with non-receptive females. Under those circumstances, Pfaus et al. showed that a moderate dose of alcohol (0.5 g/kg) could release mounting behavior from learned inhibition in approximately 70% of males. Moreover, 40% of those males ejaculated, despite never gaining vaginal intromission. In contrast, the same dose of alcohol diminished mounting behavior during trials with receptive females. Thus, the same dosage of alcohol was observed to inhibit or disinhibit sexual behavior of male rats depending on the presence or absence of conditioned sexual inhibition.

Recently, Kippin et al. (1998) found that pairing a neutral odor (almond extract) with the presentation of non-receptive females resulted in conditioned sexual inhibition. Males in that study had been trained to copulate with non-scented receptive females and to suppress copulation with almond-scented non-receptive females on alternating trials. During a final triad-mating test in an open field, the males were presented with two receptive females, one with and one without the odor. It was found that the unscented female was chosen significantly more often for mounts, intromissions and ejaculations

despite repeated solicitations from the scented female (indicating that the odor had become a CS- for copulation). Moreover, 80% of the males chose the unscented female for the first ejaculation. Thus, through classical conditioning, almond odor paired over several trials with female non receptivity, was able to inhibit male copulatory behaviors toward receptive females bearing the odor.

The Present Experiments

Studies that have previously examined the effects of cocaine have done so using either acute or chronic administration. Behavioral studies employing daily cocaine administration are of limited generalizability since human use is most often intermittent. Another limit to generalizability to human situations in previous animal studies is the use of only receptive females to examine sexual effects in males. Therefore, three experiments were conducted for the present thesis in order to provide the first systematic account of the inhibitory and disinhibitory effects of cocaine on sexual behavior in male rats. The first study addressed acute and long-term effects of cocaine on sexual responsiveness using a paradigm where cocaine was administered intermittently prior to sexual behavior and was followed by tests during withdrawal and subsequent drug challenge. The subsequent studies attempted to use the models of sexual inhibition described above to determine whether acute administration of cocaine could disrupt first-order and second-order conditioned inhibition of sexual behavior. Experiment 1 examined the acute and long-term effects of cocaine (5, 10, 20 or 40 mg /kg) on baseline rates of sexual behavior of male rats in bi-level chambers. These results were used to determine the effective doses for use in the disinhibition studies. Experiments 2 & 3 used first and second order conditioning, respectively, with inhibitory cues to train male rats to

inhibit their copulatory responses towards females. Experiment 2 employed the same methodology used in Pfaus et al. (1989). Male rats were trained to inhibit mounting of non-receptive females, and then tested with three different doses (10, 20, 40 mg/kg) of cocaine. Experiment 3 employed the almond-odor conditioning paradigm developed by Kippin et al., (1998). Following the odor conditioning trials, cocaine (20 and 40 mg/kg) was administered on the triad-mating test to examine whether the drug disrupted conditioned mate preference.

Experiment 1: Acute and Long-term Effects of Cocaine on Sexual Function

Experiment 1 examined the acute and long-term effects of systemic cocaine, cocaine withdrawal, and subsequent cocaine challenge. Cocaine (5, 10, 20 or 40 mg/kg, i.p.) was administered every 4 days in keeping with the estrous cycle of the stimulus female rats, and in order to more accurately model the intermittent use engaged in by humans and to increase the likelihood of inducing sensitization effects (Stewart and Badiani, 1993).

Methods

Animals

Males: Eighty Long-Evans rats were obtained from Charles River Canada, Inc., (St. Constant, Québec). The males weighed approximately 250 g and were sexually naïve at the start of the experiment. They were housed in groups of two in standard Plexiglas shoebox cages in a colony room maintained at approximately 21°C on a reversed 12:12-h light:dark cycle, with lights off at 0800 h. All rats had as lib access to food (LabDiet 2000) and water.

Females: One month prior to the beginning of the experiment, sixty female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions while anesthetized. The rats were anesthetized with ketamine hydrochloride (50 mg/ml; Ayerst Veterinary Laboratories, Guelph, Ontario) and xylazine hydrochloride (4 mg/ml; Bayer Inc., Etobicoke, Ontario) mixed at a ratio of 4:3 ml, respectively. The anesthetic was injected IP in a volume of 0.90 ml/kg of body weight. The females were sexually experienced, having been allowed to copulate several times with to a different set of sexually experienced males prior to the start of the experiment. Sexual receptivity was induced by subcutaneous administration of estradiol benzoate (10μg) 48 hours prior and progesterone (500μg) 4 hours prior to each trial. Females were housed under the same conditions as the males.

Apparatus

Baseline trials of copulatory behavior and Conditioning Trials took place in bi-level chambers constructed of Plexiglas (18 cm x 25 cm x 65 cm) with a centered platform elevated by a set of ramps and a narrow landing at each end, dividing each chamber into two levels (as described in Pfaus et al., 1990). Male rats were pre-exposed to the bi-level chambers once a day for 15 minutes in order to habituate them to the training environment. The habituation procedure lasted for 7 days and has been shown previously to increase the proportion of males that become vigorous copulators (Pfaus and Wilkins, 1995).

Procedure

Baseline Acquisition of Sexual Behavior: Prior to the initiation of drug trials, males were pre-exposed to the copulatory chambers for 15 minutes for 7 days and then

given baseline copulatory training trials conducted on a 4-day schedule. For the training phase, each male was placed in a bi-level chamber for 5 minutes prior to the introduction of a receptive female. Both rats were then allowed to copulate for 30 minutes. Baseline testing was conducted during the middle third of the dark cycle and continued until all males displayed stable latencies to mount and ejaculate. Males were injected with isotonic saline intraperitoneally 30 minutes prior to being placed in the chamber for the last three training trials to acquaint them with the injection procedure. All behavioral tests were videotaped and scored for sexual behaviors in accordance with the criteria described in Meisel and Sachs (1994), using a PC based program (Cabilio, 1996).

Behavioral Testing: Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in physiological saline to yield concentrations of 5, 10, 20, or 40 mg/kg. Thirty minutes prior to each test, males were weighed and injected IP with the appropriate dose of cocaine. Males were assigned to one of four drug conditions (n=10 per group) using stratified randomized blocking to equate them on their ejaculation latencies in the baseline phase.

Thirty minutes after cocaine administration, males were placed alone into the bi-level chamber for 5-minutes after which time a sexually receptive female was introduced for a 30-minute test of copulation. Testing was repeated over 15 trials at 4-day intervals. Animals within all groups were given a total of 13 drug trials, followed by a single Withdrawal trial, where saline was delivered rather than the expected drug. Four days following the Withdrawal trial, a final Drug Challenge trial was conducted, where the same dose of cocaine (5, 10, 20 or 40 mg/kg) was once again administered to the respective groups.

Statistical Analysis

A scorer blind to the animals' group membership coded the videotapes using the previously described computerized event recorder. Appetitive level change frequency (ALC) was defined the total number of level changes the male rats engaged in while alone in the chamber in anticipation of the introduction of the female. The total number of level changes (LCF) in pursuit of the female was also recorded. Copulatory behaviors recorded consisted of the number of mounts (MF), intromissions (IF) and ejaculations (EF), and were scored for each male during successive ejaculatory series. Latencies were recorded for the first mount (ML), intromission (IL), and ejaculation (EL) during the first ejaculatory series. Three secondary measures were calculated from these primary measures: the post-ejaculatory interval (PEI) was calculated as the time from the first ejaculation to the next intromission. The intromission ratio was calculated as the number of intromissions divided by the total frequency of both mounts and intromissions (IR=IF/MF+IF). The inter-intromission interval (III) was calculated as the ejaculation latency divided by the number of intromissions (EL/IF), and the Level Changes per Mount (LC/M) were calculated as the number of level changes divided by the number of mounts (LCF/MF).

Behavioral measures were analyzed using Mixed ANOVAs to assess the effects of the four levels of dose (5, 10, 20, 40 mg/kg) six levels of time (Baseline, Day 1, Day 7, Day 13, Withdrawal Day, Cocaine Challenge) and the dose x time interaction. The males' baseline measures were used as a control condition. Significant results were further subjected to post-hoc comparisons using Tukey's HSD test for unequal *n*. For the acute effects, Baseline was compared to Day 1 only. For long-term effects, Baseline was

compared to Day 7 and Day 13. For Withdrawal and Drug Challenge, Baseline and Day 13 were compared to Withdrawal (trial 14), and Drug Challenge (trial 15), respectively.

Results

Acute Effects of Cocaine.

The acute effects of cocaine are shown in Figures 2-11 (Day 1).

Appetitive Level Changes (ALC): No significant acute effects were found in any of the groups. Baseline was compared to first drug day.

Level Change Frequency (LCF): The total number of level changes was not significantly affected by cocaine administration.

Mount Latency (ML): The 20 and 40 mg/kg doses of cocaine produced an acute increase in mount latencies. A significant main effect of Time, $F(5,115) = 12.44$, $P<0.000001$, and a Dose x Time interaction, $F(15,115) = 5.37$, $P<0.000001$, were found. Post hocs for the Dose x Time interaction revealed that for the 20 mg/kg and 40 mg/kg group, acute cocaine administration (Day 1) lead to significantly elevated mount latencies when compared to Baseline ($P<0.034$).

Intromission Latency (IL): Similar results were obtained for the effects of cocaine on intromission latency. A significant main effect of Dose, $F(3,25) = 5.5$, $P<0.005$, and Time, . $F(5,155) = 19.8$, $P<0.000001$, as well as Dose X Time interaction, $F(15,125) = 9.44$, $P<0.000001$, were found. Post hoc tests on the interaction effect revealed that for the 20 mg kg and 40 mg/kg groups, acute cocaine administration (Day 1) lead to elevated intromission latencies as compared to Baseline ($P<0.0037$).

Ejaculation Latency (EL): Cocaine administration, at all doses, facilitated ejaculation in a time dependent fashion, as evidenced by a decrease in ejaculation latency. A significant main effect of Time was found, $F(5,115) = 3.38$, $P<0.007$. Post hoc tests showed that measures of EL were significantly lower than Baseline on Day 1 ($P<0.05$).

Post Ejaculatory Interval (PEI): No effects of cocaine on the PEI were apparent acutely.

Mount Frequency (MF): Acute cocaine administration did not significantly affect the number of mounts observed during the first ejaculatory series.

Intromission Frequency (IF): Compared to Baseline, males were found to ejaculate following fewer intromissions under acute administration of cocaine. Main effects for Time $F(5,170) = 10.55$, $P<0.00001$, and a Dose x Time interaction $F(15, 170) = 2.16$, $P<0.01$, were found. Post hoc tests showed that for the 20 mg/kg group, acute cocaine administration (Day 1) lead to significantly fewer intromissions than Baseline ($P<0.0002$). For the 40 mg/kg group, acute cocaine (Day 1) also lead to significantly less intromissions than Baseline ($P<0.0002$).

Ejaculation Frequency (EF): No significant effects were found for the effects of cocaine on the number of ejaculations on Day 1 as compared to Baseline.

Intromission Ratio (IR): The relative number of mounts and intromissions (Hit Rate) was affected in a dose and time dependent fashion. Analysis of IR detected a significant main effect of Time, $F(5, 145) = 2.78$, $P<0.02$, and a trend towards significance for the Time x Dose interaction $F(15, 145) = 1.64$, $P=0.068$. No acute significant effects were found when comparing Baseline to Day 1, although post-hocs on the Main Effect of Time show a near significant difference ($P<0.061$). Thus, given a larger sample size, a significant disruption of IR would probably be apparent.

Inter-Intromission Interval (III): Cocaine did not affect the time elapsed between successive intromissions.

Level Changes per Mount (LC/M): Acute cocaine administration did not significantly affect the relative number of mounts to level changes.

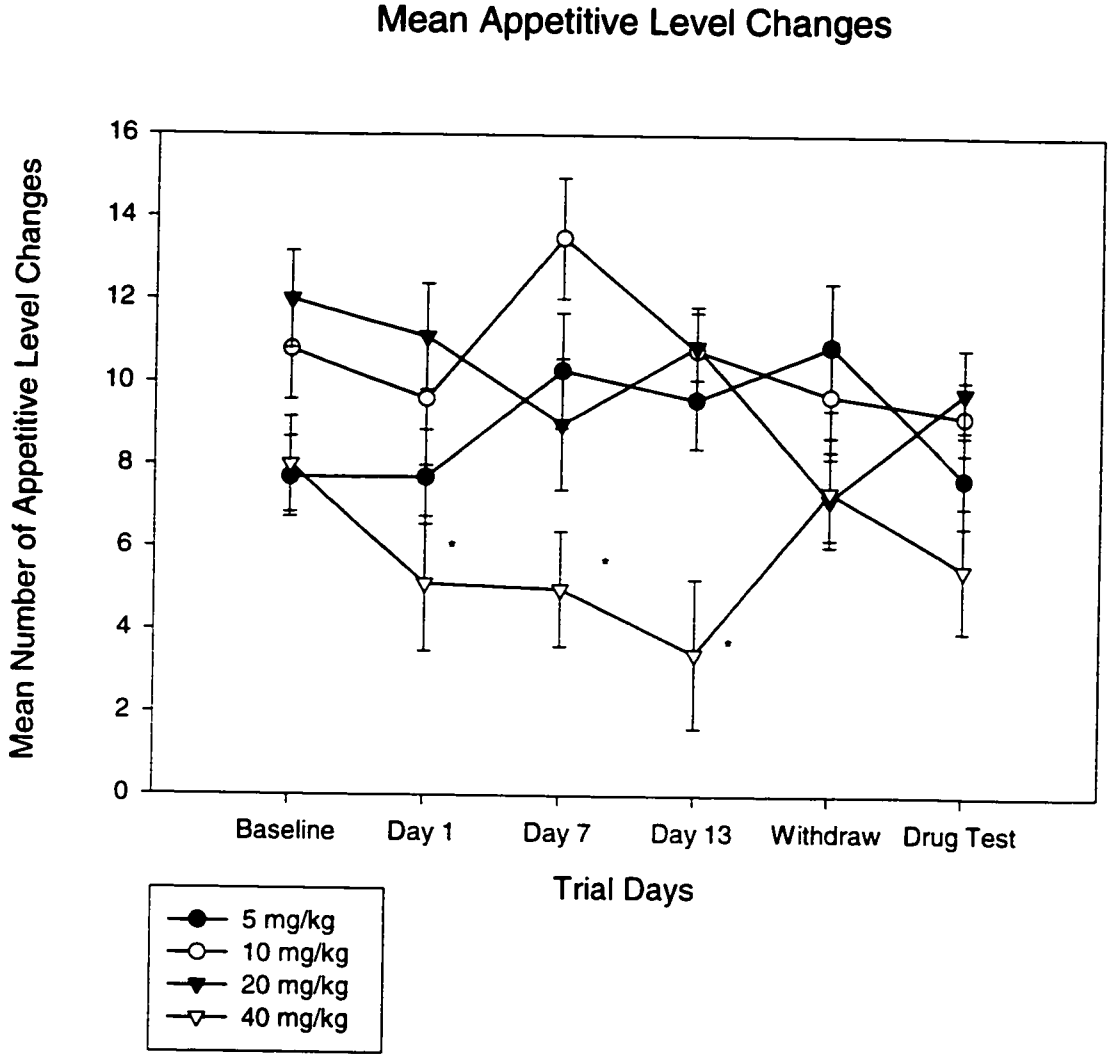

Figure 1. Average number of appetitive level changes (prior to insertion of the female) for all groups, across all drug trials.
* denotes $p < 0.05$ for Main Effect of Dose when comparing the 40 mg/kg group to the 5 mg/kg, 10 mg/kg, and 20 mg/kg groups.

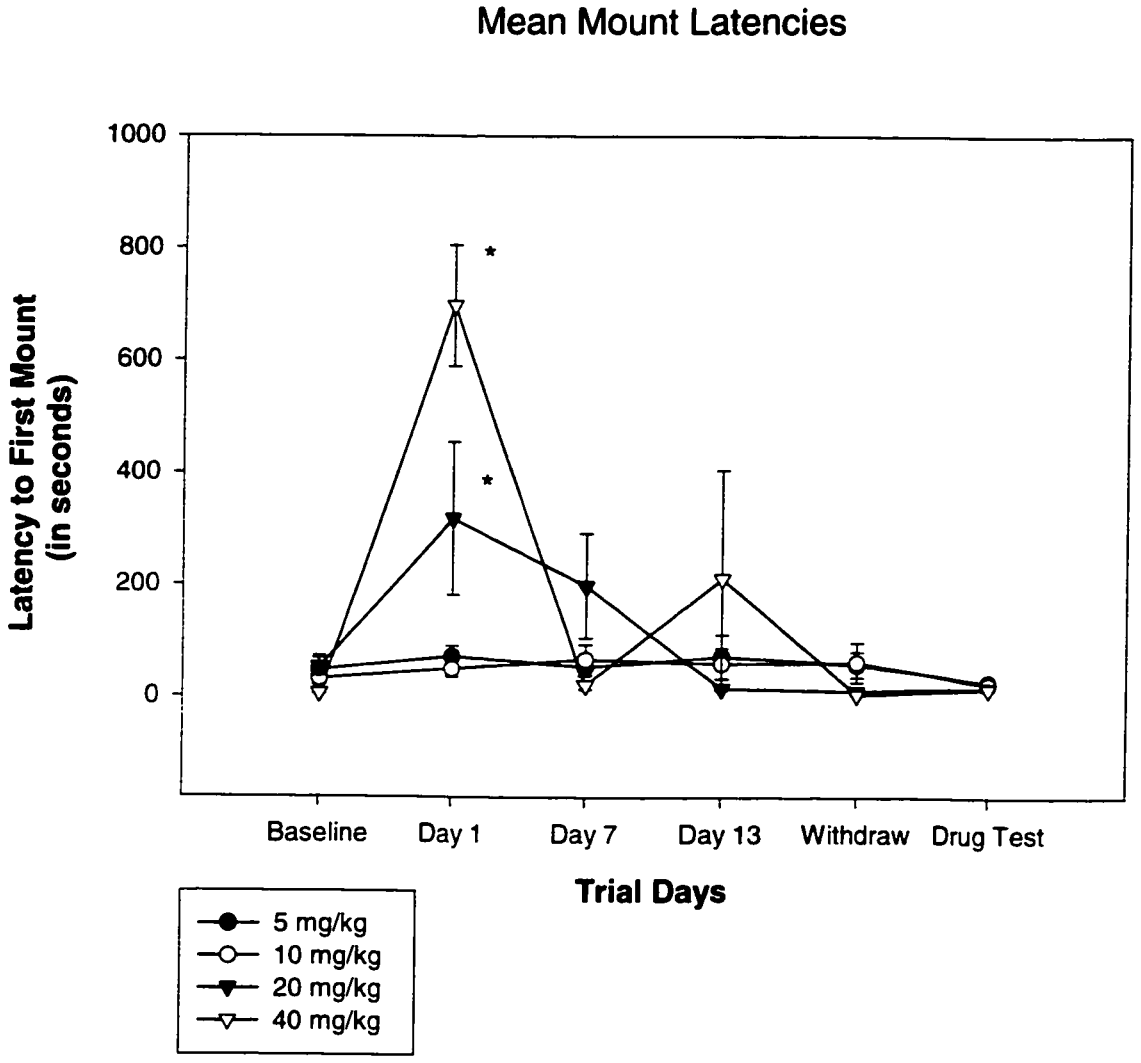

Figure 2. Average latency (in seconds) to mount for all groups, across all drug trials.
* denotes $p < 0.034$ comparing Baseline to Day 1 at 20 mg/kg and 40 mg/kg.

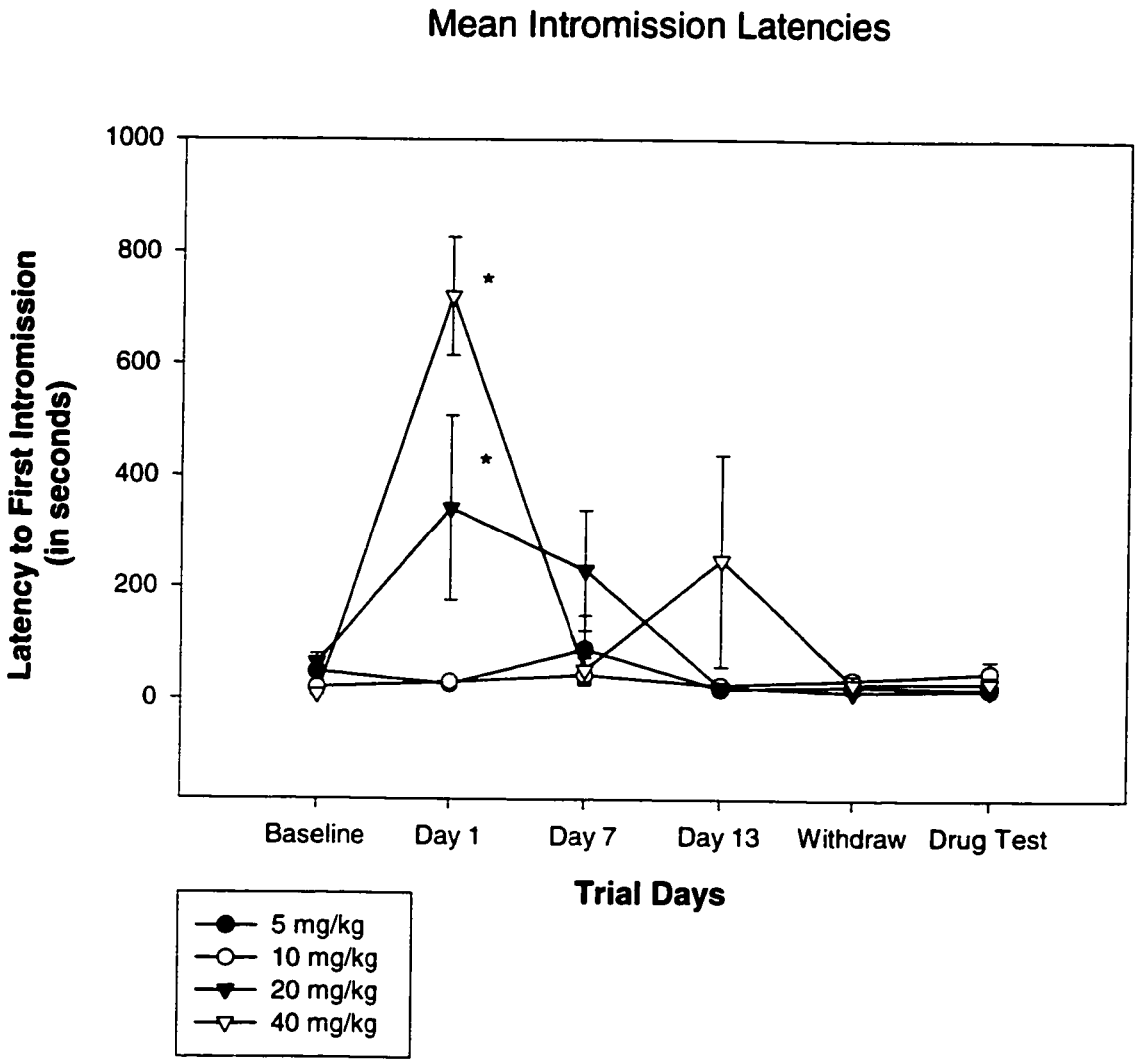

Figure 3. Average latency (in seconds) to intromit for all groups, across all drug trials.
* denotes $p < 0.0037$ comapring Baseline to Day 1 at 20 mg/kg and 40 mg/kg.

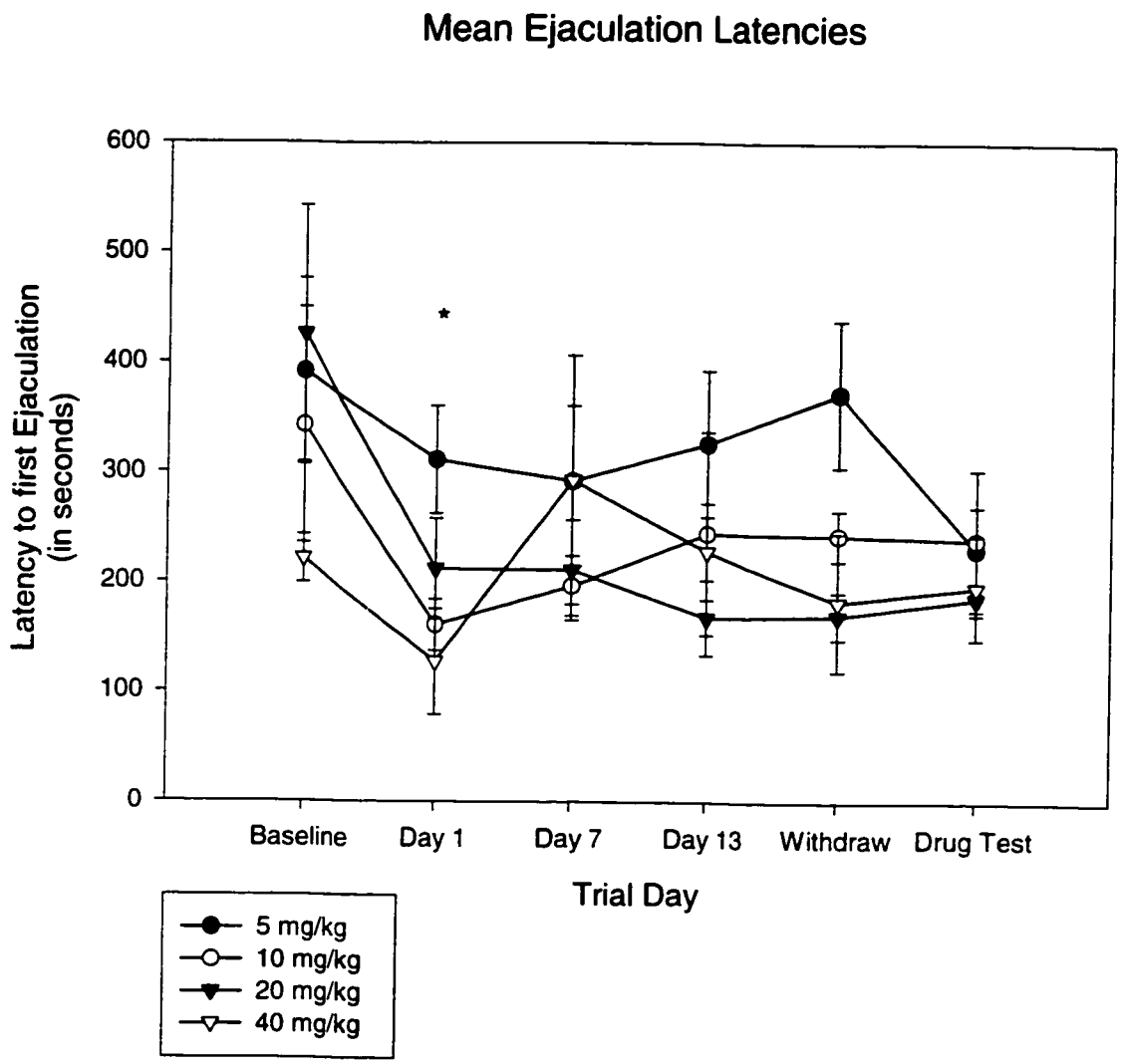

Figure 4. Average latency (in seconds) to ejaculate for all groups across all drug trials.
* denotes $p < 0.05$ for comparison between Baseline and Day 1 for all groups.

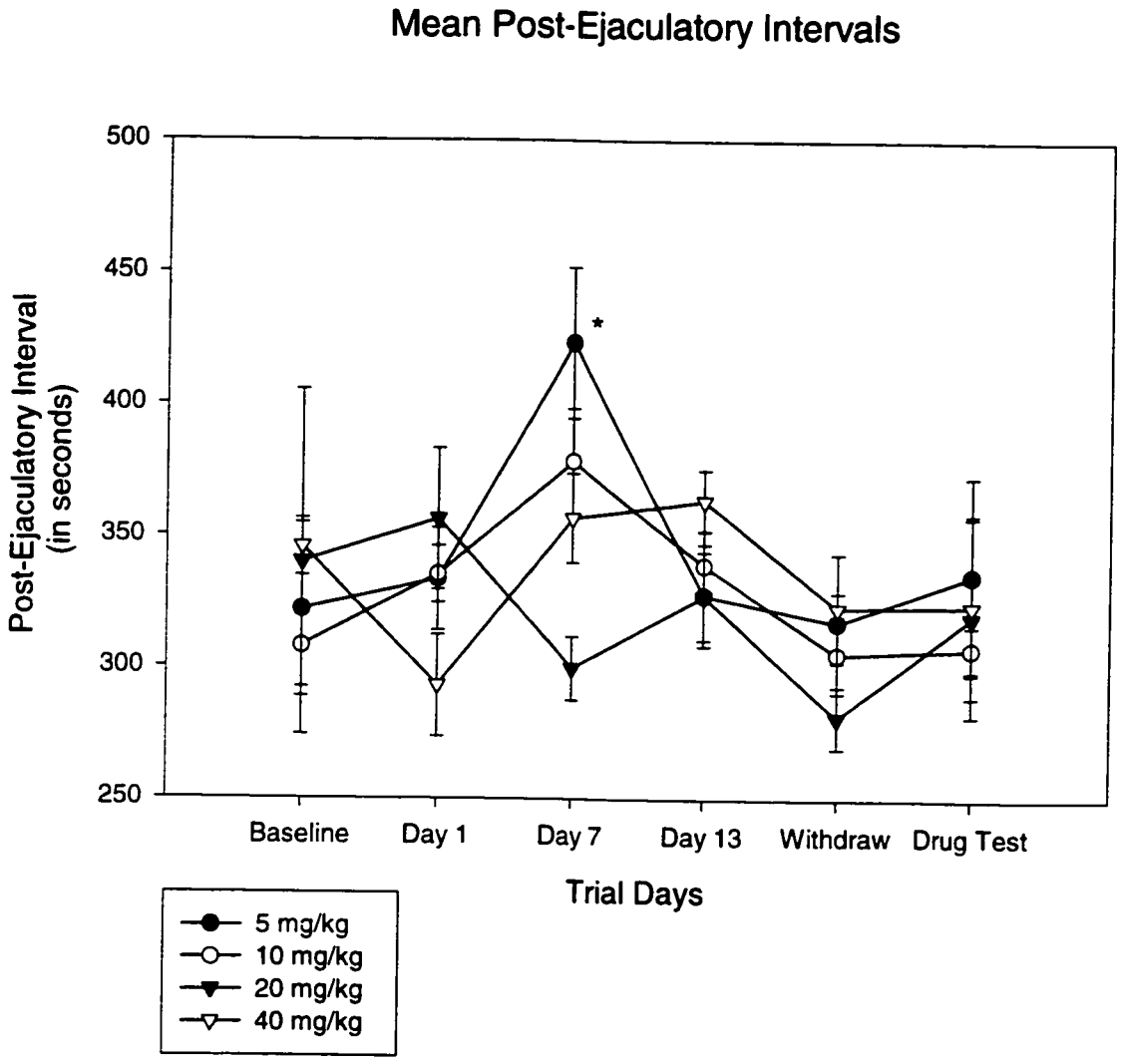

Figure 5. Average time elapsed (in seconds) between the 1st ejaculation and 1st intromission of the second ejaculatory series, for all groups across all drug trials. * denotes $p < 0.04$ comparing Day 7 to Baseline and Drug Withdrawal at 5 mg/kg.

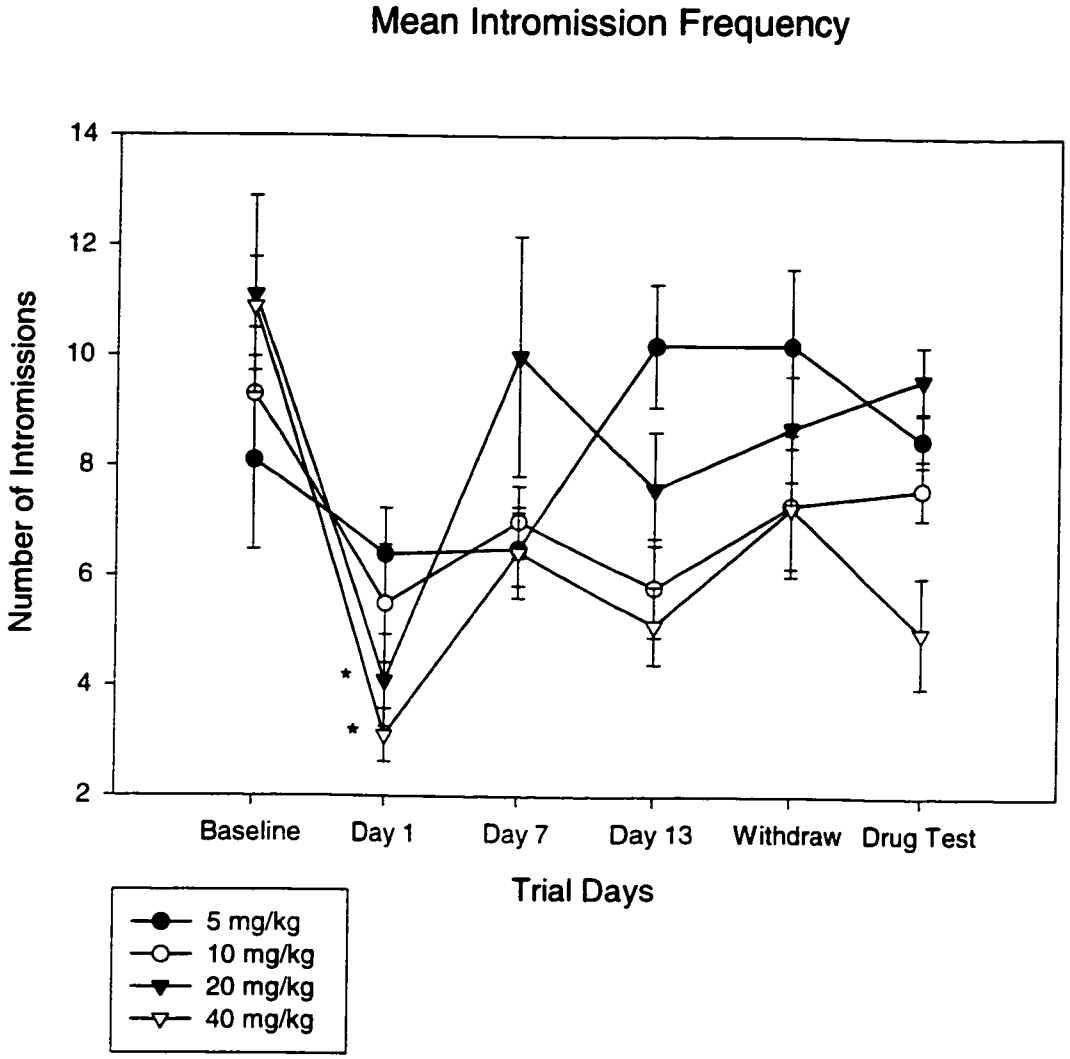

Figure 6. Average number of intromissions prior to ejaculation for all groups across all drug trials.
* denotes $p < 0.0002$ comparing Baseline to Day 1 at 20 mg/kg and 40 mg/kg.

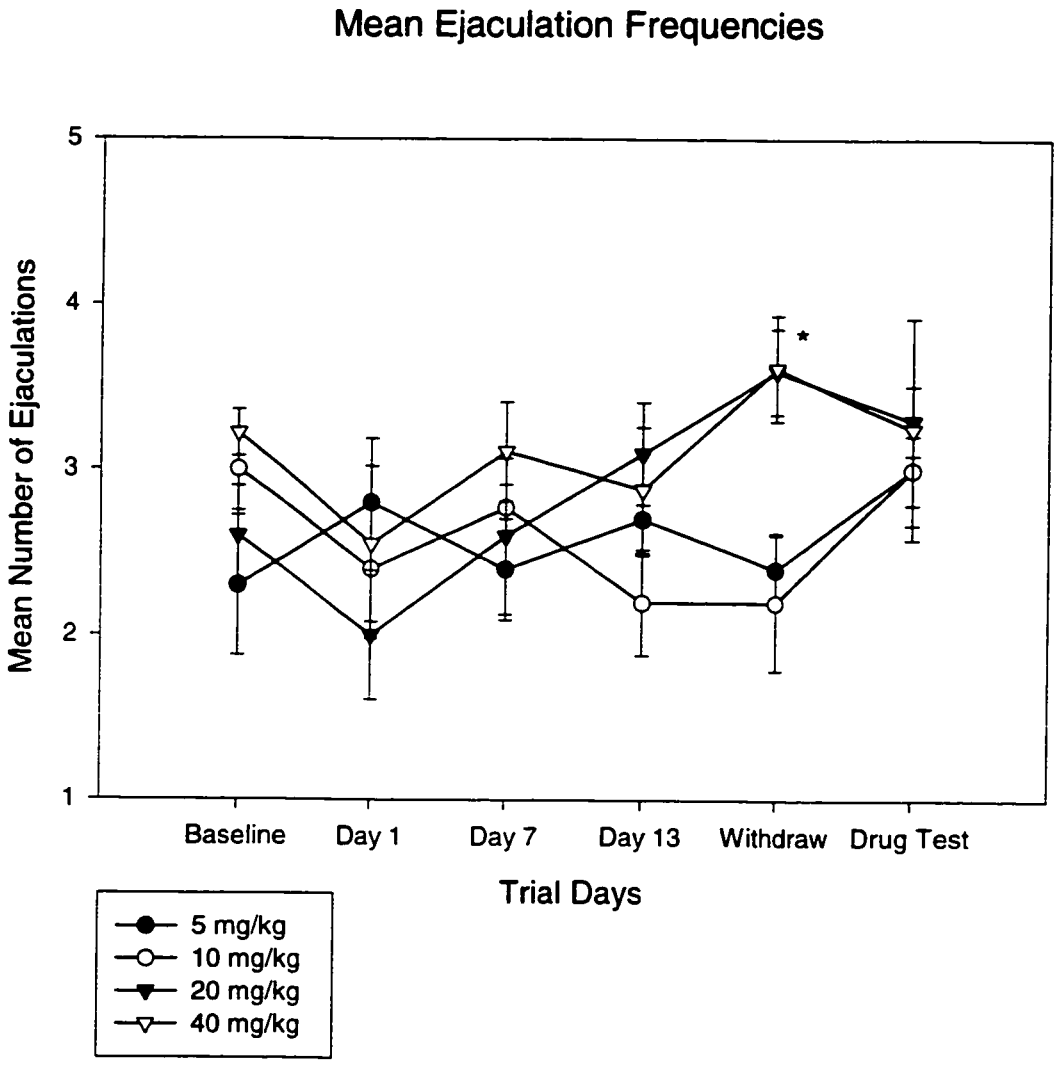

Figure 7. Average number of ejaculations for all groups, across all drug trials.
* denotes $p < 0.03$ for comparison between Withdrawal Day and Day 1 at 20 mg/kg.

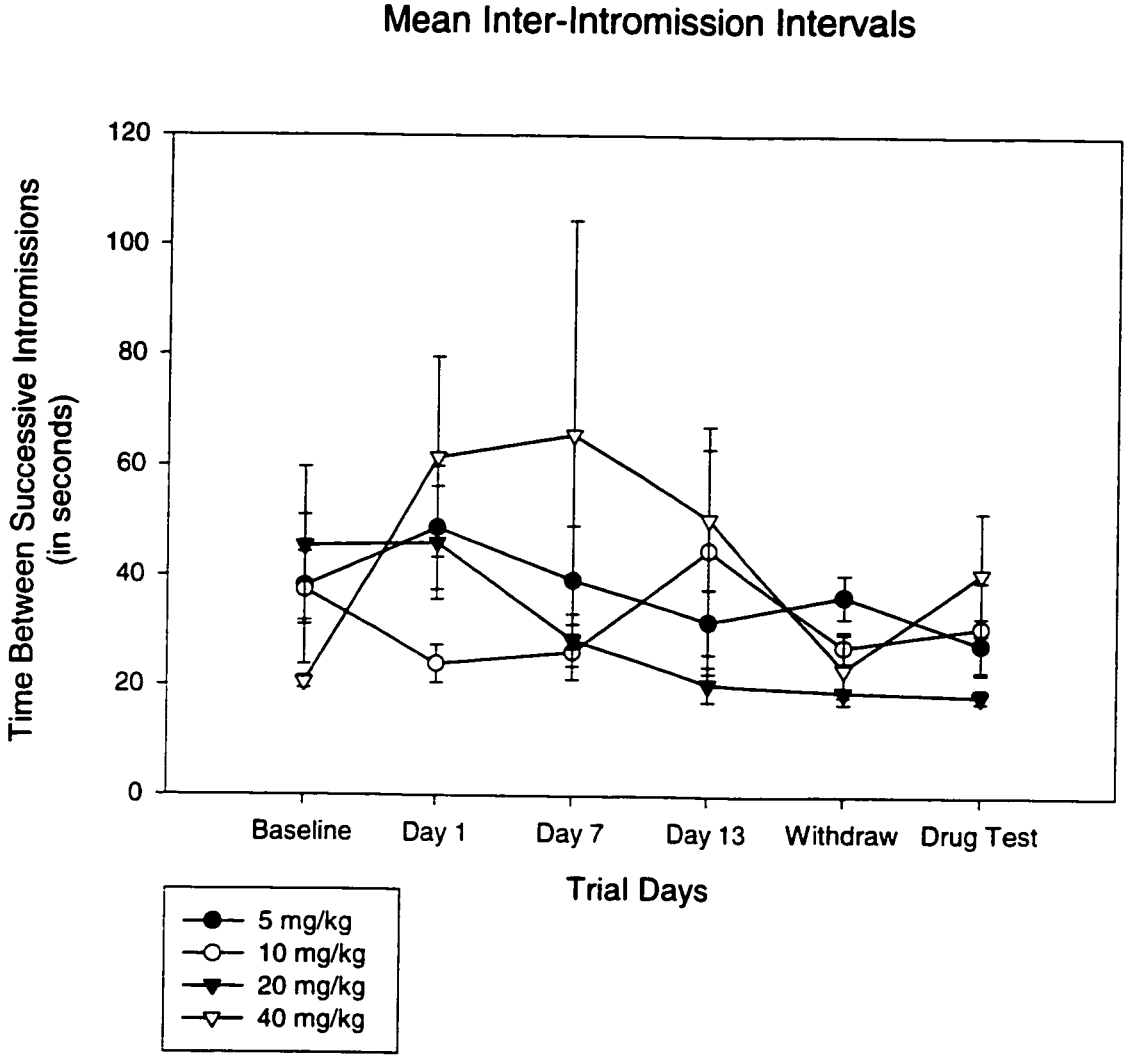

Figure 8. Average time (in seconds) between successive intromissions for all groups across all drug trials.

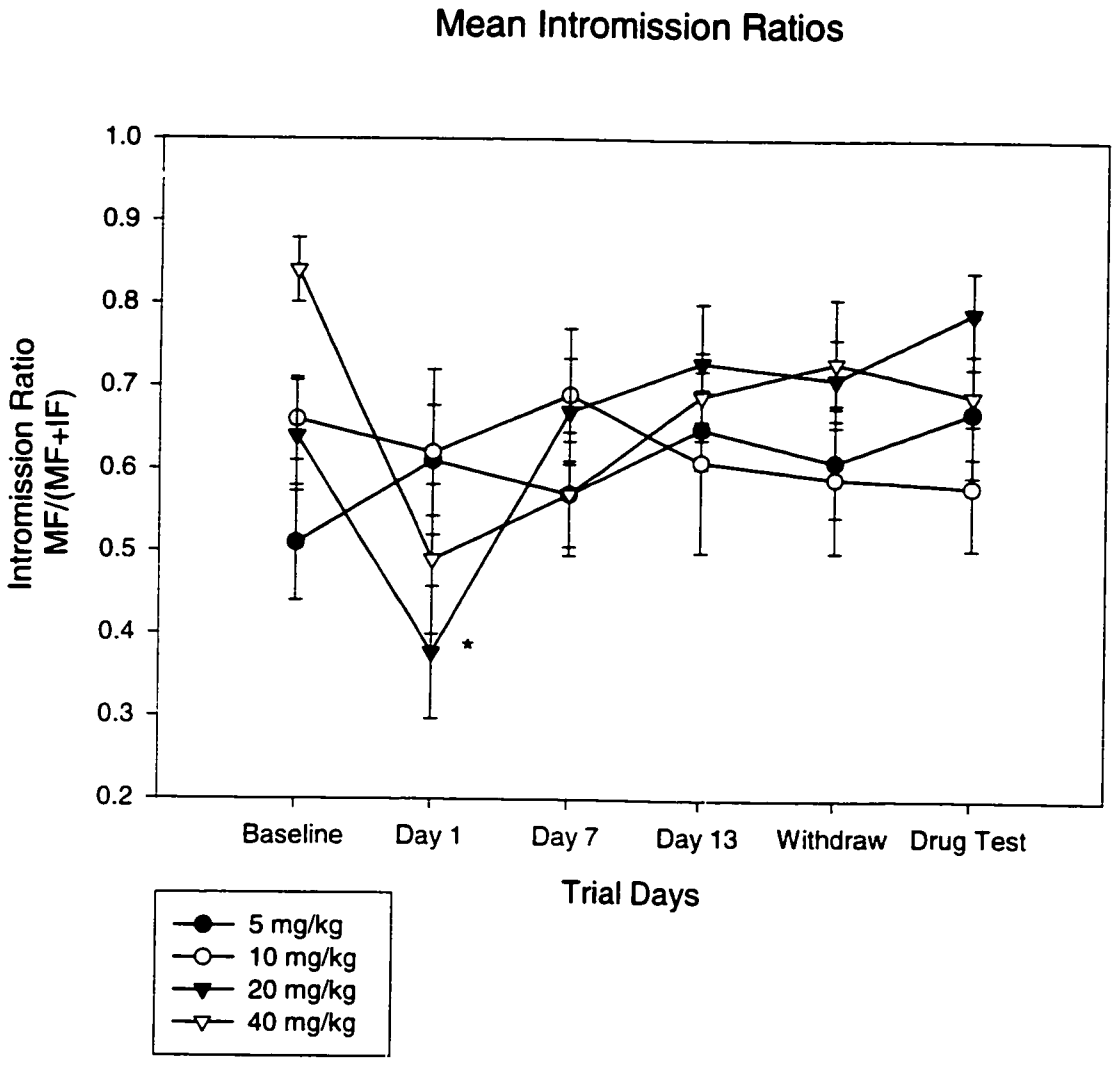

Figure 9. Average number of mounts relative to mounts + intromissions for all groups, across all drug trials.
* denotes $p < 0.02$ comparing Baseline to Day 1.

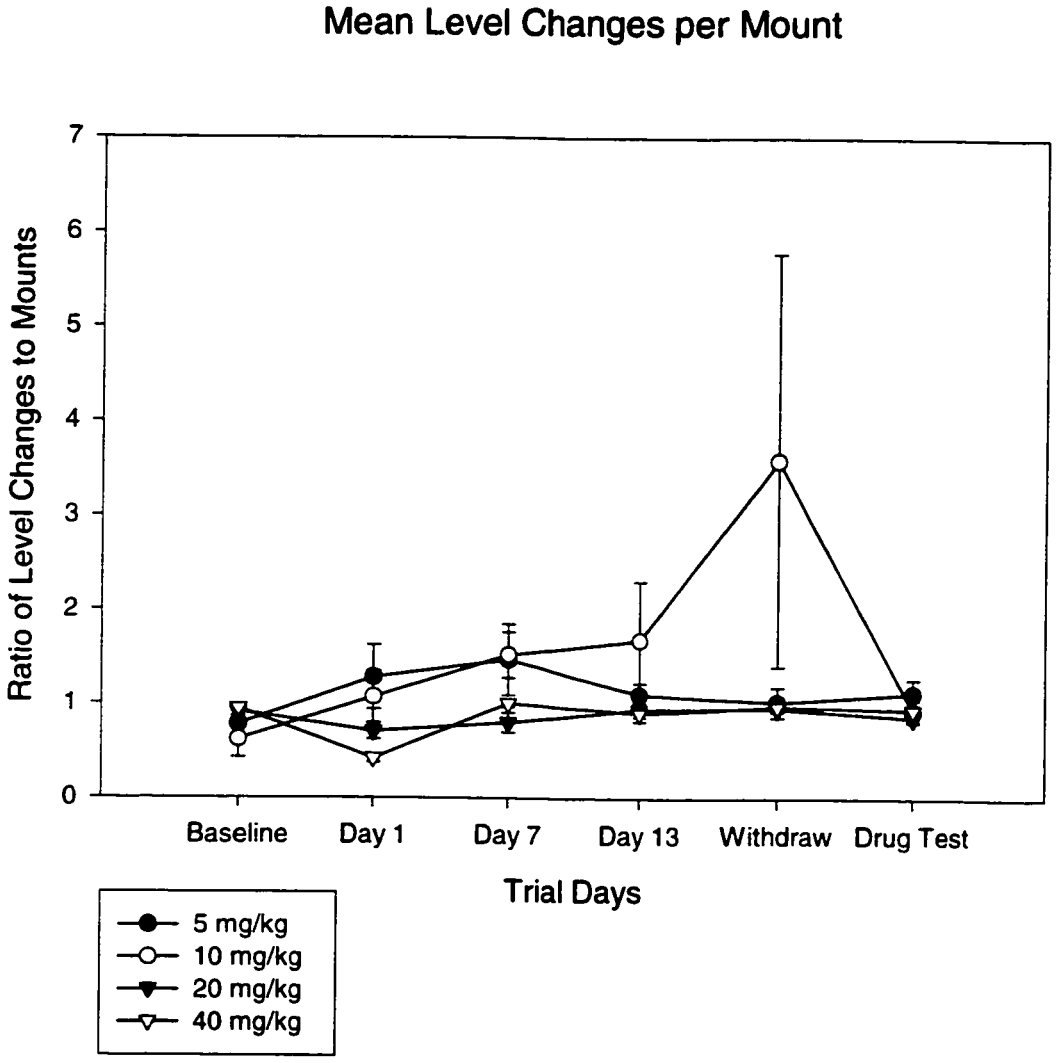

Figure 10. Average number of level changes relative to mounts for all groups across all drug trials.

Long-term effects of cocaine.

The long-term effects of cocaine are shown in Figures 2-11, (Day 7, and Day 13).

Appetitive Level Changes (ALC): Cocaine treatment lead to a dose dependent decrease in the number of level changes observed prior to the insertion of the female. A main effect of drug dose was found for ALC, $F(3,34)=8.61$, $P<0.0002$. Post hoc tests revealed that the 40mg/kg group displayed significantly less appetitive level changes throughout the experiment than the 5mg/kg ($P< 0.03$), the 10 mg/kg ($P< 0.0006$), and the 20 mg/kg group ($P<0.003$). Tolerance or sensitization did not appear to accrue to the effects of cocaine on ALC.

Mount Latency (ML): The 20 and 40 mg/kg doses of cocaine produced an acute increase in mount latencies to which tolerance accrued over the course of treatment. This is evidenced by the results of the post hoc tests which revealed that for the 20 mg/kg and 40 mg/kg group, acute cocaine administration (Day 1) lead to significantly elevated mount latencies when compared to Baseline ($P<0.034$) and every other test day, which did not significantly differ from each other.

Intromission Latency (IL): A significant main effect of Dose, $F(3,25) = 5.5$, $P<0.005$, and Time, , $F(5,155) = 19.8$, $P<0.000001$, as well as Dose X Time interaction, $F(15,125) = 9.44$, $P<0.000001$, were found. Cocaine increased latencies to intromit with females acutely in both the 20 mg/kg and 40 mg/kg groups, however tolerance accrued to this effect over repeated administrations since Day 7 and Day 13 were not significantly different from Baseline.

Ejaculation Latency (EL): Cocaine administration, at all doses, facilitated ejaculation in a time dependent fashion, as evidenced by a decrease in ejaculation latency. A significant main effect of Time was found, $F(5,115) = 3.38$, $P<0.007$. Measures of EL remained lower than Baseline throughout the course of the experiment, (Day 7 $P<0.03$; Day 13 – near-significance, $P<0.067$).

Post Ejaculatory Interval (PEI): Significant main effects for Dose, $F(3,22) = 3.49$, $P<0.04$, Time, $F(5,110) = 4.07$, $P<0.002$ and Dose x Time Interaction, $F(5,110) = 1.8$, $P<0.05$, were found. Post hoc tests revealed that this effect was limited to the 5mg/kg group in which PEI measures for Day 7 were significantly higher than Baseline ($P<0.04$) and Drug Withdrawal Day ($P<0.02$). Thus, sensitization to the effects of cocaine on PEI may have occurred in the 5 mg/kg group.

Intromission Frequency (IF): Tolerance accrued to the effect of cocaine on intromission frequency. Main effects for Time $F(5,170) = 10.55$, $P<0.00001$, and a Dose x Time interaction $F(15, 170) = 2.16$, $P<0.01$, were found. Post hoc tests showed that for the 20 mg/kg group, acute cocaine administration (Day 1) lead to significantly less intromissions than Baseline ($P<0.0002$), and Day 7 ($P<0.006$).

Ejaculation Frequency (EF): Post hoc tests showed that at 20 mg/kg the mean number of ejaculations on Day 1 was less than that on Drug Withdrawal. Figure (8) suggests that tolerance accrued to the acute effect of cocaine to decrease EF, although only the aforementioned comparison was statistically significant.

Intromission Ratio (IR): The relative number of mounts and intromissions (Hit Rate) decreased in a dose and time dependent fashion. Analysis of IR detected a significant main effect of Time, $F(5, 145) = 2.78$, $P<0.02$, and a trend towards significance for the

Time x Dose interaction $F(15, 145) = 1.64$, $P=0.068$. Post hoc analysis of the interaction effect revealed that, at 20 mg/kg, the IR on Day 1 was lower than on Day 13, Withdrawal Day, and Drug Challenge as apparent from Figure (10). The 40 mg/kg group showed a similar albeit non-significant trend. Thus, tolerance accrued to the effect of cocaine (20 mg/kg) on the relative number of successful mounts with intromission.

Inter-Intromission Interval (III): Cocaine did not affect the time elapsed between successive intromissions over the course of treatment.

Level Changes per Mount (LC/M): Long-term cocaine administration did not significantly affect the relative number of mounts to level changes.

Withdrawal and Drug Challenge

The effects of withdrawal from long-term cocaine and subsequent drug challenge are shown in Figures 2-11, (Withdrawal, and Drug-Test).

Appetitive Level Changes (ALC): Drug Withdrawal and Drug Challenge did not induce any significant changes in appetitive level changes.

Mount Latency (ML) & Intromission Latency: Drug Withdrawal and Drug Challenge did not induce any significant changes in mount and intromission latencies.

Ejaculation Latency (EL): Post hoc tests on the Main Effect of Time showed that measures of EL were significantly (or near-significantly) lower than Baseline throughout the entire experiment including Drug Withdrawal ($P<0.04$) and Drug Challenge ($P<0.0605$). Ejaculation latencies were not significantly different during withdrawal or drug challenge when compared to acute or long-term cocaine administration.

Post Ejaculatory Interval (PEI): In the 5mg/kg group, PEI measures for Day 7 were significantly higher than Drug Withdrawal ($P<0.02$). However, because Drug Withdrawal did not differ from Day 13, one cannot infer that withdrawal effects were apparent for that group.

Intromission Frequency (IF): No significant withdrawal and drug-challenge effects were observed for the number of intromissions preceding ejaculation.

Ejaculation Frequency (EF): Post hoc tests revealed that in the 20mg/kg group, males showed a significantly greater number of ejaculations on Withdrawal Day (Day 14) than on Day 1 ($P<0.03$).

Intromission Ratio (IR): As displayed in Figure (10), no significant withdrawal or drug challenge effects were observed for the relative number of mounts to mounts with intromissions.

Level Changes per Mount (LC/M): Withdrawal and Drug Challenge did not significantly affect the relative number of mounts to level changes as compared to Baseline and each other.

Discussion

Overall, cocaine administration lead to decreased ejaculation latencies compounded with fewer intromissions prior to ejaculation. This pattern of effects could indicate that cocaine increased sexual arousal and may have rendered the male rats more sensitive to sexual stimulation and ejaculation.

It is important to note that male rats under the influence of cocaine were not more likely to engage in mounting behavior; their total number of mounts was unchanged, and their mount and intromission latencies increased. Thus any disruption in conditioned

inhibition of copulatory behavior in the subsequent experiments could not be interpreted as a simple facilitation of mounting. Acute cocaine administration also disrupted the "hit rate", interfering with the activation of musculo-skeletal copulatory behaviors.

Experiment 2: Effects of Cocaine on Primary Conditioned Sexual Inhibition.

Pfaus and Pinel (1989) showed that alcohol (0.5 mg/kg, i.p.) can produce sexual disinhibition in male rats in the presence of non-receptive females. Experiment 2 addressed whether cocaine could produce this effect.

Methods

Animals

Males: Thirty-two Long-Evans rats were obtained from Charles River Canada, Inc., (St. Constant, Quebec). The males weighed approximately 300 grams and were sexually naïve at the start of the experiment. They were housed in groups of 4 in standard wire-mesh cages in a colony room maintained at approximately 21°C on a reversed 12:12-h light:dark cycle. All rats had as lib access to food (LabDiet 2000) and water.

Females One month prior to the beginning of the experiment, forty female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions while anesthetized. The rats were anesthetized with ketamine hydrochloride (50 mg/ml; Ayerst Veterinary Laboratories, Guelph, Ontario) and xylazine hydrochloride (4 mg/ml; Bayer Inc., Etobicoke, Ontario) mixed at a ratio of 4:3 ml, respectively. The anesthetic was injected IP in a volume of 0.90 ml/kg of body weight. The females were sexually experienced, having been allowed to copulate several times with a separate set

of sexually experienced males prior to the start of the experiment. Sexual receptivity was induced by subcutaneous administration of estradiol benzoate (10μg) 48 hours prior and progesterone (500μg) 4 hours prior to each trial. Females were housed under the same conditions as the males. Receptive females were housed separately from non-receptive females.

Apparatus and Behavioral Screening.

Baseline trials of copulatory behavior and Conditioning Trials took place in bi-level chambers, as in Experiment 1. Male rats were pre-exposed to the bi-level chambers once a day for 15 minutes a day for 7 days in order to habituate them to the training environment. Following the habituation phase all males received 10 training trials, at 4-day intervals, with sexually experienced females in order to acquire sexual experience. For the training phase, males were placed in the bi-level chamber for 5 minutes prior to the introduction of a receptive female. Both rats were then allowed to copulate for 30 minutes.

The training phase was followed by 14 conditioning trials. In the conditioning phase, access to sexually-receptive and non-receptive females occurred on alternating trials at 2-day intervals. Males were placed in the bi-level chambers for 5 minutes after which either a receptive or non-receptive female was introduced. On receptive-training days, males were allowed to copulate up to only one ejaculation and removed after their post-ejaculatory interval (once they resumed mounting after the first ejaculation). This usually did not exceed 15 minutes. On non-receptive training days (Inhibitory training), male rats were given 15 minutes of exposure to a non-receptive female before the trial was terminated.

All conditioning trials and the final drug test were videotaped and scored for criteria of sexual behavior described in Pfaus et al, 1990: Appetitive Level Changes (ALC), Level Change Latency (LCL), Total Level Changes (LCF), Mount Latency (ML), Intromission Latency (IL), Ejaculation Latency (EL), Mount Frequency (MF), Intromission Frequency (IF), Intromission Ratio (IR), Inter-intromission Interval (III), and the Post Ejaculatory Interval (PEI), and Level Changes per Mount (LC/M). The behaviors were recorded using a PC based program (Cabilio, 1996). The percentage of rats mounting on non-receptive trials was also calculated.

Drug Treatment

Male rats were assigned to three cocaine-dose groups (0 mg/kg, 20 mg/kg and 40 mg/kg) using randomized stratified blocking based on their ejaculation latencies on their final receptive-training day. All male rats received saline for the three trials preceding the Cocaine Test Day in order to habituate them to the minor stress induced by needle injection.

The Cocaine Test day occurred 4 days following the final non-receptive conditioning trial. On the drug test day, males were injected with cocaine HCL or saline intraperitoneally 30 minutes before the beginning of the test. Males were placed in the bi-level chamber for 5 minutes prior to the introduction of a non-receptive female, and were the allowed access to the female for 15 minutes.

Statistical Analysis

Chi Square Tests for Goodness of Fit were used to compare the proportions of males in the drug treatment group to the proportion of males in the saline group that mounted non-receptive females on the Cocaine Test Day.

Results

Development of Sexual Inhibition During the Conditioning Phase:

Consistent with the results of Pfaus et al., (1986) and Pfaus and Pinel (1989), the male rats gradually learned to suppress their copulatory behavior during a series a tests with non-receptive females while maintaining their baseline rates of copulatory behavior during intervening tests with receptive females. The proportion of males that mounted non-receptive females decreased during the Conditioning Phase from 90% on the first trial to 16% on the last trial (Table 1), $\chi 2=18.89$, $p<0.000015$. In contrast, all rats maintained their baseline rates of mounting, intromitting and ejaculating during the intervening trials with receptive females.

Effects of Cocaine During the Inhibitory Test:

Cocaine at doses of 20 mg/kg and 40 mg/kg did not produce any significant disruption of conditioned sexual inhibition, as measured by the percentage of rats attempting to mount non-receptive females [0 mg/kg: $\chi 2=0$, $p<1.0$; 20 mg/kg : $\chi 2=2.0$, $p<0.157$; 40 mg/kg : $\chi 2=2.5$, $p<0.1138$]. It is also important to note that 30% of the rats in the 40 mg/kg group appeared listless or catatonic and did not display any copulatory behaviors.

Discussion

Cocaine, at the doses tested, was not effective in releasing copulatory behavior from inhibition on the inhibitory test. Thus, primary conditioned inhibition does not appear to be affected by moderate to high doses of cocaine.

Table 1: Development of Sexual Inhibition During the Conditioning Phase.

Inhibitory Conditioning	Percent Mounting
Day 1	90%
Day 2	68.8%
Day 3	59.4%
Day 4	40.6%
Day 5	25%
Day 6	25%
Day 7	18.7%
Day 8	18.7%
Day 9	18.7%
Day 10	12.5%
Day 11	15.6%
Day 12	15.6%
Day 13	15.6%
Day 14	15.6%

Experiment 3: Effects of Cocaine on Secondary Conditioned Inhibition of Mate Preference.

Kippin et al., (1998) showed that a neutral odor could be paired with female non-receptivity and thus become a second order inhibitory cue for sexual behavior in male rats. Experiment 3 addressed whether cocaine could reduce the inhibition apparent in this paradigm.

Methods

Animals

Males: Thirty Long-Evans rats were obtained from Charles River Canada, Inc., (St. Constant, Quebec). The males weighed approximately 300 grams and were sexually naïve at the start of the experiment. They were housed in groups of 4 in standard wire-mesh cages in a colony room maintained at approximately 21°C on a reversed 12:12-h light:dark cycle . All rats had as lib access to food (LabDiet 2000) and water.

Females: Forty female Long-Evans rats from the same supplier as above were ovariectomized via bilateral lumbar incisions while anesthetized. The rats were anesthetized with Ketamine hydrochloride (50 mg/ml; Ayerst Veterinary Laboratories, Guelph, Ontario) and Xylazine hydrochloride (4 mg/ml; Bayer Inc., Etobicoke, Ontario) mixed at a ratio of 4:3 ml, respectively. The anesthetic was injected IP in a volume of 0.90 ml/kg of body weight. The females were sexually experienced, having been allowed to copulate several times with to a different set of sexually experienced males prior to the start of the experiment. Sexual receptivity was induced by subcutaneous administration of

estradiol benzoate (10μg) 48 hours prior and progesterone (500μg) 4 hours prior to each trial. Females were housed under the same conditions as the males. Receptive females were housed separately from non-receptive females. Stimulus females were selected at random for use during conditioning trials and copulatory preference tests, and scented with approximately 1ml of either almond extract (Blue Ribbon, Etobicoke, Ontario, Canada) or distilled water applied to both the back of the neck and anogenital area using a cotton swab.

Apparatus

Conditioning trials took place in uni-level pacing chambers constructed using standard laboratory Plexiglas cages (36 cm x 26 cm x 19 cm) with a Plexiglas insert. The insert was made by attaching a Plexiglas divider-piece (30 cm x 20 cm x 0.5 cm) length wise to the center of a Plexiglas base (35 cm x 18 cm x 0.5 cm). The insert was then placed into the chamber, the base was covered with bedding material, and a piece of wire mesh (0.25 cm grid, 35 cm x 18 cm) with a slit cut into the center was placed over the divider insert. A cover constructed of wire mesh (0.5 cm grid, 36 cm x 20 cm) was placed over the chamber. The result was a "racetrack" cage which allowed the female to pace the copulatory sessions by running from side to side and which maintained sideways orientation of the subjects thus facilitating the identification of sexual behaviors while scoring. The racetrack chambers, and all components were cleaned with warm water and Coverage 256 (Conva Tec, St Louis, MO), and bedding was replaced prior to each conditioning trial. All conditioning sessions were recorded on videotape and later scored using a PC-based program (Cabilio, 1996). Copulatory preference tests took place in two

identical large open field boxes (123 cm x 123 cm x 46 cm) and were scored at the time of testing.

Procedure

Conditioning Phase : Sexually naïve, male rats were pre-exposed to the racetrack chambers once a day for 15 minutes in order to habituate them to the training environment. Following the habituation phase all males received a total of 18 conditioning trials at 2-day intervals during the middle third of the dark phase of the light-dark cycle. Access to sexually receptive and non-receptive females occurred on alternating trials for a total of 15 trials each. All trials with non-receptive females were with females that had almond extract applied to the back of the neck and anogenital area. All trials with receptive females were with females that had distilled water applied to the same areas.

For all conditioning trials, males were placed into a racetrack chamber for 5 minutes, after which a female of the appropriate sexual status and scent was placed into the chamber for a 30-minute test of copulation. The number of male rats that ejaculated during receptive trials as well as those that mounted during non-receptive trials was recorded for each trial.

Copulatory Preference Test: Male rats were assigned to three drug dose groups (0 mg/kg, 20 mg/kg and 40 mg/kg) using randomized stratified blocking based on their ejaculation latencies on the final receptive trial. Males were injected with cocaine HCL or saline i.p. 30 minutes prior to the start of the Copulatory Preference Test.

Four days after the final Conditioning Trial, each male was injected with the appropriate drug dosage and placed in the large open field and allowed to habituate for 5

minutes. At the end of this period , one receptive almond-scented (A+E) and one receptive unscented female (E) were placed simultaneously into two corners of the open field at approximately equal distances from the male. All mounts, intromissions and ejaculations directed towards each female, and the time at which they occurred, were recorded. Tests were terminated 30 minutes after the females were introduced. The data collected during the copulatory preference tests were used to calculate mount latencies, intromission latencies and ejaculation latencies directed towards either female for each male's test, as well as to determine which female was selected for first mount, first intromission and first ejaculation. Criteria for sexual behaviors were those described by Meisel and Sachs (1994).

Statistical Analysis

Chi Square Tests for Goodness of Fit were used for the analysis of proportions of females (almond scented VS unscented) selected for first mount, first intromission, first ejaculation for each dosage group on the Copulatory Preference Test. Behavioral measures were analyzed using mixed ANOVAs (between dose groups, between scent status, within subjects) to assess the effects of the three levels of dose (0, 20, 40 mg/kg), 2 levels of female scent (unscented vs. almond-odor) and the dose x scent interaction for mean mounts, intromissions and ejaculations.

Planned comparisons using LSD t-tests with Bonferroni correction (p adjusted to 0.0169) were conducted to compare the mean number of mounts, and intromissions per female and mean number of ejaculations directed towards the scented versus the unscented female, for each dosage group, throughout the copulatory preference test.

Results

Conditioning Phase: The proportion of males that ejaculated with receptive females increased from 64% on the first trial to 100% on the final trial. The proportion of males that mounted non-receptive females decreased from 86% on the first trial to 14% on the final trial.

Copulatory Preference Test: Figure 12 displays the selection of females for first mount, first intromission and first ejaculation for each group. More males in the control group (0mg/kg) chose unscented receptive females for first mount. Chi square analysis confirmed the statistical significance of this observation: first mount for saline group $\chi 2=5.0$, $p<0.026$. Qualitative observations made during the copulatory preference test lend credence to the notion that the males were in fact choosing which female to mate with based on conditioned cues. When the two females were placed in the open field the males in the control group were often found to initially approach both females and engage in ano-genital investigations. If the first female approached was almond scented, most males in the control group would run towards the other female, investigate her and mount her instead. No other significant differences were found for selection of female for first intromission, or first ejaculation for the saline group. Importantly, no significant differences were found for selection of female for first mount, first intromission and first ejaculation in the 20 mg/kg and 40 mg/kg groups. Thus, cocaine caused a disruption in secondary conditioned copulatory preference, as measured by the selection of female for first mount.

A main effect of dose was found for the mean number of intromissions, $F(2,55) = 7.52$, $P<0.0013$. The 40 mg/kg group engaged in significantly less intromissions (summing across females) than the saline ($p<0.029$) or 20 mg/kg group ($p<0.0157$).

A main effect of dose was also found for the mean number of ejaculations, $F(2,55) = 7.059$, $P<0.0019$. The 40 mg/kg group displayed significantly fewer ejaculations than the saline ($p<0.0035$) or 20 mg/kg group ($p<0.0143$). In fact, only 62% of males in the 40 mg/kg group achieved ejaculation. This is in contrast to Experiment 1 where total number of ejaculations was unchanged by administration of a 40mg/kg dose.

Planned comparisons on the mean number of mounts and intromissions per female revealed an unexpected finding. The saline-control group, and the 40 mg/kg group, did not show any preference for the unscented female in terms of the mean number of mounts or intromissions directed towards that female. The 20 mg/kg group, however, showed a significant preference to mount the unscented female ($p<0.0135$), thus displaying an apparent sexual inhibition towards the scented female over the course of successive ejaculations, even though the preference to choose the unscented female for first mount was abolished in that same group.

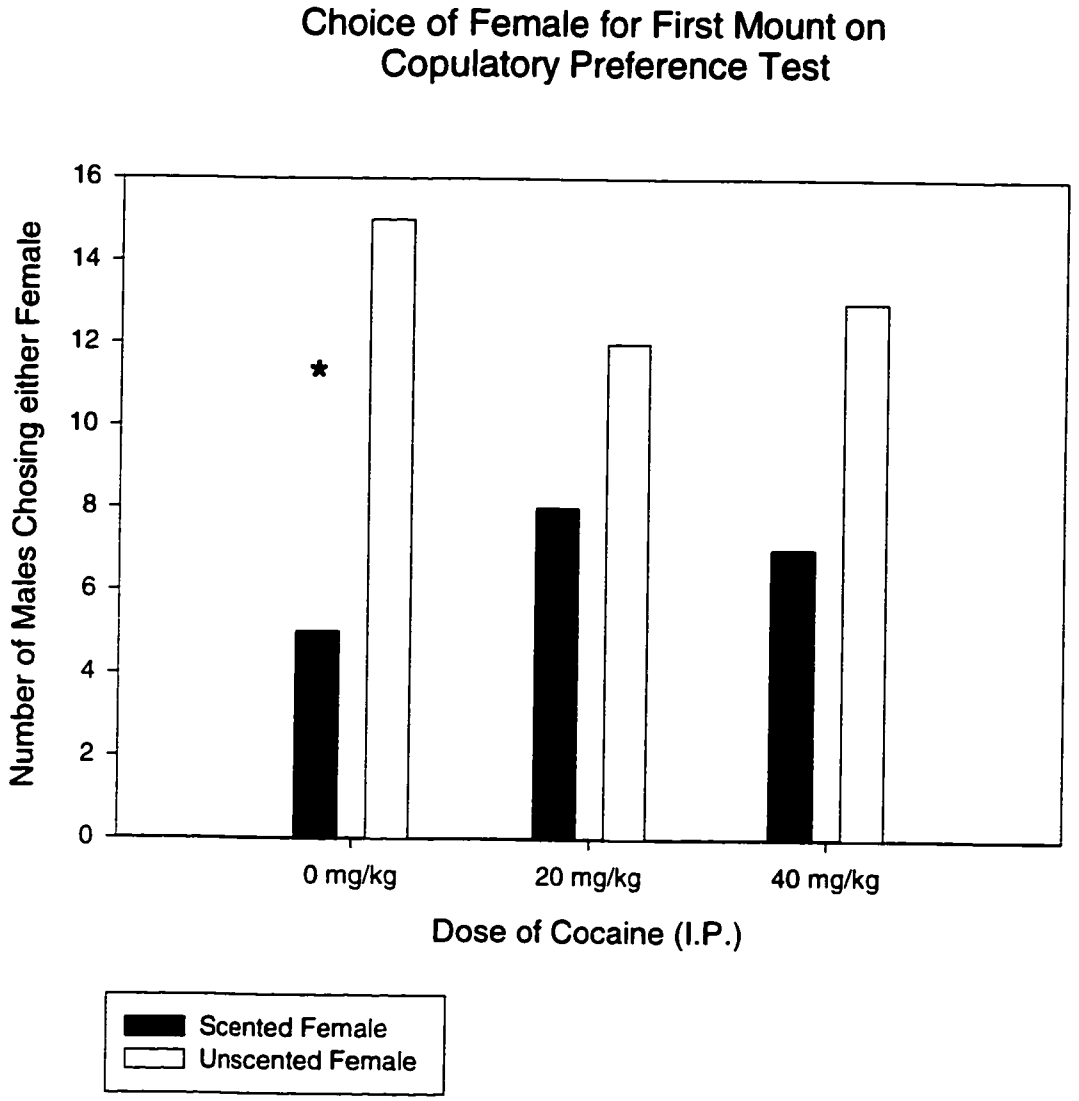

Figure 11. Number of males chosing either female for 1st mount. during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females.
* denotes $p < 0.026$ for Chi Squared test for the Saline group.

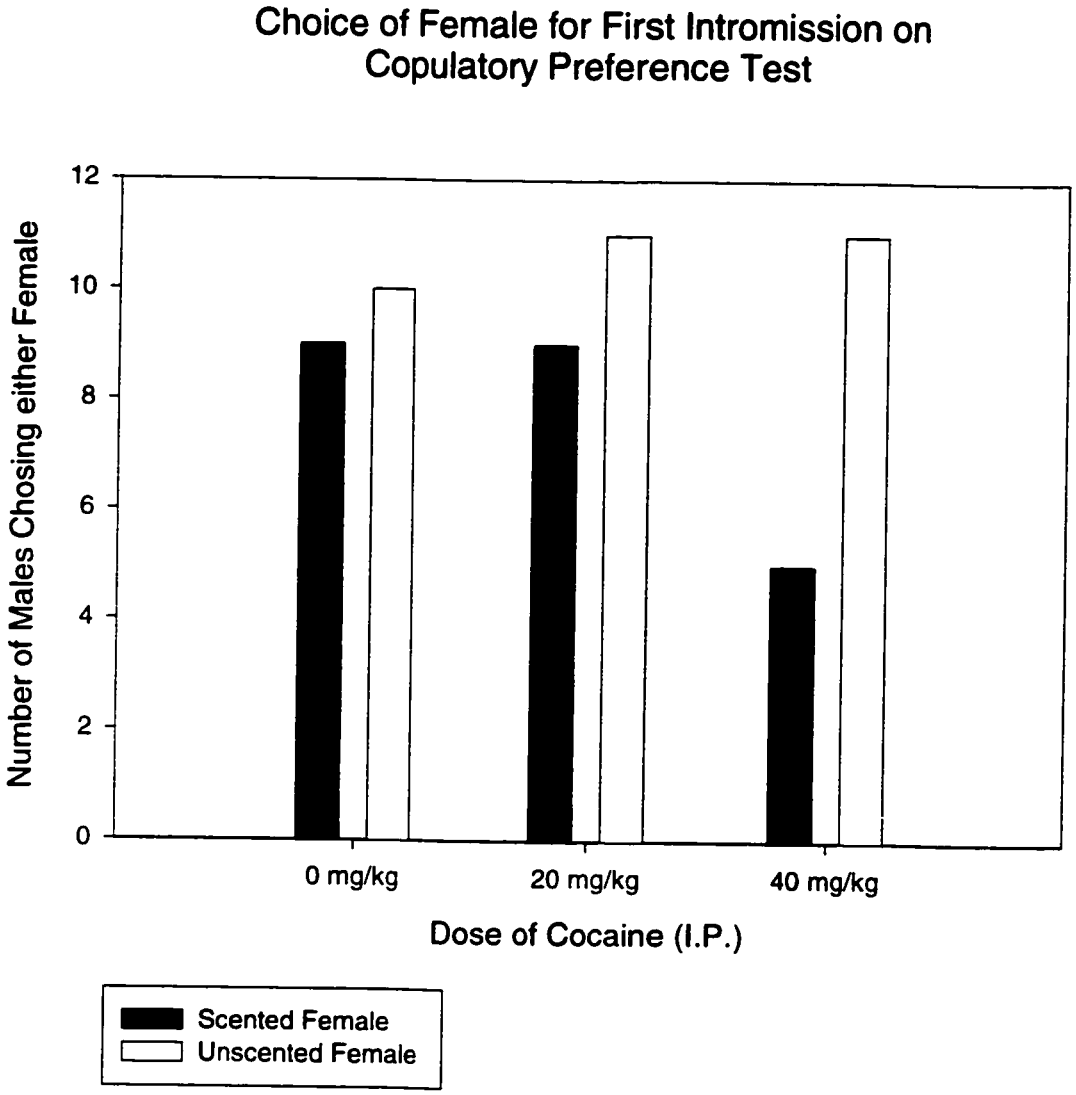

Figure 12. Number of males chosing either female for 1st intromission during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females.

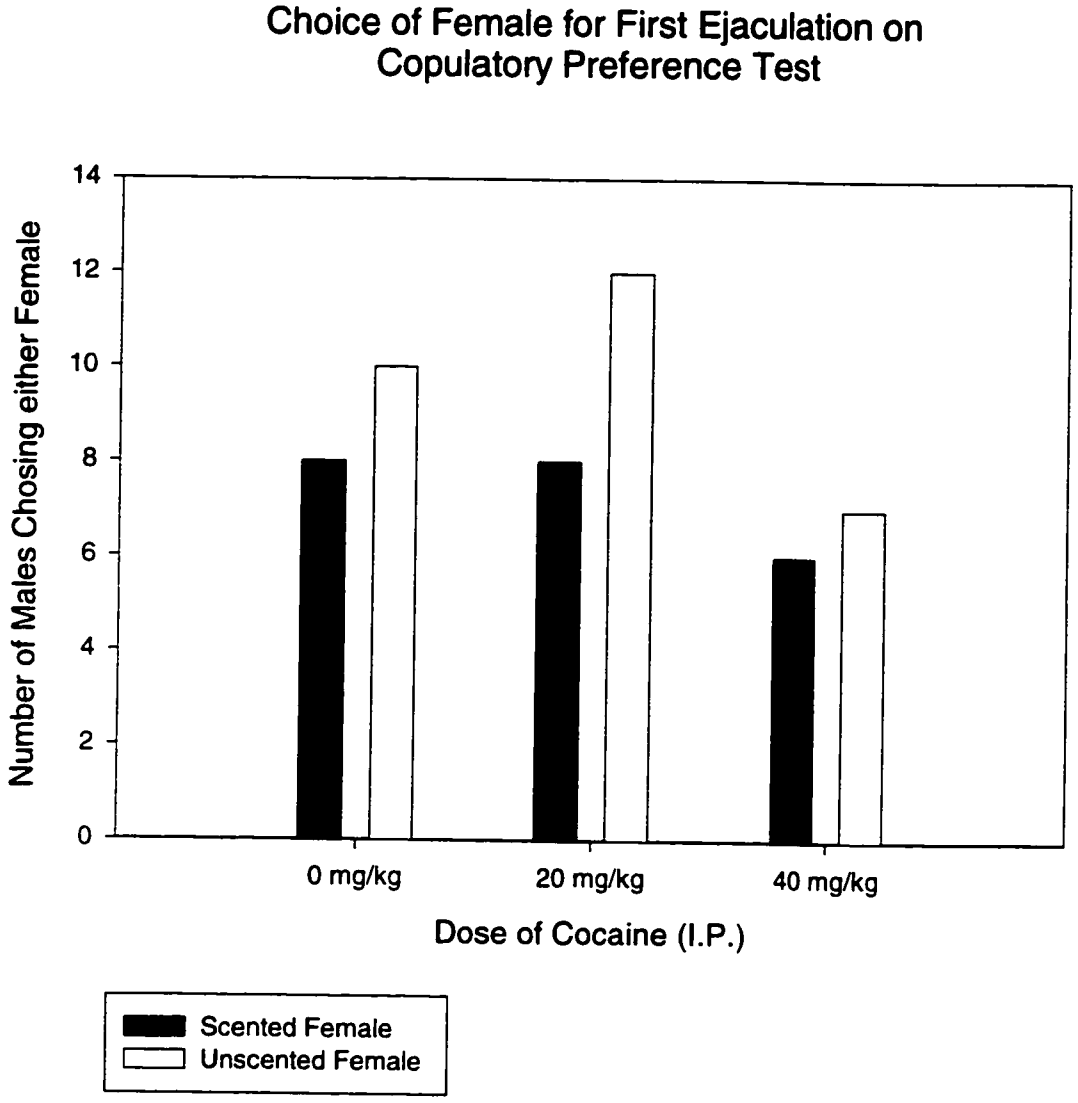

Figure 13. Number of males chosing either female for 1st ejaculation during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females.

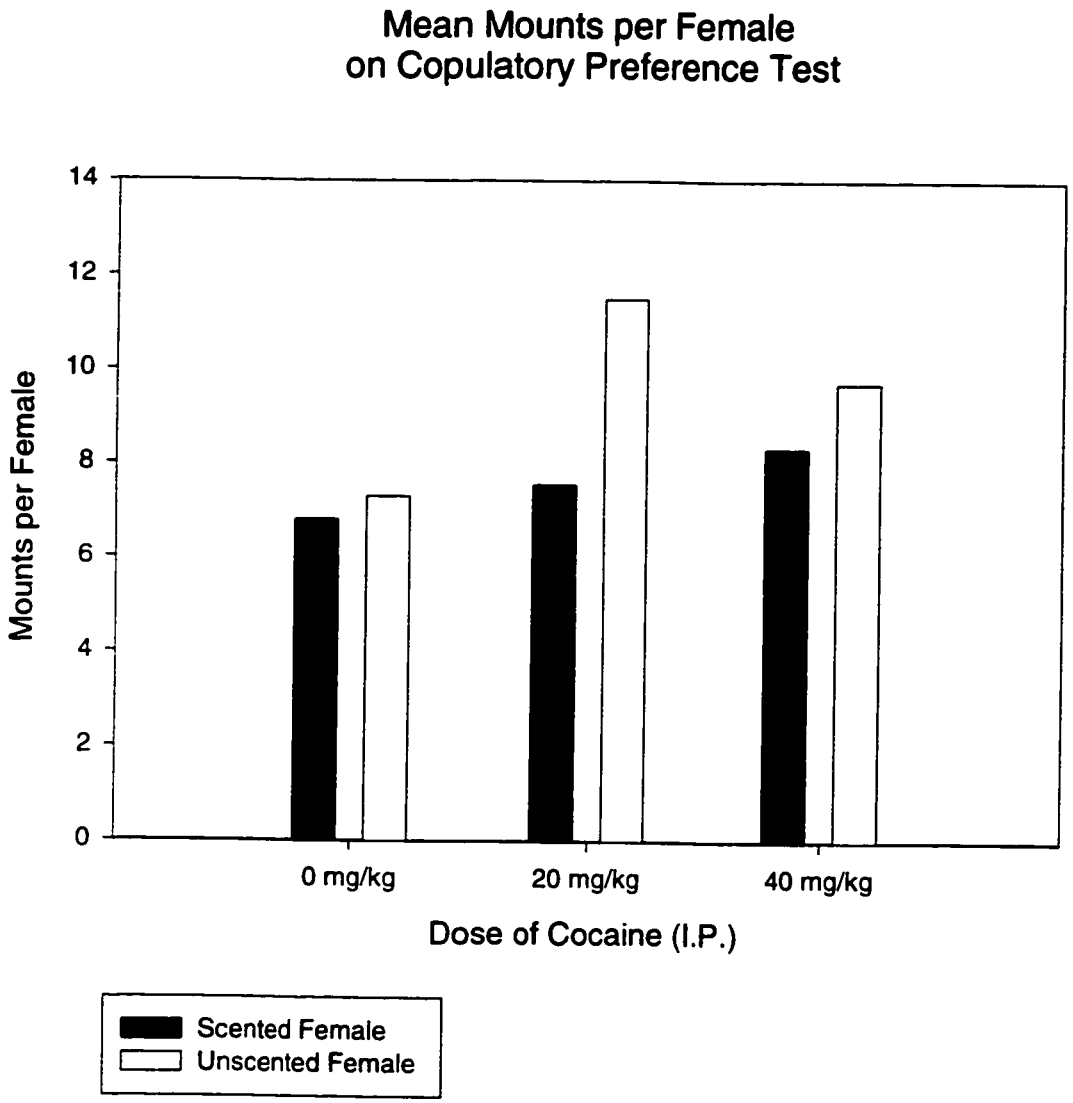

Figure 14. Mean mounts per female during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females.

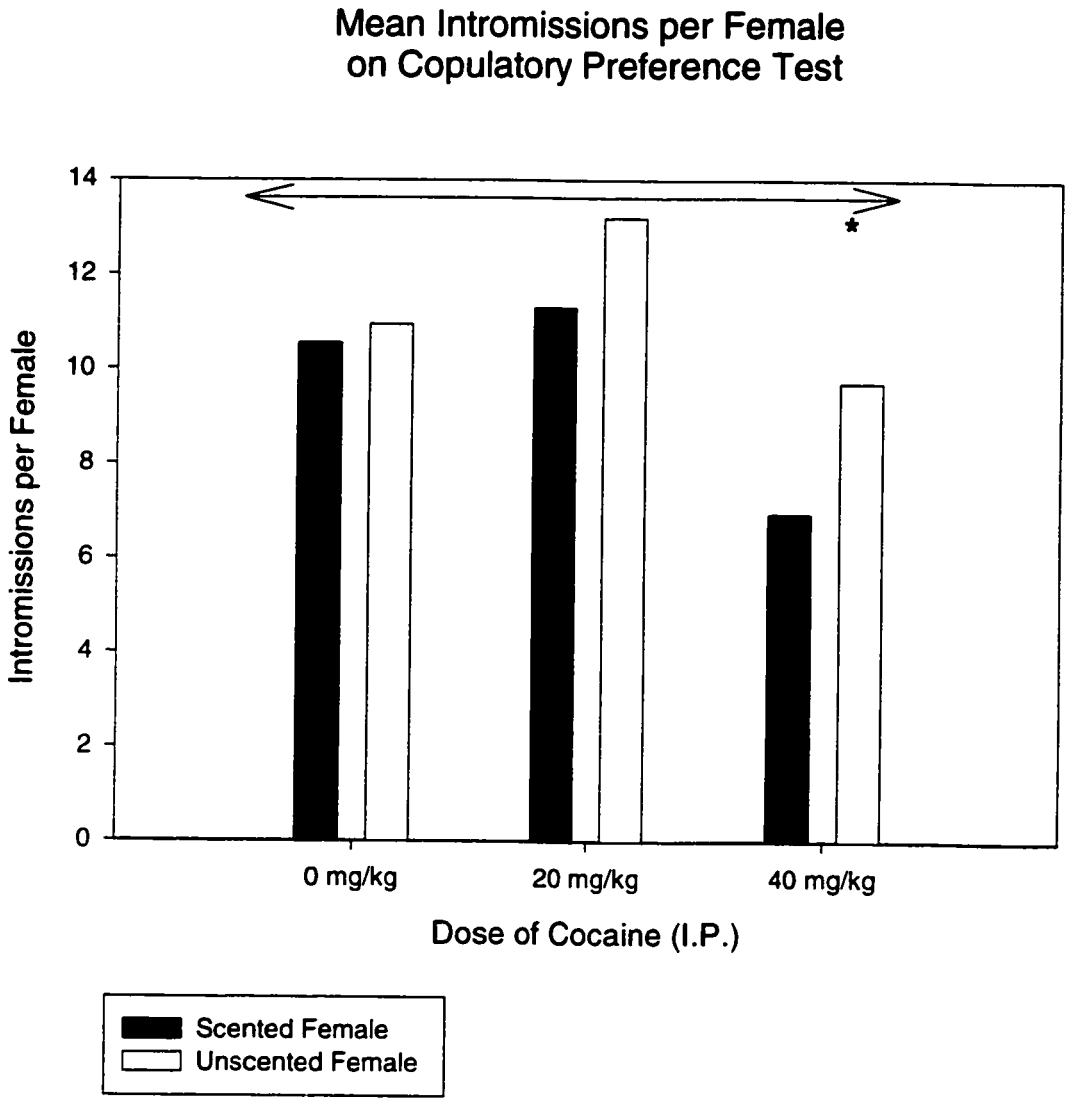

Figure 15. Mean intromissions per female during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females. * denotes $p < 0.029$ for the post hocs on the Main Effect of Dose, comparing the 40 mg/kg group to the 20 mg/kg and Saline Group.

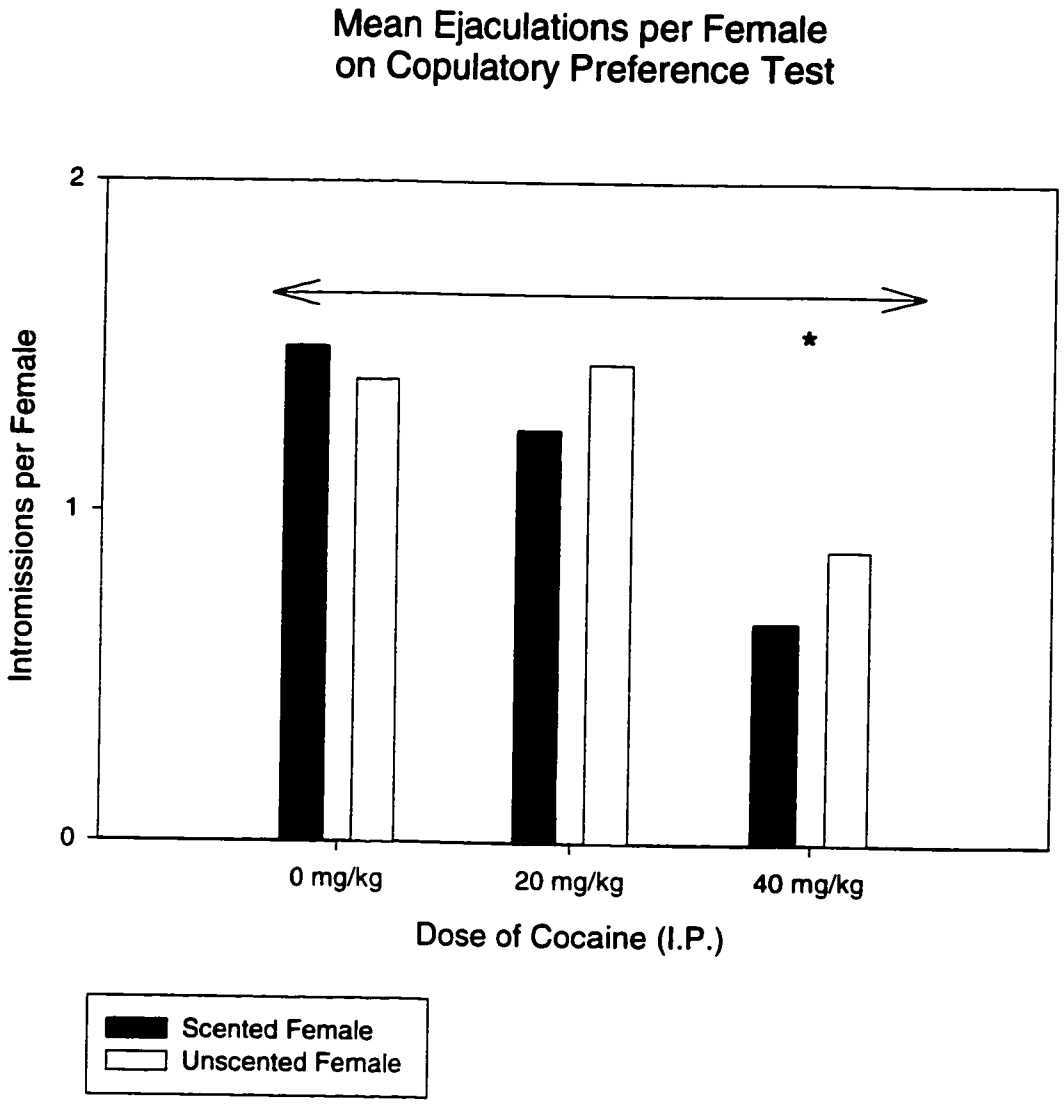

Figure 16. Mean ejaculations per female during the Copulatory Preference Test. Black bars represent scented females, white bars represent unscented females. * denotes $p < 0.01$ for the post hocs on the Main Effect of Dose, comparing the 40 mg/kg group to the 20 mg/kg and Saline Group.

Discussion

Cocaine was shown to block learned sexual inhibition against a scented female, in the choice of first mount. The control group in the present study did indeed show a significant preference for the unscented female in regards to choice for first mount. Thus, male rats in the control group can be said to have inhibited their initial directed copulatory behaviors towards the scented female. Almond odor became a conditioned cue which was associated with a female's non-receptivity, and which resulted in partial inhibition of mounting, independent of context. However, this inhibition was only temporary. As the males were met with receptivity cues from both an unscented and an almond-scented female, the males in the control group and the 40 mg/kg began to copulate with either female indiscriminately. In contrast, the males in the 20 mg/kg group moved from indiscriminant mate choice, towards a preference to copulate with an unscented female. The copulatory preference test can also be seen as an extinction paradigm in which the odor cue (CS) no longer predicts female non-receptivity (UCS). In this case, cocaine at 40 mg/kg may have facilitated extinction of the learned sexual inhibition. However, at 20 mg/kg, cocaine had dual effects: one influencing appetitive approach behaviors (disruption of a tendency to mount the unscented female first), and the other influencing the actual preference for copulation with the unscented female, as measured by mount frequency. A useful heuristic to help understand this seemingly paradoxical effect can be found by referring to Pfaus et al. (1990) who conducted a correlational and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat, and found that the latency factor and the copulatory frequency

factors were uncorrelated. This suggests that there are several discreet processes underlying appetitive and consummatory sexual behavior that can be studied separately, and can be affected differently by a given treatment. A preferred choice for first mount can be conceptualized in terms of latency to mount either female. Thus cocaine's effects were dissociated with respect to these two global factors. The 40 mg/kg group showed no evidence of this effect, consequently the dose-response curve is an inverted, U-shape with respect to cocaine's effects on mount preference. This type of curve is commonly observed in pharmacological experiments that employ behavioral indices to measure drug effects (Julien, 1995).

Interestingly, the results of the control group did not replicate the findings of conditioned ejaculatory preference reported by Kippin et al. (1998), who found that males trained to associate almond-odor with non-receptivity mounted, intromitted and ejaculated preferentially with unscented females during the copulatory preference test and chose the unscented female for first ejaculation. The males in the control group of the present experiment showed no preference in terms of mean number of copulatory behaviors or mean number of ejaculations directed towards either female. This may indicate that conditioned ejaculatory preference (or conditioned inhibition of ejaculation) using scent as a CS, as reported by Kippin (1998), may have been a transient effect. However, conditioned inhibition of sexual behavior was clearly present in Experiment #3, as reflected by the female chosen for first mount by intact males.

General Discussion

The experiments conducted for the present thesis have shown that cocaine will exert multiple effects on different aspects of sexual behavior. The main finding is that cocaine has dually dissociated effects upon conditioned sexual inhibition in male rats. In accordance with previous studies (Leavitt, 1969; Abel et al., 1989; Ferrari and Giuliani, 1997), acute administration of cocaine was shown to facilitate ejaculation by reducing the amount of intromissions necessary to achieve ejaculation. This is likely due to heightened sexual arousal, as previous studies have shown that cocaine facilitates parasympathetic penile reflexes (Rosen, 1991). Repeated administration of cocaine, in a pattern that would simulate chronic use, resulted in tolerance accruing to the facilitatory effects. Extrapolating the present findings to humans would lead to the hypothesis that the tolerance that accrues to the sexually arousing effects of cocaine could become a factor in the maintenance of a drug habit, because a higher drug dose would be needed to achieve the same effect. Additionally, if the arousal level in the acute phase is increased, then presumably sex under the influence of the drug could very easily become a preferred state and lead an individual to want to administer the drug in sexual contexts.

Disinhibition

Most drugs of abuse are widely thought to induce a general cognitive disinhibition which would facilitate the expression of otherwise inhibited behaviors. The link between stimulants, depressants and violence is particularly well documented (Graham and West, 2001; Quigley & Leonard, 2001). Additionally, there exist many scientific reports citing alcohol use as one of the key antecedent factors in rape and risky sexual behavior (Seto & Barabaree, 1995). As discussed previously, cocaine abuse has also been linked to sexual

risk taking. Presumably, risky sexual behaviors are under some sort of learned inhibitory control because most people have learned that unprotected sex is an act that can have severe negative consequences. Survey studies seem to indicate that cocaine may disrupt learned inhibition of high-risk sexual behavior. It is important to note that this purported link is correlational in nature and, as such, one cannot state empirically that cocaine use can cause an individual to engage in risky sex. However ethical standards would prohibit an experimental analysis of the effects of cocaine on human sexual inhibition, especially as it relates to risky sexual behavior. Thus, the experiments conducted for the present thesis employed animal models to test hypotheses about the effects of cocaine on sexual behavior and to test whether learned sexual inhibition, can be disrupted by cocaine. Two paradigms were employed in order to test for disruption of first-order and second-order conditioned inhibition, respectively.

First Order Conditioned Inhibition of Sexual Behavior

In the first-order model, males were conditioned to inhibit their mounting of non-receptive females. In essence, this model used operant conditioning to progressively extinguish mounting behavior, and to condition the male rats differentially with respect to the reproductive status of the female. Thus, if a female displayed estrous odors, and engaged in proceptive sexual behaviors (hopping, darting, ear wiggling, ultrasonic vocalizations), then copulation was reinforced through penile stimulation, ejaculation, and the post-ejaculatory interval. If a female displayed no estrous odor and was avoidant or relatively inactive, then mounting behavior was not reinforced and often punished through antagonistic and aggressive reactions on the part of the female. Using this model, males were gradually conditioned to inhibit their mounting of non-receptive females.

Cocaine was administered within the same conditions as the training phase to assess whether it could release mounting behavior from inhibitory control.

The results of this study indicated that cocaine (20 or 40 mg/kg) was ineffective in provoking disinhibition as measured by a release of mounting behavior. These results are in marked contrast to those obtained with alcohol in the original study by Pfaus et al. (1989). Pfaus et al. had found that a low dose of alcohol (0.5 mg/kg, i.p.) not only released mounting from inhibition, but also caused most males to ejaculate in the presence of non-receptive females. Those results were consistent with several published correlations between proximate alcohol use and rape and other problem sexual behaviors (see Seto and Barbaree, 1995, for review). Cocaine has also been examined in relation to violent sex crimes, (Giannini et al., 1993; Miller et al., 1991) however its link with rape and risky sexual behaviors has never been examined independently of concurrent alcohol use. Cocaine and alcohol are most often used concomitantly (Grant & Harford, 1990). Thus, one cannot state that any purported disinhibitory effects of cocaine are not better accounted for by concurrent alcohol use, or to a synergistic effect of both drugs or to separate additive effects. Another possible factor may be cocaethylene, a psychoactive cocaine metabolite produced in the presence of alcohol (Landry, 1992; McCance et al., 1998). McCance and colleagues conducted a placebo-controlled study in human drug abusers to compare the pharmacokinetic, physiological, and behavioral effects of cocaine, alcohol, and combined cocaine/alcohol administration. Their results indicated that cocaine-alcohol produced greater euphoria and increased perception of well-being relative to cocaine, and that heart rate significantly increased following cocaine-alcohol administration relative to either drug alone. Furthermore, cocaine concentrations were

greater following cocaine-alcohol administration. They also found that cocaethylene had a longer half-life with increasing concentrations relative to cocaine. Based on this evidence, they concluded that the concomitant use of cocaine and alcohol is more stimulating, rewarding, and more euphorigenic than cocaine or alcohol alone. Furthermore, Cunningham et al., (1995) found that cocaethylene increases dopamine levels equipotently to cocaine, but increases serotonin levels to a much lesser extent. Since dopamine and serotonin are thought to exert opposite effects upon sexual behavior (Crenshaw and Goldberg, 1996), this activational profile could prove to be quite significant when considering the effects of either compound alone.

The results of Experiment 2 suggest that cocaine does not affect first order conditioned inhibition of sexual behavior in the male rat. This suggests that other factors may be mediating the purported link between cocaine and risky or 'problem' sexual behaviors.

Second Order Inhibition of Sexual Behavior

The second order inhibition experiment differed in two important ways from the first order inhibition paradigm. In Experiment 3, an odor was paired with female non-receptivity, and sexual inhibition was in response to a conditioned cue. Moreover, the final test day was a copulatory preference test with two receptive females, one almond-scented and the other unscented. Thus, the male rat, conditioned to inhibit responses to almond-scented, non-receptive females, was met with conflicting cues (estrous odor and almond odor) from a scented receptive female. As described previously, the Copulatory Preference Test can also be considered an extinction trial.

Cocaine's effects were dissociated with respect to copulatory approach behaviors directed towards the scented female. On one hand, cocaine abolished the learned preference seen in the control group for the choice of the unscented female for first mount. However, over the course of the test, the males in the control group quickly "learned" that the scented female was, in fact, receptive and began copulating with either female indiscriminately. In fact, the males in the 20 mg/kg group instead moved from indiscriminate mounting (no preference for first mount) to discriminate mounting (significantly more mounts directed towards the unscented female). One possible explanation for these results is that cocaine administration may have lead to a momentarily heightened arousal and attention to all incentive stimuli when both females were placed in the open field. Following this, cocaine's effects can best be described as perseveration of previously learned responses – a resistance to extinction, whereby the unscented female was preferred for mounting despite the fact that almond odor was no longer predictive of non-receptivity.

Interestingly, cocaine and amphetamine have been shown to produce perseverative behaviors in rats in such tests as the Radial Arm Maze (Loh, 1997), visual discrimination tasks (Koek & Slangen, 1984) and bar switching schedules (Scheuer & Moore, 1974). This may explain the perseverative sexual behaviors displayed by the males in the copulatory preference test.

Cocaine and Risky Sexual Behaviors

The studies that have reported a link between cocaine use and risky sexual behaviors have suggested that cocaine produces a cognitive disinhibition and a lack of self-control (Solow & Solow, 1986; Leigh, 1990; Castilla et al., 1999. The results of the

present experiment do not entirely support this assumption. Certainly, cocaine abolished the preference for first mount, however it potentiated the preference for the unscented female, in comparison to the control group. How might these results be reconciled with the purported link in humans? One possible explanation is that under the influence of cocaine, it may be more difficult to ignore sexual stimuli. Consequently, hasty decisions might be made regarding the initiation of sexual behavior, despite inhibitions "conditioned" through culturally bound rules concerning appropriate sexual conduct. Similarly, the male rats were met with sexual stimuli from a scented, receptive female and initially the estrous odors and other cues indicating receptivity may have momentarily superseded the learned association between almond odor and non-receptivity.

There are also a number of different possible explanations as to why cocaine caused a momentary disruption of learned inhibition. Cocaine may have interfered with the memory processes involved in conditioned inhibition. This alternative seems unlikely because many studies have found that dopamine agonists can enhance retention of avoidant response task performance in rats (White, et al., 1995; Janak, et al., 1992). An alternative explanation is that cocaine activates sexual pathways and increases sexual arousal to the extent that all sexual incentives are magnified. This effect would be consistent with the results of Experiment 1 where acute cocaine administration lead to a decrease in the number of intromissions preceding ejaculation, which was possibly due to heightened arousal and increased penile sensitivity. This increased arousal may mediate the effects of risk perception, in risky sexual situations. However, over the course of the copulatory preference test, cocaine enhanced the preference for the unscented female.

Therefore, it would be incorrect to state that cocaine causes only sexual disinhibition. First, the concept of disinhibition is not a unitary one. Sexual behavior is comprised of discrete events such as arousal, erection, directed responding, and ejaculation. Because cocaine was administered peripherally, all regions in the nervous system that underlie these responses were affected, and thus a multitude of effects of cocaine could be observed. Cocaine's effects were dissociated with respect to choice for first mount and mount frequency. In the case of risky sexual behavior it is important to ask which of these two measures is comparable to an interference with sexual risk perception, and sexual risk management in humans as defined by Bancroft (2000). This may be too complex a question to ask from this particular animal model. However, of the two behaviors that were observed to be affected by cocaine, "first mate choice" may be more salient when speaking of sexual risk. The choice for first mate can be thought of as an approach behavior. Approach behaviors are arguably more likely to be the focus of cognitive inhibition due to their inherent risk, especially in a novel environment such as the open field where the copulatory test took place. Thus, at 20 mg/kg males were impaired at initially assessing the sexual risk involved, but not impaired once the bouts of copulation began.

Future Directions

The present thesis examined the effects of cocaine on appetitive and consummatory aspects of sexual behavior as well as on primary and secondary conditioned sexual inhibition. Primary conditioned inhibition was implemented by training male rats to become attentive to behavioral and olfactory and pheromonal cues associated with female non-receptivity. Secondary conditioned inhibition was

implemented by pairing a neutral odor with female non-receptivity, then allowing males the opportunity to copulate with two receptive females that differed with respect to the presence or absence of the odor, an olfactory conditioned stimulus. The results of the first study indicated that cocaine facilitates ejaculation possibly through a heightening of penile sensitivity or arousal. The results of the primary conditioned inhibition experiment indicated that cocaine was not effective in releasing mounting behavior from inhibition. This was in marked contrast to a similar study by Pfaus et al. (1989) who showed that a low dose of alcohol not only released mounting from inhibition but also caused the rats to ejaculate ex copula. However, cocaine was found to disrupt secondary conditioned sexual inhibition as it relates to choice of first mate in a copulatory preference test, but also to enhance inhibition as it relates to total number of mounts with the unscented female versus the scented female.

Experiment 3 was the first study to test the effects of a drug on secondary conditioned sexual inhibition. Many drugs are thought to possess prosexual and disinhibitory properties, and several (e.g., amphetamine, MDMA, morphine, caffeine, and alcohol) have been tested for their simple effects on appetitive and consummatory aspects of sexual behavior (Pfaus, Wilkins, & Schattmann, in preparation; Dornan et al., 1991; Pfaus & Gorzalka, 1987; Di Pietro & Pfaus, 2001; Pinel, Pfaus & Christensen, 1991; Pfaus & Pinel, 1989). Could this paradigm be used to test the effects of other drugs of abuse? Drugs from several different classes of action would undoubtedly produce different results. Yet, should drugs from opposing classes produce similar results, this would indicate a common mechanism of action upon brain regions involved in inhibition/disinhibition. For example, if alcohol were tested in a secondary conditioned

inhibition paradigm, its effects could be compared to cocaine. The second order inhibition paradigm could also be used to examine where sexual inhibition occurs in the brain. A relatively simple test of c-Fos activation and Fos-like immunoreactivity in response to the conditioned odor could be conducted, to reveal brain regions activated by the odor and affected by the drug in question. This could be compared to brain regions activated in response to the odor in the absence of the drug. Common regions of Fos induction by these drugs could denote a common mechanism of action upon a central sexual disinhibitory system and could be used as a guide as to the anatomical locations in which inhibitory learning might occur. Subsequent studies could employ techniques such as the reversible inactivation of candidate brain regions by the sodium channel blocker tetrodotoxin to examine the role of these regions in both inhibitory learning and its expression after the response has been learned.

Other avenues for further research could examine whether tolerance or sensitization to the effects of cocaine are contingent upon it being administered concurrently with sexual behavior. This could be done using the methodology employed by Pinel et al., (1992) who showed that tolerance to the disruptive effects of alcohol on sexual behavior was contingent upon its administration prior to and not after sexual behavior. To show that tolerance to the effects of cocaine upon sexual behavior were response contingent, one would need to administer cocaine to one group of rats just prior to copulation and to administer cocaine to a second group of rats immediately following a copulation. After several such treatments, cocaine would be administered prior to copulation to both groups. If such response contingent tolerance were shown to exist for cocaine, this would indicate that if increasingly larger doses of cocaine would need to be

ingested prior to sexual activity in order to achieve the same enhancement of sexual pleasure and performance. This would undoubtedly serve to maintain and exacerbate a drug habit.

Another common assumption is that sex under the influence of cocaine is more rewarding than without it (Crenshaw & Glodberg, 1996). Animal models could be used to investigate this purported effect by testing for place preference for an environment previously paired with either cocaine, or sexual activity (ejaculation) or concurrent cocaine and sexual activity. If ejaculation paired with cocaine is more rewarding than ejaculation alone then one would expect to find this effect reflected within measures of place preference comparing cocaine or ejaculation. This would indicate a 'dangerous' synergy between concurrent drug use and sexual behavior that may be a factor in the maintenance of a drug habit.

As discussed previously, cocaethylene effects on sexual behavior could prove to be another interesting avenue of future research, since a more appropriate model of cocaine use would include concomitant alcohol use. Therefore, future studies could test the effects of simultaneous cocaine and alcohol administration compared to either drug alone in conditioned inhibition paradigms. Subsequent studies could then test cocaethylene in comparison to cocaine, alcohol, and cocaine + alcohol, to elucidate whether the effects are additive or synergistic, or simply attributable to cocaethylene alone.

The results of the present thesis also call for an in depth examination of underlying variables that may drive the relationship between cocaine and unsafe sex in humans. Several such variables have been proposed including risk-taking personality,

impulsivity, and sensation seeking, along with other socio-cultural variables. Studies that use diary methods and self reports should not overlook alcohol use when inquiring about drug use as so-called "heavy drugs" such as cocaine and amphetamines often override alcohol use in diary reports. Additionally, it would be of interest to examine whether the route of administration, such as nasal insufflation, intravenous, or freebase smoke inhalation, would result in stronger correlations between cocaine use and sexual risk taking.

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