The Role of Testosterone in the Reward and Behavioral Responses to Cocaine

by

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Abstract

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Adviser: Vanya Quiñones-Jenab, Ph.D.

Growing evidence suggests that sex differences in cocaine reward responses are regulated by endogenous gonadal hormones. However, few studies have addressed the role of testosterone on cocaine reward and psychomotor activation. The aim of the present dissertation proposal was to determine whether testosterone influences the development of psychomotor and reward responses to cocaine. The first experiments were conducted with adult rats using chronic and acute methods of testosterone administration. Castrated 8-week old male Fischer rats received placebo or testosterone via SILASTIC capsules (1 to 3 capsules of 100% T) or subcutaneous injections (400, 800, or 1200 µg/kg) concurrent with cocaine administration. While chronic testosterone administration did not alter cocaine-induced conditioned place preference (CPP), concurrent administration of testosterone and cocaine affected the development of cocaine CPP dose-dependently; 400 µg/kg blocked the expression of cocaine-induced CPP. However, testosterone did not affect cocaine-induced locomotor activity. Furthermore, testosterone-saline-treated controls did not develop CPP, suggesting that at these doses, testosterone does not produce rewarding or motor responses. Anabolic steroids are synthetic derivatives of testosterone which have

a high abuse potential. Recent finding indicate that anabolic steroid use is becoming increasingly prevalent among adolescents, a population that has previously been associated with cocaine use. Although both substances lead to complex behavioral responses, little is known about the physiological and behavioral effects of testosterone when co-administered with cocaine. As such, subsequent experiments involved studying whether testosterone might differentially affect cocaine-induced locomotor behavior within adolescent and adult male rats. To test this postulate, intact adolescent (4-week old) and adult (8-week old) male Fischer rats were pre-treated with vehicle (sesame oil) or testosterone (5mg/kg or 10 mg/kg) 45 minutes prior to saline or cocaine (20mg/kg) administration. Testosterone was found to differentially affect cocaine-induced locomotor behavior between adult and adolescent male rats. Testosterone did not have an effect at any dose, on ambulations, rearing, or total locomotor counts among adults. However, testosterone decreased both ambulatory and rearing behaviors among adolescents. No effects were found for total locomotor counts. These findings suggest that testosterone may act within the adolescent DA system, thereby altering the response to cocaine.

Acknowledgments

The following dissertation manuscript is dedicated to the memory of my father,

Charles W. Minerly (June 23, 1916 – January 16, 2004).

For the walks on the beach in the rain at 4am, the trips to Haddam and Newport

For the laughing and dreaming, talking and crying

For the drinks of fine scotch on a cold winter's night,

and mourning the great ones we've lost

For giving me everything you had… I thank you

with all my heart… "You'll be in symphony"

I would like to thank my mother Encarnacion Garcia Torres Minerly for her endless love and support, and for helping me to find my own strength during the darkest days of my life. Without her I would be nothing.

I would like to thank Mary Margaret Minerly for being the most special person in my life: my sister, my friend, and above all, my inspiration.

To all my wonderful family and friends: thank you. The laughing, the comfort, the strength of our friendships… I couldn't have gone on without you.

When I felt like I was falling, you held me up so high…

even if it meant you had to stand on each other.

Sebastian, Kayla, Bailey and Charlie: you are always in my heart, always making me smile. God blessed me with each of you, how did I get so lucky?

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To my doctoral dissertation committee members, Dr. Vanya Quiñones-Jenab, Dr. Shirzad Jenab, Dr. Victoria N. Luine, Dr. Jim Gordon, and Dr. Ann Ho: I thank you for your participation and guidance during my preparation for the doctoral defense. Your commitment and creativity have had an immeasurable impact on me both as a student and as a member of the scientific community.
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Alex, although you are last, I cannot leave you out. You have been, after all, a significant part of my life. Despite everything, a part of me will always love you. I could not have taken this step without your encouragement. Thank you for pushing me and making me realize my full potential. My time with you has made me stronger, and I know that I can endure just about anything that is ahead of me now. I know that although we have changed you are still proud of me and this accomplishment. I will never forget the time we broke into the Columbia library so I could get my hands on that journal that no one else had. There is a lot I won't forget.... but I'll sure try to work on the pain.

JMAT – you made me whole again. And that could not have been easy.

First Fig

My candle burns at both ends
It will not last the night;
But ah, my foes, and oh, my friends,
It gives a lovely light

Edna St. Vincent Millay

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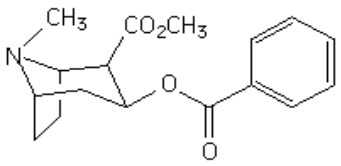
Chapter 1. Introduction

History and Background



Cocaine

Cocaine is a powerfully addictive stimulant that directly affects various deep structures in the brain. In its various forms it is derived from (*Figure* 1, [135]) the coca plant (Figure 2), which is native to the high mountain ranges of South America [123]. Cocaine is an active alkaloid found in the leaves of *Erythroxylon coca*, a tree indigenous to Peru and Bolivia. The chemical synthesis of these leaves led to the creation of white crystal powdered cocaine, and the invention of newer methods for magnifying the euphoric effects of cocaine has led to the form of the drug known as crack [123].

Figure 2. Coca plant [75]

Cocaine has been used as a psychomotor stimulant among the people of Colombia for 2,500 – 5,000 years [135]. In the United States however, its use remained relatively limited until the late 1800's [135]. From the 1850's to the early 1900's, cocaine and opium laced elixirs, tonics, and wines were broadly used by people of all social classes [123]. Around this time cocaine began to

see heightened popularity when a young physician by the name of Sigmund Freud advocated its use as a treatment for a multitude of personality disorders, morphine addiction [113], depression and sexual impotence [123]. Cocaine became even more popular in 1886, when John Pemberton included the drug as the main ingredient in his new soft drink, Coca-Cola, named for the two main ingredients of coca leaf and kola nuts [123]. It was the euphoric and energizing effects on the consumer that led to its place as the most popular soft drink in history [123]. The amount of cocaine in the soft drink became less and less as concerns over the addictive properties began to surface, and in 1929, Coca-Cola was officially declared cocaine-free [123]. Reports of cocaine addiction threatened its new founded popularity and by 1894, the American Medical Association was beginning to question its use [135]. Finally on December 17, 1914 the use of cocaine was banned by the Harrison Narcotic act [135]. This act, adopted under President Woodrow Wilson, made narcotics illegal except for medicinal purposes. In 1919, the Supreme Court changed the Harrison Narcotics Act; thereafter it was illegal for doctors to prescribe narcotic drugs to addicts. Since the ban of this substance as a narcotic and potentially addictive substance, its use has fluctuated affecting all classes and races of people. Today there is a lack of understanding about the unequal distribution of cocaine use between the sexes.

In 2003 the National Household Survey on Drug Abuse reported that approximately 30% of the 2.3 million Americans that currently used cocaine were women [166]. While men have a higher rate of current cocaine use overall than do women [123, 166], this may be due to the fact that males are more likely than females to have an initial opportunity to use drugs, as there seem to be no differences in the progression to intense drug use following the initial use [123].

In recent years there has been accumulating evidence suggesting that there are sex differences in cocaine-induced behavioral and subjective responses. Gonadal hormones have a profound effect on brain function, indicating that the hormonal state of cocaine abusers at the time of drug ingestion may affect their behavioral response to the drug. However, the roles of each of these hormones and their interactions with drugs in the brain is not completely understood.

Cocaine's Mechanism of Action

Cocaine exerts its reinforcing properties through three neuronal systems: dopamine, serotonin and norepinephrine, and by the activation of the endogenous opioid system [6; 155]. It produces an inhibition of the reuptake of neuronal monoamines by binding with dopamine, serotonin, and norepinephrine transporters, thereby increasing the synaptic concentrations of these substrates [6; 20; 45; 57; 93; 155] (see Figure 3). However, due to the

pivotal role of the mesocorticolimbic dopaminergic system, this discussion will mainly concentrate on the dopamine system. The potency of cocaine as a dopamine uptake inhibitor can be seen by its competition with specific ligands for striatum binding sites [49].

Dopamine Pathway and Reward in the Brain

The role of dopamine receptors in brain mechanisms and behaviors commonly assumed to be involved in the addicting properties of cocaine needs to be addressed in order to further understand the pathways by which cocaine exerts its mechanisms of action. Long-term alterations in dopamine transmission may be an important neurochemical substrate of stress and cocaine sensitization [81; 136].

A great amount of research has been devoted to understanding the way cocaine produces its pleasurable effects, and the reasons it is so addictive [52; 178]. One mechanism is through its effects on structures in the brain, which, when stimulated, produce feelings of pleasure. As shown in Figures 4 and 5, there is a complex network of central nervous system pathways that affect cocaine behavioral responses. One neural system that modulates most of cocaine's addictive effects is the ventral tegmental area (VTA) [4; 28; 64; 159; 172] in the striatal pathway. Drugs of abuse activate the mesocorticolimbic dopamine system where cell bodies in the VTA project to forebrain structures including the nucleus accumbens and the prefrontal cortex [reviewed in 18].

The VTA- nucleus accumbens have been called the "reward pathway". PET and fMRI studies have implicated these specific brain regions within the dopaminergic system in the induction of the cocaine "high" and craving, respectively [37; 61; 129].

When a pleasurable event is occurring, it is accompanied by a large increase in the amounts of dopamine released in the nucleus accumbens by neurons originating in the VTA [122]. In the normal communication process, dopamine is released by a neuron into the synapse, where it binds with dopamine receptors on the neighboring neuron, thereby sending a signal to that neuron [122]. Dopamine is then recycled back into the presynaptic neuron by the dopamine transporter. When cocaine is administered, it binds to the dopamine transporter, blocking the reuptake of dopamine. This in turn results in a build-up of dopamine in the synapse which contributes to the pleasurable effects of cocaine [178]. As cocaine abuse continues, higher doses and more frequent use of cocaine are required for the brain to register the same level of pleasure experienced during initial use [122]; this is referred to as tolerance. Low doses of cocaine require longer periods of time to acquire self-administration behavior, suggesting that lower doses of cocaine may not be initially reinforcing but may become so with repeated exposure [154].

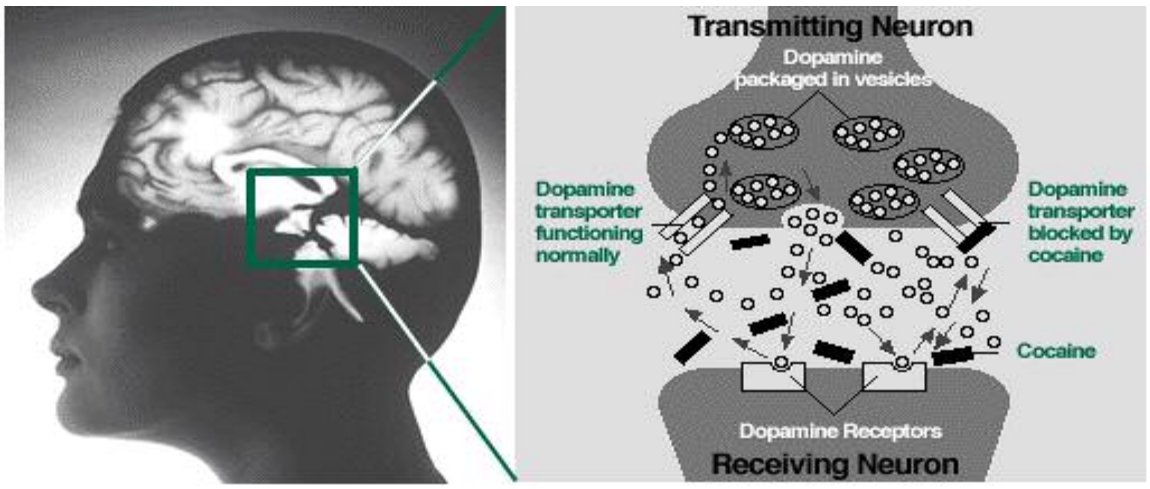


Figure 3: Cocaine's effects on dopamine transmission

adapted from the National Institute on Drug Abuse: Web report. (2004). http://www.drugabuse.gov. [123]

Behavioral sensitization is a progressive increase in the observable effect of a drug which occurs after repeated administration of the drug. The mechanisms underlying this phenomenon seem to be rooted in the mesolimbic dopaminergic projections from the VTA to the nucleus accumbens, suggesting that repeated administration of addictive drugs produces a neural change in these structures which alters the response to the drug [82].

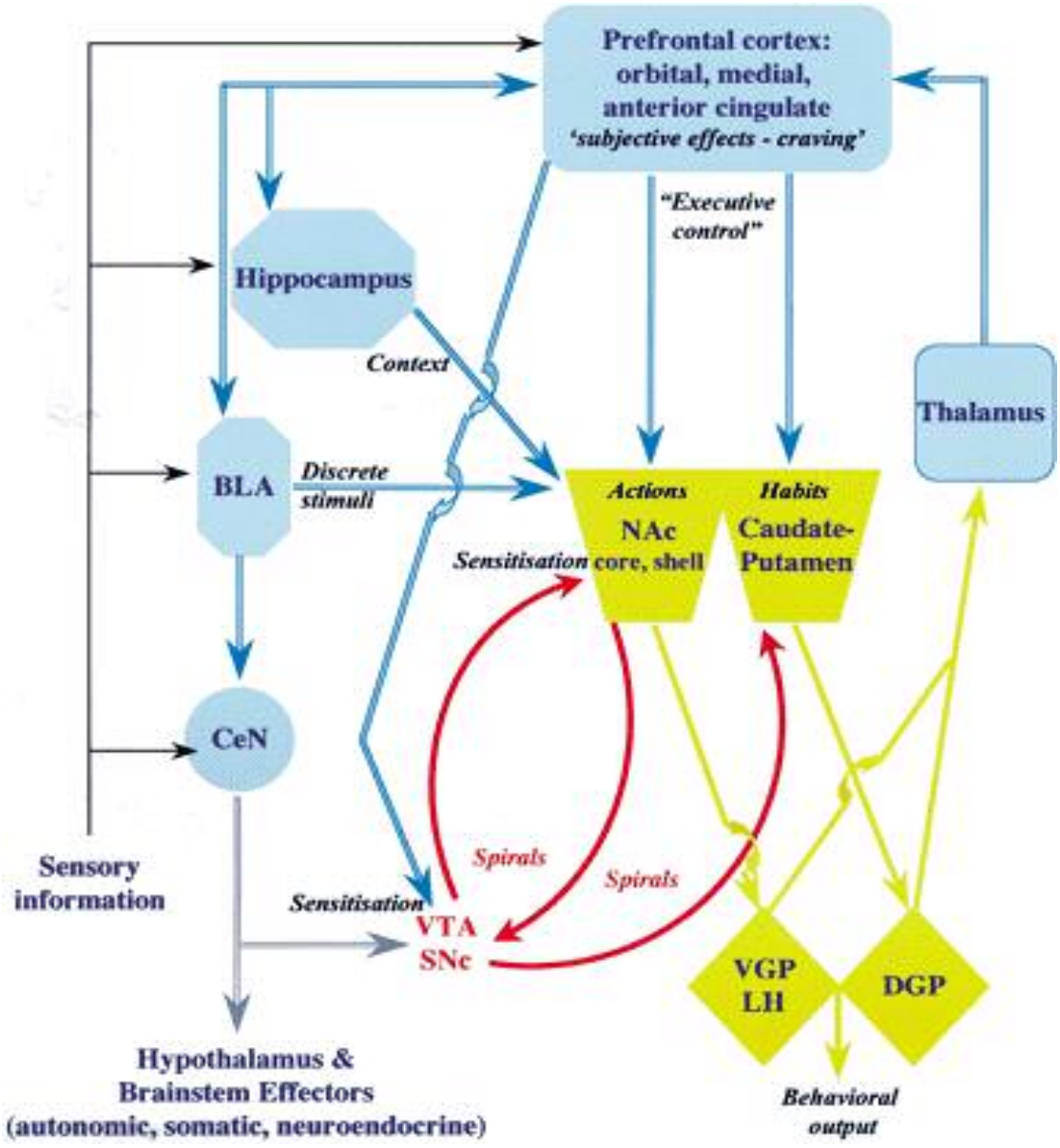


Figure 4: Addiction Pathway

adapted from Everitt, B.J. & Wolf, M.E. (2002), Psychomotor Stimulant Addiction: A Neural Systems Perspective. *The Journal of Neuroscience, 22* (9), pp. 3312-3320. [52]

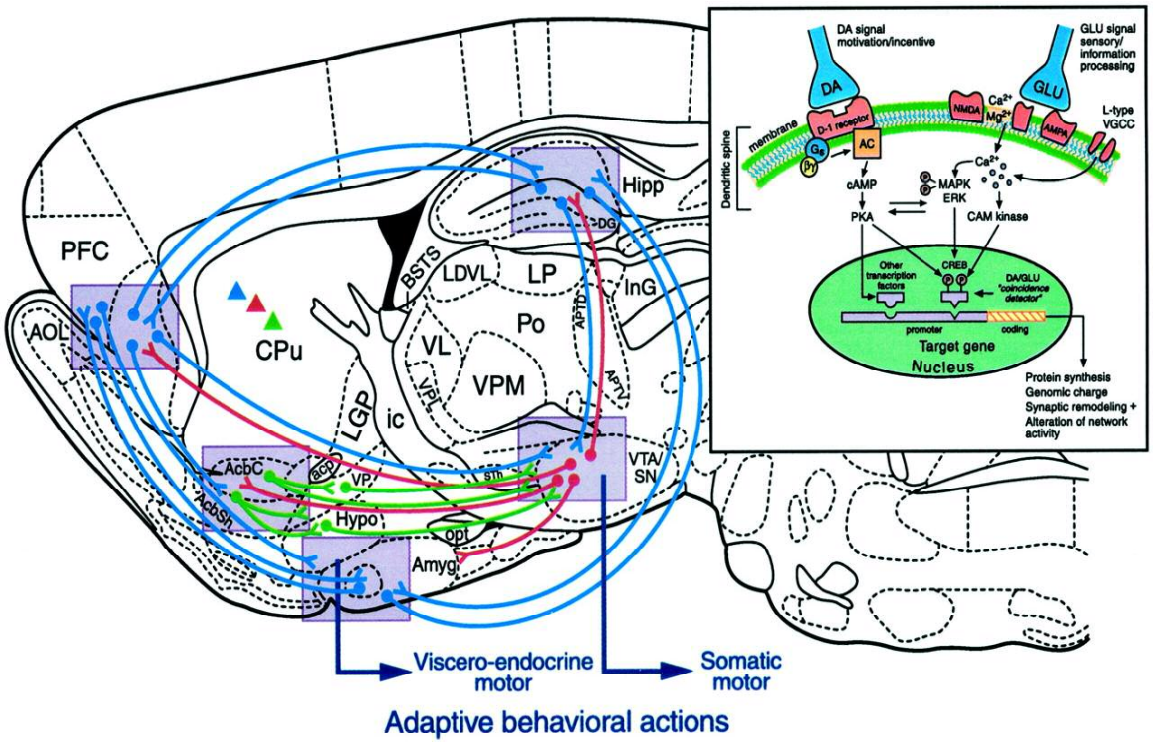


Figure 5: Reward Pathway

adapted from Kelley, A.E. & Berridge, K.C. (2002). The Neuroscience of Natural Rewards: Relevance to Addictive Drugs. *The Journal of Neuroscience,* 22 (9), pp. 3306-3311. [88]

Cocaine and Locomotor Activity

Cocaine causes a rapid increase of locomotor activity that lasts 30 minutes, with these activities involving rearing and stereotypic behavior [56; 54]. The increases of locomotion and rearing, head circling, and body shaking induced by cocaine may involve the indirect activation of postsynaptic D1 and D2 receptors, presumably via dopamine release, resulting from inhibition of the presynaptic D2 receptors [175]. However other findings suggest that these responses may be regulated by changes in striatal dopamine transporter binding site densities and not necessarily by dopamine-releasing mechanisms or D1/D2 receptor modification [43; 81].

Cocaine, Dopamine and Conditioned Place Preference (CPP)

The inhibition of dopamine transporter (DAT) is the mechanism most often associated with the rewarding properties of cocaine in CPP studies [43; 67; 136; 175]. However, the deletion of this transporter alone did not eliminate cocaine reward responses in mice [67]. Blockade of serotonin and norepinephrine transporters has been shown to acquire reward when DAT is absent [67].

D1 antagonists block the expression of both cocaine-induced CPP and sensitization [124], whereas D1 and D2 antagonists block cocaine-induced locomotor behavior and stereotypy, and at higher doses self-administration as well [124]. In male rats, D1 but not D2 receptors are involved in the

acquisition of cocaine CPP [12; 13; 36; 137]. Female rats have lower D1 receptor densities in the nucleus accumbens, and greater dopamine release, reuptake and dopamine transporter density in the striatum [6; 19; 146]. It has been suggested that differences in D1 receptor activity in male and female rats may be involved in the differential gender development of learned associations between environmental cues and cocaine reward [124]. The activation of D1 and D2 receptors is regulated by gonadal hormones [24; 53; 99; 101]; these same hormones also have effects in dopamine and serotonin activity and are involved in learning and memory processes [104; 112].

Conditioned Place Preference

CPP is a behavioral test model commonly used to study the rewarding and incentive motivational effects of drugs and drug-paired stimuli [31; 63; 108; 110]. After examining the actions of different receptor antagonists, a number of studies have suggested that the expression of cocaine induced CPP can be pharmacologically dissociated from that of cocaine-induced conditioned locomotor activity and behavioral sensitization [24; 72; 100; 54; 110; 144; 164]. CPP studies have important clinical implications for the examination of environmental cue – elicited drug-seeking behaviors.

The modification of CPP expression

When considering treatment options it is important to examine measures that can affect the development of CPP. Numerous events can obstruct the acquisition of cocaine-induced CPP. Injections of cocaine into the ventral pallidum produced a CPP effect [64]. The number of crossings between sides of the place preference chamber increased after cocaine conditioning [92]. This did not occur with injections of cocaine into the nucleus accumbens septi [63]. This suggests that dopaminergic concentration in the ventral pallidum may be working independently of dopaminergic concentration in the nucleus accumbens septi to produce the reward responses to cocaine. 6-hydroxydopamine lesions in the ventral pallidum blocked CPP at a 5 mg/kg dose of cocaine, but not at a 10 mg/kg dose [64], however when the initial lesion effect of lowered dopamine concentration in the ventral pallidum was potentiated by a depletion of more than 60%, CPP failed to develop for either dose [64]. This supports the notion that dopaminergic mechanisms of action are crucial in the regulation of behavioral responses to cocaine. Blocking the opioid receptors of the ventral pallidum also attenuates the acquisition of cocaine-induced CPP [146] and in some cases will produce a conditioned place aversion. Administration of non-selective opioid antagonists such as naloxone and naltrexone [24; 89; 95; 167] and dopamine antagonists, such as haloperidol, raclopride, and SCH 23390 [73] all attenuate the rewarding effects of cocaine [1; 24; 89; 95; 167]. The prelimbic subregion of the medial

prefrontal cortex has been implicated in the reinstatement of previously extinguished cocaine-CPP by priming injections of cocaine [186].

Once established, CPP effects can linger long after the absence of additional drug exposure. This can be maintained by occasional testing up to several weeks [106]. If rewarding effects can be felt continually for some time after the end of drug exposure, serious implications can exist as a result for human drug addicts that attempt to cease their drug use.

Sex differences in the behavioral response to cocaine

Male and female rats differ in physiological, neurochemical, and behavioral responses to stress or drug exposure, including locomotor responses and the development of CPP, with females exhibiting typically larger responses to cocaine [18; 38; 39; 54; 55; 56; 118; 124; 141; 149; 150].

Sex differences in CPP acquisition

Sex differences in cocaine-induced CPP have been observed [149; 150]. Female rats have acquired CPP after fewer pairing sessions and at lower doses of cocaine than males [149; 150]. These sex differences appear to be regulated to some extent by gonadal hormone-mediated mechanisms, since the intensity of CPP effects can be modulated by gonadectomy and hormone replacement.

Sex differences in locomotor activity, sensitization, and self-administration

Cocaine induces larger physiological responses in female rats, including the acquisition, maintenance, and reinstatement of cocaine self-administration, stereotypic behaviors and locomotor activity, including ambulations and rearing [38; 48; 139].

Acute cocaine administration

Pattern of administration has been shown to affect both cocaine-stimulated behavioral and endocrine responses in male and female rats [54], with both single and "binge" pattern cocaine administration resulting in a transient decrease in testosterone levels (at 30 min but not 3 hr following a single injection) [54]. In both male and female rats, "binge" cocaine increases plasma progesterone levels [140]. It is possible then, that progesterone's effects on CNS plasticity may be affected by cocaine administration. It has been found that acute cocaine administration led to increased progesterone levels transiently in female rats [54]. Much of the literature indicates that acute cocaine administration leads to higher overall hyperactivity (ambulations, rearing and total counts) in females than in males [38; 39; 53; 139].

Chronic cocaine administration

Repeated administration of low to moderate doses of cocaine has been shown to enhance stereotypy [reviewed in 8] and an increase in behavioral response to cocaine is observed [32; 130]. During repeated administration of cocaine, behavioral sensitization with increased stereotyped behavior occurs [130; 183]. During the later stages of the cocaine-induced sensitization process there is an enhanced dopaminergic response which may be a key factor in the long-term expression of sensitization [71].

It has been shown that cocaine produces dose-dependent locomotor stimulant effects and elicits behavioral sensitization with repeated and intermittent exposures [81; 83]. The sensitivity to cocaine and the extent of sensitization is sex dependent, with numerous studies indicating that females are more susceptible to cocaine sensitization [38; 139; 151; 156; 160; 180]. Chronic cocaine administration has resulted in the development of sensitization to cocaine-induced ambulations and total locomotor counts across sexes, but studies have found that while the female condition persisted after cocaine challenge in both ambulations and total locomotor counts, the condition in males persisted only in measures of total locomotor counts [39].

The role of gonadal hormones

Gonadal hormones have been postulated to be modulators in the sexually dimorphic responses to cocaine. They influence neuronal activity and

plasticity in the brain, thereby mediating the effects of cocaine on behavior [55; 138; 141; 149], including locomotor behavior and the development of cocaine-induced CPP. When attempting to understand the sex differences in the behavioral and neuroendocrine responses to cocaine, the role of gonadal hormones cannot be overlooked, since both castration and ovariectomy have profound effects of the neurochemical and behavioral responses to cocaine, as does subsequent hormone replacement in a dose-dependent manner [131; 142; 149].

Repeated exposure to psychostimulants appears to enhance the activity of the HPA axis and lead to a decrease in the activity of the HPG axis [27]. The importance of the role of the HPG axis as a regulator of the sex differences in the acquisition and expression of cocaine CPP has been implied [150]. The HPG axis has been previously found to play a role in cocaine-induced behavioral activation [18; 106; 131; 158] since ovariectomy has been shown to have effects on CPP while adrenalectomy was shown to have no effect on CPP for either sex.

Effects of Gonadectomy

The larger physiological responses to cocaine in female rats have been held constant in ovariectomized rats that were pretreated with different steroid replacement treatments [142] and also in intact male and female rats [139; 142; 161], but varied in females across different estrous cycle phases

[106]. While castration of male rats has relatively few effects on acute sensitivity to cocaine, ovariectomy of females significantly attenuates locomotor activity induced by cocaine [69; 94]. This suggests that while still important, gonadal hormones in males seem to play a more limited role in the expression of cocaine-induced reward responses.

Testosterone and its metabolites

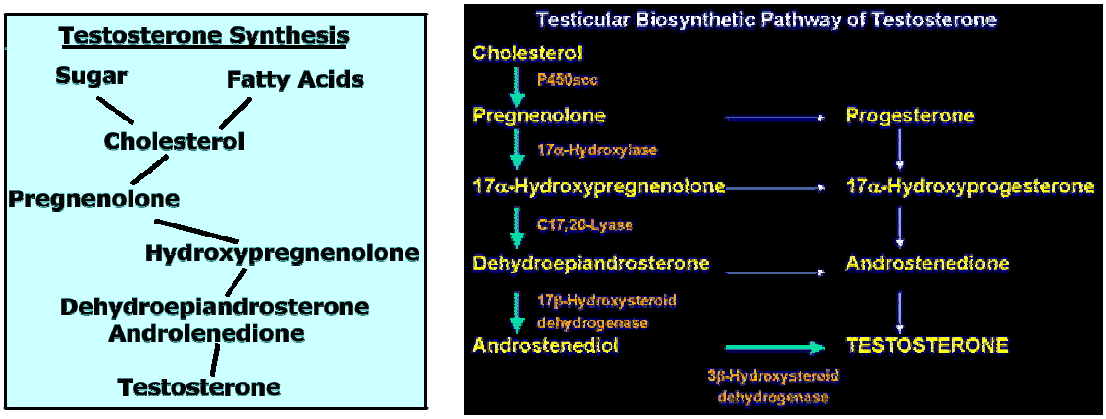


Figure 6: Testosterone Synthesis Pathway. Adapted from the web: www.endotext.org (2004). [76]

Testosterone is an androgen produced by the Leydig cells of the testes (see Figure 6). It is responsible for masculinization [85] and overall sexual differentiation of specific brain regions such as the medial preoptic nucleus (in conjunction with estradiol) [138].

Testosterone has been postulated to play a role in the behavioral and neurochemical responses to cocaine. Testosterone enhances dopamine and

serotonin turnover and also affects the binding and uptake sites of the latter [3]. Cocaine may stimulate significant increases in estradiol and testosterone in females but not in males [115]. These rapid hormonal changes may contribute to cocaine's abuse-related effects, as well as to disruptions of the menstrual cycle during chronic cocaine administration [115].

An important characteristic to remember when studying testosterone and its effects is that it has numerous metabolites, the roles of which are not completely understood. In some cases, testosterone may metabolize very rapidly, exerting an effect during the transformation that will not be continued into its new configuration, but each metabolic alteration may have its own distinctive characteristics [25]. Some conversions to metabolite are reversible [25].

As shown in Figure 7, testosterone and androstenedione may be converted to estradiol by metabolic actions [114]. The process of metabolism occurs via the activation of monooxygenases and other steroid-metabolizing enzymes [23; 165]. Some common metabolic pathways are as follows: 5-alpha-reductase metabolizes testosterone to dihydrotestosterone [165], 3-alpha-hydroxysteroid oxidoreductase activity reduces 5-alpha-dihydrotestosterone to 5-alpha-androstane-3-alpha.17-beta–diol [165], 3-oxidoreductase metabolizes testosterone into 3-alpha-androstanediol (3-alpha-diol) [59; 60; 148], aromatase activity metabolizes testosterone to

estradiol-17b [165], and 17-beta-hydroxysteroid oxidoreductase activity metabolizes testosterone to 4-androsterone-3.17-dione [59; 60; 148; 165]. One of the most potent testosterone metabolites is estradiol, which causes sexual differentiation of the brain [68; 85]. Major testosterone metabolites detected in adult rat thymus are 6 beta-hydroxytestosterone (HT), 7 alpha-HT, 16 alpha-HT, 2 alpha-HT, and androstenedione [23]. 3-alpha-diol, another testosterone metabolite, is a neurosteroid considered by some to be the most effective androgen for eliciting a CPP effect [59; 60; 148]. As such, androgen regimens that increase the concentration of 3-alpha-diol in the brain may enhance CPP. Direct implants of this metabolite, as well as DHT and testosterone into the nucleus accumbens shell have already been found to produce a CPP effect [59; 60; 148].

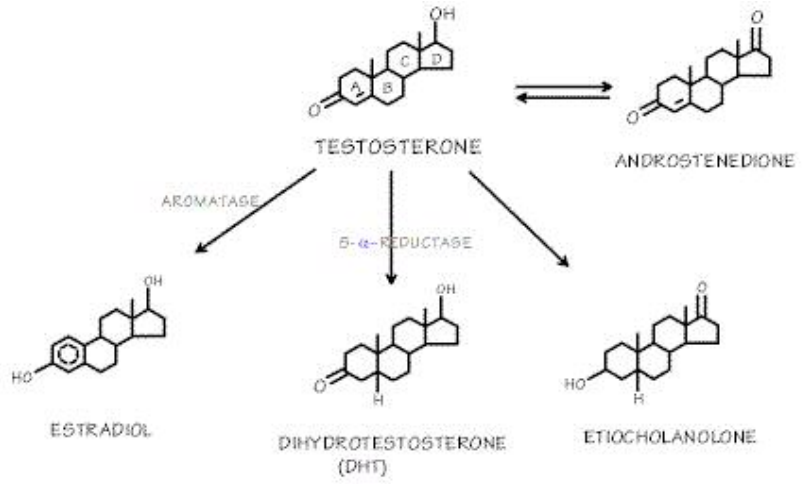


Figure 7: Testosterone Metabolism. Adapted from the web: www.antiaging.com (2004). [73]

The binding properties differ for various metabolites. For instance, testosterone and DHT bind to intracellular androgen receptors, while 3-alpha-diol binds to $GABA_A$/benzodiazepine receptor complexes [60]. Thus, the GABA system may be a site through which testosterone can exert its behavioral effects.

Testosterone metabolism can be affected by a number of variables, including mode and route of administration, type of vehicle, dose, and bioavailability, as well as the influence of other hormonal actions [60]. For instance, testosterone metabolism has been inhibited by dihydrotestosterone and progesterone in rat epididymal epithelium cells [25].

General effects of testosterone

At puberty, the hypothalamic secretion of GnRH stimulates the secretion of FSH and LH from the pituitary gland. These, in turn, stimulate the Leydig cells to secrete testosterone. In most mammalian species, motivational aspects of male sexual behavior are regulated by testosterone [60]. It has been suggested that the brain areas that mediate copulatory behavior are independent of those that mediate sexual motivation [2]. One study supporting this theory found that castration of male rats abolished an acquired preference for an environment previously paired with a receptive female, whereas medial preoptic lesions did not [78]. In contrast,

6-hydroxydopamine lesions of the mesolimbic dopamine system produced a decrease in sexual appetite behavior but not copulation [51].

Behavioral effects of testosterone

There has been some conflict in the study of testosterone as related to CPP. Some studies have shown a CPP effect only with low doses of testosterone [111], while others have shown no effect at any dose [2; 30; 90; 127; 157]. Still others have demonstrated that the pairing of systemic testosterone with a distinctive chamber has produced a CPP [9; 10; 60; 90; 127]. Further, infusions of testosterone into the nucleus accumbens or the medial preoptic area and immediate pairing with the nonpreferred side of the CPP apparatus reliably produced a preference for that side [60; 90; 127]. These effects appear to have been dose-dependent [2].

Testosterone has a distinct mechanism of action. It works by stimulating receptor molecules in muscle cells, which activate specific genes to produce proteins (see Figure 8) [74]. Testosterone also affects the activation rate of enzyme systems involved in protein metabolism, thus enhancing protein synthesis and inhibiting protein degradation [74; 182].

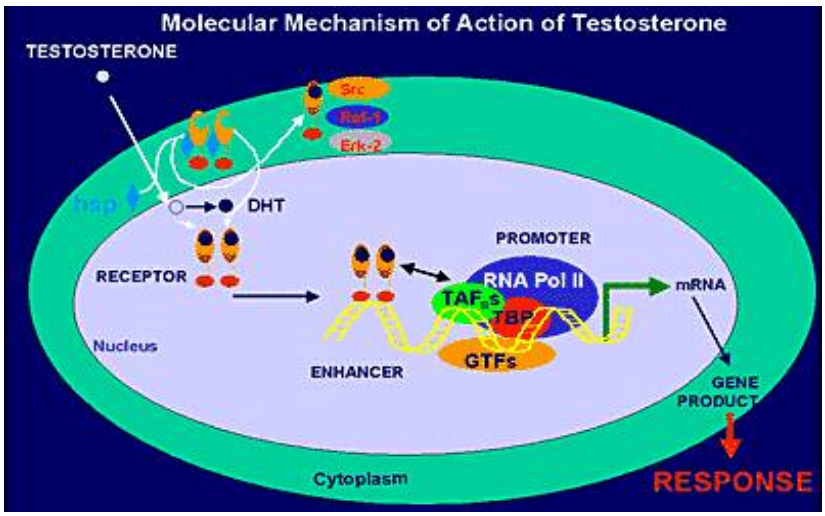


Figure 8: Testosterone's Mechanism of Action. Adapted from the web: www.bioalternatives.com (2004). [74]

When administered by itself, it has been shown to have rewarding properties in both rats [2; 90] and mice [9; 10]. Androgens may motivate continued drug seeking and consumption behavior because of their primary rewarding properties [2; 127; 157; 182]. Activation of D1 and D2 receptors has been thought to mediate the primary rewarding effects of testosterone [157]. Testosterone CPP has been blocked by an injection of dopamine receptor antagonists such as alpha-flupenthixol, SCH 23390, and sulpiride [157].

Long-term effects of testosterone and cocaine

Testosterone inhibits electroencephalogram (EEG) output and the overall behavioral effects of acute cocaine administration [176]. Chronic exogenous testosterone administration has reduced the behavioral effects of cocaine

[103]. Studies have also found that long-term treatment with testosterone prevented the enhancement of cocaine-induced locomotor activity produced acutely in male rats [103; 111] and in mice [152], possibly via increases in noradrenaline and dopamine and decreases in serotonin [111], since these monoamines appear to be affected by androgen action in the CNS [42].

Chronic treatment of testosterone was found to delay and reduce cocaine-induced stereotypical behavior in castrated rats [40], while castrated chronic cocaine-treated rats who did not receive testosterone showed a robust sensitization effect [40]. Postmortem examination revealed that testosterone significantly restored levels of striatal plasmalemmal dopamine, indicating that there may be a possible interactive effect between chronic testosterone and chronic cocaine administration [40]. Specifically, testosterone may regulate the homeostasis of dopamine uptake, which is disrupted by the administration of chronic cocaine. This has important implications for the study of sex differences in drug abuse and the sex-dependent reactions to gonadal hormone interactions with these drugs.

Dopamine and testosterone

Pharmacological and biochemical evidence suggests that the mesolimbic dopamine system may be an important factor in the regulation of the affective properties of testosterone and of androgenic effects on CPP [60; 128]. Testosterone CPP has been established in gonadectomized male rats [46].

Castration of male rats reduces dopamine concentrations in the nucleus accumbens, but pretreatment with testosterone hindered this effect [3]. Alternatively, it has been found that castration decreases locomotion in male rats when compared to intact males [38; 54; 149; 150]. Castration of male rats causes a decrease in the level of mesolimbic dopamine and subsequent testosterone replacement restores these levels [3]. This is an important factor to take into account when assessing the possible cross-effects between testosterone and cocaine and their actions on dopaminergic levels during drug administration.

Interactions of cocaine and testosterone on reward

It is not yet fully known whether cocaine affects the actions of steroids, or whether steroid plasma levels affect the actions of cocaine. Nongenomic variations, such as in the levels of extracellular monoamines, may mediate the interactions between steroids and cocaine. Such alterations may be important factors in the cocaine-induced HPA and HPG response, as these neurochemicals regulate the structures which produce and secrete gonadal hormones [141]. Testosterone exerts varying effects on neurotransmitters that are also affected by cocaine administration [155]. A single dose of testosterone has been found to abolish the sensitivity to norepinephrine in the young rat vas deferens organ [96].

Forman, et. al. [58] determined that cocaine and testosterone interact to modulate concentrations of immunoreactive beta-endorphin in the anterior pituitary. Gonadal steroid environment can therefore modulate the regulation of this polypeptide, which is vastly associated with feelings of "high". This raises interesting questions about the combined role of testosterone and cocaine in these other types of rewarding effects.

Cocaine's effects on testosterone serum levels

The precise effect of cocaine administration on testosterone serum levels is not yet well understood. Cocaine administration has led to an initial sharp increase in testosterone levels, which later falls significantly below normal levels [21; 65; 114]. Chronic low doses of cocaine have been found to cause no hormonal changes, while high doses result in the depression of both testosterone and LH levels [21; 27]. This indicates an acute biphasic effect of cocaine on testosterone. Lower doses of cocaine (0.5 mg/kg) have increased testosterone serum levels while higher doses of cocaine (10 mg/kg) have not [147], suggesting that cocaine may have an acute effect on the structure of the rat testis [147]. Further, chronic administration of cocaine has caused testicular lesions, which in turn may be responsible for the testosterone diminution [15]. One study in which a single cocaine dose significantly lowered testosterone levels did not result in the elevation of LH levels to above those of control animals [21], suggesting that cocaine effects on

testosterone levels may occur directly during the synthesis or secretion of testosterone [21]. However, in another study where cocaine was found to produce no change in testosterone levels, LH and to a lesser degree, FSH, were significantly increased after cocaine administration [70].

The co-abuse of anabolic steroids and cocaine

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone. The abuse of these substances is a growing problem in Western society. These were initially abused by super-athletes to add an edge to a performance which was already close to perfection [148]. Currently however, this phenomenon has spread from elite Olympic, professional, college or high school athletes [26; 148; 181] to the general population [26]. As a result, testosterone was banned as a controlled substance in 1991 [182]. Estimates indicate that approximately 375,000 adolescent boys and 175,000 adolescent girls are steroid users [26]. Fluctuations in gonadal hormones due to the onset of puberty may have an interesting role in the development of addiction and dependence to AAS in both males and females.

General effects of AAS

AAS abusers may develop addiction and dependence, and the withdrawal from these substances is similar to that of stimulant withdrawal in that it is characterized by depressive symptoms [60]. AAS abuse has been attributed

to high levels of aggression, known in humans as "roid rage" [182]. AAS abuse can contribute to kidney and liver damage, liver cancer, heart disease, hypertension, hypogonadism, decreased sperm production, and gynomastia [41; 60] or ("bitch tits" [182]). Their use is particularly problematic in women because some of the physical effects may be irreversible. Specifically, masculine traits such as facial hair and a deepening of the voice may persist after the cessation of AAS use [reviewed in 148].

The abuse of AAS has been correlated with an increased likelihood of abuse of other drugs, including cocaine [103; 174]. In a recent study of 227 men treated for cocaine dependence, 9.3% were also steroid abusers [123], with many of them indicating that their initial use of cocaine had been in an effort to minimize the negative effects of steroids, including insomnia, depression, and irritability [123]. AAS are believed to exert their effects via certain areas of the brain that are also associated with cocaine reward, via androgen target structures [111] in the sites that are associated with aggression and sexual behavior and well as to addiction and affective properties [59; 60].

General effects of cocaine

It is important to address the physiological effects that cocaine exerts when administered by itself in order to understand the possible consequences of its co-administration with AAS. Physical effects of cocaine use include

constricted peripheral blood vessels, dilated pupils, and increased temperature, heart rate, and blood pressure [123]. Several studies provide evidence that the CNS plays an important role in the cardiovascular effects of cocaine [8; 174]. Cocaine is a powerful stimulant, therefore increased use results in severe sleep deprivation and loss of appetite, psychotic behavior, hallucinations, delusional behavior [123]. Addicts in withdrawal from cocaine do experience anxiety and depression, repetitive, compulsive movements, the sense of insects crawling under the skin, and severe depression and delirium. Withdrawal may lead to severe depression and suicidal thoughts [91].

The duration of cocaine's immediate euphoric effects, which include hyper-stimulation, reduced fatigue, and mental clarity, depends on the route of administration [123]. The faster the absorption, the more intense the high. On the other hand, the faster the absorption, the shorter the duration of action [123]. The high from snorting may last 15 to 30 minutes, while that from smoking may last 5 to 10 minutes [123]. Increased use can reduce the period of stimulation [123].Scientific evidence suggests that the powerful neuropsychologic reinforcing property of cocaine is responsible for an individual's continued use, despite harmful physical and social consequences [91].

Effects of co-administration

Although cocaine and AAS have been independently associated with cardiovascular toxic effects, the cardiovascular consequences of their concomitant abuse are not well understood [174]. Testosterone, at concentrations that have no toxic effect on their own, was shown to have the capacity to potentiate the noxious effects of cocaine on cardiovascular function [174], suggesting that co-abuse of testosterone and cocaine could lead to a marked risk of cocaine-induced thrombosis. This is particularly noteworthy since AAS abuse by itself has been known to cause heart disease and other cardiovascular ailments [41], yet the doses of testosterone that were used had no effect alone. Sex differences have been observed in the response to AAS, even at this level, with males developing cocaine-induced cardiovascular toxicity at lower plasma concentrations of cocaine than females [118].

Testosterone is able to modify the reward effects of cocaine by reducing the area of activation within reward-associated areas in the brain [170]. This indicates that testosterone may indeed play a role in the modulation of cocaine reward effects. An added danger exists with the co-abuse of AAS with cocaine, including a possibility for the magnified effects of both substances, or the synthesis of a new toxic substance as a result of their combination [123]. For instance, when people mix cocaine and alcohol

consumption, they are compounding the danger each drug poses and unknowingly forming a complex chemical experiment within their bodies [123]. The human liver combines cocaine and alcohol and manufactures a third substance, cocaethylene, that intensifies cocaine's euphoric effects, while possibly increasing the risk of sudden death [123]. It is possible that similar reactions could occur after the co-abuse of AAS with cocaine.

Adolescent susceptibility to drugs of abuse

The period of adolescence is characterized by a vulnerability to drugs [107], a higher likelihood of initial drug use [107], high risk behaviors [163], alterations in novelty-seeking and exploratory behavior [31], and changes in the behavioral responsiveness to many drugs of abuse [31; 163]. Adolescents respond differently than adults to a number of drugs that act on dopamine neural systems [31]. For instance, nicotine has been found to significantly decrease locomotor activity in the adolescent rat as compared to adults [145], particularly in males [44]. Animals at this age also show a reduced responsiveness to the locomotor effects of psychostimulants, including cocaine [22; 162]. In particular, periadolescents have been found to lack the cocaine-induced stereotyped behaviors that are characteristic of adults, including head scanning and focused sniffing [98].

Adolescence is also characterized by increased levels of stress [107]. High levels of stress have been postulated to increase the behavioral responsivity to

psychostimulant drugs; these responses can in turn modulate the rewarding effects of drugs of abuse [134].

Adolescent rats displayed an exaggerated behavioral response to cocaine administered in two binge patterns [34]. Total locomotor counts were the same in adult and adolescent rats, however adolescents engaged in more intense stereotypic behaviors, suggesting that equivalent tissue concentrations of cocaine produce a greater behavioral response in young rats [34].

In a study comparing cocaine-induced locomotion in adolescent and adult female rats, female adolescents were found to exhibit significantly higher levels of locomotor activity than adults [35]. Another study found a more pronounced locomotor response in female adolescent rats as compared to male adolescents [34], a trend which is observed between adult male and female rats as well [18; 55; 56; 141; 149; 150]. A possible factor in this discovery is that estradiol has been found to be a more influential determinant of susceptibility to the effects of cocaine than testosterone [48].

AAS and the adolescent population

AAS abuse in nonathletes is particularly common among adolescent boys (7% of high school seniors) [184]. The misuse of AAS among adolescents is increasingly concomitant to the use of other drugs [11]. Among adolescents, the frequency of anabolic steroid use was significantly associated with the

frequency of cocaine use [50]. Prevalence of cocaine dependence has been shown to be higher in adolescent females and adolescent males [84]. Furthermore, cocaine was the drug most likely to be co-abused by adolescent AAS abusers [50].

Androgen reinforcement is not comparable to that of cocaine, but more like other mild reinforcers, such as caffeine or nicotine [182]. However, although nicotine and caffeine are mild reinforcers, it is remarkably difficult for many habitual users to quit; AAS may have similar effects [182]. Nicotine has been found to significantly decrease locomotor activity in the adolescent rat as compared to adults [145], particularly in males [182]. Testosterone has been found to have similar effects [103; 111].

Testosterone and the adolescent brain

Important structural and functional changes in the brain occur during adolescence [34], but there has been little direct study of how this impact on drug abuse vulnerability. In the brain testosterone can affect neural function by binding to classical androgen receptors, through classical estrogen receptors after aromatization to estrogen, or through nongenomic receptors [42]. Androgens modulate behavior through organizational or activational effects either directly or via the action of its metabolites estradiol or dihydrotestosterone [59; 60; 126; 148].

The interaction between AAS and other psychoactive substances most likely involves the reward system, since dopamine and serotonin systems involved in cocaine reward are also affected by androgen action at the central nervous system [171]. One important characteristic of the adolescent brain is the extensive overproduction and pruning of dopaminergic, cholinergic, serotonergic, GABAergic, and adrenergic receptors [79; 102; 143]. Most notably is the increase in DA receptor densities in the rat striatum throughout development which peaks around postnatal day 28-30 [120; 121]. Furthermore, basal DA levels change during adolescence in both the striatum [5] and the nucleus accumbens [133], with a rate of DA synthesis and turnover in the prefrontal cortical reward areas that peaks early in adolescence and then declines into adulthood [169]; these changes are likely to play a role in the drug-induced alterations in adolescent drug responses.

Adolescent vs. adult sex differences in cocaine response

Sex differences in cocaine-induced locomotor responses can also be mediated by sexual development. Locomotor responses of prepubertal males and females to cocaine were similar in one study [94], however, the postpubertal sex difference resulted from a fall in cocaine-stimulated locomotion in males rather than a rise in females.

Much of the literature points to a higher affinity for sensitization among females, both as adults [38; 98; 139; 151; 156; 160; 180] and periadolescents [98], the latter indicating that sensitization may be a function of age-specific alterations in sensitivity to cocaine. Neonatal testosterone treatment of female rat pups decreased the response to cocaine in adulthood, suggesting that the activational and organizational effects of gonadal steroids contribute to the greater response of females to cocaine [94]. This presents significant implications for treatment of abuse.

Overview and Rationale:

The Office of National Drug Control Policy estimates the number of chronic cocaine users at 3.6 million today [123]. This includes occasional users as well as repeated or compulsive users, and a variety of use patterns between these extremes [123]. Last year, there were approximately 170,000 visits to emergency rooms in the U.S. due to cocaine [168]. This suggests that cocaine abuse is still quite prevalent in Western society.

Another dangerous trend is the growing number of androgenic-anabolic steroid abusers. This phenomenon is no longer limited to superior athletes [26; 148] but currently affects many other members of the population [181]. The interactive effects of each of these substances are not yet well understood and require further study. Previous findings on sexually dimorphic responses to cocaine-induced neuronal and behavioral responses indicate that

much is to be learned with respect to the effects of testosterone and cocaine in combination. The investigation of these effects may shed more light on sex differences in the gonadal hormone mediated response pathways.

Past research seems to have focused primarily on the consequences of drug interactions with cocaine in females, probably because of the finding that many of the disparities in the behavioral responses could be explained by fluctuating hormonal status as determined by the estrous cycle. Hormonal fluctuations in pre- and post-pubescent boys, however, have not typically been investigated with respect to the same behavioral responses. While this is understandable in studies of cocaine abuse, the trend for boys to begin drug experimentation at a younger age cannot be ignored, particularly since the majority of both AAS and cocaine abusers are male. Further, the examination of the co-abuse of AAS and cocaine may lead to advances in the development of pharmacological treatment for drug abusers at a younger age.

Significance:

In recent years there has been accumulating evidence suggesting that there are sex differences in cocaine-induced behavioral and subjective responses. Gonadal hormones have a profound effect on brain function, indicating that the hormonal state of cocaine abusers at the time of drug ingestion may affect their behavioral response to the drug. For example,

cocaine cravings may vary for females during different stages of their menstrual cycles. Further, adults 18 to 25 years old have a higher rate of current cocaine use than those in any other age group [123]. The behavioral responses to cocaine may then vary as hormone levels fluctuate in an age-related manner. There is growing interest in the differences between male and female responses to cocaine and the effects of gonadal hormones on these responses. Several studies have reported that estrogen and progesterone mediate sex differences in locomotor activity, self-administration and CPP responses to cocaine [106; 131; 149]. However, to date there have been few studies conducted that address the role of testosterone in the behavioral, endocrinological and neurochemical responses to cocaine.

Overall, men have a higher rate of cocaine use than women. Additionally, male athletes may have a high incidence of testosterone steroid abuse which may coincide with their use of cocaine. Anabolic steroids are synthetic derivatives of testosterone which have a high abuse potential. Concurrently, some steroid abusers also abuse cocaine. Although both substances lead to complex behavioral responses, little is known about the physiological and behavioral effects of testosterone when co-administered with cocaine.

This study seeks to understand the role that testosterone plays in the regulation of male behavioral and reward responses to cocaine and the possible contribution of this hormone to sexually dimorphic responses to

cocaine. We postulate that testosterone affects cocaine-induced endocrinological and behavioral responses in a dose dependent manner. We further hypothesize that since during male development different levels of testosterone influence overall behavioral activity, significant differences in cocaine-induced behavioral responses will be observed across different stages of development. In particular, we postulate that testosterone will differentially alter cocaine-induced responses between adults and adolescents, with adolescent males exhibiting a lower response to cocaine as compared to adult males. To test these postulates the following specific aims are proposed:

Specific Aim I: To determine the effect of testosterone on the locomotor response to cocaine, cocaine-induced CPP, and serum levels of corticosterone and testosterone in castrated male rats.

Specific Aim II: To determine whether different methods of testosterone administration will have a differential effect on cocaine-induced CPP and locomotor activity.

Specific Aim III: To assess how testosterone's effects on cocaine-induced behavioral activity in intact adolescent male rats compare with testosterone's effects on cocaine-induced behavioral activity in intact adult male rats.

Chapter 2: Testosterone Plays a Limited Role in Cocaine-Induced Conditioned Place Preference and Locomotor Activity in Male Rats

1. Introduction

In recent years there has been accumulating evidence suggesting that there are sex differences in cocaine-induced behavioral and subjective responses [18; 38; 39; 54; 55; 56; 118; 124; 141; 149; 150]. Male and female rats differ in physiological, neurochemical, and behavioral responses to stress or drug exposure, including locomotor responses and the development of CPP, with females typically exhibiting more pronounced responses to cocaine [18; 38; 39; 54; 55; 56; 118; 124; 141; 149; 150]. Moreover, females develop cocaine-induced CPP with lower doses and after fewer pairing sessions than males [149; 150]. These sex differences in cocaine reward and psychoactive stimulation appear to be regulated to some extent by gonadal hormones.

For example, gonadectomy of female rats decreased overall cocaine-induced behavioral responses, while in male rats it has no effect [149; 150]. Cocaine-induced CPP is also affected by gonadectomy; in female rats there is an attenuation of CPP after ovariectomy [149; 150]. Furthermore, in female rats gonadal hormone replacement alters cocaine responses; i.e. estrogen increases while progesterone decreases or has no effect on acute cocaine-induced behavioral activity [151]. Similarly, cocaine-induced CPP is altered by the hormonal replacement paradigm; in females, progesterone attenuates while estrogen and progesterone coadministration enhances cocaine-induced CPP

[151]. However, to date there have been few studies conducted that address the role of testosterone in the behavioral, endocrinological and neurochemical responses to cocaine.

Testosterone has been postulated to play a role in the behavioral and neurochemical responses to cocaine. When administered by itself, testosterone has been shown to have rewarding properties [2; 90]. Testosterone has been found to delay and reduce cocaine-induced stereotypical behavior in castrated rats [40], and testosterone serum increases after cocaine administration. Although the current literature postulates that testosterone contributes to the sexually dimorphic responses to cocaine, few studies have demonstrated a direct link between testosterone and cocaine.

Testosterone is able to modify the reward effects of cocaine by reducing the area of activation within reward-associated areas in the brain [9; 60; 170]. This indicates that testosterone may indeed play a role in the modulation of cocaine reward effects.

CPP is a behavioral test model commonly used to study the rewarding and incentive motivational effects of drugs and drug-paired stimuli [31; 63; 108; 110]. CPP studies have important clinical implications for the examination of environmental cue – elicited drug-seeking behaviors. Once established, CPP effects can linger long after the absence of additional drug exposure.

Cocaine, when administered alone, has rewarding properties. The strong environmental cues between "high feelings" and cocaine may be further reinforced or inhibited by the coadministration of testosterone. This in turn may have an impact on the rate of abuse of both substances.

The present study sought to understand the role that testosterone plays in the regulation of male behavioral and reward responses to cocaine. Using a systematic approach, we aimed to determine to what extent testosterone contributes to cocaine-induced locomotor responses and the acquisition/expression of reward associations. We postulated that testosterone would dose dependently affect cocaine-induced endocrinological and behavioral responses.

2. Method

2.1 Animals

Eight week old Fischer rats (Charles River Laboratories, Kingston, NY) were singly housed in animal cages with free access to food and water, and were maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.). Animals were handled daily after their arrival. Animals were randomly assigned to either saline- or cocaine- treatment and further subdivided into hormone-replacement groups. All experimental manipulations were conducted in at least 3 cohorts with a total of 8-10 animals per group. All NIH guidelines for the care and use of

laboratory animals were followed, as well as those in a protocol approved by the IACUC of Hunter College.

2.2 Drugs

Cocaine (20 mg/kg) (Sigma Chemical Co., St. Louis, MO) or saline was given via intraperitoneal injection. This dose was chosen because it has produced cocaine-induced CPP during a 4-day paradigm [150]. Testosterone (Innovative Research of America, Sarasota, FL; Sigma Chemical Co., St. Louis, MO), was administered in pellet form. For the silastic implant procedure testosterone (Sigma Chemical Co., St. Louis, MO) was subsequently administered in the form of packed Silastic capsules (100% T).

2.3 Testosterone Replacement

Pellets were implanted subcutaneously after the animals were briefly sedated with isofluorane using the following doses: 0 mg (control, where all surgical procedures were followed except for the actual implantation), 10, 25, 50, 100 and 200 mg. For Silastic implants, rats were subcutaneoulsly implanted with either empty capsules (30mm) or 0.5, 1, 2 or 3 30-mm (dose equivalents of 0, 15, 30, 60 or 90mm) testosterone-filled capsules [0.058 in. ID x 0.077 in. OD; Dow Corning (Midland, MI)], after brief sedation with isofluorane.

2.4 CPP apparatus

For behavioral assays, a Single Station Package with computer-automated Guillotine Doors, White and Black Place Preference Apparatus from Med Associates (Georgia, VT) was used. This device consisted of a white chamber, a black chamber, and a gray neutral chamber in the middle. The three chambers were separated by computer-automated guillotine doors to allow the animals access to all three chambers. The central chamber was 12 cm long and had a smooth PVC floor. The black chamber was 28 cm long and had a stainless steel grid rod floor, while the white chamber, also 28 cm long, had a stainless steel mesh floor. This was to provide different visual and tactile cues on either side of the apparatus. Spontaneous locomotor activity was monitored by a Photobeam Activity System within each conditioning chamber. The length of time spent in each chamber, and the total locomotor activity (the sum of all horizontal counts), entrances, and explorations into each chamber were also recorded.

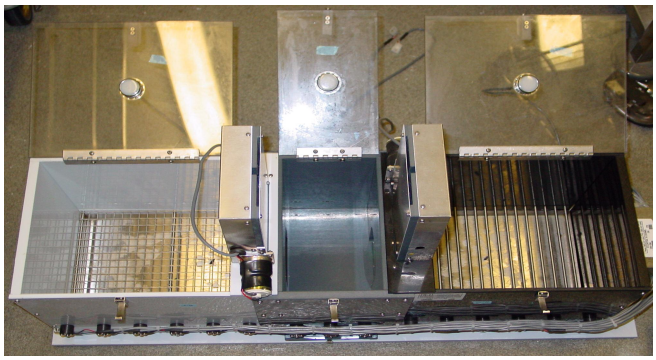


Figure 9: Conditioned Place Preference Apparatus

2.5 CPP Procedure

The conditioning procedure was conducted one week after surgery as previously described [150]. For preconditioning, rats were placed into the neutral gray area for a 5-minute acclimation period and then allowed free access to all three chambers for 15 minutes. Rats were then randomly assigned to either cocaine/saline conditioning groups or saline/saline control groups. The second phase consisted of four conditioning days (two cocaine/saline pairings). Thirty minutes after the lights were turned on, rats (n = 5-10 animals/group) received intraperitoneal injections of saline (0.9%) or cocaine (20 mg/kg) in the conditioning chambers. On conditioning days 1 and 3, rats were injected with cocaine and immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes. Control rats received saline in both chambers on alternate days. On the testing day, rats were placed into the neutral gray chamber for a 5-minute acclimation period and then allowed 15 minutes of free access to all three chambers in a drug-free state. Time spent and total locomotor activity (sum of all horizontal counts) were recorded using a computerized photo-beam system run with MED-PC software.

DAY	1	2	3	4	5	6
Preconditioning	▲					
Cocaine		▲		▲		
Saline			▲		▲	
Test						▲

Figure 10 : Cocaine and Saline Administration paradigm for acute conditioned place preference procedure

Animals are tested in a drug-free state and sacrificed 20 minutes after behavioral assay

2.6 Testosterone radioimmunoassay

Animals were sacrificed by rapid decapitation after a brief exposure to CO_2. Trunk blood was centrifuged (at 3000 rpm for 30 minutes at 4˚C); serum was then collected and stored at -80˚C. Analysis of testosterone serum levels was conducted using a Coat-A-Count radioimmunoassay kit from Diagnostic Product Corporation (CA). Intra-assay coefficient of variance averaged <10%. Results were determined using a log-logit analysis within GRAPHPAD PRISM (GraphPad Software, CA, USA). Testosterone serum levels are expressed as ng/ml.

2.7 Data analysis

A dependent measures t-test (two-tailed) within each group was used to determine statistically significant differences between the time spent in each side

of the place preference apparatus. CPP score was calculated in order to determine the magnitude of the place preference, and differences across the different testosterone doses were assessed by means of a one-way ANOVA. Total locomotor counts are represented as the sum of photobeam breaks due to the animal's crossover in all three chambers of the place preference apparatus. To examine the effects of cocaine conditioning on total locomotor activity, a dependent measures t-test (two-tailed) within each group was used to determine statistically significant differences among the total locomotor counts between saline-treated and cocaine-treated animals.

3. Results

Throughout the study, control rats (saline/saline) did not differ in the time spent of either side of the chamber, confirming the unbiased nature of the procedure (data not shown). Data collected using testosterone pellet implantation had to be discarded due to the development of severe infections at the surgical site. It is important to note that previous studies in our lab [116] as well as others [86; 87; 117] utilizing testosterone pellet (Innovative Research of America, Sarasota, FL) implantation as a method of hormone replacement have shown a consistent problem with the integrity of the pellets. Extrusion of the pellets was commonly seen in these studies, and this condition persisted even after washing of the pellets prior to implantation was performed [87]. In some cases, use of these pellets led to severe infections at the implant site [116; 117],

resulting in the premature sacrifice of over 60% of the experimental animals [116]. As a result of this problematic circumstance, the following results reflect only the data collected using the implantation of the SILASTIC capsules.

3.1 Serum levels of testosterone increased with increased replacement doses

Serum levels after chronic testosterone replacement were found to increase dose-dependently ($F(1, 83) = 26.618$, $p < 0.01$; see Figure 11). As the SILASTIC length increased (thereby increasing the dose of testosterone), serum testosterone levels also increased. Thirty mm SILASTIC capsules produced higher levels of testosterone than 0 and 15 mm, while 60 and 90 mm lengths resulted in higher testosterone levels than 15 and 30mm lengths.

3.2 Chronic testosterone administration did not affect the development of conditioned place preference to cocaine

As seen in Figure 12, paired t-tests conducted for each dose of testosterone administered revealed a significant difference in the amount of time spent on the cocaine-paired side and the unpaired side**: (0 mm** of testosterone: t (12) = 4.402, $p < 0.05$; **15 mm**: t (6) = 2.924, $p < 0.05$; **30 mm**: t (13) = 2.047, $p < 0.05$; **60 mm**: t (4) = 2.792, $p < 0.05$; **90 mm**: t (6) = 4.930, $p < 0.05$.) However, there was a lack of significant differences across groups. CPP scores did not differ significantly across all testosterone treated rats: $F (4, 47) = 0.8658$, $p > 0.05$ (see Figure 13).

3.3 Chronic testosterone administration does not affect cocaine-induced locomotor activity

As seen in Figure 14, paired t-tests conducted for each dose of testosterone administered revealed a significant difference in the total number of locomotor counts for conditioning day 1 between cocaine-treated and saline-treated animals: (**0 mm** of testosterone: t (10) = 5.101, $p < 0.05$; **15 mm**: t (6) = 2.685, $p < 0.05$; **30 mm**: t (9) = 4.503, $p < 0.05$; **60 mm**: t (4) = 3.059, $p < 0.05$; **90 mm**: t (2) = 7.926, $p < 0.05$.) However, no significant differences were observed across all the doses of testosterone.

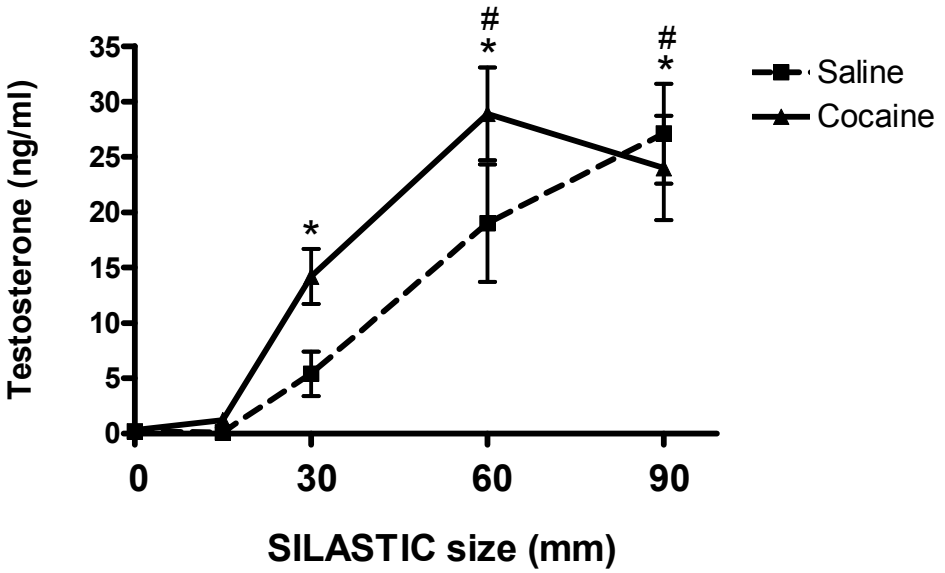


Figure 11. The effects of chronic testosterone administration in castrated male rats via Silastic capsule implant. Data represents changes in serum levels of testosterone in cocaine-treated (solid line) and saline-treated (dotted line) animals. * denotes significant differences ($p < 0.05$) in serum levels between rats that received 30mm capsules and those that received 0 and 15mm; # denotes significant differences ($p < 0.05$) in serum levels between rats that received 60 and 90mm capsules and those that received 15 and 30mm lengths.

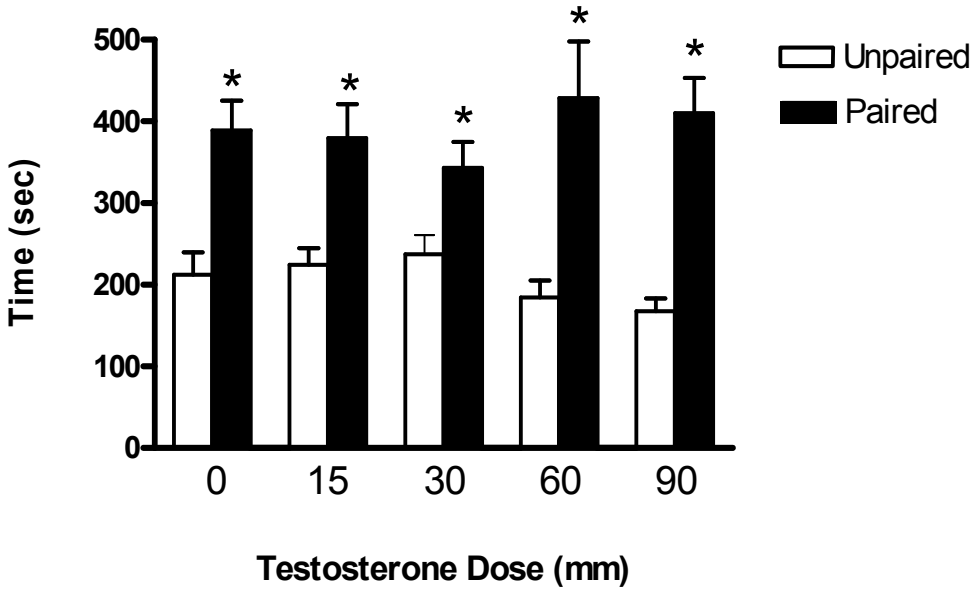


Figure 12. The effects of chronic testosterone administration on cocaine-induced CPP in castrated male rats. Data represents the time spent in seconds in saline-paired (white bars) and cocaine-paired (black bars) compartments of the CPP chamber on the test day. *Indicates significant difference ($p < 0.05$) between saline- and cocaine-paired compartments within testosterone dose.

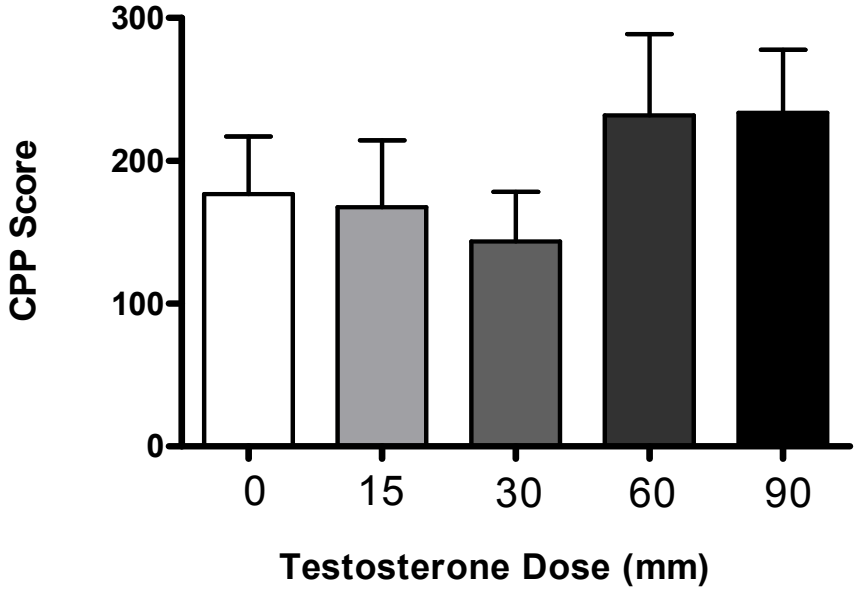


Figure 13. The effects of chronic testosterone administration on a calculated CPP score in castrated male rats. Data represents the difference in time spent (in seconds) on the saline-paired and cocaine-paired compartments of the CPP chamber on the test day for each testosterone dose.

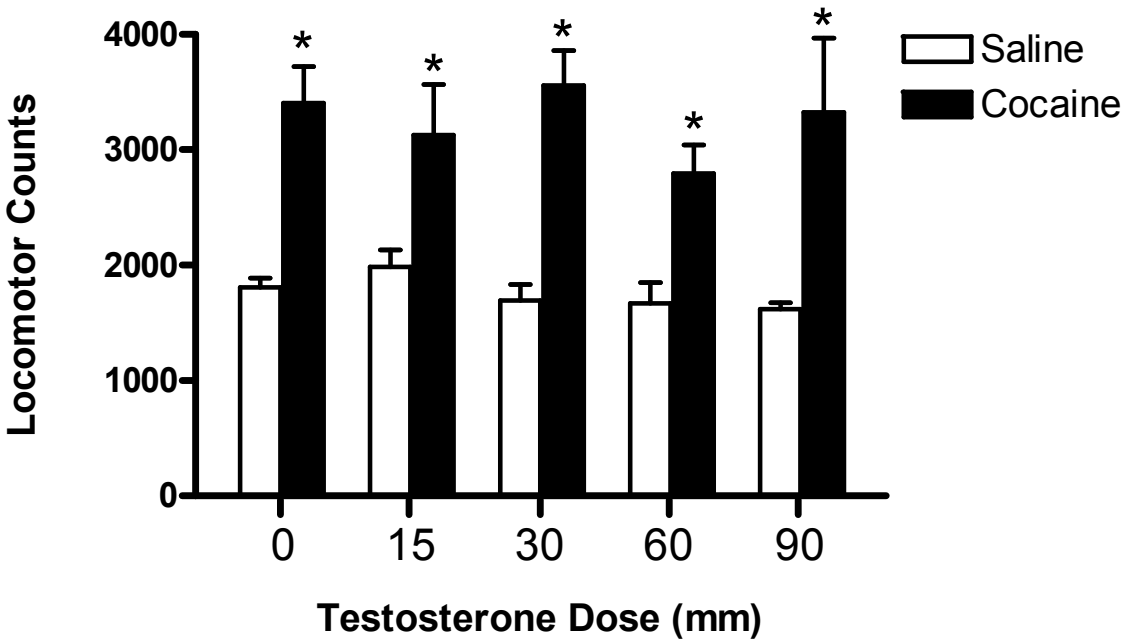


Figure 14. The effects of chronic testosterone administration on cocaine-induced locomotor activity in castrated male rats on Day 1. Data represents the total locomotor counts recorded among saline-treated (white bars) and cocaine-treated (black bars) animals on Day 1 of conditioning. *Indicates significant difference ($p < 0.05$) between saline- and cocaine-treated rats within testosterone dose.

4. Discussion

In this study we demonstrated that testosterone alone does not produce CPP. Some studies have shown the development of CPP after high doses of testosterone [106], while others have shown no effect at any dose [30; 90; 127; 157]. Still others have demonstrated that the pairing of systemic testosterone with a distinctive chamber has produced a CPP [60]. Further, infusions of testosterone into the nucleus accumbens or the medial preoptic area and immediate pairing with the nonpreferred side of the CPP apparatus reliably produced a preference for that side in a dose-dependent manner [2].

After chronic exogenous testosterone, neither cocaine-induced conditioned place preference nor locomotor activity was affected at any of the doses administered. Long, et. al. [103] reported that testosterone implantation reduced cocaine-induced locomotor effects in Wistar rats. Specifically, an 80mg/kg dose of oral cocaine enhanced locomotor activity among all the experimental groups except the intact testosterone-treated group [103]. Conversely, Martinez-Sanchis, et. al. [111] found that the administration of exogenous testosterone resulted in an enhancement of cocaine-induced locomotor activity in Swiss-Webster mice. An enhancement was observed in locomotor behavior by a 2mg/kg dose of testosterone, however among all other groups testosterone did not stimulate locomotor activity when compared with vehicle [111]. Furthermore, all animals that received cocaine and peanut oil

displayed a higher locomotor activity dose-dependently when compared with controls [111].

It is important to note the differences in animal strain and method of drug administration among testosterone studies, as well as the different compounds of testosterone which have been used. Differences in behavioral responses to cocaine have been observed across different rodent strains, as well as within the same strain from different vendors; for instance, Fischer rats from different strains [29] and vendors [132] using similar experimental paradigms do not respond to cocaine in the same behavioral manner. Furthermore, the source and doses of testosterone differ among various studies. Therefore experimental differences in rodent models may account for the discrepancies among these studies.

An important characteristic to remember when studying testosterone and its effects is that it has numerous metabolites, the roles of which are not completely understood. In some cases, testosterone may metabolize very rapidly, exerting an effect during the transformation that will not be continued into its new configuration, but each metabolic alteration may have its own distinctive characteristics [25]. Some conversions to metabolite are reversible [25]. Animals in the present study received testosterone one week prior to the conditioned place preference procedure, which is conducted over a 6-day period. Therefore, post-test sacrifice and subsequent trunk blood collection did not occur

until 2 weeks after the administration of testosterone. This time period may have allowed for the administered testosterone to convert to any one or more of its metabolites, making precise analysis difficult. A possible alternative to the chronic administration would be to administer testosterone acutely, possibly via a subcutaneous injection prior to testing, which would eliminate the need for the 7 day surgical recovery period, thereby lessening the likelihood of more metabolic conversions. This method of administration has previously resulted in a dose-dependent effect of testosterone on cocaine-induced reward responses [94].

Based on our observations we postulate that testosterone plays a limited role in the development of environmental cue associations in adult male rats. Moreover, non-subjective effects, such as locomotor activity, were not activated by testosterone.

Chapter 3: The Role of Testosterone in Cocaine-Induced Conditioned Place Preference and Locomotor Activity: Chronic vs. Acute Administration

1. Introduction

Sex differences in cocaine-induced behavioral and subjective responses have been reported, where females typically exhibit more pronounced locomotor responses and development of cocaine-induced CPP than males [18; 38; 39; 54; 55; 56; 118; 124; 141; 149; 150]. The prevailing theory suggests that gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine (reviewed in 56). However, although the current literature postulates that testosterone contributes to the sexually dimorphic responses to cocaine, a direct link between testosterone and cocaine has yet to be established.

To date there have been few studies conducted that address the role of testosterone in male responses to cocaine. Indirect evidence, via the study of castration on cocaine-induced activity, has revealed a direct link between testosterone and cocaine effects. For example, in castrated males, testosterone has been found to delay and reduce cocaine-induced stereotypical behavior [40; 103]. After testosterone replacement there has been reduced cocaine-induced brain activity within reward-associated areas in the brain [170] which suggests that testosterone is able to modify the reward effects of cocaine by reducing the area of activation [170]. Further, testosterone serum levels increase after cocaine

administration, suggesting an interaction between testosterone and cocaine at different levels.

Conditioned place preference (CPP) has been used to determine the rewarding effects of drugs by establishing associations between the rewarding and incentive motivational effects of drugs and environmental stimuli [31; 63; 108; 110]. Using this experimental technique, the present study aims to determine if testosterone dose-dependently affects locomotor activity and the formation of reward associations with cocaine.

2. Method

2.1 Animals

Eight week old castrated male Fischer rats (Charles River Laboratories, Kingston, NY) were singly housed in animal cages with free access to food and water, and maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.). Animals were handled daily after their arrival. Animals were randomly assigned to either saline- or cocaine- treatment and further subdivided into hormone-replacement groups. The experimental manipulations were conducted in at least 3 cohorts with a total of 8-10 animals per group. All National Institute of Health (NIH) and Institutional Animal Care and Use Committee guidelines for the care and use of laboratory animals were strictly followed. All chemicals, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2 Testosterone replacement paradigms

Testosterone (Innovative Research of America, Sarasota, FL or Sigma Chemical Co., St. Louis, MO), was administered using two paradigms (see Figure 15). For chronic testosterone replacement, after sedation with isofluorane, rats were implanted with one to three 30mm, or one 15mm Silastic capsule [0.058 in. ID x 0.077 in. OD; Dow Corning (Midland, MI)], filled with 100% T. Control rats were implanted with empty capsules. Rats were behaviorally tested one week after hormone replacement. For acute testosterone administration, testosterone (0, 400, 800, or 1200 μg/kg) or vehicle (DMSO) was administered via subcutaneous injections at the nape of the neck concurrently with drug administration. These doses and manner of administration have been previously shown to produce physiological and pharmacokinetic testosterone serum levels [2]. Cocaine was prepared daily in 0.9% saline solution and administered via intraperitoneal injection at a concentration of 20 mg/kg. This dose was chosen because in males it reliably produces cocaine-induced CPP using a 4-day conditioning paradigm [150] and enhances locomotor activity [55; 56].

2.3 CPP apparatus and procedure

The CPP apparatus used was a Single Station Package Place Preference Apparatus from Med Associates (Georgia, VT) with three chambers (a white chamber, a black chamber, and a central gray neutral chamber). The central chamber was 12 cm long and had a smooth PVC floor. The black chamber was

28 cm long and had a stainless steel grid rod floor, while the white chamber, also 28 cm long, had a stainless steel mesh floor. The three chambers were separated by computer-automated guillotine doors. Spontaneous locomotor activity was monitored by a Photobeam Activity System within each conditioning chamber.

A 4-day conditioning paradigm was used as previously described [150]. For preconditioning, rats were placed into the neutral gray area for a 5-minute acclimation period and then allowed free access to all three chambers for 15 minutes. The conditioning phase consisted of a 4-day paradigm (two cocaine/saline pairings). Thirty minutes after the lights were turned on, rats received intraperitoneal injections of saline or cocaine in the conditioning chambers. On conditioning days 1 and 3, rats in the acute testosterone replacement group received a co-administration of cocaine and testosterone, while rats in the chronic testosterone replacement group received only cocaine; rats were then immediately confined to one chamber for 30 minutes. On alternate days, rats were injected with saline and immediately confined to the opposite chamber for 30 minutes (see Figure 15). Control rats received saline on all four days. On the testing day, rats were placed into the neutral gray chamber for a 5-minute acclimation period and then allowed 15 minutes of free access to all three chambers in a drug-free state. In the chronic testosterone group, testosterone was consistently present throughout the conditioning and testing days. Time spent in each chamber and total locomotor activity (sum of all

horizontal counts) were recorded using a computerized photo-beam system run with MED-PC software.

DAY	1	2	3	4	5	6
Preconditioning	▲					
Cocaine		▲		▲		
Saline			▲		▲	
Test						▲

Figure 10 : Cocaine and Saline Administration paradigm for acute conditioned place preference procedure

Animals are tested in a drug-free state and sacrificed 20 minutes after behavioral assay

2.4 Testosterone radioimmunoassay

After the final behavioral test, rats were sacrificed by rapid decapitation (after a 20 sec exposure to CO_2). Trunk blood was collected, centrifuged (at 3000 rpm for 30 minutes at 4˚C), and serum was stored at -80˚C until used. Testosterone serum levels were determined with a Coat-A-Count radioimmunoassay kit from Diagnostic Product Corporation (Los Angeles, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit analysis within GRAPHPAD PRISM (GraphPad Software, CA, USA). Testosterone serum levels are expressed as ng/ml.

2.5 Data analysis

For CPP, behavioral data is presented as the mean time ± SEM in seconds spent in each side of the place preference apparatus. A dependent measures t-test (two-tailed) within each group was used to determine statistically significant differences between the time spent in each chamber. The CPP score represents the magnitude of the place preference (subtraction of the amount of time spent on the saline-paired side of the chamber from the amount of time spent on the cocaine-paired side of the chamber). Total locomotor counts are represented as the mean sum of photobeam breaks ± SEM. One-way ANOVAs were used to determine differences in serum levels, CPP scores, and locomotor activity. When appropriate, a Fisher LSD post hoc test was conducted. In all cases $p < 0.05$ was considered significant. Serum levels are expressed as ng/ml.

3. Results

3.1 Testosterone serum levels increased after chronic and acute hormone replacement

After chronic testosterone administration, serum levels were found to increase dose-dependently ($F(3, 79) = 28.34$, $p < 0.001$) (see Figure 11). However, no differences in testosterone serum levels between cocaine/saline treatment were observed. Testosterone serum levels increased dose dependently, where 30, 60 and 90 mm Silastic capsules produced significantly higher serum levels than 0, and 60 and 90 mm capsules increased serum levels to a greater degree than 30mm capsules ($F(1, 83) = 26.618$, $p < 0.01$). However, after acute

testosterone administration, 48 hours after the last testosterone dose, no significant changes in testosterone serum levels were observed (Figure 16).

3.2 Cocaine-induced CPP in control rats

In both replacement paradigms, control rats (saline/saline–treated) did not differ in the time spent on either side of the chamber, confirming the unbiased nature of the procedure (data not shown). Overall, cocaine increased the time spent in the drug-paired side as compared to the saline-paired side ($F(1, 60) = 36.609$, $p < 0.01$). Moreover, both replacement paradigms produced equivalent CPP scores. Furthermore, testosterone administration did not alter CPP or locomotor activity in any of the testosterone-saline–treated rats (data not shown).

3.3 Chronic testosterone administration did not affect cocaine-induced CPP or locomotor activity

As seen in Figure 12, regardless of the testosterone dose, rats spent more time in the cocaine-paired side of the apparatus as compared to the saline-paired side (**0 mm**: t (12) = 4.402, $p < 0.05$; **15 mm**: t (6) = 2.924, $p < 0.05$; **30 mm**: t (13) = 2.047, $p < 0.05$; **60 mm**: t (4) = 2.792, $p < 0.05$; **90 mm**: t (6) = 4.930, $p < 0.05$). Furthermore, no significant differences in the CPP scores were observed between any of the experimental groups: [$F (4, 47) = 0.8658$, $p > 0.05$, (Figure 13)]. As seen in Figure 14, cocaine increased overall locomotor activity

(F (1, 60) = 36.6, $p < 0.05$). However testosterone administration did not alter cocaine-induced locomotor activity.

3.4 Acute testosterone administration blocked development of conditioned place preference to cocaine at the lowest dose but had no effect on locomotor behavior

As shown in Figure 17, acute testosterone administration dose-dependently altered cocaine-induced CPP; only 800 and 1200 µg/kg produced cocaine-induced CPP (**0 µg/kg** of testosterone: t (7) = 2.923, $p < 0.05$; **400 µg/kg**: t (7) = 1.133, $p > 0.05$, *ns*; **800 µg/kg**: t (6) = 4.467, $p < 0.05$; **1200 µg/kg**: t (6) = 5.512, $p < 0.05$). However, no significant differences in CPP scores were observed [(F (3, 27) = 1.126, $p > 0.05$), (see Figure 18)]. Although overall, cocaine increased locomotor activity (F (1, 51) = 56.4, $p < 0.05$), no significant interaction between testosterone replacement doses and cocaine was observed (Figure 19).

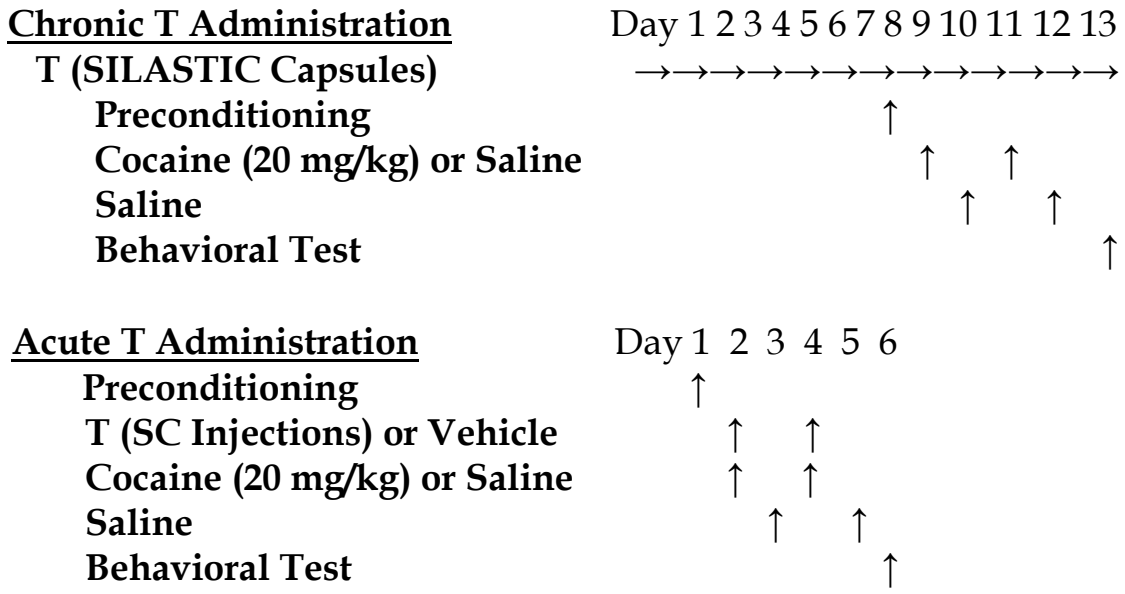


Figure 15: Testosterone administration paradigms. Arrows represent injection administrations.

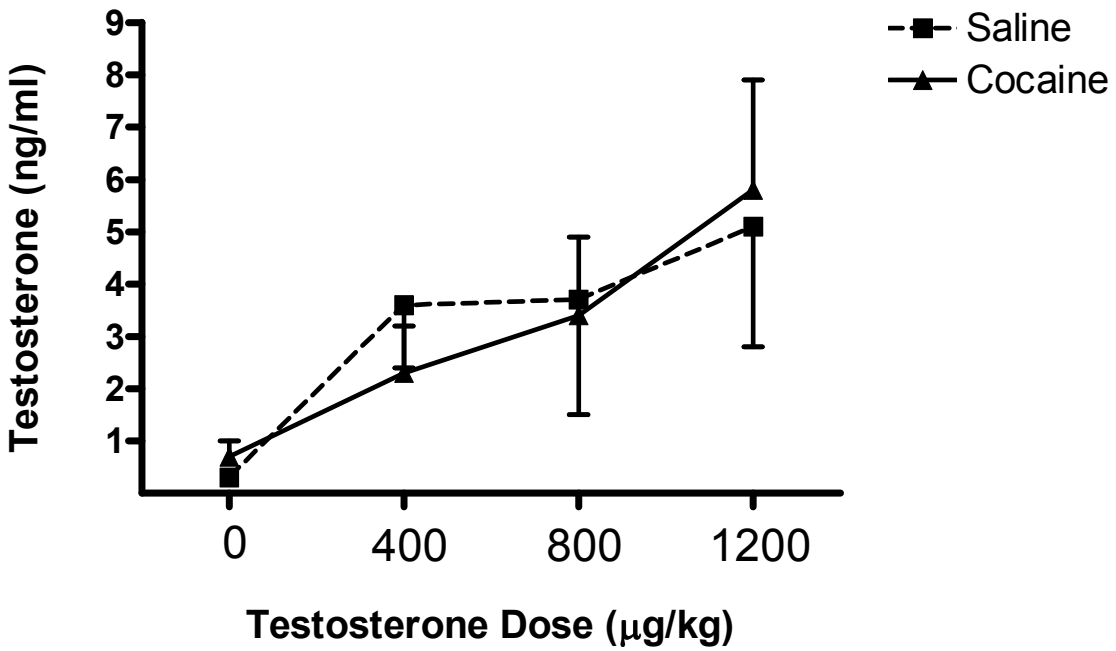


Figure 16: Testosterone serum levels after acute testosterone administration. Data represents changes in serum levels of testosterone in cocaine-treated (solid line) and saline-treated (dotted line) animals.

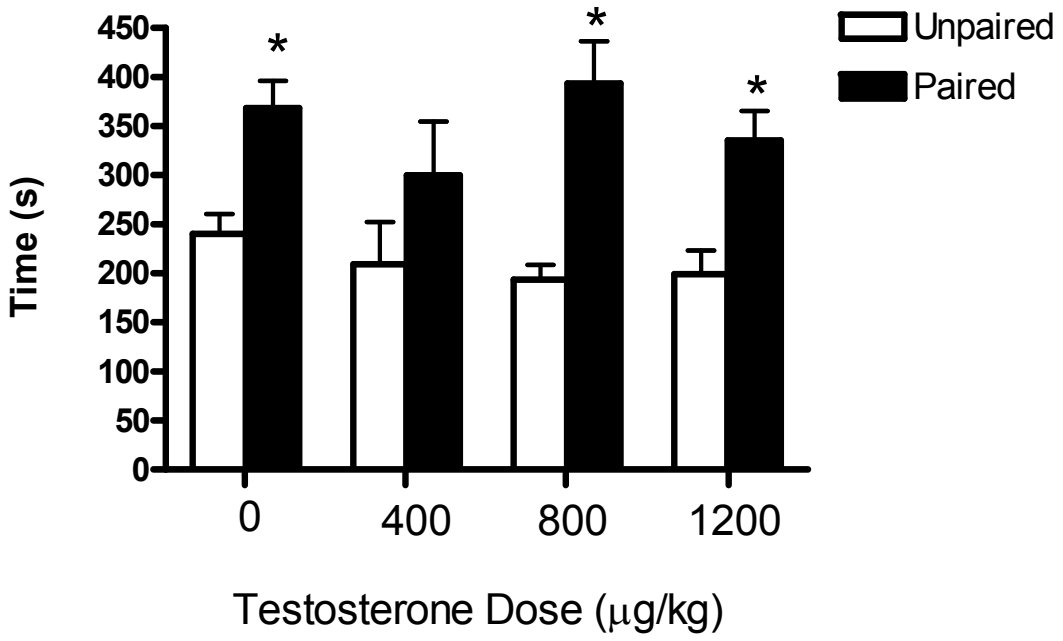


Figure 17: Acute testosterone administration effects on cocaine-induced CPP in castrated male rats. Data represents the time spent in seconds in saline-paired (white bars) and cocaine-paired (black bars) compartments of the CPP chamber on the test day. *Indicates significant difference ($p < 0.05$) between saline- and cocaine-paired compartments within testosterone dose.

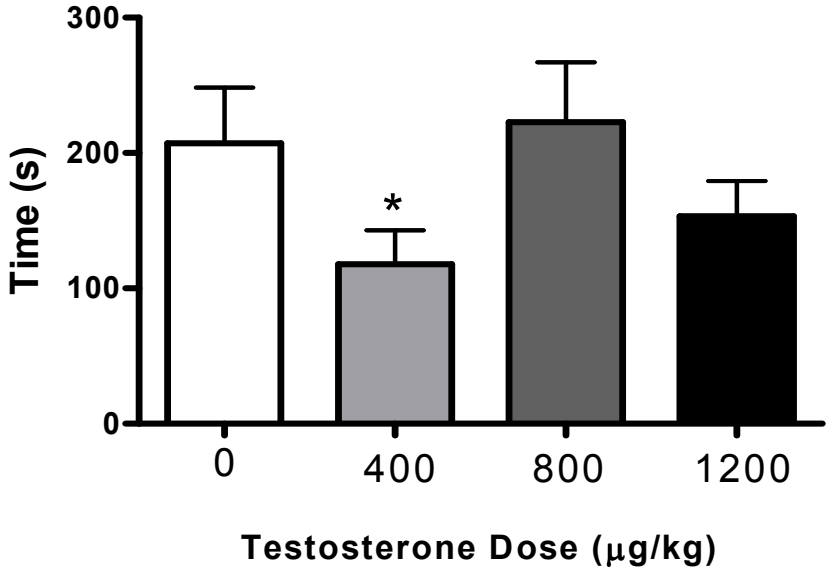


Figure 18. CPP Score. Data represents the difference in time spent (in seconds) on the saline-paired and cocaine-paired compartments of the CPP chamber on the test day for each testosterone dose. *Indicates significant difference ($p < 0.05$) between the difference in time spent between the cocaine-paired and saline-paired side of the chamber within testosterone dose.

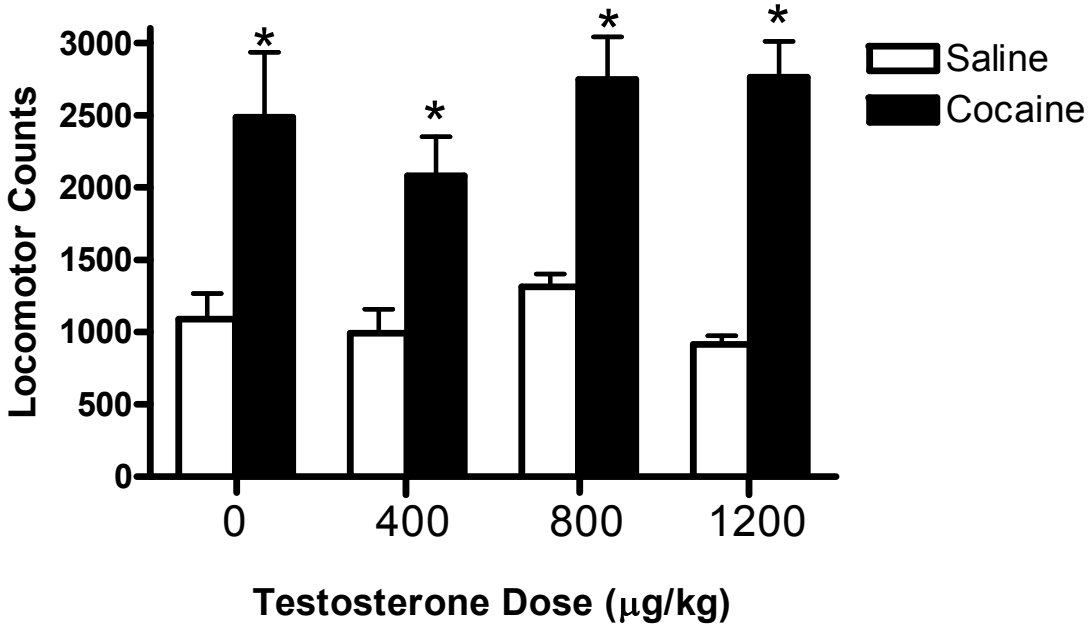


Figure 19: Acute testosterone administration effects on cocaine-induced locomotor activity in castrated male rats. Data represents the total locomotor counts recorded among saline-treated (white bars) and cocaine-treated (black bars) animals on Day 1 of conditioning. *Indicates significant difference ($p < 0.05$) between saline- and cocaine-treated rats within testosterone dose.

4. Discussion

The manner of testosterone replacement affected the outcome of cocaine CPP development and expression in male rats. Acute testosterone administration disrupted the formation of environmental associations with cocaine reward effects. This is consistent with previous results [2], where testosterone blocked cocaine CPP dose-dependently.

Testosterone has a distinct mechanism for activating behavioral reponses. Chronic testosterone administration produces a genomic activation which in turn results in new protein formation [42; 74]. On the other hand, transient/acute testosterone replacement produces an activation of membrane receptors which in turn produces transient activation of intracellular responses [42; 126].

Based on our observations we postulate that testosterone effects on the development of cocaine reward associations occur through membrane-mediated mechanisms. Moreover, in the current literature there are inconsistent findings regarding the effects of testosterone on cocaine reward, although in part these discrepancies may be due to differences in the type of testosterone selected for administration. Based on our observations, we further postulate that the manner of testosterone replacement has an impact on the hormonal regulation of cocaine reward effects. We further demonstrate a dissociation of cocaine-induced CPP and locomotor responses. Indeed, it has been postulated that cocaine-induced CPP is a measurement of recalling reward associations and thus a subjective measurement, while locomotor responses are a measurement of physiological

effects of cocaine. These two behavioral measurements are controlled by distinct neuronal pathways, thereby suggesting that testosterone effects may be distinct to areas of the brain which regulate reward.

Long, et. al. [103], found that the implantation of 100 mg pellets of testosterone in Wistar rats reduced cocaine-induced locomotor effects using a 80 mg/kg dose of oral cocaine, which increased motor activity among all rats except the testosterone-treated group. Conversely, Martinez-Sanchis, et. al. [111] found that the administration of exogenous testosterone resulted in an enhancement of cocaine-induced locomotor activity using a 2mg/kg dose of testosterone in Swiss-Webster mice when administered via acute injection, using a 10mg/kg dose of cocaine [111]. However among all other groups testosterone did not stimulate locomotor activity when compared with vehicle [111]. Furthermore, all animals that received cocaine and peanut oil displayed a higher locomotor activity dose-dependently when compared with controls [111].

Discrepancies between these observations and ours may in part be indicative of differences in animal strain. Indeed in female rats, estrogen + progesterone administration produces different effects in cocaine-induced activations depending the animal strain used for study.

While in our study chronic hormone replacement led to a significant increase in testosterone levels, acute replacement did not. Rats in the acute testosterone replacement group did not receive any testosterone for 48 hours

prior to behavioral testing and sacrifice. The chemical degradation of exogenous testosterone may proceed differently within an organism depending on the manner of administration.

The use of DMSO as the vehicle solvent may have been affected behavioral outcome. This substance may have caused some irritation at the injection site and may have interfered with the natural course of cocaine-induced locomotor reactions. Furthermore, DMSO has been shown to alter plasma corticosterone concentration in male rats [80]. DMSO was chosen as the vehicle because the oils that are typically used to dissolve hormones for replacement therapy do not effectively break down pure testosterone molecules. In future studies, the use of a different form of testosterone, such as testosterone propionate, (which can be successfully dissolved by non-irritating vehicles such as sesame or peanut oils) may be a favorable alternative to using pure testosterone (which would require the use of harsher substances as vehicles).

Although further investigation is needed to determine whether testosterone replacement affects the expression of cocaine-induced reward responses, including CPP and locomotor activity, the present findings suggest that testosterone effects may contribute to another mechanism by which sex differences in behavioral and subjective responses to cocaine are regulated.

Sex differences in cocaine reward and psychoactive stimulation appear to be regulated to some extent by gonadal hormones. For example, gonadectomy of

female rats decreased overall cocaine-induced behavioral responses, while in male rats it has no effect. Cocaine-induced CPP is also affected by gonadectomy; after ovariectomy, female rats demonstrate less cocaine-induced CPP [141; 142]. Furthermore, in female rats gonadal hormone replacement alters cocaine responses; i.e. estrogen increases while progesterone decreases or has no effect on acute cocaine-induced behavioral activity [38; 125]. Moreover, in females, progesterone attenuates while estrogen and progesterone coadministration enhances cocaine-induced CPP [125; 141]. Testosterone effects on cocaine-induced CPP further lend support to these findings.

Many cocaine users report having started their habitual cocaine use during their adolescent years [123]. Given the newfound trend among adolescents of abusing anabolic steroids [26], it may be worthwhile to investigate whether testosterone's affects on cocaine-induced reward responses differ between adolescents and adults. There may be a critical point during development where the vulnerability to addiction is stronger; therefore determining the point where cocaine-induced reward responses begin to show a heightened intensity may propose helpful clinical solutions to the problems of cocaine addiction and anabolic steroid use in our society.

Chapter 4: Effects of Testosterone on Cocaine Induced Locomotor Behavior: Adult vs. Adolescent Intact Male Fischer Rats

1. Introduction

Cocaine abuse is an increasing problem in Western society. Approximately 67% of cocaine users are male [123], with many reporting their first use during adolescence. The abuse of anabolic-androgenic steroids (AAS), which are synthetic derivatives of testosterone, has spread from elite Olympic, professional, college or high school athletes to the general population [26; 148; 181]. Estimates indicate that approximately 375,000 adolescent boys and 175,000 adolescent girls are steroid users [26]. Some cocaine users co-abuse anabolic steroids. AAS abuse in nonathletes is particularly common among adolescent boys (7% of high school seniors) [184]. The misuse of AAS among adolescents is increasingly concomitant to the use of other drugs [11]. Among adolescents, the frequency of anabolic steroid use was significantly associated with the frequency of cocaine use [50]. Furthermore, cocaine was the drug most likely to be co-abused by adolescent AAS abusers (33%) [50]. Moreover, the abuse of AAS has been correlated with an increased likelihood of abuse of other drugs, including cocaine [103; 108]. In a recent study of 227 men treated for cocaine dependence, 9.3% were also steroid abusers [123], with many of them indicating that their initial use of cocaine had been in an effort to minimize the negative effects of steroids, including insomnia, depression, and irritability [123].

In adult male rats, testosterone inhibits electroencephalogram (EEG) output and the overall behavioral effects of acute cocaine administration [176]. Chronic exogenous testosterone administration has reduced the behavioral effects of cocaine [103]. Studies have also found that long-term treatment with testosterone prevented the enhancement of cocaine-induced locomotor activity produced acutely in male rats [103; 111] and in mice [152], possibly via increases in noradrenaline and dopamine and decreases in serotonin [111], since these monoamines appear to be affected by androgen action in the CNS [42], and delayed/reduced cocaine-induced stereotypical behavior in castrated rats [38]. Cocaine administration has also been shown to decrease circulating testosterone levels [54], suggesting an interaction between both substances.

The period of adolescence is characterized by a vulnerability to drugs [107], a higher likelihood of initial drug use [107], high risk behaviors [163], alterations in novelty-seeking and exploratory behavior [31], and changes in the behavioral responsiveness to many drugs of abuse [31; 163]. Furthermore, adolescents respond differently than adults to a number of drugs that also act on dopamine neural systems [31]. For instance, nicotine has been found to significantly decrease locomotor activity in the adolescent rat as compared to adults [145], particularly in males [44]. Animals at this age also show a reduced responsiveness to the locomotor effects of psychostimulants, including cocaine [22; 162]. In particular, periadolescents have been found to lack the cocaine-

induced stereotyped behaviors that are characteristic of adults, including head scanning and focused sniffing [98]. Adolescence is also characterized by increased levels of stress [107]. High levels of stress have been postulated to increase the behavioral responsivity to psychostimulant drugs; these responses can in turn modulate the rewarding effects of drugs of abuse [134].

Important structural and functional changes in the brain occur during adolescence [34], but there has been little direct study of how this impact on drug abuse vulnerability. Studies have shown that adolescent rats display an exaggerated behavioral response to cocaine administered in two binge patterns. Total locomotor counts were the same in adult and adolescent rats, however adolescents engaged in more intense stereotypic behaviors, suggesting that equivalent tissue concentrations of cocaine produce a greater behavioral response in adolescent rats as compared to adults [34].

Past research seems to have focused primarily on the consequences of drug interactions with cocaine in females, probably because of the finding that many of the disparities in the behavioral responses can be explained by fluctuating hormonal status as determined by the estrous cycle. Hormonal fluctuations in pre- and post-pubescent boys, however, have not typically been investigated with respect to the same behavioral responses.

The present study seeks to determine the differences in testosterone's effects on cocaine-induced locomotor behavior between adult and adolescent

male rats. In addition, we seek to determine whether testosterone's effects are organizational or activational. However it must be noted that there is a lack of systematic study and consistency within findings in the literature regarding the effects of testosterone on cocaine-induced responses. Moreover, the extent to which developmental aspects impact the relationship between testosterone and cocaine has not yet been fully established.

2. Method

2.1 Animals

Adult (8 week old) and adolescent (4 week old) intact male Fischer rats (Charles River Laboratories, Kingston, NY) were singly housed in animal cages with free access to food and water. (These ages were chosen in accordance with previous studies that have designated adolescence as rats aged postnatal day 21-34 and adulthood as rats ages postnatal day 46-59 [173]). Rats were maintained on a 12-hour light/dark cycle (lights on at 9:00 a.m.) and handled daily after their arrival. Animals were randomly assigned to either saline- or cocaine- treatments and further subdivided into hormone-replacement groups. Each experimental group had a total of n=10 animals per group. All National Institutes of Health (NIH) and Institutional Animal Care and Use Committee (IACUC) guidelines for the care and use of laboratory animals were strictly followed.

2.2 Drug and hormone administration

Chemicals were purchased from Sigma Chemical Co (St. Louis, MO). Prior to cocaine or saline administration, rats were pretreated for 45 minutes with testosterone propionate (0, 5, or 10 mg/kg) or vehicle (sesame oil) via subcutaneous injection. These doses and timing of administration are consistent with previous studies which focus on the physical and behavioral effects of AAS abuse, where a significant locomotor effect using these doses was demonstrated after 45 minutes of pretreatment [111]. Furthermore these doses have been characterized as within a range of doses commonly used by abusers [105; 179]. Cocaine was prepared daily in 0.9% saline solution and administered via intraperitoneal injection at a 20 mg/kg dose. This dose was chosen because it has reliably produced a significant increase in locomotor activity in male rats without producing a maximal effect [55; 56].

2.3 Behavioral activity

Behavioral measurements were performed in the rats' home cage for 30 min after drug treatment. Locomotive activity was monitored with a Photobeam Activity System from San Diego Instruments (CA), as previously described [125]. Ambulatory activity represents the number of counts produced by the interruption of two consecutive photobeams in the horizontal frame. Rearing activity represents the total counts of vertical motion. Total activity represents the sum of counts in the horizontal frame.

To assess stereotypic activity, rats were videotaped for 30 seconds, 30 minutes after cocaine or saline administration. The videotapes were later analyzed for behavioral stereotypy by three trained observers blinded to each animal's treatment group. The rating for cocaine-induced stereotypic behavior was based on a modification of the Creese and Iversen scale [125]. As summarized in Table 1, this scale consists of 10 scores ranging from 1, given to an animal that was asleep or inactive, to 10, given to an animal that exhibited splayed hind limbs. In the present study, a score of 10 was never seen.

2.4 Testosterone radioimmunoassay

Animals were sacrificed by rapid decapitation after a brief (20 sec) exposure to CO_2. Trunk blood was collected, centrifuged (at 3000 rpm for 30 minutes at 4˚C), and serum was extracted and stored at -80˚C until used. Testosterone serum levels were determined with a Coat-A-Count radioimmunoassay kit from Diagnostic Product Corporation (Los Angeles, CA). Intra-assay coefficient of variance averaged less than 10%. Results were determined using a log-logit analysis within GRAPHPAD PRISM (GraphPad Software, CA, USA). Testosterone serum levels were expressed in ng/ml.

2.5 Data analysis

Behavioral and testosterone serum level data are presented as mean±SEM. Stereotypic data are presented as median score±semi-interquartile range. To analyze locomotive activity, ANOVAs were used to determine the effects of cocaine and hormone on behavioral responses within each age group as follows: Drug (saline or cocaine) × Testosterone dose (0, 5, 10)]. Fisher LSD post hoc tests were used when appropriate. For stereotypic behavior, a Kruskal–Wallis test followed by a Dunn's post hoc analysis was used to assess the effects of T dose or drug treatment. Differences in testosterone serum levels were analyzed via a three-way ANOVA: drug (saline or cocaine) x testosterone dose (0, 5, 10) x age (adult or adolescent). A p-value of <0.05 was considered significant in all statistical analyses.

3. Results

3.1 Effects of acute testosterone administration on testosterone serum levels

Serum levels of testosterone were found to increase dose-dependently after acute administration, ($F(2, 83) = 440.41$, $p < 0.001$). Significant interactions were found between testosterone dose and drug ($F(2, 83) = 50.48$, $p < 0.05$) and testosterone dose and age ($F(2, 83) = 235.00$, $p < 0.001$) (see Figure 20). In control rats receiving 0 mg/kg of testosterone, adult rats had significantly higher testosterone serum levels than adolescents regardless of the drug treatment ($p > 0.05$). Moreover, at the 5 mg/kg testosterone dose, significant differences in

testosterone serum levels were observed between saline- and cocaine-treated rats. Adolescent rats receiving 5 mg/kg and cocaine had higher testosterone serum levels than saline-treated adults and secondly, cocaine administration significantly increased testosterone serum levels in adult rats as compared to saline controls.

3.2 Effects of acute testosterone administration on ambulatory, rearing, and total locomotor activity

Regardless of the dose, testosterone did not alter cocaine-induced behavioral responses in adult rats. However, in adolescent male rats, while no significant effects were observed in total locomotor counts, testosterone dose-dependently altered cocaine induced ambulatory and rearing counts ($F(2, 24) = 3.925$, $p < 0.05$; $F(2, 24) = 8.013$, $p < 0.05$, respectively). These responses were lowered in the presence of testosterone. Adolescent rats treated with vehicle and cocaine had significantly higher rearing responses than adults receiving the same treatment ($F(1, 16) = 10.938$, $p < 0.05$). Ambulatory responses were also higher in adolescents than adults, but this value did not reach significance.

As shown in Figure 21, regardless of age, cocaine increased overall ambulatory, rearing , and total locomotor counts in rats ($F(1, 91) = 62.230$, $p < 0.01$; $F(1, 88) = 63.732$, $p < 0.01$; $F(1, 91) = 91.371$, $p < 0.01$, respectively). Across time, no statistically significant differences were observed between adolescent

and adult rats. Within the doses of testosterone used, no significant effects were observed among adolescents across time (see Figure 22).

After testosterone administration a baseline effect was observed in adolescent rats ($F(1, 47) = 34.841$, $p < 0.01$, see Figure 23), where, after 5 mg/kg of testosterone, ambulatory activity increased as compared to the 0 and 10 mg/kg doses of testosterone. To compensate for this effect data was converted to percent control format (summarized in Figure 24).

After receiving a 10 mg/kg dose of testosterone, adolescent rats demonstrated significantly higher ambulatory activity than after the 5 mg/kg dose. On the other hand, adolescents who received 5 or 10 mg/kg of testosterone had significantly lower ambulatory and rearing activity as compared to vehicle-treated rats.

With respect to the analysis of stereotypical data, cocaine increased stereotyped behavior as expected ($F (1, 96) = 13.392$, $p < 0.01$). However no statistically significant differences in the effects of testosterone were observed across any of the experimental groups (see Figure 25).

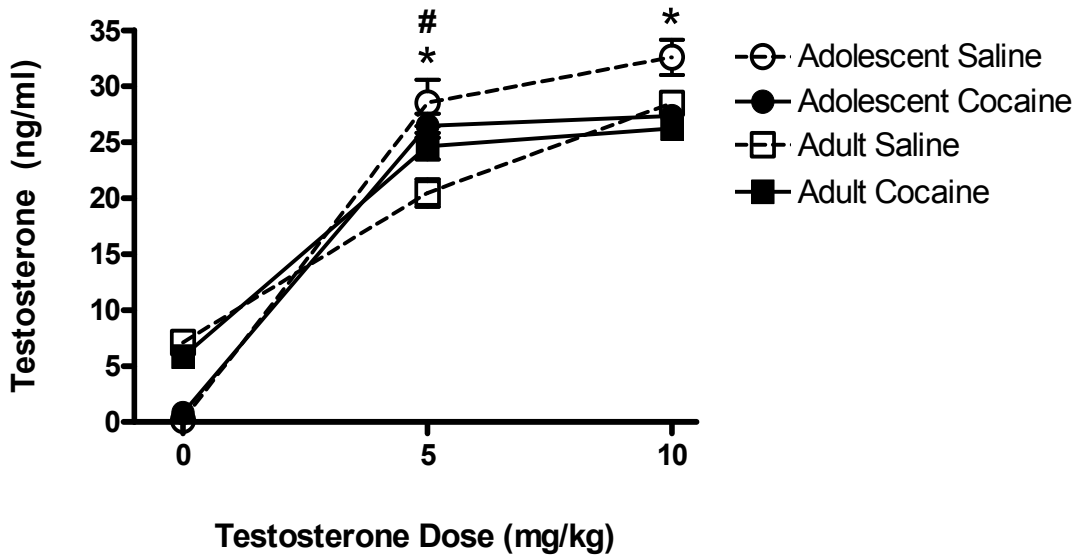


Figure 20: Testosterone serum levels after acute testosterone administration. Data represent changes in serum levels of testosterone for both adult and adolescent intact male rats in cocaine-treated (solid line) and saline-treated (dotted line) animals. * denotes significance ($p < 0.05$) between saline- and cocaine-treated rats receiving 5 mg/kg of T and vehicle; # denotes significance ($p < 0.05$) between adults and adolescents receiving 5 mg/kg of T and vehicle.

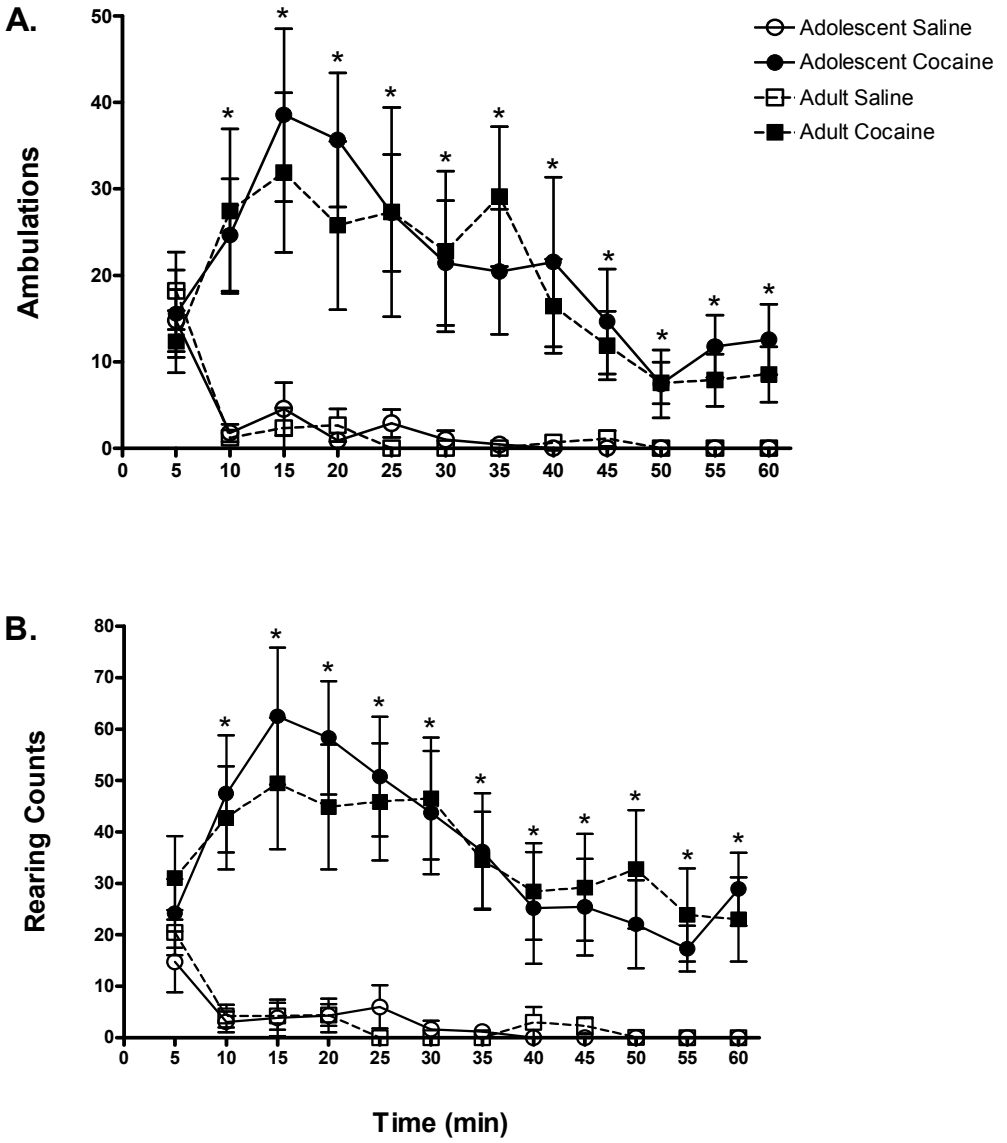


Figure 21: Time course analysis of ambulatory (A) and rearing counts (B) in vehicle-treated adult and adolescent intact male rats after cocaine administration. Data represent changes in behavioral response over a 1-hr period (5-minute intervals). * denotes a significant difference between cocaine and saline treated groups at the $p < 0.05$ level.

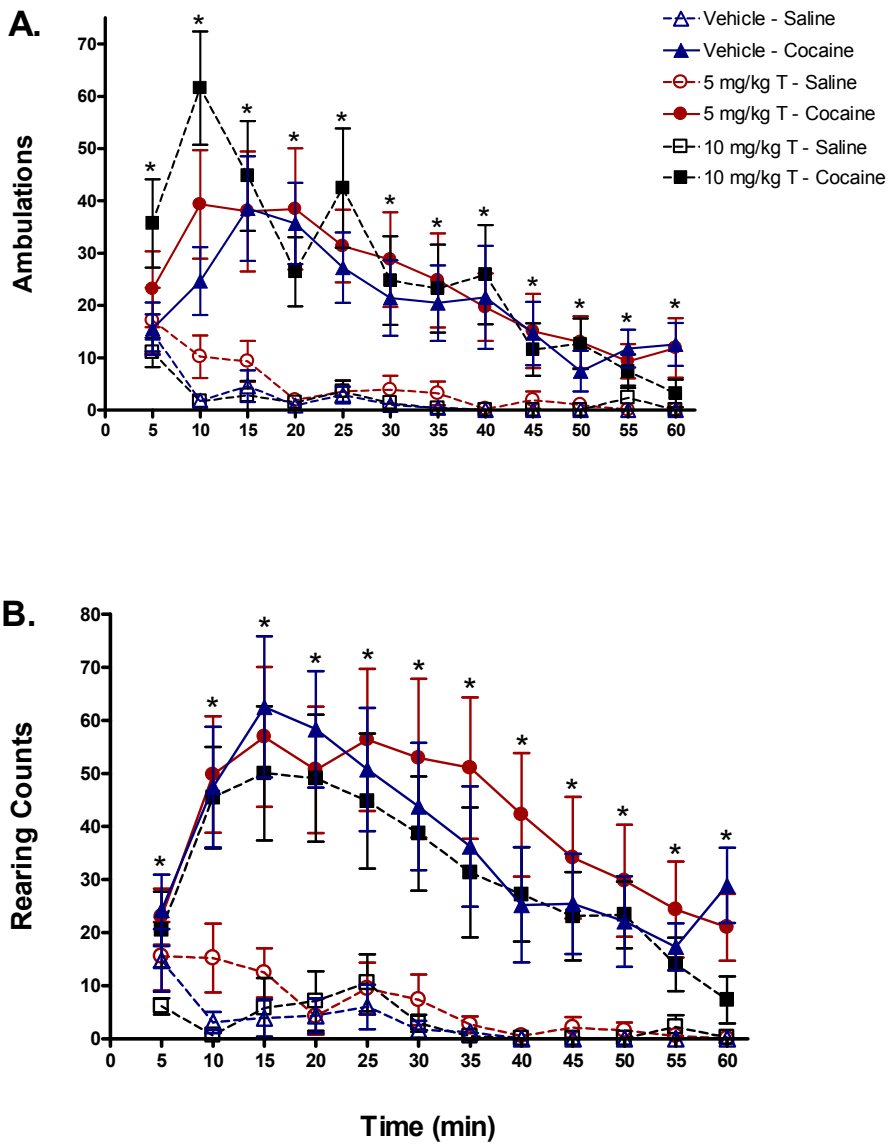


Figure 22: Time course analysis of ambulatory (A) and rearing counts (B) in adolescent intact male rats after cocaine administration. Data represent changes in behavioral response over a 1-hr period (5-minute intervals). * denotes a significant difference between cocaine and saline treated groups at the $p < 0.05$ level.

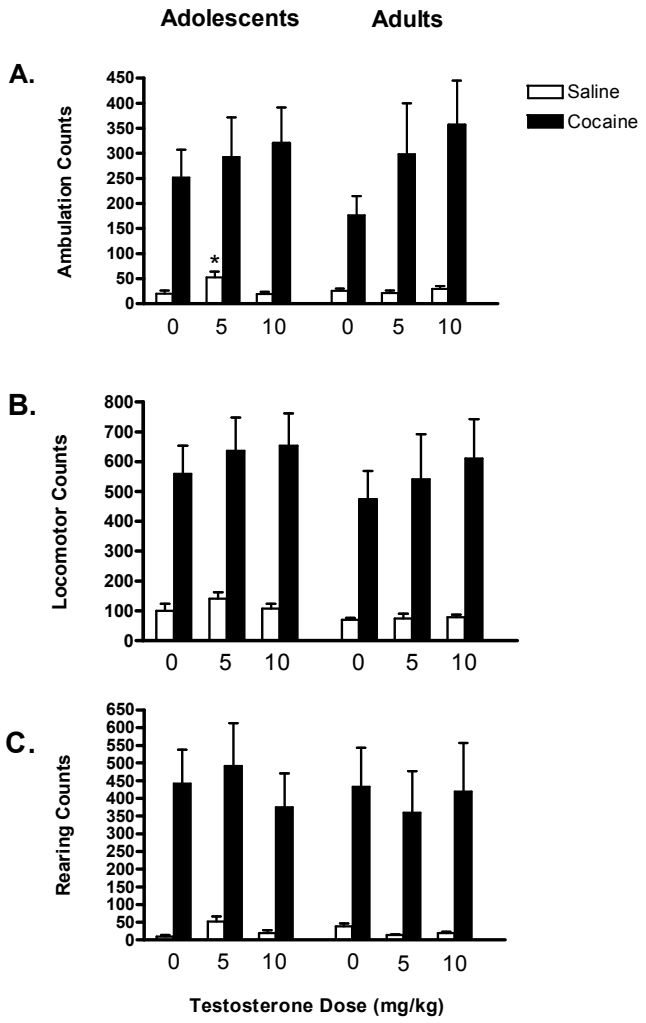


Figure 23: Ambulatory (A), total locomotor (B), and rearing (C) behavioral responses in adult and adolescent intact male rats after acute testosterone and cocaine administration. Intact rats received testosterone (5 or 10 mg/kg, s.c., 45 min before cocaine), or vehicle and cocaine (20 mg/kg) or saline. N= 8-12 per group. Behavior was recorded for 1hr after cocaine/saline administration. *denotes a significant difference ($p < 0.05$) in baseline ambulations between the adolescent 0 and 5 mg/kg T dose.

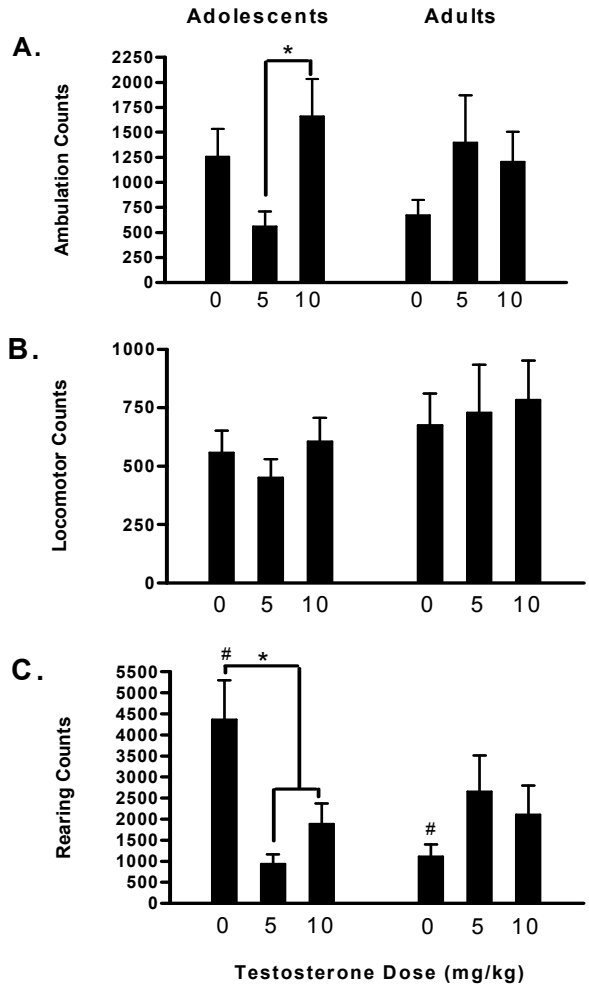


Figure 24: Ambulatory (A), total locomotor (B), and rearing (C) behavioral responses in adult and adolescent intact male rats after acute Tand cocaine administration, expressed as percent control. Rats received T (5 or 10 mg/kg, s.c., 45 min before cocaine), or vehicle and cocaine (20 mg/kg) or saline. N= 8-12/group. Behavior was recorded for 1hr after cocaine/saline administration. * denotes significance ($p < 0.05$) between adolescents receiving 5 and 10 mg/kg T and vehicle; # denotes significant difference between adults and adolescent rearing.

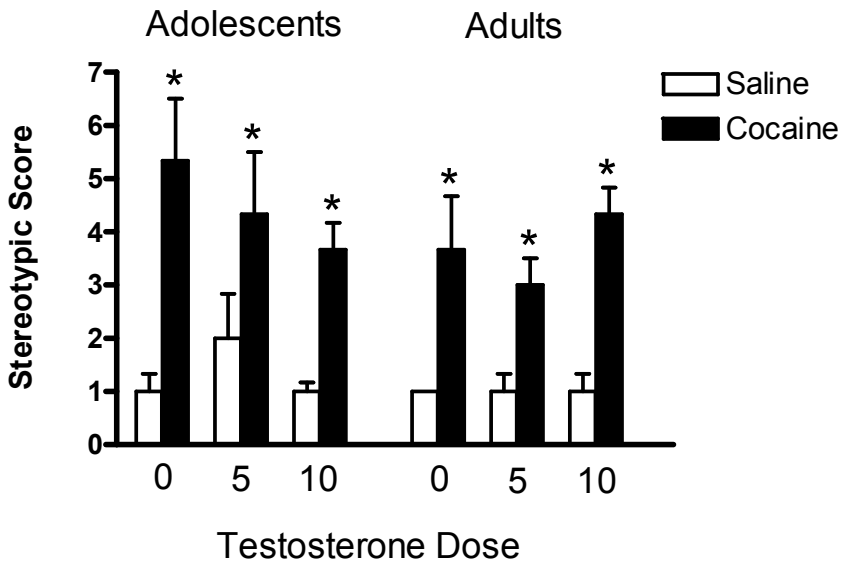


Figure 25: Sterotypic activity, expressed as mean + SEM of the median stereotypic scores for adolescent and adult male rats after acute testosterone and cocaine administration. Activity was recorded for 30 seconds, 30 minutes after cocaine injection. Data represents stereotypic score as designated by three blind observers for saline-treated (white bars) and cocaine-treated (black bars) animals. * denotes a significant difference between cocaine and saline groups at the $p < 0.05$ level.

Score	Behavior
1	Asleep, inactive
2	Alert, actively grooming
3	Increased sniffing in one location
4	Intermittent rearing and sniffing
5	Increased locomotion and sniffing
6	Intense sniffing in one location
7	Continuous pivoting and sniffing
8	Continuous rearing and sniffing
9	Maintained rearing and sniffing for >25 seconds
10	Splayed hind limbs

Table 1. Rating scale from Daunais and McGinty (1995) [125]

4. Discussion

In the present study we demonstrated that testosterone differentially affected cocaine-induced locomotor behavior between adult and adolescent rats. Specifically, we found that testosterone lowered cocaine-induced locomotor responses dose-dependently in adolescent rats but had no effect on those responses in adult rats.

Long, et. al. [103] reported that testosterone implantation reduced cocaine-induced locomotor effects in Wistar rats. Specifically, an 80mg/kg dose of oral cocaine enhanced locomotor activity among all the experimental groups except the intact testosterone-treated group [103]. Conversely, Martinez-Sanchis, et. al. [111] found that the administration of exogenous testosterone resulted in an enhancement of cocaine-induced locomotor activity in Swiss-Webster mice. The enhancement was observed in locomotor behavior by a 2mg/kg dose of testosterone, however among all other groups testosterone did not stimulate locomotor activity when compared with vehicle [111]. Furthermore, all animals that received cocaine and peanut oil displayed a higher locomotor activity dose-dependently when compared with controls [111]. These studies, however, included only adult animals.

Even within parallel findings in the literature it is important to note the differences in animal strain and method of drug administration among testosterone studies, as well as the different compounds of testosterone which have been used. Differences in behavioral responses to cocaine have been

observed across different rodent strains, as well as within the same strain from different vendors; for instance, Fischer rats from different vendors using similar experimental paradigms do not respond to cocaine in the same behavioral manner [132]. Furthermore, the source and doses of testosterone differ among various studies. While these explanations may account for the lack of consistency among studies involving the effects of testosterone on cocaine-induced motor behavior, a growing trend in the current literature indicates a propensity for adolescents to exhibit differences in testosterone and cocaine-induced responses as compared to adults. Several studies have indicated that adolescents exhibit higher levels of locomotion than adults in a cocaine-induced state [22; 162; 163]. Our results further support these findings, where higher locomotor responses were observed among adolescents as compared to adult controls (vehicle-cocaine treated). Aside from differences in levels of endogenous testosterone between adolescent and adult males, an interesting recent finding indicates that handling increases cocaine-induced locomotor activity in adolescent but not adult animals [107]. In the presence of testosterone, these enhanced cocaine-induced responses are attenuated [103].

In the brain testosterone can affect neural function by binding to classical androgen receptors, through classical estrogen receptors after aromatization to estrogen, or through nongenomic receptors [42]. The interaction between AAS and other psychoactive substances most likely involves the reward system, since

dopamine and serotonin systems involved in cocaine reward are also affected by androgen action at the central nervous system [171]. A significant characteristic of the adolescent brain is the widespread overproduction and pruning of many receptors [79; 102; 143]. Most notably is the increase in DA receptor densities in the rat striatum throughout development which peaks around postnatal day 28-30 [120; 121]. The adolescent rats used in this study were 4 weeks old, therefore they may have been at the height of the DA receptor density peak. It is possible that testosterone is acting upon these receptors by initiating a blockade of cocaine binding to the DA receptors, thereby inhibiting the effects of cocaine as they would otherwise occur.

Testosterone's rewarding properties have been characterized as less powerful than those of psychoactive drugs such as cocaine [182]. However a growing body of literature indicates that an increasing number of adolescents are becoming anabolic-androgenic steroid abusers, and many of them are also abusing other drugs [50; 84], with cocaine being the most common [50]. Further studies are needed to pinpoint how cocaine's mechanism of action is altered by testosterone in both adolescent and adult neural systems in order to develop effective treatment methods for the abusers of these substances.

Chapter 5: Conclusion

1. Introduction

Cocaine abuse is a growing problem in Western society, with recent reports indicating that approximately 2.3 million Americans are cocaine users, 30% of which are female. In recent years there has been accumulating evidence suggesting that there are sex differences in cocaine-induced behavioral and subjective responses. A growing body of literature suggests that the sex differences in cocaine reward responses are regulated by endogenous gonadal hormones. These hormones have a profound effect on brain function, indicating that the hormonal state of cocaine abusers at the time of drug ingestion may affect their behavioral response to the drug. However, the roles of each of these hormones and their interactions with drugs in the brain is not well understood. Past research seems to have focused primarily on the consequences of drug interactions with cocaine in females, probably because of the finding that many of the disparities in the behavioral responses could be explained by fluctuating hormonal status as determined by the estrous cycle. As such, numerous studies have focused on the roles of estrogen and progesterone, alone or in combination, in cocaine-induced responses. However few studies have addressed the role of testosterone in the development and regulation of cocaine reward and psychomotor activation responses. Those that have investigated this issue have

yielded conflicting results, with some reporting no effect, others an enhancement, and still others an inhibition of cocaine-induced responses.

The focus of this dissertation was to investigate and clarify the role of testosterone in cocaine-induced behavioral responses, specifically cocaine-induced conditioned place preference and locomotor activity. CPP is a behavioral test model commonly used to study the rewarding and incentive motivational effects of drugs and drug-paired stimuli by using associations between environmental cues and psychoactive drugs to examine drug-seeking behavior. We chose to incorporate this measure in our study because it has consistently yielded reliable results among studies that focus on behavior induced by the rewarding responses of psychoactive substances.

2. Chronic testosterone replacement

In order to address the role of testosterone in cocaine-induced behavioral response, we initially conducted studies where castrated male rats received chronic testosterone replacement via the implantation of testosterone pellets (Innovative Research of America, Sarasota, FL). All data collected using testosterone pellet implantation had to be discarded due to the development of severe infections at the surgical site. Studies in our lab as well as utilizing testosterone pellet implantation as a method of hormone replacement have shown a consistent problem with the integrity of the pellets, including extrusion of the pellets after implantation surgery and severe infections at the implant site.

This resulted in the premature sacrifice of over 60% of the experimental animals in our study.

We then switched to the implantation of 100% testosterone filled SILASTIC capsules as our method of testosterone replacement. Castrated 8-week old male Fischer rats received placebo (empty capsule) or testosterone via SILASTIC capsules (1 to 3 capsules of 100% T). No effects were observed in either cocaine-induced CPP or locomotor behavior across all doses of testosterone.

Based on our observations we postulated that testosterone plays a limited role in the development of environmental cue associations in adult male rats. Since different methods of testosterone administration may yield different results, we thought it useful to conduct an investigation of the effects of testosterone on cocaine-induced responses using varying administration paradigms in order to determine which method most consistently indicates a significant effect. Furthermore, we decided to investigate whether using lower doses of testosterone would alter cocaine-induced responses.

3. Comparison between chronic and acute testosterone replacement

The aim of the present study was to determine whether different methods of testosterone administration would influence the development of psychomotor and reward responses to cocaine. For the chronic testosterone replacement condition, we implanted SILASTIC capsules in the following doses: 0 (empty capsule), 15mm or 30mm, filled with 100% testosterone. We then combined

these data with those obtained in the first chronic testosterone administration study to provide a wider range of doses. For the acute testosterone replacement condition, we administered subcutaneous injections (400, 800, or 1200 µg/kg) of testosterone concurrent with cocaine administration. While chronic testosterone administration did not alter cocaine-induced CPP, concurrent administration of testosterone and cocaine affected the development of cocaine CPP dose-dependently; 400 µg/kg blocked the expression of cocaine-induced CPP. However, testosterone did not affect cocaine-induced locomotor activity. Furthermore testosterone-saline-treated controls did not develop CPP suggesting that at these doses testosterone does not produce rewarding or motor responses. Overall, these data suggest that testosterone may regulate behavioral responses to cocaine via membrane-mediated mechanisms.

Based on our observations we postulated that testosterone may play a limited role in cocaine-induced reward associations and locomotor responses, thus having a limited role in the previously reported sexually dimorphic responses to cocaine. A possible flaw of the chronic and acute testosterone studies was the lack of a gonadally intact control group. This may have provided crucial insight into the effects of cocaine on testosterone levels, as well as the effects of testosterone on the behavioral responses that were examined. As a result we opted to include this control in our subsequent experiments, particularly since its comparison to the castrated subjects could serve as an

important indicator of clinical relevance; most individuals that would co-abuse anabolic steroids and cocaine would most likely be gonadally intact.

4. Comparison of cocaine-induced locomotor activity between intact adolescent and adult male rats after acute testosterone administration

Anabolic steroids are synthetic derivatives of testosterone (T), which have a high abuse potential. Recently it has been found that anabolic steroid use is becoming increasingly prevalent among adolescents, a population that has previously been associated with cocaine use. Although both substances lead to complex behavioral responses, little is known about the physiological and behavioral effects of testosterone when co-administered with cocaine. The specific effects of anabolic steroids and cocaine on adolescents are still poorly understood. In the present study, we tested the hypothesis that among male rats, testosterone influences the development of psychomotor responses to cocaine depending on their developmental stage. To test this postulate, intact adolescent (4-week old) and adult (8-week old) male Fischer rats were pre-treated with vehicle (sesame oil) or testosterone (5mg/kg or 10 mg/kg) 45 minutes prior to saline or cocaine (20mg/kg) administration. Testosterone did not have an effect at any dose, on ambulations, rearing, or total locomotor counts for adult male rats. Additionally, total locomotor counts were not affected by testosterone in adolescents. However, testosterone was found to inhibit the ambulatory and rearing locomotor responses dose-dependently among adolescents. Specifically,

5 mg/kg of testosterone significantly lowered these responses as compared to vehicle-treated rats. Overall, these data suggest that activational versus organizational differences may account for some of the observed variations in cocaine-induced effects; i.e., differences in dopaminergic levels and/or metabolic processes of testosterone. The data further suggest that exogenous testosterone is differentially absorbed or metabolized when co-administered with cocaine. Conversely, testosterone lowered cocaine effects. This may have clinical implications; when adolescents self-administer cocaine and testosterone, higher doses of either substance may be required to achieve the previous rewarding effects of each. In adolescents the co-administration of cocaine and testosterone may lead to a desirable anxiolytic effect that each substance does not elicit on its own. As such, cocaine abusers who initiate the use of anabolic androgenic steroids may experience a reduced rewarding effect of cocaine, thereby turning to increased doses of cocaine to achieve their usual "high". Likewise, anabolic androgenic steroid abusers who later abuse cocaine may notice a decrease in the potency of the steroid effects; they may then abuse greater doses of anabolic androgenic steroids, creating a dangerous circular pattern of co-abuse.

In order to understand the findings presented in the aforementioned experiments it is imperative that the different characteristics between adolescent and adult rats be considered. In addition to differences in endogenous hormone levels at different developmental stages, one particularly important trait of the

adolescent brain is the increase in dopamine receptor densities throughout development. Studies have shown that estrogen and progesterone can alter dopamine release, dopamine receptor levels, and dopamine transporter densities. It is possible that testosterone has a similar ability to alter dopaminergic functions. Previous studies have found that relatively high doses of anabolic-androgenic steroids increase dopaminergic metabolism in male rat brain, possibly because of enhanced turnover in these monaminergic systems [171]. Treatment with testosterone in older rats has restored the age-related decline in dopamine function [3]. These testosterone-induced alterations in dopamine function may be occurring in the medial preoptic area, which has been implicated in the regulation of male sexual behavior [3]. It has recently been found that neither castration nor ovariectomy altered dopamine receptor density, although enhanced testosterone levels increased D1 receptor binding 4.2% and 19.5% in males and females, respectively [7]. This finding suggests that the endogenous rise in gonadal steroid hormones during puberty may not responsible for the overproduction of receptors in males or the lack of overproduction in females [7]. In the future it may be worthwhile to investigate the selectivity of the dopamine receptor ligands that are studied [66], as variations in their selective properties can in turn lead to variations in their mechanism of action. It may also be useful to conduct future experiments using measures that can account for the metabolic processes in both substances. For

instance, replication should include a dose response curve analysis using a range of doses of cocaine, particularly since others have found cocaine-induced behavioral responses using 10 mg/kg [111] and 15 mg/kg [53] of cocaine. It would be interesting to use these doses in combination with a range of testosterone doses as well, as differential effects have been found at 2mg/kg of testosterone [111] as compared to our chosen doses of 5 and 10 mg/kg of testosterone, with 2mg/kg resulting in an enhancement of locomotor responses [111] and 5 mg/kg resulting in an attenuation of locomotor responses. Further, a U-shaped curve was observed where vehicle-treated and 10 mg/kg treated rats showed neither an enhancement nor an attenuation. Varying the doses of both substances may be useful in finding an optimal combination of doses for future experiments. Analyses should then entail an intracellular examination of neural structures for testosterone and both testosterone and cocaine metabolic products.

In light of the interesting locomotor activation findings within adolescent rats, conducting future experiments that involve a measurement of conditioned place preference and reward may be a worthwhile follow up to examine whether similar effects are seen among adolescents in reward responses. However caution should be used when comparing the locomotor effects obtained using a CPP apparatus with those obtained using a locomotor photobeam system, as the latter apparatus measures ambulatory and rearing counts in addition to total locomotor counts, whereas the CPP apparatus used for these experiments

measures only horizontal beam breaks in a total locomotor count measure. Since a notable anxiolytic effect was observed among adolescents, it would be imperative to use an apparatus that encompasses both vertical and horizontal beam break counting capabilities.

While the literature investigating the effects of testosterone on cocaine-induced reward and behavioral responses has been sparse, a number of potential mechanisms of action have been suggested. Most of these imply an involvement of the dopamine system due to its crucial role in reward and behavioral associations. A complex interaction between cocaine and testosterone may work to alter the behavioral responses to cocaine; perhaps the testosterone-induced increases in dopaminergic function may interfere with cocaine's ability to bind to dopamine receptors, thereby reducing the level of synaptic dopamine and the rewarding effects of the drug. Higher levels of circulating testosterone may be acting upon the HPG and HPA axes, perhaps by signaling the cessation of its own production at the HPG inhibitory feedback loop and initiating a premature inhibition of testosterone release. This in turn may be offsetting the complex testosterone and cocaine interaction and ultimately altering cocaine-induced behavioral responses. Clinical treatment strategies may be effectively developed for abusers of these drugs based on their mechanisms of action within neural reward pathways.

Figure 26: "Will Work for Testosterone" ☺

Adapted from the web: bztoons.com, by Brian Zaikowski (2004) [185]

Appendix

List of Abbreviations

AAS	Anabolic-androgenic steroids
ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
CNS	Central nervous system
CORT	Corticosterone
CPP	Conditioned place preference
CRF	Corticotropin-releasing factor
DAT	Dopamine transporter
DHT	Dihydrotestosterone
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
EEG	Electroencephalogram
fMRI	Functional magnetic resonance imaging
FSH	Follicle-stimulating hormone
GABA	Gamma aminobutyric acid
HPA	Hypothalamic Pituitary Adrenal
HPG	Hypothalamic Pituitary Gonadal
HSD	Honestly Significantly Different

LH	Luteinizing hormone
NIH	National Institutes of Health
NMDA	N-methyl-D-aspartate
OVX	Ovariectomized
PET	Positron emissions tomography
RIA	Radioimmunoassay
T	Testosterone
VTA	Ventral tegmental area

Reference List

1. Adams, J.U., Careri, J.M., Efferen, T.R. & Rotrosen, J. (2001). Differential effects of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. Behavioral Pharmacology, 12(8), pp. 603-611.

2. Alexander, G.M., Packard, M.G. & Hines, M. (1994). Testosterone has rewarding affective properties in male rats: Implications for the biological basis of sexual motivation. Behavioral Neuroscience, 108 (2), pp. 424-428.

3. Alderson, L.M. & Baum, M.J. (1981). Differential effects of gonadal steroids on dopamine metabolism in mesolimbic and nigro-striatal pathways of male rat brain. Brain Research, 218 (1-2), pp. 189-206.

4. Almodovar-Fabregas, L.J., Segarra, O., Colon, N., Dones, J.G., Mercado, M., Mejias-Aponte, C.A., Vazquez, R., Abreu, R., Vazquez, E., Williams, J.T. & Jimenez-Rivera, C.A. (2002). Effects of cocaine administration on VTA cell activity in response to prefrontal cortex stimulation. Annals of the N Y Academy of Science, 965, pp. 157-171.

5. Andersen, S.L. & Gazzara, R.A. (1993). The ontogeny of apomorphine-induced alterations of neostriatal dopamine release: Effects of spontaneous release. Journal of Neurochemistry, 61, pp. 2247-2255.

6. Andersen, S.L., Rutstein, M., Benzo, J.M., Hostetter, J.C. & Teicher, M.H. (1997). Sex differences in dopamine receptor overproduction and elimination. Neuroreport, 8, pp. 1495-1498.

7. Andersen, S.L., Thompson, A.P., Krenzel, E. & Teicher, M.H. (2002). Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. Psychoneuroendocrinology, 27(6), 683-91.

8. Ansah, T.A., Wade, L.H. & Shockley, D.C. (1996). Changes in locomotor activity, core temperature, and heart rate in response to repeated cocaine administration. Physiology and Behavior, 60 (5), pp. 1261-1267.

9. Arnedo, M.T., Salvador, A., Martinez-Sanchis, S. & Gonzalez-Bono, E. (2000). Rewarding properties of testosterone in intact male mice: A pilot study. Pharmacology Biochemistry and Behavior, 65, pp. 327-332.

10. Arnedo, M.T., Salvador, A., Martinez-Sanchis, S. & Pellicer, O. (2002). Similar rewarding effects of testosterone in mice rated as short and long attack latency individuals. Addiction Biology, 7(4), pp. 373-9.

11. Bahrke, M.S., Yesalis, C.E., Kopstein, A.N. & Stephens, J.A. (2000). Risk factors associated with anabolic-androgenic steroid use among adolescents. Sports Medicine, 29 (6), pp. 397-405.

12. Baker, D.A., Fuchs, R.A., Specio, S.E., Khroyan, T.V. & Neisewander, J.L. (1998). Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. Synapse, 30(2), pp. 181-193. 101.

13. Baker, D.A., Khroyan, T.V., O'Dell, L.E., Fuchs, R.A., Neisewander, J.L. (1996). Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. Journal of Pharmacology and Experimental Therapeutics, 279(1), pp. 392-401.

14. Bardo, M.T., Rowlett, J.K. & Harris, M.J. (1995). Conditioned place preference using opiate and stimulant drugs: A meta-analysis. Neuroscience & Biobehavioral Reviews, 19(1), pp. 39-51.

15. Barroso-Moguel, R., Mendez-Armenta, M. & Villeda-Hernandez, J. (1994). Testicular lesions by chronic administration of cocaine in rats. Journal of Applied Toxicology, 14 (1), pp. 37-41.

16. Baumann, M.H. & Rothman, R.B. (1998). Alterations in serotonergic responsiveness during cocaine withdrawal in rats: Similarities to major depression in humans. Biological Psychiatry, 44, pp. 578-591.

17. Becker, J.B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacology, Biochemistry and Behavior, 64, pp. 803-812.

18. Becker, J.B., Molenda, H. & Hummer, D.L. (2001). Gender differences in the behavioral responses to cocaine and amphetamine: Implications for mechanisms mediating gender differences in drug abuse.
Annals of the N Y Academy of Science, 937, pp. 172-87.

19. Becker, J.B., & Ramirez, V.D. (1981). Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro. Brain Research, 204, pp. 361-372.

20. Belej, T., Manji, D., Sioutis, S., Barros, H.M. & Nobrega, J.N. (1996). Changes in serotonin and norepinephrine uptake sites after chronic cocaine: Pre- vs. post-withdrawal effects. Brain Research, 736(1-2), pp. 287-296.

21. Berul, C. I. & Harclerode, J.E. (1989). Effects of cocaine hydrochloride on the male reproductive system. Life Sciences, 45, pp. 91-95.

22. Bolanos, C.A., Glatt, S.J. & Jackson, D. (1998). Subsensitivity to dopaminergic drugs in periadolescent rats: A behavioral and neurochemical analysis. Developmental Brain Research, 111, pp. 25-33.

23. Borlak, J., Schulte, I. & Thum, T. (2004). Androgen metabolism in thymus of fetal and adult rats. Drug Metab Dispos, 32 96, pp. 675-679.

24. Bosse, R. & DiPaolo, T. (1996). The modulation of brain dopamine and $GABA_A$ receptors by estradiol: A clue for CNS changes occurring at menopause. Cellular Molecular Biology, 16, pp. 199-212.

25. Brown, D.V. & Amann, R.P. (1984). Inhibition of testosterone metabolism in cultured rat epididymal principal cells by dihydrotestosterone and progesterone. Biology of Reproduction, 30 (1), pp. 67-73.

26. Buckley, W.E., Yesalis, C.E., Friedl, K.E., Anderson, W.A., Streit, A.L. & Wright, J.E. (1988). Estimated prevalence of anabolic steroid use among male high school seniors. Journal of the American Medical Association, 260 (23), pp. 3441-5.

27. Budziszewska, B., Jaworska-Feil, L. & Lason, W. (1996). The effect of repeated amphetamine and cocaine administration on adrenal, gonadal and thyroid hormone levels in the rat plasma. Experimental and Clinical Endocrinology and Diabetes, 104 (4), pp. 334-8.

28. Bunney, E.B., Appel, S.B. & Brodie, M.S. (2001). Electrophysiological effects of cocaethylene, cocaine, and ethanol on dopaminergic neurons of the ventral tegmental area. Journal of Pharmacology and Experimental Therapeutics, 297(2), pp. 696-703.

29. Cailhol, S. & Mormède, P. (1999). Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. Brain Research, 842, pp. 200-205.

30. Caldarone, B., Stock, H., Abrahamsen, G., Boechler, M., Svare, B. & Rosellini, R. (1996). Non-associative processes and place preferences conditioned by testosterone. The Psychological Record, 46, pp. 373-90.

31. Campbell, J.O., Wood, R.D., & Spear, L.P. (2000). Cocaine and morphine-induced place conditioning in adolescent and adult rats. Physiology and Behavior, 68, pp. 487-493.

32. Carey, R.J., Damianopoulos, E.N. & DePalma, G. (1994). Differential temporal dynamics of serum and brain cocaine: relationship to behavioral, neuroendocrine and neurochemical cocaine induced responses. Life Sciences, 55 (22), pp. 1711-6.

33. Carr, G.D., Fibiger, H.C. & Phillips, A.G. (1989). Conditioned place preference as a measure of drug reward. In J.M. Leibman & S.J. Cooper (Eds.), Oxford reviews in psychopharmacology: Vol. 1 Neuropharmacological basis of reward, pp. 265-319. New York: Oxford University Press.

34. Caster, J.M., Walker, Q.D. & Kuhn, C.M. (2005). Enhanced behavioral reponse to repeated-dose cocaine in adolescent rats. Psychopharmacology (Berl.), 21, pp. 1-8.

35. Catlow, B.J. & Kirstein, C.L. (2005). Heightened cocaine-induced locomotor activity in adolescent compared to adult female rats. Journal of Psychopharmacology, 19(5), pp. 443-447.

36. Cervo, L. & Samanin, R. (1995). Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Research, 673(2), pp. 242-50.

37. Childress, A.R., Mozley, P.D., McElgin, W., Fitzgerald, J., Reivich, M. & O'Brien, C.P. (1999). Limbic activation during cue-induced cocaine craving. American Journal of Psychiatry, 156(1), pp. 11-18.

38. Chin, J., Sternin, O., Wu, H.B.K., Burrell, S., Lu, D., Jenab, S., Perrotti, L.I. & Quinones-Jenab, V. (2002). Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. Brain Research, 945 (1), pp. 123-30.

39. Chin, J., Sternin, O., Wu, H.B.K., Fletcher, H., Perrotti, L.I., Jenab, S. & Quinones-Jenab, V. (2001). Sex differences in cocaine-induced behavioral sensitization. Cellular and Molecular Biology, 47 (6), pp. 1089-1095.

40. Chen, R., Osterhaus, G., McKerchar, T., & Fowler, S.C. (2003). The role of exogenous testosterone in cocaine-induced behavioral sensitization and plasmalemmal or vesicular dopamine uptake in castrated rats. Neuroscience Letters, 351 (3), pp. 161-164.

41. Clark, A.S. & Henderson, L.P. (2003). Behavioral and physiological responses to anabolic-androgenic steroids. Neuroscience and Biobehavioral Review, 27 (5), pp. 413-36.

42. Clark, J.H., Schrader, W.T. & O'Malley, B.W. (1992). Mechanisms of action of steroid hormones. In: Wilson, J.L., Foster, D.W., editors. William's textbook of endocrinology, 8th ed., pp. 35-90. Philadelphia: Saunders.

43. Claye, L.H., Akunne, H.C., Davis, M.D., DeMattos, S. & Soliman, K.F. (1995). Behavioral and neurochemical changes in the dopaminergic system after repeated cocaine administration**.** Molecular Neurobiology, 11 (1-3) pp. 55-66.

44. Collins, S.L. & Izenwasser, S. (2003). Chronic nicotine differentially alters cocaine-induced locomotor activity in adolescent vs. adult male and female rats. Neuropharmacology, 46, pp. 349-362.

45. Cunningham, K.A., Bradberry, C.W., Chang, A.S. & Reith, M.E. (1996). The role of serotonin in the actions of psychostimulants: Molecular and pharmacological analyses. Behavioral Brain Research, 73 (1-2), pp. 93-102.

46. De Beun, R., Jansen, E., Slangen, J.L. & Van de Poll, N.E. (1992). Testosterone as appetitive and discriminative stimulus in rats: Sex- and dose-dependent effects. Physiology and Behavior, 52, pp. 629-34.

47. Dewey, S.L., Morgan, A.E., Ashby, C.R. Jr., Horan, B., Kushner, S.A., Logan, J., Volkow, N.D., Fowler, J.S., Gardner, E.L. & Brodie, J.D. (1998). A novel strategy for the treatment of cocaine addiction. Synapse, 30 (2), pp. 119-29.

48. Dickerson, G.A., Waters, I.W., Wilson, M.C. & Davis, W.M. (1991). Effect of sex steroids on cocaine lethality in male and female mice. General Pharmacology, 22 (3), pp. 553-7.

49. Di Paolo, T., Rouillard, C., Morissette, M., Levesque, D. & Bedard, P.J. (1989). Endocrine and neurochemical actions of cocaine**.** Canadian Journal of Physiological Pharmacology, 67 (9), pp. 1177-81.

50. DuRant, R.H., Rickert, V.I., Ashworth, C.S., Newman, C. & Slavens, G. (1993). Use of multiple drugs among adolescents who use anabolic steroids. New England Journal of Medicine, 328 (13), pp. 922-6.

51. Everitt, B.J. (1990). Sexual motivation: A neural and behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. Neuroscience and Biobehavioral Reveiws, 14, pp. 217-232.

52. Everitt, B.J. & Wolf, M.E. (2002). Psychomotor stimulant addiction: A neural systems perspective. The Journal of Neuroscience, 22 (9), pp. 3312-3320.

53. Febo, M., Gonzalez-Rodriguez, L.A., Capo-Ramos, D.E., Gonzalez-Segarra, N.Y. & Segarra, A.C. (2003). Estrogen-dependent alterations in D2/D3-induced G protein activation in cocaine-sensitized female rats. Journal of Neurochemistry, 86(2), pp. 405-12.

54. Festa, E.D., Jenab, S., Chin, J., Gazi, F.M., Wu, H.B.K., Russo, S.J. & Quinones-Jenab, V. (2003). Frequency of cocaine administration affects behavioral and endocrine responses in male and female Fischer rats. Cellular and Molecular Biology (Noisy-le-grand), 49 (8), pp. 1275-80.

55. Festa, E.D., Russo, S.J., Gazi, F.M., Niyomchai, T., Kemen, L.M., Lin, S.N., Foltz, R., Jenab, S. & Quinones-Jenab, V. (2004). Sex differences in cocaine-induced behavioral responses, pharmacokinetics, and monoamine levels. Neuropharmacology, 46 (5), pp. 672-87.

56. Festa, E.D. & Quinones-Jenab, V. (2004). Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. Hormones and Behavior, 46(5), pp. 509-19.

57. Fleckenstein, A.E., Gibb, J.W. & Hanson, G.R. Differential effects of stimulants on monoaminergic transporters: Pharmacological consequences and implications for toxicity. European Journal of Pharmacology, 406(1), pp. 1-13.

58. Forman, L.J., Harwell, M.F. & Cater, J. (1990). Beta-endorphin in the male rat pituitary: testosterone influences the effect of cocaine. Brain Research Bulletin, 25 (1), pp. 65-68.

59. Frye, C.A., Park, D., Tanaka, M., Rosellini, R. & Svare, B. (2001). The testosterone metabolite and neurosteroid 3alpha-androstanediol may mediate the effects of testosterone on conditioned place preference. Psychoneuroendocrinology, 26 (7), pp. 731-50.

60. Frye, C.A., Rhodes, M.E., Rosellini, R. & Svare, B. (2002). The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5alpha-reduced metabolites. Pharmacology, Biochemistry and Behavior, 74, pp. 119-127.

61. Garavan, H., Pankiewicz, J., Bloom, A., Cho, J.K., Sperry, L., Ross, T.J., Salmeron, B.J., Risinger, R., Kelley, D. & Stein, E.A. (2000). Cue-induced cocaine craving: Neuroanatomical specificity for drug users and drug stimuli. American Journal of Psychiatry, 157(11), pp. 1789-1798.

62. Goeders, N.E. (1997). A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology, 22 (4), pp. 237-259.

63. Gong, W., Neill, D. & Justice, J.B. Jr. (1996). Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. Brain Research, 707 (1), pp. 64-74.

64. Gong, W., Neill, D. & Justice, J.B. Jr. (1997). 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Research, 754 (1-2), pp. 103-112.

65. Gordon, L.A., Mostofsky, D.I. & Gordon, G.G. (1980). Changes in testosterone levels in the rat following intraperitoneal cocaine HCl. International Journal of Neuroscience, 11(2), pp. 139-141.

66. Gyertyan, I., & Gal, K. (2003). Dopamine D3 receptor ligands show place conditioning effect but do not influence cocaine-induced place preference. Neuroreport, 14 (1), pp. 93-98.

67. Hall, F.S., Li, X.F., Sora, I., Xu, F., Caron, M., Lesch, K.P., Murphy, D.L., & Uhl, G.R. (2002). Cocaine mechanisms: Enhanced cocaine, fluoxetine and nisoxetine place preferences following monoamine transporter deletions. Neuroscience, 115 (1), pp. 153-161.

68. Han, T.M. & De Vries, G.J. (2003). Organizational effects of testosterone, estradiol, and dihydrotestosterone on vasopressin mRNA expression in the bed nucleus of the stria terminalis. Journal of Neurobiology, 54 (3), pp. 502-510.

69. Haney, M., Castanon, N., Cador, M., Le Moal, M. & Mormede, P. (1994). Cocaine sensitivity in Roman high and low avoidance rats is modulated by sex and gonadal hormone status. Brain Research, 645, pp. 179-185.

70. Heesch, C.M., Negus, B.H., Bost, J.E., Keffer, J.H., Snyder, R.W. II & Eichhorn, E.J. (1996). Effects of cocaine on anterior pituitary and gonadal hormones. Journal of Pharmacology and Experimental Therapeutics, 278 (3), pp. 1195-1200.

71. Heidbreder, C.A., Thompson, A.C., & Shippenberg, T.S. (1996). Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. Journal of Pharmacology and Experimental Therapeutics, 278 (2), pp. 490-502.

72. Hemby, S.E., Jones, G.H., Justice, J.B. Jr. & Neill, D.B. (1992). Conditioned locomotor activity but not conditioned place preference following intra-accumbens infusions of cocaine. Psychopharmacology (Berl), 106 (3), pp. 330-336.

73. Http://www.antiaging.com . Testosterone Metabolism. Taken from the web (2004).

74. Http://www.bioalternatives.com . Mechanism of action of testosterone. Taken from the web (2004).

75. Http://www.cnn.com/interactive/specials/0008/coca.gallery/. Coca plant. Taken from the web (2004).

76. Http:// www.endotext.org. Testosterone Synthesis. Taken from the web (2004).

77. Http://www.snopes.com/cokelore/cocaine.asp. Photo of the chemical composition of cocaine. Taken from the web (2004).

78. Hughes, A.M., Everitt, B.J. & Herbert, J. (1990). Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behaviour in male rats: studies of instrumental behaviour, conditioned place preference and partner preference. Psychopharmacology, 102, pp. 243-256.

79. Huttenlocher, P.R. (1984). Synapse elimination and plasticity in developing human cerebral cortex. American Journal of Mental Deficiency, 88, pp. 488-496.

80. Ivanović Matić, S., Dinić, S., Mihailović, M., Grigorov, I., Bogojević, D. & Poznanović, G. (2004). Acute-phase protein expression in DMSO-intoxicated rats. Toxicology Letters, 147, pp. 153-159.

81. Izenwasser, S., French, D., Carroll, F.I. & Kunko, P.M. (1999). Continuous infusion of selective dopamine uptake inhibitors or cocaine produces time-dependent changes in rat locomotor activity. Behavioural Brain Research, 99, pp. 201-208.

82. Janak, P.H., Hernandez, R.V., Rule, R.R., & Martinez, J.L. Jr. (1997). Rapid decay of cocaine-induced behavioral sensitization of locomotor behavior. Behavioral Brain Research, 88 (2), pp. 195-9.

83. Kalivas, P.W., Sorg, B.A. & Hooks, M.S. (1993). The pharmacology and neural circuitry of sensitization to psychostimulants. Behavioural Pharmacology, 4, pp. 315-334.

84. Kandel, D., Chen, K., Warner, L.A., Kessler, R.C., & Grant, B. (1997). Prevalence and demographic correlates of symptoms of last year drug dependence on alcohol, nicotine, marijuana and cocaine in the U.S. population. Drug and Alcohol Dependency, 44, pp. 11-29.

85. Kandel, E.R., Schwartz, J.H. & Jessell, T.M. (2000). Principles of Neural Science, 4th Ed. McGraw-Hill / Appleton & Lange.

86. Kelleher, S., Howe, C., Conway, A. J. & Handelsman, D. J. (2004). Testosterone release rate and duration of action of testosterone pellet implants. Clinical Endocrinology, 60 (4), pp. 420.

87. Kelleher, S., Turner, L., Howe, C., Conway, A.J. & Handelsman, D.J. (1999). Extrusion of testosterone pellets: a randomized controlled clinical study. Clinical Endocrinology (Oxf), 51 (4), pp. 469-471.

88. Kelley, A.E. & Berridge, K.C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. The Journal of Neuroscience, 22 (9), pp. 3306-3311.

89. Kim, H.S., Park, W.K., Jang, C.G., Oh, K.W., Rheu, H.M., Cho, D.H. & Kang, S.Y. (1997). Blockade by naloxone of cocaine-induced hyperactivity, reverse tolerance and conditioned place preference in mice. Behavioral Brain Research, 85, pp. 37-46.

90. King, B.E., Packard, M.G. & Alexander, G.M. (1999). Affective properties of intra-medial preoptic area injections of testosterone in male rats. Neuroscience Letters, 269, pp. 149-152.

91. Kleber, H.D. & Gawin, F.H. (1984). Cocaine abuse: A review of current and experimental treatments. In: Cocaine: Pharmacology, effects and treatment of abuse, (Ed.) J. Grabowski, pp. 111-129.

92. Kosten, T.A. & Miserendino, M.J.D. (1998). Dissociation of novelty- and cocaine-conditioned locomotor activity from cocaine place conditioning. Pharmacology Biochemistry and Behavior, 60 (4), pp. 785-91.

93. Kreek, M.J. (1996). Cocaine, dopamine and the endogenous opioid system. Journal of Addictive Disorders, 15 (4), pp. 73-96.

94. Kuhn, C.M., Walker, Q.D., Kaplan, K.A. & Li, S.T. (2001). Sex, steroids, and stimulant sensitivity. Annals of the New York Academy of Sciences, 937, pp. 188-201.

95. Kuzmin, A.V., Gerrits, M.A., van Ree, J.M. & Zvartau, E.E. (1997). Naloxone inhibits the reinforcing and motivational aspects of cocaine addiction in mice. Life Sciences, 60, pp. 257-264.

96. Lamas, J.L. & Spadari, R.C. (1993). Effect of testosterone on the response of young rat vas deferens to norepinephrine. General Pharmacology, 24 (1), pp. 185-189.

97. Larrson, K. (1979). Features of the neuroendocrine regulation of masculine sexual behavior. In C. Beyer (Ed.), Endocrine control of sexual behavior, pp. 77-163. New York: Raven Press.

98. Laviola, G., Wood, R.D., Kuhn, C., Francis, R. & Spear, L.P. (1995). Cocaine sensitization in periadolescent and adult rats. Journal of the American Society for Pharmacology and Experimental Therapeutics, 275 (1), pp. 345-57.

99. Lee, S.H. & Mouradian, M.M. (1999). Up-regulation of D1A dopamine receptor gene transcription by estrogen. Molecular and Cellular Endocrinology, 156(1-2), pp. 151-157.

100. Le Pen, G., Duterte-Boucher, D.,& Costentin, J. (1996). Place conditioning with cocaine and the dopamine uptake inhibitor GBR12783. Neuroreport, 7 (18), pp. 2839-42.

101. Levesque, D. & Di Paolo, T. (1993). Modulation by estradiol and progesterone of the GTP effect on striatal D-2 dopamine receptors. Biochemical Pharmacology, 45(3), pp. 723-733.

102. Lidow, M.S. & Rakic, P. (1992). Scheduling of monoaminergic neurotransmitter receptor expression in the primate neocortex during postnatal development. Cerebral Cortex, 2, pp. 401-416.

103. Long, S.F., Dennis, L.A., Russell, R.K., Benson, K.A. & Wilson, M.C. (1994). Testosterone implantation reduces the motor effects of cocaine. Behavioral Pharmacology, 5(1), pp. 103-106.

104. Luine, V.N. (1997). Steroid hormone modulation of hippocampal dependent spatial memory. Stress, 2, pp. 21-36.

105. Lukas, S.E. (1996). CNS effects and abuse liability of anabolic-androgenic steroids. Annual Review of Pharmacology and Toxicology, 36, 333-57.

106. Lynch, W.J., Roth, M.E., Mickelberg, J.L. & Carroll, M.E. (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. Pharmacology Biochemistry and Behavior, 68, pp. 641-646.

107. Maldonado, A.M. & Kirstein, C.L. (2005). Handling alters cocaine-induced activity in adolescent but not adult male rats. Physiology and Behavior, 84, pp. 321-6.

108. Markou, A., Weiss, F., Gold, L.H., Caine, S.B., Schulteis, G. & Koob, G.F. (1993). Animal models of drug craving. Psychopharmacology, 112, pp. 163-182.

109. Martin-Iverson, M.T. & Reimer, A.R. (1994). Effects of nimodipine and/or haloperidol on the expression of conditioned locomotion and sensitization to cocaine in rats. Psychopharmacology (Berl), 114 (2), pp. 315-20.

110. Martin-Iverson, M.T., Reimer, A.R. & Sharma, S. (1997). Unbiased cocaine conditioned place preferences (CPP) obscures conditioned locomotion, and nimodipine blockade of cocaine CPP is due to conditioned place aversions. Psychopharmacology (Berl), 130 (4), pp. 327-333.

111. Martinez-Sanchis, S., Aragon, C.M.G. & Salvador, A. (2002). Cocaine-induced locomotor activity is enhanced by exogenous testosterone. Physiology and Behavior, 76, pp. 605-609.

112. McEwen, B.S., Alves, S.E., Bulloch, K. & Weiland, N.G. (1997). Ovarian steroids and the brain: Implications for cognition and aging. Neurology, 48, pp. S8-S15.

113. McKim, W.A., Psychomotor stimulants. In: McKim, W.A. (Ed.), Drugs and behavior: An introduction to behavioral pharmacology. Prentice Hall, Upper Saddle River, NJ, 1996, pp. 212-234.

114. Mello, N.K. & Mendelson, J.H. (1997). Cocaine's effects on neuroendocrine systems: clinical and preclinical studies. Pharmacology, Biochemistry and Behavior, 57 (3), pp. 571-99.

115. Mello, N.K., Mendelson, J.H., Negus, S.S., Kelly, M., Knudson, I. & Roth, M.E. (2004). The effects of cocaine on gonadal steroid hormones and LH in male and female rhesus monkeys**.** Neuropsychopharmacology. [Epub ahead of print]

116. Minerly, A.C.E., Russo, S.J., Nazarian, A., Akhavan, A., Jenab, S. & Quinones-Jenab, V. (2002). Unpublished data.

117. Mitchell, J.A. & Wilson, M.C. (1987). The behavioral and toxicological effects of testosterone pellet implantation. Physiology and Behavior, 41 (5), pp. 427-32.

118. Morishima, H.O., Abe, Y., Matsuo, M., Akiba, K., Masaoka, T., & Cooper, T.B. (1993). Gender-related differences in cocaine toxicity in the rat. The Journal of Laboratory and Clinical Medicine, 122, pp. 157-163.

119. Mueller, D. & Stewart, J. (2000). Cocaine-induced conditioned place preference: Reinstatement by priming injections of cocaine after extinction. Behavioural Brain Research, 115, pp. 39-47.

120. Murrin, L.C. & Zeng, W.Y. (1986). Postnatal ontogeny of dopamine D2 receptors in rat striatum. Biochemistry and Pharmacology, 35, 1159-62.

121. Murrin, L.C. & Zeng, W.Y. (1990). Ontogeny of dopamine D1 receptors in rat forebrain: A quantitative autoradiographic study. Developmental Brain Research, 57, pp. 7-13.

122. National Institute on Drug Abuse. (1999). Cocaine: Abuse and addiction. Research Report Series. NIH Publication Number 99-4342.

123. National Institute on Drug Abuse: Web report. (2004). http://www.drugabuse.gov.

124. Nazarian, A., Russo, S.J., Festa, E.D., Kraish, M. & Quinones-Jenab, V. (2004). The role of D1 and D2 receptors in the cocaine conditioned place preference of male and female rats. Brain Research Bulletin, 63 (4), pp. 295-9.

125. Niyomchai, T., Chin, J., Russo, S.J., Festa, E.D., Perrotti, L.I., Lu, D. Cornejo, S., Jenab, S. & Quinones-Jenab, V. Estrogen and progesterone affect behavioral and endocrinological responses to acute cocaine administration in a dose-dependent manner. (manuscript in preparation).

126. O'Connor, L. & Cicero, T. (1993). Anabolic steroids: Misuse or abuse. In: Schulkin, J., editor. Hormonally induced changes in mind and brain, pp. 129-57. San Diego: Academic Press.

127. Packard, M., Cornell, A. & Alexander, G. (1997). Rewarding affective properties of intra-nucleus accumbens injections of testosterone. Behavioral Neuroscience, 111 (1), pp. 219-24.

128. Packard, M.G., Schroeder, J.P. & Alexander, G.M. (1998). Expression of testosterone conditioned place preference is blocked by peripheral or intra-accumbens injection of alpha-flupenthixol. Hormones and Behavior, 34, pp. 39-47.

129. Panikkar, G.P. (1999). Cocaine Addiction: Neurobiology and Related Current Research in Pharmacotherapy. Substance Abuse, 20 (3) pp. 149-166.

130. Partridge, B. & Schenk, S. (1999). Context-independent sensitization to the locomotor-activating effects of cocaine. Pharmacology, Biochemistry and Behavior, 63 (4), pp. 543-548.

131. Perrotti, L.I., Russo, S.J., Fletcher, H., Chin, J., Webb, T., Jenab, S. & Quinones-Jenab, V. (2001). Ovarian hormones modulate cocaine-induced locomotor and stereotypic activity. Annals of the New York Academy of Sciences, 937, pp. 202-216.

132. Perrotti, L.I., Russo, S.J., Lagos, F. & Quinones-Jenab, V. (2001). Vendor differences in cocaine-induced behavioral activity and hormonal interactions in ovariectomized Fischer rats. Brain Research Bulletin, 54(1), pp. 1-5.

133. Philpot, R.M. & Kirstein, C.L. (2004). Developmental differences in the accumbal dopaminergic response to repeated ethanol exposure. Annals of the New York Academy of Science, 1021, pp. 422-426.

134. Piazza, P.V. & Le Moal, M.L. (1996). Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annual Review of Pharmacology and Toxicology, 36, pp. 359-78.

135. Platt, J.J. (1997). The problem of cocaine abuse and addiction, pp. 3-21. Cambridge, Massachusetts: Harvard University Press.

136. Prasad, B.M., Sorg, B.A., Ulibarri, C. & Kalivas, P.W. (1995). Sensitization to stress and psychostimulants: Involvement of dopamine transmission versus the HPA axis. Annals of the New York Academy of Science, 771, pp. 617-25.

137. Pruitt, D.L., Bolanos, C.A. & McDougall, S.A. (1995). Effects of dopamine D1 and D2 receptor antagonists on cocaine-induced place preference conditioning in preweanling rats. European Journal of Pharmacology, 283(1-3), pp. 125-131.

138. Quadros, P.S., Lopez, V., De Vries, G.J., Chung, W.C. & Wagner, C.K. (2002). Progesterone receptors and the sexual differentiation of the medial preoptic nucleus. Journal of Neurobiology, 51 (1), pp. 24-32.

139. Quinones-Jenab, V., Ho, A., Schlussman, S.D., Franck, J. & Kreek, M.J. (1999). Estrous cycle differences in cocaine-induced stereotypic and locomotor behaviors in Fischer rats. Behavioural Brain Research, 101, pp. 15-20.

140. Quinones-Jenab, V., Perrotti, L.I., Ho, A., Jenab, S., Schlussman, S.D., Franck, J. & Kreek, M.J. (1999). Cocaine affects progesterone plasma levels in female rats. Pharmacology, Biochemistry and Behavior, 66 (2), pp. 449-53.

141. Quinones-Jenab, V., Perrotti, L.I., Fabian, S.J., Chin, J., Russo, S.J. & Jenab, S. (2001). Endocrinological basis of sex differences in cocaine-induced behavioral responses. Annals of the New York Academy of Sciences, 937, pp. 140-171.

142. Quinones-Jenab, V., Perrotti, L.I., Ho, A. & Kreek, M.J. (2000). Ovarian hormone replacement affects cocaine-induced behaviors in ovariectomized female rats. Pharmacology Biochemistry and Behavior, 67 (3), pp. 417-22.

143. Rakic, P., Bourgeois, J.P. & Goldman-Rakic, P.S. (1994). Synaptic development of the cerebral cortex: Implications for learning, memory, and mental illness. Progress in Brain Research, 102, pp. 227-243.

144. Reimer, A.R. & Martin-Iverson, M.T. (1994). Nimodipine and haloperidol attenuate behavioural sensitization to cocaine but only nimodipine blocks the establishment of conditioned locomotion induced by cocaine. Psychopharmacology (Berl), 113 (3-4), pp. 404-410.

145. Rezvani, A.H. & Levin, E.D. (2004). Adolescent and adult rats respond differently to nicotine and alcohol: Motor activity and body temperature. International Journal of Developmental Neuroscience, 22, pp. 349-35.

146. Rivest, R., Falardeau, P. & DiPaolo, T. (1995). Brain dopamine transporter: Gender differences and effect of chronic haloperidol. Brain Research, 692, pp. 269-272.

147. Rodriguez, M.C., Sanchez-Yague, J. & Paniagua, R. (1992). Effects of cocaine on testicular structure in the rat. Reproductive Toxicology, 6 (1), pp. 51-5.

148. Rosellini, R., Svare, B., Rhodes, M.E. & Frye, C.A. (2001). The testosterone metabolite and neurosteroid 3alpha-androstanediol may mediate the effects of testosterone on conditioned place preference. Brain Research Reviews, 37, pp. 162-171.

149. Russo, S.J., Festa, E.D., Fabian, S.J., Gazi, F.M., Kraish, M., Jenab, S. & Quinones-Jenab, V. (2003). Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience, 120, pp. 523-533.

150. Russo, S.J., Jenab, S., Fabian, S.J., Festa, E.D., Kemen, L.M., & Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. Brain Research, 970 (1-2), pp. 214-20.

151. Russo, S.J., Nazarian, A., Festa, E.D., Niyomchai, T., Akhavan, A., Jenab, S. & Quinones-Jenab, V. Progesterone blocks the acquisition and expression of cocaine-induced CPP in intact female rats. (manuscript in preparation).

152. Salvador, A., Moya-Albiol, L., Martinez-Sanchis, S. & Simon, V.M. (2000). Lack of effects of anabolic-androgenic steroids on locomotor activity in intact male mice. Perceptual and Motor Skills, 88(1), pp. 319-328.

153. Sarnyai, Z., Dhabhar, F.S., McEwen, B.S. & Kreek, M.J. (1998). Neuroendocrine-related effects of long-term, 'binge' cocaine administration: diminished individual differences in stress-induced corticosterone response. Neuroendocrinology, 68 (5), pp. 334-44.

154. Schenk, S. & Izenwasser, S. (2002). Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. Pharmacology Biochemistry and Behavior, 72, pp. 651-657.

155. Schlussman, S.D., Nyberg, F., & Kreek, M.J. (2002). The effects of drug abuse on the stress responsive hypothalamic-pituitary-adrenal axis and the dopaminergic and endogenous opioid systems. Acta Psychiatr Scand Suppl. (412) pp. 121-4.

156. Schindler, C.W., & Carmona, G.N.. (2002). Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. Pharmacology Biochemistry and Behavior, 72, pp. 857-863.

157. Schroeder, J.P. & Packard, M.G. (2000). Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. Neuroscience Letters, 282, pp. 17-20.

158. Sell, S.L., Scalzitti, J.M., Thomas, M.L. & Cunningham, K.A. (2000). Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. Journal of Pharmacology and Experimental Therapeutics, 293, pp. 879-886.

159. Shi, W.X., Pun, C.L. & Zhou, Y. (2004). Psychostimulants induce low-frequency oscillations in the firing activity of dopamine neurons. Neuropsychopharmacology, 29(12), pp. 2160-2167.

160. Sircar, R. & Kim, D. (1999). Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. Journal of Pharmacology and Experimental Therapeutics, 289, pp. 54-65.

161. Spangler, R., Zhou, Y., Schlussman, S.D., Ho, A., & Kreek, M.J. (1997). Behavioral stereotypies induced by 'binge' cocaine administration are independent of drug-induced increases in corticosterone levels. Behavioural Brain Research, 86, pp. 201-204.

162. Spear, L.P. & Brick, J. (1979). Cocaine-induced behavior in the developing rat. Behavioral and Neural Biology, 26, 401-15.

163. Stansfield, K.H. & Kirstein, C.L. (2005). Neurochemical effects of cocaine in adolescence compared to adulthood. Developmental Brain Research, 159, pp. 119-125.

164. Steketee, J.D. & Goeders, N.E. (2002). Pretreatment with corticosterone attenuates the nucleus accumbens dopamine response but not the stimulant response to cocaine in rats. Behavioral Pharmacology, 13 (7), pp. 593-601.

165. Stuerenburg, H.J., Breivik, M.J.A. Puchner, Lohmann, F. & Kunze, K. (1997). Testosterone is locally metabolized in human brain tumors, with possible qualitative and quantitative changes in its effects. Neuroendocrinology Letters, 18 (4), pp. 203-213.

166. Substance Abuse and Mental Health Services Administration. National survey on drug use and health. (formerly: National household survey on drug abuse). 2003. Rockville, M.D., U.S. Department of Health and Human Services.

167. Suzuki, T., Mori, T., Tsuji, M., Misawa, M. & Nagase, H. (1994). The role of delta-opioid receptor subtypes in cocaine- and methamphetamine-induced place preferences. Life Sciences, 55, pp. 339-344.

168. Syndistar, Inc. Taken from the web (2004): http://www.intheknowzone.com/cocaine/testresults.asp

169. Teicher, M.H., Andersen, S.L. & Hostetter, Jr., J.C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not NAcc. Developmental Brain Research, 89, pp. 167-72.

170. Tenney, J.R., Febo, M., Segarra, A.C., Messenger, T. & Ferris, C.F. (manuscript in preparation). Functional MRI in conscious rats shows testosterone alters cocaine-induced brain activity.

171. Thiblin, I., Finn, A., Ross, S.B. & Stenfors, C. (1999). Increased dopaminergic and 5-hydroxytryptaminergic activities in male rat brain following long-term treatment with anabolic-androgenic steroids. British Journal of Pharmacology, 126, pp. 1301-6.

172. Thomas, M.J. & Malenka, R.C. (2003). Synaptic plasticity in the mesolimbic dopamine system. Philosophical Transactions,Royal Society of London, Series B, Biological Sciences, 358 (1432), pp. 815-9.

173. Tirelli, E., Laviola, G. & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neuroscience and Biobehavioral Reviews, 27, 163-178.

174. Togna, G.I., Togna, A.R., Graziani, M. & Franconi, M. (2003). Testosterone and cocaine: vascular toxicity of their concomitant abuse. Thrombosis Research, 109, pp. 195-201.

175. Ushijima, I., Carino, M.A. & Horita, A. (1995). Involvement of D1 and D2 dopamine systems in the behavioral effects of cocaine in rats. Pharmacology Biochemistry and Behavior, 52 (4), pp. 737-41.

176. van Luijtelaar, E.L., Dirksen, R., Vree, T.B. & van Haaren, F. (1996). Effects of acute and chronic cocaine administration on EEG and behaviour in intact and castrated male and intact and ovariectomized female rats. Brain Research Bulletin, 40 (1), pp. 43-50.

177. Vlachou, S., Nomikos, G.G. & Panagis, G. (2003). WIN 55,212-2 decreases the reinforcing actions of cocaine through CB1 cannabinoid receptor stimulation. Behavioural Brain Research, 141 (2), pp. 215-22.

178. Volkow, N.D., Wang, G.J., Fischman, M.W., Foltin, R.W., Fowler, J.S., Abumrad, N.N., Vitkun, S., Logan, J., Gatley, S.J., Pappas, N., Hitzemann, R. & Shea, C.E. (1997). Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature, 386(6627), pp. 827-30.

179. Wadler, G.I. & Hainline, B. (1989). Drugs and the athlete. Philadelphia: FA Davis.

180. Walker, Q.D., Francis, R., Cabassa, J., Kuhn, C.M. (2001). Effect of ovarian hormones and estrous cycle on stimulation of the hypothalamo-pituitary-adrenal axis by cocaine. Journal of Pharmacology and Experimental Therapeutics, 297 (1), pp. 291-8.

181. Wilson, J. (1988). Androgen abuse by athletes. Endocrine Reviews, 9,181-9.

182. Wood, R.I. (2004). Reinforcing aspects of androgens. Physiology and Behavior, 83(2) pp. 279-89.

183. Yamaguchi, M., Suzuki, T., Abe, S., Baba, A., Hori, T. & Okado, N. (2002). Repeated cocaine administration increases GABA(B(1)) subunit mRNA in rat brain. Synapse, 43 (3), pp. 175-80.

184. Yesalis, C.E., Anderson, W.A., Buckley, W.E., & Wright, J.E. Incidence of the nonmedical use of anabolic-androgenic steroids. In: Lin, G.C., Erinoff, L., editors. Anabolic steroid abuse. NIDA Research Monograph, 102, pp. 97-112.

185. Zaikowski, B, (2004). "Will work for testosterone". Taken from the web: bztoons.com.

186. Zavala, A.R., Weber, S.M., Rice, H.J., Alleweireldt, A.T. & Neisewander, J.L. (2003). Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. Brain Research, 990 (1-2), pp. 157-64.