THE SOCIAL AND NONSOCIAL IMPACT OF ANABOLIC ANDROGENIC STEROIDS AND LOW SEROTONIN IN PUBERTAL MALE RATS: A BEHAVIORAL AND NEUROCHEMICAL ANALYSIS.

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ABSTRACT

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This study's purpose was to determine the behavioral and neurochemical impact of low serotonin (5-HT), via p-chlorophenylalanine (PCPA) injections, in males exposed to anabolic androgenic steroids (AAS) during puberty. Social and non-social behavioral tests were conducted and 5-HT-related neurochemical measurements were taken. Behavioral and neurochemical measures were also assessed following discontinuation of PCPA in combination with the AAS testosterone propionate (TP) to determine the

permanence of these effects. Beginning at 26 days of age, 30 gonadally intact male pubescent rats received injections of PCPA (50 mg/kg) or saline three times a week for 9 weeks. Starting at puberty (Day 40), animals received injections of TP (5 mg/kg) five days a week for 7 weeks. Five groups were studied: TP, PCPA, TP + PCPA, Control and Withdrawal. Behavioral tests began at 61 days of age. The withdrawal group received a second set of behavioral tests 10 days after their last injection of TP + PCPA. At the end of behavioral testing the brainstem, striatum, hypothalamus, hippocampus and cortex were removed and levels of 5-HT, 5-hydroxyindoleaceticacid (5-HIAA), and tryptophan (TRP) were determined using high performance liquid chromatography (HPLC). Results indicated that locomotor activity was reduced by PCPA, whether alone or in combination with TP. PCPA alone also elicited a longer latency to nose poke in a separate non-social and novel environment. No significant differences were found between any of the sexual behavior measures. The first aggression tests were conducted with and without provocation, which was induced using a mild tail pinch. Without provocation the PCPA group displayed significantly more dominance mounts than controls. With provocation, PCPA and TP+PCPA treated males exhibited the highest levels of aggression compared to controls. In the social aggression test, animals receiving TP alone and TP + PCPA exhibited heightened aggression compared to controls. Following withdrawal, there were no significant differences in any behavioral measure. HPLC revealed that 5-HT levels were depleted approximately 80% in each region examined for the TP+PCPA and PCPA alone groups. In the withdrawal group, levels of 5-HT were similar to those observed in controls. TP was found to significantly increase 5-HT levels in the frontal cortex and also decreased 5-HIAA levels in the hypothalamus. Taken together these data suggest

that AAS exposure during adolescence increases the likelihood of an animal to react to provocation or a threatening environment aggressively. When AAS exposure during adolescence is combined with low 5-HT animals also tend to exhibit behaviors found in animals receiving just PCPA, such as reacting aggressively toward non-threatening animals and exhibiting decreases in locomotor activity. It also provides evidence that the observed behavioral and neurochemical effects may be reversible.

CHAPTER I

INTRODUCTION

Anabolic steroid use is a growing concern in today's society. It has been estimated that over one million Americans have abused anabolic androgenic steroids (AAS) (Sturmi & Diorio, 1998). In human studies, the use of AAS has been linked to an increase in unprovoked aggression and impulsivity (Elliot & Goldman, 1996), high risk behavior (Middleman et al., 1995), and enhanced sexual desire (Moss, Clutton-Brock, & Monfort, 2001). The population that may be the most susceptible to the harmful effects of AAS abuse could be adolescent individuals who are using AAS to enhance their athletic performance and physical appearance. The 2003 Youth Risk Behavior Surveillance System, administered by the Centers for Disease Control and Prevention, found that 6.8% of adolescent males and 5.3% of adolescent females had experimented with anabolic steroids at some point in their lifetime. Common among today's society are reports of AAS abuse by professional athletes. However, AAS use is no longer limited to professional or elite athletes and the rise in publicity of AAS may be responsible for the increase in adolescent AAS abuse (Denham, 2006). AAS abuse during the hormonally sensitive time of adolescence may permanently alter normal physiological development as well as the development of normal adult behavior patterns. A better understanding of the possible consequences of AAS abuse may help adolescent individuals make more informed decisions about AAS use.

One of the key problems in studying AAS abuse in humans is that athletes often administer cocktails of different AASs known as "stacking" (Strauss & Yesalis, 1991). This makes it difficult to determine the behavioral and physiological effect of each individual AAS. Because of this and other ethical limitations in the study of AAS abuse in adolescent individuals, animal models have been used to aid in the understanding of the behavioral and physiological effects of AAS abuse.

In previous studies, testosterone exposure during adolescence has been shown to produce significantly increased levels of aggression in male hamsters (Melloni & Ferris, 1996; Melloni & Conner, 1997), as well as in male rats (Farrell and McGinnis, 2003). However, some studies have shown that adolescent male rats only exhibit a significant increase in aggression, relative to controls, when they are physically provoked (Farrell & McGinnis, 2004). Furthermore, a recent study indicated that when physically provoked, adolescent male rats exposed chronically to AAS also exhibited an increase in aggression towards ovariectomized females but not females treated with estrogen (Cunningham & McGinnis, 2006), thus providing evidence that testosterone might heighten the animal's reactivity to a variety of social stimuli that would not normally elicit an increase in aggression.

Research has also investigated a possible link between adolescent AAS exposure and low 5-HT (Grimes & Melloni, 2004; Grimes & Melloni, 2006; Keleta et al., 2007; Ricci, Catizone, Esposito, & Galdieri, 2004). A recent study suggests that neural signaling through 5-HT1 receptors may represent a mechanism responsible for AAS induced aggression (Grimes & Melloni, 2006). Grimes & Melloni (2006) have also suggested that adolescent AAS exposure may have long term effects on 5-HT neural

systems. Because 5-HT abnormalities have been implicated in a variety of psychiatric disorders, further research into the long term effects AAS abuse on 5-HT systems is critical.

Adolescent male rats chronically exposed to AAS did have significantly lower concentrations of 5-HT and/or 5-HIAA in several brain regions compared to controls (Keleta et al., 2007). For example, testosterone was shown to significantly deplete 5-HT in the striatum, but raise 5-HT in the frontal cortex. The combination of testosterone and PCPA was shown to significantly decrease 5-HT in all brain regions. Keleta et al. (2007) combined the biochemical data with the behavioral data to assess whether exposure to AAS during adolescence in combination with the 5-HT synthesis inhibitor PCPA would alter the expression of psychosocial behavior patterns. Both nonsocial and social behavioral tests were performed. The non-social tests measured the irritability and locomotor activity of the animal, while the social behaviors tests measured copulatory behavior and aggression. Results showed that a 100mg/kg dose of PCPA was sufficient enough to produce dramatic decreases in brain 5-HT, 5-HIAA and tryptophan. The group receiving both testosterone and PCPA displayed significantly greater levels of aggression following provocation compared to the control group. The group receiving testosterone alone did not exhibit significantly more aggression than controls in the aggression test. This suggests that adolescent males with low 5-HT may be even more at risk for aggression while taking AAS (Keleta et al., 2007).

Based on the work of Keleta et al. (2007) this study will provide a comprehensive behavioral and neurochemical assessment of both the short and long term effects of AAS alone and in combination with low 5-HT in adolescence. This study had three aims. The

first aim was to assess the reactivity, in both social and non-social environments, of adolescent male rats chronically exposed to AAS alone and in combination with low 5-HT. Through the manipulation of different social and non-social environments this experiment attempts to uncover the possible cues which might initiate the onset of AAS-induced aggression. The second aim was to assess the neurochemical effects of a 50mg/kg dose of PCPA alone and in combination with AAS. The third aim of this experiment was to assess the behavioral and neurochemical impact of adolescent male rats exposed to AAS alone and in combination with PCPA following withdrawal from chronic exposure.

The first aim was to assess the reactivity, in both social and non-social environments, of adolescent male rats chronically exposed to AAS alone and in combination with low 5-HT. Berton, Ramos, Chaouloff, and Mormde (1997) suggest that social and non-social reactivity are specific and dissociable responses. Specifically, this means that animals will exhibit different responses based on the context of the environment they are introduced to. Therefore, by assessing animals in a variety of both social and non-social environments we can obtain a better view of the myriad of behavioral effects related to low 5-HT and AAS abuse during adolescence.

An open field test was administered in order to measure the animal's locomotor activity in a novel environment. Locomotor activity also correlates to the animal's adaptability (Kulikov, 1990). It has been reported that animals administered PCPA exhibit decreases in locomotor activity (Dringenberg et al., 1995; Genot, Conan, Barthelemy, & Peyraud, 1984; Keleta et al., 2007; Matte & Turnow, 1978). Keleta et. al

(2007) reported a decrease in locomotor activity relative to controls by adolescent animals receiving PCPA.

A nose-poke test was designed and performed in a novel environment with a rewarding stimulus present. This test was designed to gain further understanding into the reactivity of animals exposed to AAS and low 5-HT and to look into whether the treatments would elicit maladaptive responses even in a potentially rewarding and novel environment.

Using the resident-intruder paradigm, aggression tests were performed with and without physical provocation. It has been previously reported that provocation, produced by tail pinching the animals, increased aggression levels of adolescent male rats administered PCPA and testosterone (Keleta et al., 2007). In contrast to prior studies on AAS induced aggression, the current study used smaller opponent males in order to determine whether aggression would still be elicited against a non-threatening intruder. Previous research has shown that male Long-Evans rats normally do not attack foreign immature animals (Thor & Flannelly, 1976). Therefore, this opponent should elicit very low levels of normal species specific aggression. However, following provocation animals administered

To further delineate the impact of AAS and PCPA in a potential socially competitive situation, animals were placed in the home of a long-term paired resident male and female (Flannelly & Lore, 1977). Animals administered testosterone normally exhibit heightened levels of aggression in environments in which social rank is not established (Albert, Petrovic & Walsh, 1989). Resident males paired with intact females generally attack intruders significantly more (Flannelly & Lore, 1977). Therefore, unlike

the previous aggression test using smaller non threatening opponent males, this test attempted to understand the reactivity of animals placed with a threatening opponent. The test was performed once with a sexually receptive paired female present and once with a non-receptive paired female present in order to assess the possible social cues, such as vaginal odors, which may elicit an increase in aggression.

To obtain an even broader view of the role of AAS and low 5-HT in a social setting, a separate sexual behavior test was also administered. The sexual behavior test provides information into the possible sexual side-effects associated with the administration of AAS with low 5-HT and to determine possible alterations in normal sexual function.

The second aim of this study was to assess the neurochemical effects of a 50mg/kg dose of PCPA alone and in combination with AAS. Studies using acute administration of a similar dose of PCPA have failed to produce significant behavioral effects (eg. Guemene & Etches, 1989). However, chronic exposure to a 100 mg/kg dose of PCPA has been shown to dramatically deplete central 5-HT in numerous brain regions and produce significant behavioral effects when combined with AAS (Keleta et al., 2007). Using HPLC we were able to compare 5-HT, tryptophan, and 5-HIAA concentrations in the frontal cortex, striatum, hippocampus, hypothalamus and brain stem of each group. By comparing these biochemical changes in the brain to results obtained through the behavioral tests, we gain insight into the possible neurochemical mechanisms affecting AAS-induced aggression.

The third aim of this experiment was to assess the impact of withdrawal from chronic exposure of AAS alone and in combination with PCPA. There is a lack of

research involving chronic 5-HT depletion throughout adolescence and its long term effects. There is also a lack of neurochemical evidence regarding AAS and their neurochemical effects following withdrawal. Behaviorally, it has been shown that aggression in animals administered AAS during adolescence remained elevated following five weeks of withdrawal (Farrell & McGinnis, 2004). Therefore, the behavioral and neurochemical results from the present study's withdrawal group provide further information about the long term effects of AAS use and low 5-HT during puberty.

CHAPTER II

PUBERTY

Neural Development and Triggers of Puberty

Gonadal hormones have a dramatic effect in the development of organisms. In an effort to categorize how gonadal hormones affect the maturing brain, researchers have developed the organizational/activational hypothesis in which hormones organize and later activate neural machinery. The traditional organizational/activational hypothesis for the effects of steroid hormones suggests that puberty is a time in which gonadal steroids exhibit activational effects. However, recent literature suggests that adolescence is also a period in which hormones produce organizational effects, particularly in the development of adult-like behaviors (Romeo, 2003). For example, castration of male rats just before puberty produces significant reductions in adult typical testosterone-mediated behaviors compared to male rats castrated following puberty (Larrson, 1967). Therefore, the behavioral responsiveness to testosterone changes during puberty (Romeo, 2003). Sisk and Foster (2004) suggest that gonadal maturation and behavioral maturation are two separate but interacting processes that work together toward the reproductive maturation of an organism and there is no single trigger for the onset or termination of puberty.

The onset of puberty is generally marked by a rise in testosterone, mediated by an increase in the secretion of gonadotropin-releasing hormone (GnRH) into the median

eminence from the hypothalamus, as shown in Figure 1. This, in turn, signals the pituitary gland to release lutenizing hormone (LH) and follicle stimulating hormone (FSH) which mediate the secretion of steroid hormones.

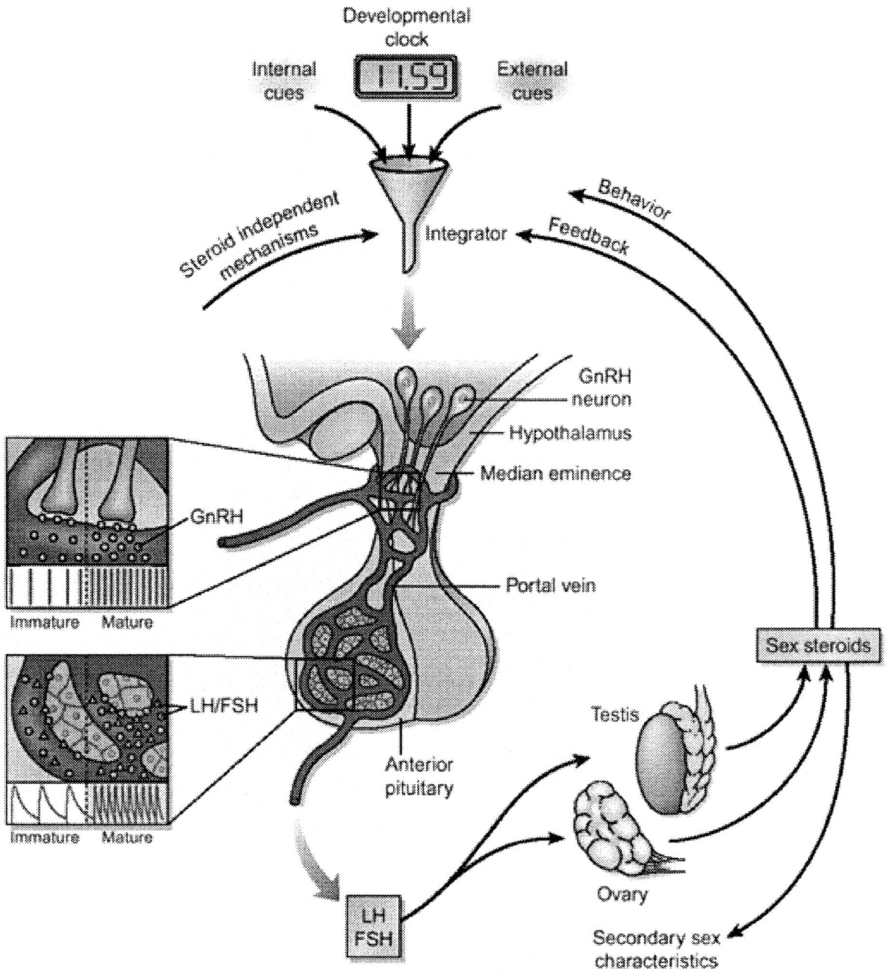


Figure 1. The HPG Axis. Internal and external cues occurring during puberty produce an increase in GnRH neuronal activity, which in turn stimulates the release of LH and FSH. GnRH neurons are then inhibited by the circulating steroid hormones. The steroid hormone increase at the onset of puberty facilitates the gonadal maturation and behavioral maturation of the organism. Adapted from "The neural basis of puberty and adolescence", by C.L. Sisk, and D.L. Foster, 2004, *Nat and Neurosci, 7*, p. 1041.

The regulation of steroid hormones such as testosterone is provided via the negative feedback loop of the hypothalamic-pituitary-gonadal (HPG) axis where circulating steroid hormones inhibit the release of GnRH. During puberty a progressive decrease in the inhibition of testosterone occurs (Sisk & Turek, 1983). This decrease in inhibition allows for more secretion of GnRH and the rise in circulating steroid levels which initiate the onset of pubertal development.

In addition to marked changes in the function of the HPG axis, maturation of the hypothalamic-pituitary-adrenal (HPA) axis also occurs. One of the key functions of the HPA access is in the modulation of stress-response. Compared to adult males, prepubertal male rats have been shown to take significantly longer to recover from acute stressors (Romeo & McEwen, 2004). This suggests that the HPA axis is further shaped during pubertal development to allow for the emergence of a more tightly regulated stress response in adulthood.

The pre-frontal cortex also continues to develop during adolescence. For example, myelination and synaptic pruning continue to occur during puberty (Schneider & Koch, 2005). In the frontal cortex the volume of gray matter increases until adolescence and then decreases until early adulthood (Jernigan, Hesselink, & Tallai, 1991; Sowell et al., 1999). This change in volume has been correlated with an increase in cognitive ability (Sowell et al., 2004). Adult animals which have undergone neonatal lesions in the pre-frontal cortex serve as models for the study of neurodevelopmental deficits that have been proposed to underlie neuropsychiatric disorders (Lipska & Weinberger, 1995). In humans, prefrontal cortex damage has been shown to impair many

social behaviors including social interactions and social cognition (Anderson, Kelly, & Wu, 1999; Eslinger, Flaherty-Craig, & Benton, 2004).

Serotonin has also been implicated in the development of the adult organism. It has been suggested that 5-HT interacts with testosterone to produce organizing effects in pre-pubertal animals and has an inhibitory effect on the action of neonatal testosterone (Gonzalez, Farabollini, Albonetti & Wilson, 1994). It has also been shown that PCPA given during the second week of life produces an increase in reactivity to environmental and social cues (Farabollini, Hole, & Wilson, 1988). Cholinergic innervation of the prefrontal cortex also increases during adolescence (Gould, Woolf, & Butcher, 1991). Reorganization at the receptor level also occurs. For example, 5-HT2A receptors reach peak expression in the cortex just before adolescence and decline to adult levels in adulthood (Morilak & Ciaranello, 1993).

Drug Abuse During Puberty

In a recent review Crews and Hodge (2007) suggest that adolescence represents a critical period in which drugs may significantly modulate brain development. Crews and Hodge (2007) described critical period as, "a specific window during development when both genetic driven processes and environmental processes interact to establish functional characteristics" (p. 190). Thus, both nature and nurture are integrated toward the goal of establishing adult-like behaviors. Unfortunately, this critical period of development also corresponds to a time when high risk behaviors, including reckless driving, unprotected sex, and substance abuse are prevalent (Merrick et al., 2004). A variety of studies have reported long term behavioral effects with individuals who abuse drugs during adolescence (Aberg, Wade, Wall, & Izenwasser, 2007; Grimes & Melloni, 2006;

McGinnis, Lumia, Breuer, & Possidente, 2002). Substance abuse in adolescence has also been shown to accurately predict substance abuse later in life (Merline et. al, 2004), which supports the hypothesis that many adult behavioral patterns are formed during adolescence.

CHAPTER III

ANABOLIC ANDROGENIC STEROIDS

Neurochemical Analysis of AAS

All steroids have the chemical structure characterized by three six-carbon rings plus one five-carbon ring, as shown in Figure 2. The adrenal glands and the gonads are the most common sources of steroid hormones. The precursor for all steroid hormones is cholesterol. Steroids bind to water-soluble proteins and are transported through the blood to their target tissues. The chemical structure of steroids enable them to diffuse through cell membranes to bind to steroid receptors, generally located in the cytosol (or nucleus) of the cell. The steroid-receptor binds to a hormone response element on the DNA which produces mRNA. The mRNA then migrates to the endoplasmic reticulum and is transcribed. It has been suggested that certain co-activators regulate the transcription process (C. Smith, Nawaz, & O'Malley, 1997). Structural proteins are then formed which produce physiological responses. Steroid hormones may also produce more rapid, non-genomic, effects by acting on receptors that are located in cell membranes (Wehling & Losel, 2006).

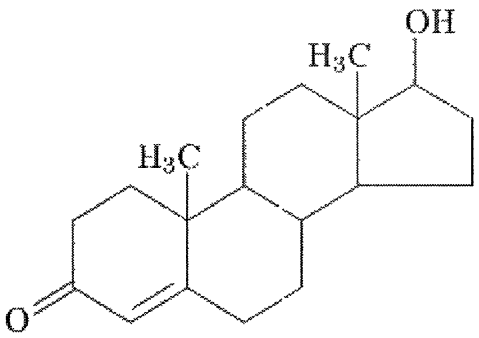


Figure 2. Chemical Structure of Testosterone. (adapted from http://www.knowledgerush.com/kr/encyclopedia/Testosterone)

Substances such as testosterone belong to a class of steroid hormones known as androgens. Androgens are produced in Leydig cells in the testes and are carried by Sertoli cells. Androgens serve many physiological and behavioral functions throughout the body. For example, physiologically androgens are necessary for spermatogenesis and the maintenance of the genital tract, accessory sex organs, and secondary sex characters. Androgens affect copulatory, aggressive, and other social behaviors.

Anabolic Androgenic Steroids (AAS) are synthetic derivatives of testosterone. The term "anabolic" refers to the tissue building properties of the chemicals. The term "androgenic" refers to the masculanizing effects of the chemicals. The structure of this group of chemicals is split into three classes based on synthetic modifications, as shown in Figure 3. Class A anabolic steroids are modified at the 17-β-Hydroxyl group of testosterone and include testosterone propionate (TP) and testosterone cypionate. This modification increases the hydrophobic properties of the AAS by decreasing the polarity of the molecule. This decrease in polarity causes slower absorption when given by intramuscular injection (Basaria, Wahlstrom, & Dobs, 2001). Class B AAS undergo alkylation at the 17-hyrdoxy position (Basaria et. al, 2001). Class C AAS are alkylated at

the 17th carbon, including 17α -methyltestosterone, oxymetholone, methandrostenone, and stanozolol. The modifications of testosterone derivatives contribute to the varying effects found in different AASs.

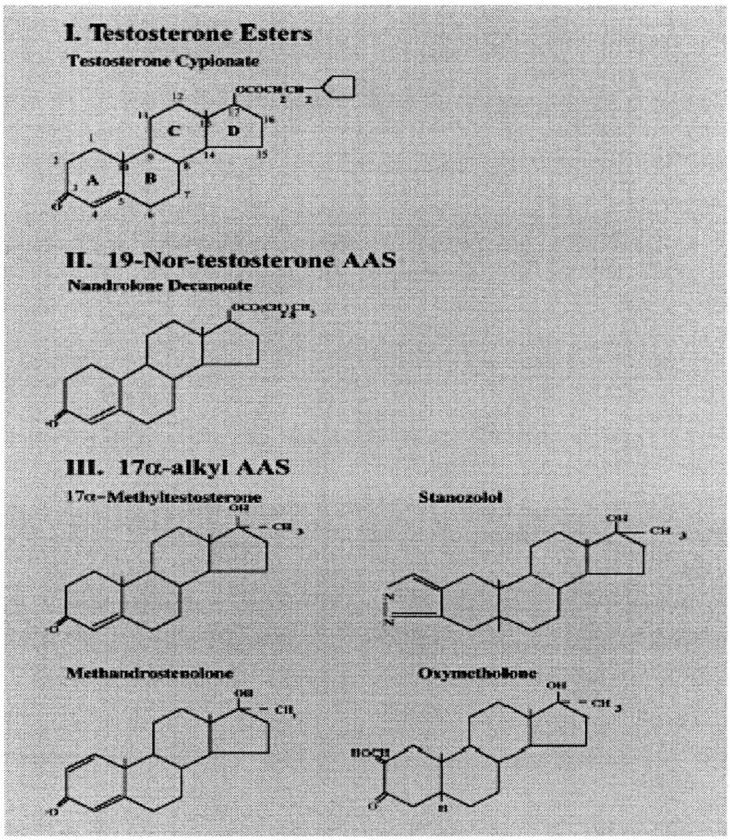


Figure 3. **The Three Classes of AAS.** Adapted from "Behavioral and physiological responses to anabolic-androgenic steroids" by A.S. Clark and L. P. Henderson. *Neurosci Biobehav Rev, 27*, p. 414.(Clark, 2003).

Effects of AAS on Locomotor Activity

Studies have provided very little evidence for a clear link between AAS exposure and locomotor activity in adult male rats, (Clark & Barber, 1994; Clark & Fast, 1996, Salvador, Moya-Albiol, Martinez-Sanchis, & Simon, 1994) as well as in adolescent male

rats (Keleta, Lumia, Anderson, & McGinnis, 2007). The only effects tend to come from studies using AAS in combination with other treatments. For example, Keleta et al. (2007) found that testostosterone administration throughout adolescence had no significant difference in locomotor activity relative to controls. However, when testosterone was combined with PCPA, a non-significant decrease in locomotor activity was found. It has also been suggested that concurrent administration of testosterone and cocaine do interact to increase locomotor activity (Martinez-Sanchis, Aragon & Salvador, 2002). Together these results suggest that AAS alone plays a minimal role in the facilitation of locomotor activity.

Effects of AAS on Sexual Behavior

Several studies have investigated the effects of different AAS's on male sexual behavior (Clark & Fast, 1996; Clark, Harold, & Fast, 1997; Farrell & McGinnis, 2003; Lumia, Thornor, & McGinnis, 1994; Wesson & McGinnis, 2006). Results seem to vary depending on the chemical structure of the AAS administered. Research has found that the AAS nandrolone, methandrostenone, and testosterone cypionate have no effects on sexual behavior, measured by mounts, intromissions and ejaculations (Clark & Barber, 1994; Farrell & McGinnis, 2003). However, stanozolol, oxymetholone, and 17α-methyltestosterone suppressed sexual behavior and suppressed serum testosterone levels (Clark & Barber, 1994). Other animal studies have shown that the AAS testosterone will enhance ejaculatory behavior in sexually inexperienced pubertal male rats, (Feinberg, Lumia, & McGinnis, 1997) but not in sexually experienced males (Farrell & McGinnis, 2003). It has also been reported that following fifteen weeks of withdrawal from chronic exposure to testosterone throughout adolescence no changes in sexual behavior were

reported (Farrell & McGinnis, 2004). This suggests that testosterone's effects on sexual behavior may be reversible. Keleta et al. (2007) found that chronic exposure to testosterone during adolescence combined with PCPA-induced low 5-HT concentrations produced no effects on mount frequency, intromission frequency, mount latency, intromission latency, ejaculation latency or post-ejaculatory interval in sexually experienced male rats.

Effects of AAS on Aggression

Adult human studies have shown that one of the common behavioral effects of AAS use is a reported increase in aggression (Anderson & Kelly, 1997; Strauss & Yesalis, 1991). Using the resident-intruder paradigm, animal studies have yielded further evidence of the effects of different kinds of AAS. For example, testosterone has been shown to increase aggressive behavior (Breuer & McGinnis, 2001; Lumia et al. 1994), stanozolol has been shown to decrease aggressive behavior (Breuer & McGinnis, 2001; McGinnis et al., 2002), and the results are equivocal as to whether or not nandrolone has any impact on aggressive behavior (Breuer & McGinnis, 2001; McGinnis et al., 2001; Melloni & Conner, 1997). The varying effects of different anabolic steroids make human studies difficult to conduct because it has been observed that athletes commonly use cocktails of different steroids (Rogol & Yesalis, 1992; Strauss & Yesalis, 1991). Also, some human studies are based on subjective measures such as verbal reports or questionnaires. It is also unethical to conduct blind studies of AAS on humans. Therefore, better controlled human studies need to be performed.

The question remains as to whether the enhanced aggression following AAS exposure to testosterone is unprovoked, often called "roid rage", or whether it is due to an

enhanced reactivity to provocation. In rats, mild physical provocation, using a tail pinch procedure, has been reported to exaggerate the effects of testosterone-induced aggression (Cunningham & McGinnis, 2006; McGinnis et al., 2002a). Smith & Stewart (1997) suggest that tail pinching rats produces arousal and increases the animal's sensitivity to external incentives, which then could stimulate a variety of behaviors, including aggression. McGinnis et al. (2002a) suggest that testosterone lowers the animal's threshold to elicit aggression and dominance in response to provocation.

Recent research has focused on the effects of AAS on aggression in adolescent rats. Farrell and McGinnis (2003) found that chronic testosterone administration during adolescence increases aggression in male rats. Furthermore, tail pinch-induced intermale aggression in pubertal male rats was found to remain above control levels for 5 weeks following withdrawal from the AAS (Farrell & McGinnis, 2004). A separate study found an increase in aggression in hamsters after 12 days of withdrawal from AAS (Grimes & Melloni, 2006). With physical provocation, using tail-pinch, Cunningham & McGinnis (2006) reported that AAS exposed pubertal male rats exhibited heightened aggression towards ovariectomized females, suggesting that the AAS exposed animals may exhibited heightened aggression even towards non-threatening conspecifics.

It has been proposed that the aggression associated with testosterone serves the purpose of establishing dominance relationships (Ruiz-de-la-torre & Manteca, 1999). Studies on social aggression have found that testosterone implanted males display more aggression and are more successful in maintaining access to food than their cagemates (Albert & Petrovic, 1989). Conversely, castrated alpha males become subordinate and lose dominance (Albert & Walsh, 1986). These results suggest that testosterone plays a

key role in the development and maintenance of a social hierarchy. Furthermore, animals with higher levels of testosterone could have an advantage over opponents in competitive situations.

CHAPTER IV

SEROTONIN (5-HT)

Neurochemical Analysis of Serotonin

Serotonin, also known as 5-Hydroxytryptamine (5-HT) is a neurotransmitter which has been implicated in a wide variety of behaviors including aggression, locomotor activity, depression, appetite and sexual behavior (Valzelli, 1984). Perhaps one of the reasons why 5-HT produces such a wide variety of behavioral effects is because it is projected from the raphe nuclei to most areas of the brain. For example, ascending axons from the raphe nuclei project to many regions in the brain including the hippocampus, hypothalamus, amygdala, basal ganglia, striatum and many parts of the cortex. Another possible reason for the wide variety of effects produced by 5-HT is the variety of receptor subtypes. There are at least 15 serotonergic receptor subtypes. Through the use of certain agonists and antagonists, which act at specific receptors, current research is unveiling the specific effects of each receptor subtype (Gorzalka et al., 1990; Milan et al., 2003).

Tryptophan is the precursor for the synthesis of 5-HT. Tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. This is considered the rate limiting step in the synthesis of serotonin. PCPA prevents the production of tryptophan hydoxylase and thereby inhibits the production of 5-HT. 5-hydroxytrypophan is

converted to 5-hydroxytryptamine or 5-HT by the L-amino acid decarboxylase. 5-HT is released from presynaptic neurons and stimulates postsynaptic receptors. It is then removed from the synapse by active transport, or reuptake, back into the presynaptic neuron where it is repackaged for release or catalyzed by monoamine oxidase which converts the 5-HT to 5-hydroxyindoleacetic acid (5-HIAA). SSRIs such as paroxetine, fluoxetine, and fluvoxamine interfere with the reuptake of 5-HT into the terminal button. The 5-HT therefore stays in the synaptic cleft and can maintain activation of the postsynaptic receptors for a prolonged period (Coppen & Harnett, 1991).

Effects of 5-HT on Locomotor Activity

Kulikov (1990) suggested that locomoter activity is a manifestation of adaptive behavior to new environments. His studies found that animals with low locomotor activity in an open field test also spent less time orienting themselves to new environments and displayed more emotional reactivity to novel environments (Kulikov, 1990). Previous animal studies have shown a decrease in locomotor activity in an open field test by animals with low 5-HT (Dringenberg et al., 1995; Genot et al., 1984; Keleta et al., 2007; Matte & Turnow, 1978). Dringenberg et. al (1995) also found that acute administration (150-1000 mg/kg) of PCPA produces a dose-dependant decrease in exploratory locomotor activity in an open field test. Conversely, high levels of 5-HT, produced through the administration of SSRI's have been shown to increase locomotor activity (Brocco et al., 2002). This SSRI-induced hyperactivity has also been shown to be attenuated by concurrent administration of PCPA (Slattery et al., 2005). Together these results suggest a directly proportional relationship between central 5-HT levels and locomotor activity.

Recent research has looked into certain localizations of projecting 5-HT neurons and certain 5-HT receptor subtypes and their role in locomotor activity. For example, Kusilic and Van den Buuse (2004) suggest that 5-HT projections from the median raphe nucleus to the dorsal hippocampus play an important role in the facilitation of locomotor activity. Other research has focused on the possibility that certain individual 5-HT receptors might mediate locomotor activity. For example, using selective 5-HT receptor antagonists Milan et al. (2003) found that increases in locomotor activity produced by the SSRI's citalprom and fluvaxamine were mediated by 5-HT1B and 5-HT2A receptors. Thus, 5-HT2A and 5-HT1B receptors might play a key role in the facilition of locomotor activity.

Effects of 5-HT on Sexual Behavior

Clinical studies have reported that SSRI's commonly have induced sexual dysfunction in both men and women (Clayton et al., 2002; Rosen et al., 1999). Animal studies have also shown that serotonin (5-HT) may inhibit sexual behavior (Bitran and Hull, 1987; Gorzalka et al., 1990). For example, Taylor et al. (1996) report that chronic treatment of male rats with the SSRI fluoxetine produced prolonged ejaculation latency, and reduced ejaculation frequencies. Alterations of 5-HT during development might also lead to changes in sexual behavior. For example, neonatal stimulation of 5-HT 1A receptors, by the administration of citalprom, has been shown to produce delayed ejaculation in adult males (Macciag et al., 2006). The delayed ejaculation produced by SSRI's has recently been related to the gradual desensitization of 5-HT 1A receptors on oxytocin neurons (de Jong et al., 2007), thus implying an interaction between serotonin and oxytocin in the expression of sexual behavior.

Conversely, decreasing 5-HT levels by the administration of PCPA has been shown to increase mounting, intromissions and ejaculatory behaviors in male rats (Ahlenius et al., 1971). This effect is dramatically less pronounced in studies using sexually active animals (McIntosh & Barfield, 1984; Paxinos et al., 1977). It has also been shown that PCPA acts on median raphe nucleus neurons to facilitate ejaculatory behavior (Kondo and Yamanouchi, 1997). Using unisexual whiptail lizards Dias and Crews (2006) found that PCPA facilitated male sexual behavior, but that it was dependant on the circulating hormonal environment, specifically estradiol, progesterone and testosterone. This suggests a possible interaction between these hormones and 5-HT in the expression of sexual behavior (Dias and Crews, 2006).

Effects of 5-HT on Aggression

Previous human and animal studies have related low 5-HT levels to increased aggression (Matte and Turnow, 1978; Miczek et al., 2002; Sewell & Koch, 1982), more specifically offensive aggression (Vergnes et al., 1986), and impulsive aggression (Coccaro, 1989). For example, depletion of 5-HT using PCPA has been shown to induce mouse killing behavior in male rats (Miczek et al., 1975), but this effect seems to be suppressed by concurrent administration of the SSRI fluoxetine (Molina et al., 1987). Lower levels of 5-HIAA have also been linked to increased aggression (Mann, 2003). Thus, previous results suggest an inverse relationship between 5-HT and aggression. But, the relationship does not seem to be that simple. For example a recent study suggested that significant 5-HT depletion, produced by the injection of PCPA, may be necessary, but not sufficient, to elicit aggression (Keleta et al., 2007). Keleta (2007) also found that the combination of testosterone and PCPA significantly increased aggression

relative to controls, while separately, testosterone and PCPA did not elicit heightened aggression compared to controls. This suggests a possible interaction between low 5-HT and high levels of AAS. Other studies have shown that administration of the SSRI fluoxetine may actually trigger violent or aggressive behavior (Troisi et al. 1995; Bondurant et al., 1998). Similar results were found in clinical studies using adolescent children on SSRIs (Constantino et al., 1997). In this clinical study 13 patients were assessed both on and off SSRI's. Verbal aggression ($p = 0.04$), physical aggression toward objects ($p = 0.05$), and physical aggression toward self ($p < 0.02$) were shown to occur significantly more frequently on SSRIs than off. This result indicates a possible differing effect of SSRI use by adolescent individuals versus adults.

Using selective drugs that act on specific 5-HT receptor subtypes it has been found that the 5-HT1B receptors play a vital role in the modulation of offensive aggression (Olivier & Oorschot, 2005). Rodriguez-Arieas et al. (1998) also demonstrated that administration of risperidone, a 5-HT2A receptor antagonist decreases aggressive behavior. Future studies into the receptor subtypes involved in aggression could help clarify which 5-HT receptor subtypes are the most involved in the onset and propagation of aggressive encounters.

Recent studies have attempted to determine how 5-HT affects aggression using a variety of new techniques including in vivo microdyalysis in freely moving animals. For example Van Erp and Miczek (2000) measured extracellular serotonin in the nucleus accumbens and the prefrontal cortex of male rats before, during, and after a 10 minute aggression interaction. While fighting, 5-HT levels decreased in the prefrontal cortex and remained lowered for an hour after the confrontation. No effect was found in the nucleus

accumbens. Future in vivo aggression studies could shed some light on serotonin's role in aggression and which 5-HT neuron innervation areas are most responsible for the increased aggression associated with lowered 5-HT.

CHAPTER V

HYPOTHESIS

This study's purpose was to determine the behavioral and neurochemical impact of low serotonin (5-HT) in males exposed to anabolic androgenic steroids (AAS) during puberty. It is hypothesized that the 50 mg/kg dose of PCPA will be sufficient in producing significant depletion of 5-HT, 5-HIAA and tryptophan in the hypothalamus, striatum, cortex, hippocampus and frontal cortex. It is also expected that testosterone will induce similar changes in 5-HT and 5-HIAA levels as previously shown (Keleta et al., 2007). Locomotor activity and exploratory behavior in the nose-poke test should parallel 5-HT levels. It is expected that AAS will elicit an increase in aggression following provocation and in the presence of a paired male and female. AAS plus low 5-HT could cause an increase in aggression even in the presence of a smaller opponent. It is believed that AAS combined with low 5-HT will elicit behavioral and neurochemical changes following withdrawal.

CHAPTER VI

METHODS

Setting

All experiments were performed at the University of Texas at San Antonio under the supervision of Dr. Augustus R. Lumia and Dr. Marilyn Y. McGinnis. HPLC analysis of brain samples was performed at Yale University medical school under the supervision of Dr. George M. Anderson.

Subjects

Twenty-nine gonadally intact male Long-Evans rats were received from Charles River Laboratories (Wilmington, MA) ranging from approximately 75 grams to 100 grams. The males were twenty-six days old upon arrival. The thirtieth male was received seven days after the first 29 males. Subject males were paired with each other for the first two weeks and then individually housed once puberty was reached. Day 40 corresponds closely to the day of preputial separation for male Long-Evans rats, which occurs just before the increase in endogenous testosterone levels (Korenbrot et al., 1977). Female rats weighing between 225-250 grams were used for the sexual behavior test and separately caged. They were ovariectomized and implanted with one silastic capsule (1.47-mm i.d. × 1.96-mm o.d. × 5-mm length) packed with 50% crystalline estradiol as described before (McGinnis et al., 1981). Forty-seven day old, intact opponent males weighed 250 grams and were separately housed upon arrival. All animals were housed in

Nalgene cages with cardboard bedding. Food and water were provided ad libitum. The room in which the animals were kept was maintained a 12 hour light-dark cycle with the lights turned off at noon. Cages were cleaned weekly and the bedding was changed in each cage, unless specified otherwise by the procedure for a particular behavior test. All procedures followed guidelines established for the care and use of laboratory animals by the National Institute of Health.

Experimental Groups

The experimental animals were assigned to one of five groups based on the smallest weight deviation possible between each group. The TP group received a combination of the AAS testosterone propionate (TP) plus PEG (Polyethyleneglycol mol weight 200). The PCPA group received PCPA (4-Chloro-DL-phenlalanine methyl ester) plus PEG. The TP + PCPA group received both TP and PCPA. The Withdrawal group also received both TP and PCPA and then went through 15 days of withdrawal. The control group received the vehicle substances, PEG and saline. Animals were ear punched for identification and weighed to the nearest gram weekly in order determine the correct treatment dose.

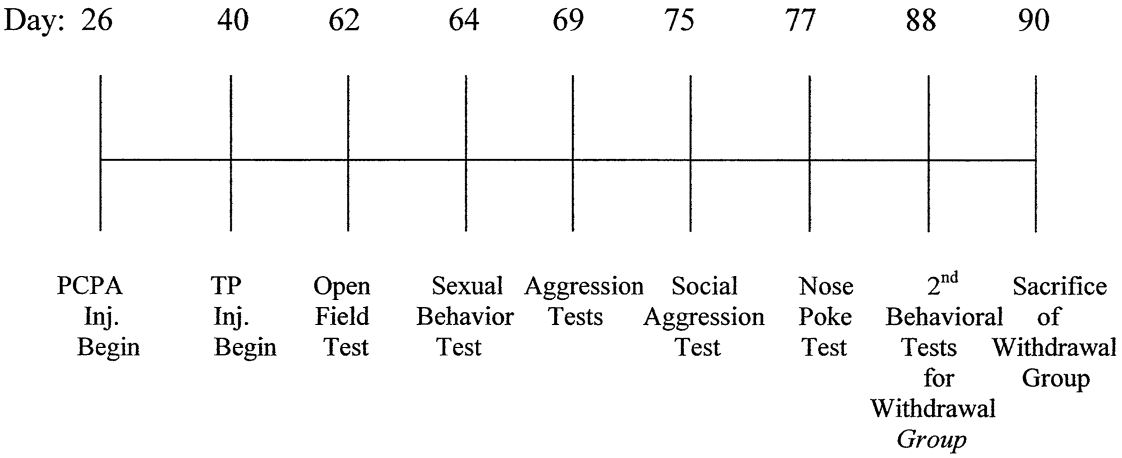
PCPA and AAS Treatment

PCPA injections, given intraperitoneally (ip), began on the day 26 of the experiment. Each of the animals either received 50 mg/kg of PCPA (Sigma Chemicals, St. Louis, MO) or the same volume of saline ip. This dose was lower than what had been used in previous studies (Keleta et al., 2006), but it was still expected to be sufficient to produce significant depletions of 5-HT in experimental subjects since the previous study had produced such a dramatic decrease in 5-HT using a 100 mg/kg dose. The lower dose

was also used so that a less dramatic and more physiological depletion range could be obtained. The PCPA injections were administered three times a week for the duration of the experiment for all groups except for the withdrawal group. The volume of the injection was determined at the beginning of the week, based on each group's calculated average weight.

On week number three, which corresponds to the first day of puberty for the experimental animals, TP or PEG were injected subcutaneously (5 mg/kg). This dose of the AAS, TP had been previously shown to produce heightened levels of aggression (Breuer & McGinnis, 2001; McGinnis et al., 2002a). TP injections were given five days a week for the duration of the experiment for all groups except for the withdrawal group. The volume of the injection was determined at the beginning of each week, based on the group's calculated average weight.

Treatments and behavioral tests occurred in accordance with this timeline:



Behavioral Tests

All behavior tests were conducted in the afternoon, which corresponds to the animal's 12 hour dark schedule. The tests were independently scored by two experimenters. All tests were video taped in case discrepancies occurred between scorers. Behavioral tests and injections occurred in accordance with this timeline:

Open Field Test

After five weeks of PCPA treatment and three weeks of AAS treatment the open field test was conducted. It took place in a square (60cm × 60cm × 30cm) plexi-glass container which was divided on the bottom into four (30 × 30 cm) quadrants. In the middle of these quadrants a 5 inch diameter circle was drawn. Each rat was placed in the appropriate quadrant and observed for 5 minutes. During this time the number of center crosses, vertical crosses, rearing behaviors, and grooming behaviors were recorded. The container was cleaned with alcohol after each trial and the frequency of each of these behaviors was recorded for the duration of the test. Behaviors were operationally defined as:

a.) Vertical crosses – the number of times the experimental animal moved from one quadrant to another with all four paws crossing into the next quadrant.

b.) Center crosses – the number of times the animal placed all four paws in the center circle.

c.) Grooming – the number of times the animal cleaned itself.

d.) Rearing - the number of times the animal stood on two feet.

e.) Total Crosses – the sum of vertical and center crosses.

Sexual Behavior Test

Animals were 64-65 days old and sexually naïve at the time of the sexual behavior test. Sexual behavior tests were performed in accordance with previous studies (Farrell & McGinnis, 2003: Vagell & McGinnis, 1998). Ten females, weighing between 225 and 250 grams were received two days before testing and were ovariectomized. Stimulus females were implanted with a 5mm capsule filled with 50% estradiol. The females were injected with 1 mg of progesterone on the day of the sexual behavior test. The experimental male and females were placed in an aquarium that was filled with fresh bedding. The frequency of mounts and intromissions, as well as the mount latency, intromission latency, ejaculation latency and the post-ejaculatory interval were recorded. Five females were used for each of the two days of sexual behavior testing. The test was terminated if an animal did not intromit, or mount in fifteen minutes. The behaviors were operationally defined as:

a.) Mount - the number of times the experimental animal mounted the female from behind with pelvic thrusts, but without penile insertion.

b.) Intromission – number of mounts with insertion of penis into vagina.

c.) Post-ejaculatory interval – the time period between the ejaculation and the next mount or intromission.

d.) Mount latency – the amount of time it takes for the first mount to occur.

e.) Intromission latency – the amount of time it takes for the first intromission to occur.

f.) Ejaculation latency – the amount of time it takes for the first ejaculation to occur.

Aggression Tests

Aggression tests were performed on week seven of the experiment. The bedding inside of the experimental animal's cage was not cleaned for two weeks prior to the aggression test. Ten intact males were received the day aggression tests began. These males ranged from 200 – 250 grams. Experimental animals ranged from 300 – 325 grams at this time. In the first series of aggression tests smaller males were placed in the cages of the experimental animals for ten minutes. During the test the number of dominance mounts, threats, boxing interactions, dominance postures, lateral kicks, dominance mount latency, and threat latency were recorded and operationally defined as:

a.) Dominance mount -the number of times the experimental animal attempted to mount the opponent male.

b.) Threats – an experimental animal threatening an opponent, defined by pilo-erection and threatening stances by the experimental animal.

c.) Boxing - both experimental male and opponent standing on two legs and fighting with their front limbs.

d.) Dominance postures – a posture by the experimental animal that is the result of fighting in which the opponent lies in a supine position with the experimental animal on top.

e.) Lateral kicks - kicks occurring from the experimental animal on the opponent male.

f.) Combined Aggression Score – the sum of dominance mounts, threats, boxing, dominance postures and lateral kicks.

A second series of aggression tests were conducted in the same manner as the first but included a tail pinch procedure, as used in previous studies (Farell & McGinnis, 2004; McGinnis, 2004; McGinnis et al, 2002a). Experimental males were physically provoked by pinching the tail of the animal every 60 seconds for the duration of the 10 minute test. Aggression measures were recorded in the same manner as previously described.

Social Aggression Test

The animals were 75 days old when the social aggression test was conducted. In the social aggression test four pairs of stimulus males and females were housed together two weeks prior to the test. Three hours before the behavior test the paired females were injected with progesterone to induce receptivity and then they were separated from the males. Each experimental male was placed into the cage of the paired animals and observed for ten minutes. Sexual and aggressive behaviors were operationally defined and recorded in the same manner as they were in the separate sex and aggression behavior tests.

A separate social aggression test was performed when the animals were 82 days old, in the same manner as the original test, except that the stimulus females were not injected with progesterone to induce sexual receptivity.

Nose-Poke Test

The nose-poke behavior test was performed when the animals were 77 days old. Animals acquired both smell and taste of the oreo cookies by placing a piece of the cookie in the experimental cage the day before the test and allowing the animal to eat the small piece of oreo. The animals were food deprived for 24 hours prior to the nose-poke

behavior test. The experimental setup was a 16 inch × 6 inch plexi-glass cage. One of the sides had a small hole size cut out in it at the level of the animal's nose. On the other side of the hole a funnel was taped to the wall with oreo cookies in it. This device allowed the animal to smell the oreo but not eat it. Animals were placed inside the experimental cage and the frequency and duration of the nose pokes were recorded. Behaviors were operationally defined as:

a.) Nose poke – the number of times each animal put its nose in the hole containing the oreo crumbs.

b.) Freezing – the amount of time the experimental animal stood stationary without moving.

c.) Nose poke latency – the amount of time it takes before the animal first nose pokes.

Withdrawal Group's Second Set of Behavioral Tests

The withdrawal group received a second set of behavioral tests 10 days after their last injection of TP + PCPA. Sexual behavior, open field, aggression, and paired impulsivity tests were performed in the same manner as was explained previously.

Tissue Samples and HPLC Analysis

When the animals were 85 days old the TP, PCPA, TP + PCPA and the Control group was sacrificed using a lethal dose (3mL) of chloral hydrate. Animals received their final injections one hour before they were sacrificed. Five brain regions (frontal cortex, striatum, hypothalamus, dorsal hippocampus, and brain stem) that are known for having high concentrations of serotonin were removed and separated from each of the animals sacrificed. These were placed in pre-weighed 2 mL ependorph fliptop tubes and stored at

– 80 degrees Celsius. The withdrawal group was sacrificed in the same manner as the first four groups 15 days after their last injection.

After shipping samples in dry ice to Yale University tube weights were recorded with the samples in them. The internal standard solution (ISS) was then made by combining 99mL of DI water, 100mg of Absorbic Acid, and 1 g of MBS, 50 ul of N-methyl serotonin. Based on the weight of the sample .5 – 2 mls were added to each of the samples, depending on the brain region being analyzed. After the addition of the ISS all samples were sonified. Perchloric acid was then added to each sample and the sample was then vortexed. The supernatant was then poured into separate ependorf tubes and the pellet was thrown away. The samples were then placed into injection tubes and loaded into the HPLC machine. Reverse-phase HPLC was then used to determine the 5-HT, 5-HIAA and tryptophan concentrations in the frontal cortex, striatum, hypothalamus, dorsal hippocampus, and brain stem of the experimental animals. The HPLC machine was made up of the following sections:

a) Mobile Phase: The mobile phase is continuously pumped through the column. The mobile phase was made up of 80% (.1M phosphoric acid, 150 mg/L Na-octyl sulfate, 50 mg/L Na-2-EDTA). 20% MeOH (2500ml HPLC water, 20.25ml phosphoric acid, 450mg octyl sulfate, 150mg EDTA). This mixture was then brought to a pH of 4.65.

b) Stationary Phase and Column: The stationary phase, which was inside the 25 × 4 cm column, was made up of C18 polar resin. Separation of chemicals occurred within the column based on the hydrophobic characteristic of each chemical.

c) Pump: the pump cycled the mobile phase solution through the column. The flow rate was set to 1ml/min. The pressure inside the column remained close to 2130 PSI.

d) Automatic injector: The automated injector injected 25 micro liters of STDs and samples into the column.

e) Column Heater: The column heater heated the column to 40°c.

f) Fluorometer: Selected out the fluorescent 5-HT and metabolites. The excitation wavelength was set to 285 nm. The emission wavelength was set at 342 nm.

g) Chromatographic Chart Recorder: The chromatographic chart recorder processed the output from the flourometer and provided a quantifiable measurement of the amount of 5-HT and its metabolites. This measurement was a series of four peaks corresponding to 5-HT, tryptophan, 5-HIAA and the known standard solution.

Statistical Analysis

Comparisons of the groups by analysis of variance (ANOVA) was performed using the software package, Statview. Fisher's PLSD post hoc analysis was used for detecting significant main effects. Comparisons between groups were based on a two-tailed test with the significance value set at $p<.05$.

CHAPTER VII

Results

Behavioral Tests

Open Field Test

The number of center and vertical crosses were combined to yield the total number of crosses for each animal. Both the PCPA alone group and the TP + PCPA group exhibited significantly fewer total crosses ($p<.05$) than controls, as shown in Figure 4. The TP + PCPA group also exhibited significantly ($p<.05$) fewer vertical crosses than controls, as shown in Table 1. No significant differences in rearing behavior were found between any of the groups. The TP alone group did not differ significantly from controls in any of the open field measures. Following withdrawal from the combination of TP + PCPA locomotor activity returned to control levels.

Sexual Behavior Test

The latency to mount was found to vary depending on treatment. Both PCPA and TP + PCPA groups exhibited significantly ($p<.05$) shorter mount latencies compared to controls, as shown in Table 2. Neither the PCPA alone group nor the TP + PCPA group significantly differed from controls in any of other sexual behaviors tested. The TP treated males did not significantly differ from controls in any of the sexual behaviors measured. Following withdrawal from the combination of TP + PCPA mount latency returned to control levels and all other measures remained at control levels.

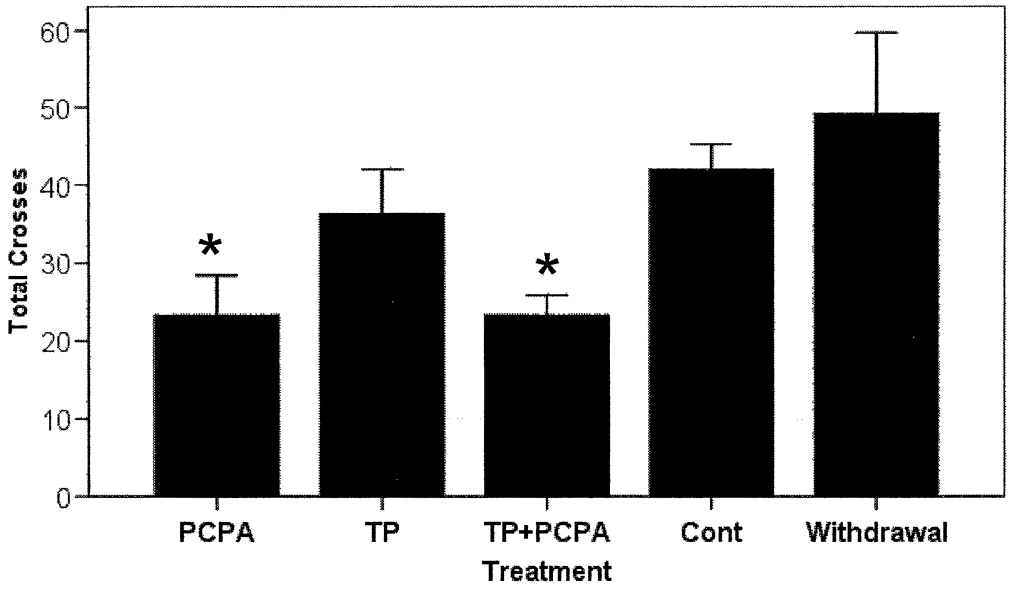


Figure 4. Total Crosses in Open Field Test. Mean (± SEM) number of total crosses for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p < .05$) difference relative to controls.

Table 1. Open Field Test. Mean number of vertical crosses, and rearings for each treatment group during the open field test, followed by the SEM. N's are provided in parenthesis.
* Indicates a significant ($p<.05$) difference relative to controls.

	PCPA	TP	TP + PCPA	Cont	Withdrawal
Vertical Crosses	25.5 ± 6.0 (6)	26.2 ± 5.5 (6)	*23.0 ± 2.5 (12)	39.4 ± 2.5 (5)	49.2 ± 10.5 (5)
Rearings	27.0 ± 5.7 (6)	33.5 ± 6.9 (6)	22.4 ± 3.0 (12)	31.2 ± 2.5 (5)	36.8 ± 8.5 (5)

Table 2. Sexual Behavior Test. Shown are the mean mount latency, intromission latency, ejaculation latency, number of mounts, number of intromissions, and PEI for each treatment group during the sexual behavior test. The table also shows the SEM, followed by the N in parentheses.
* Indicates a significant ($p<.05$) difference relative to controls.

	PCPA	TP	TP + PCPA	Cont
Mount Latency (s)	*550.2 ± 188.5 (6)	794.0 ± 198.7 (5)	*416.2 ± 70.9 (10)	1180.0 ± 320 (2)
Intromission Latency (s)	592.8 ± 195.1 (6)	758.0 ± 188.1 (5)	445.6 ± 102.2 (10)	883.0 ± 436.0 (2)
Ejaculation Latency	902.8 ± 243.5 (6)	1320.6 ± 173.3(5)	976.8 ± 83.3 (10)	1438.0 ± 195.0 (2)
Mounts	2.7 ± .6 (6)	2.3 ± 1.1 (6)	3.3 ± .7 (12)	.8 ± .6 (5)
Intromissions	8.3 ± 1.9 (6)	7.0 ± 2.1 (6)	7.0 ± 1.2 (12)	6.4 ± 4.8 (5)
PEI (s)	1121.7 ± 199.9 (6)	1771.6 ± 195.0 (5)	1584.2 ± 264.0 (10)	1873.5 ± 231.5 (2)

Aggression Test Without Provocation

In the aggression test without provocation the TP + PCPA group exhibited significantly ($p<.05$) more dominance mounts than controls, as shown in Figure 5A. The PCPA alone group did not significantly differ from controls in number of dominance mounts they exhibited, although their mean was the highest. TP alone were not significantly different from controls in any of the aggression measures. None of the groups differed significantly in composite aggression score compared to controls, as shown in Figure 5B. Following withdrawal from the combination of TP + PCPA aggression measures returned to control levels.

Aggression Test With Provocation

In the aggression test with provocation the PCPA alone, and the TP +PCPA groups elicited significantly ($p<.05$) more dominance mounts than controls, as shown in Figure 5C. TP alone, however, did not elicit a significant difference from controls in the frequency of dominance mounts. Following withdrawal from the combination of TP and PCPA the number of dominance mounts returned to control levels and was significantly less than the TP+PCPA group. All other aggression measures remained at control levels for the withdrawal group.

The composite aggression score was determined by summing all aggressive behaviors including: dominance mounts, threats, boxing encounters, dominance postures and lateral kicks. Both the PCPA alone and the TP + PCPA groups exhibited significantly ($p<.05$) greater composite aggression scores than controls, as shown in Figure 5D. The TP group did not exhibit a significant increase in composite aggression

score relative to controls. Following withdrawal from the combination of TP + PCPA the composite aggression score returned to control levels.

There were no significant differences between any of the treatment groups in any other aggression measure for this test. However, an interesting effect was obtained by comparing the frequency of dominance mounts within the TP group between the aggression test without provocation and the aggression test with provocation. The number of dominance mounts by TP males differed significantly ($p<.05$) between the two aggression tests, as shown in Figure 5E. The dominance mounts exhibited following provocation was significantly greater than when tested without tail pinch. This effect was not found in any of the other groups.

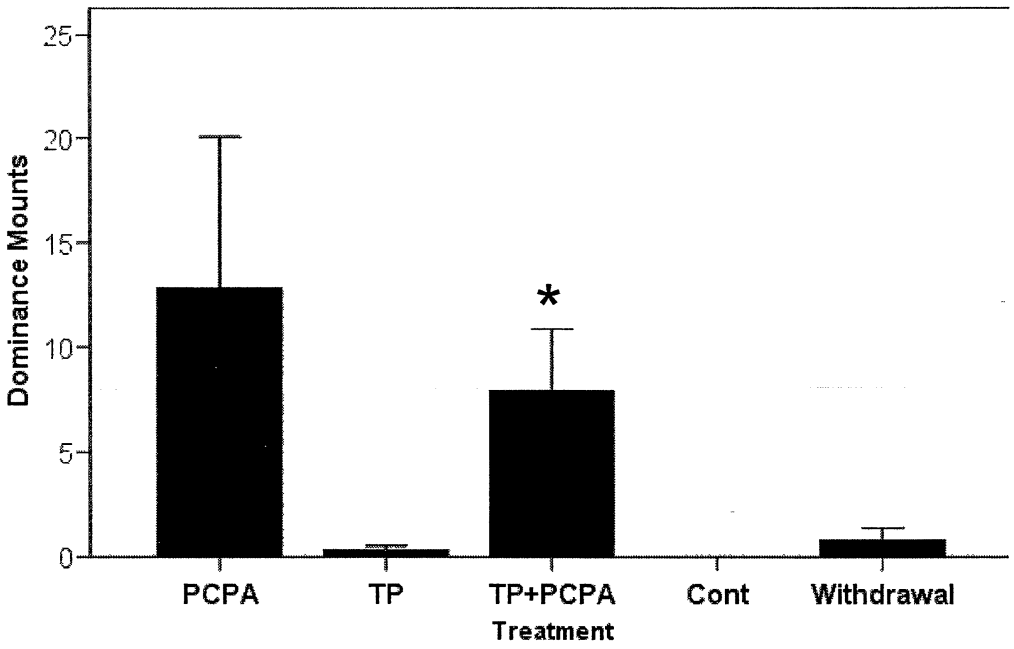


Figure 5A. Dominance Mounts in Aggression Test Without Provocation. Mean (± SEM) number of dominance mounts for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p < .05$) difference relative to controls.

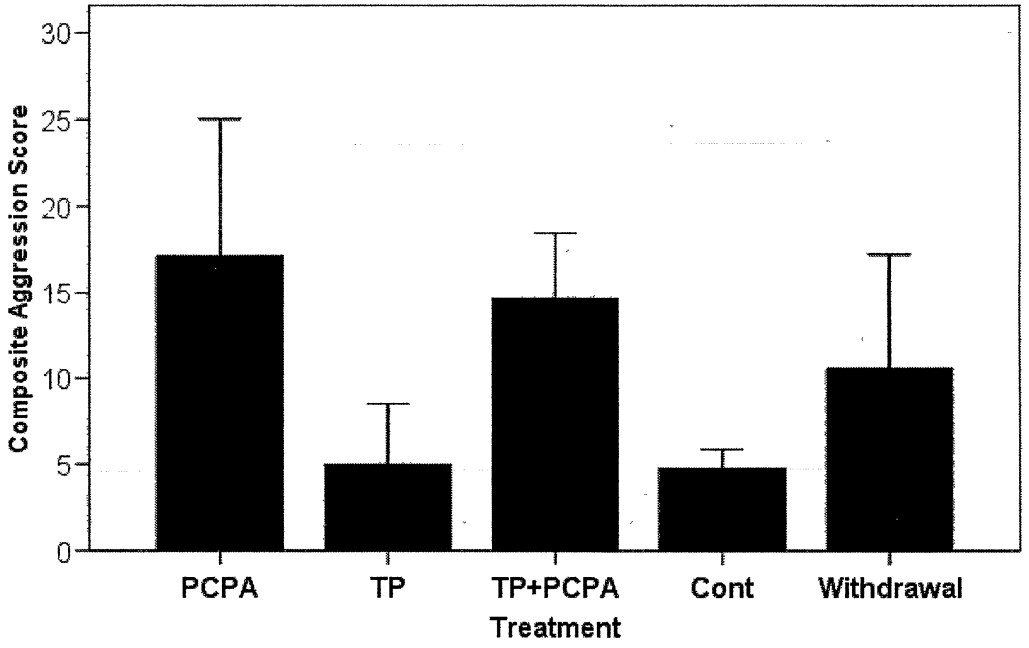


Figure 5B. Composite Aggression Scores in Aggression Test Without Provocation. Mean (± SEM) composite aggression score for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p<.05$) difference relative to controls.

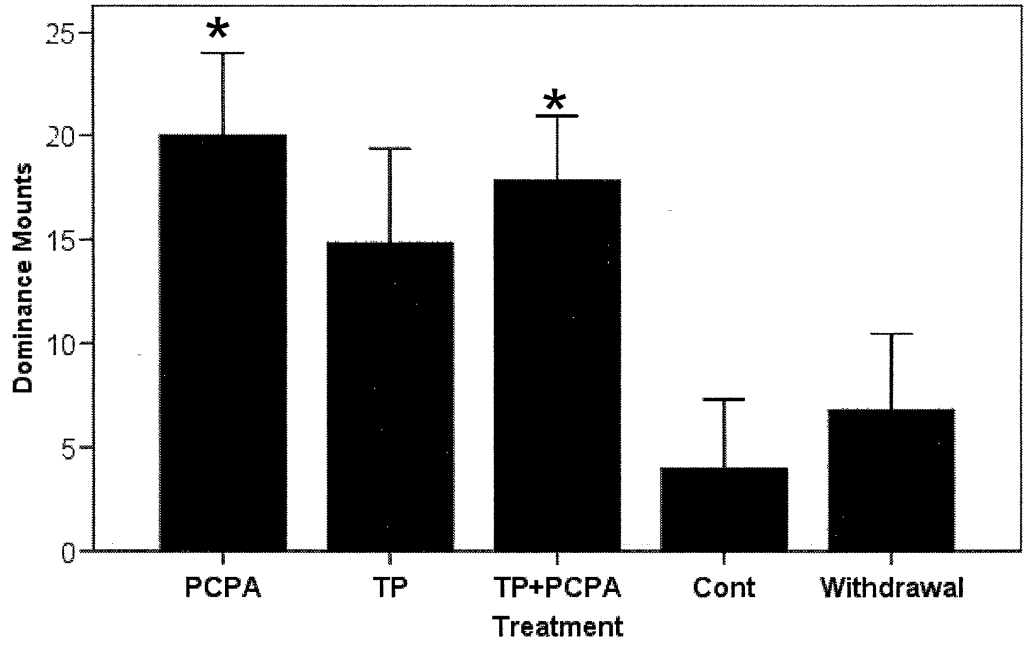


Figure 5C. Dominance Mounts in Aggression Test With Provocation. Mean (± SEM) number of dominance mounts for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p < .05$) difference relative to controls.

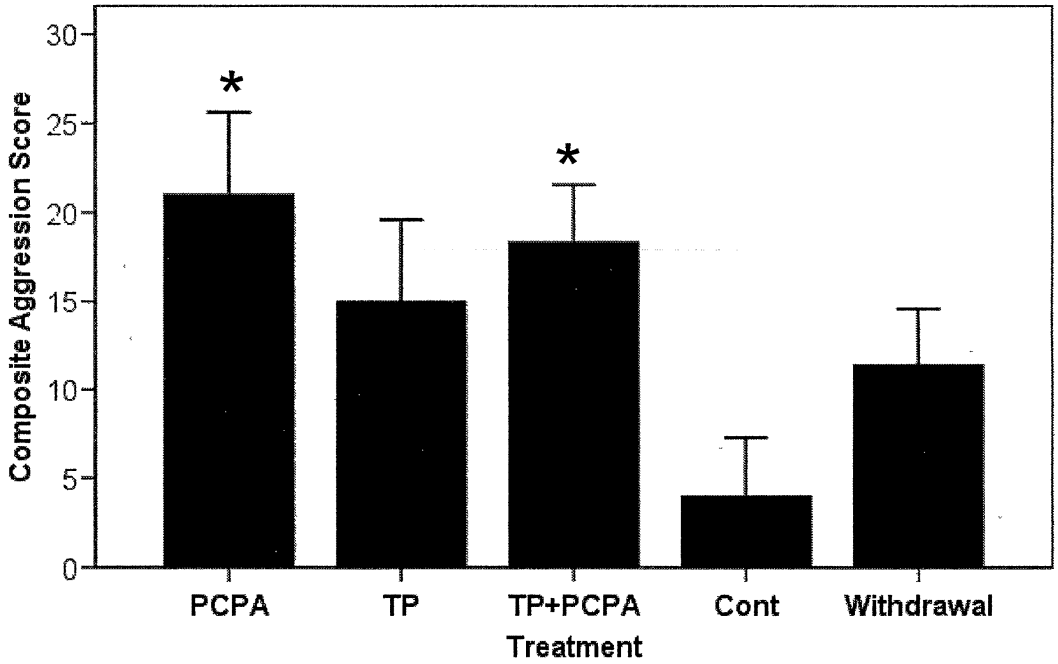


Figure 5D. Composite Aggression Scores in Aggression Test With Provocation. Mean (± SEM) composite aggression score for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).

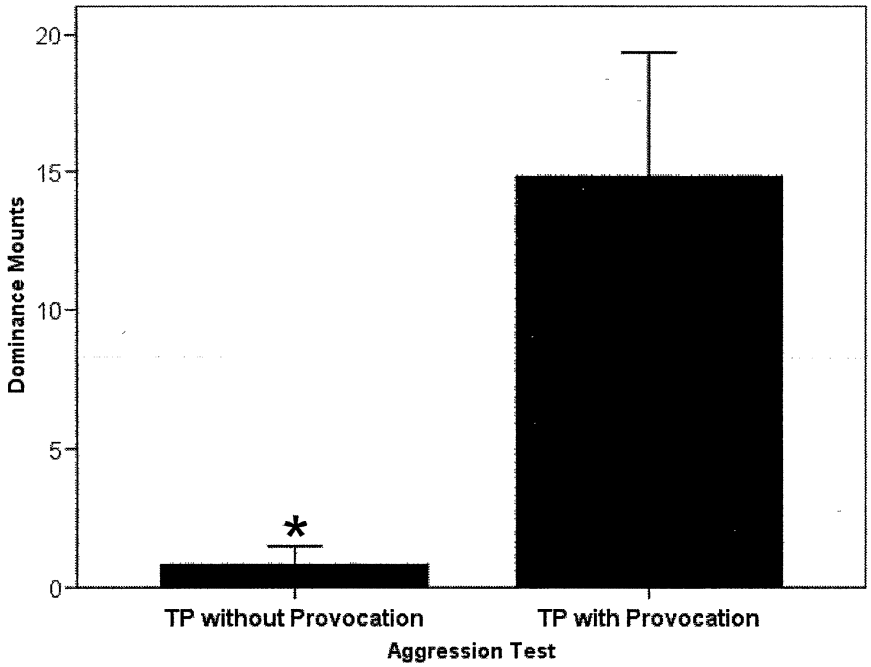


Figure 5E. Dominance Mounts in Aggression Test With and Without Provocation for the TP Group. Mean (± SEM) number of dominance mounts for the TP group for aggression tests with and without provocation.

* Indicates a significant ($p<.05$) difference between aggression tests within the same

Social Aggression Test With Receptive Female

In the social aggression test, using a receptive female, both the TP alone group and the TP+PCPA group displayed significantly ($p<.05$) greater composite aggression scores compared to controls, as shown in Figure 6A. There was no significant difference from the control group in the composite aggression score for the group receiving only PCPA. Following withdrawal from the combination of TP + PCPA composite aggression scores returned to control levels. There were no significant differences between any of treatment groups in any other aggression measures observed during this social aggression test.

There were no significant differences between any of the treatment groups in any of the sexual measures observed during the social aggression test with the receptive female (Fig 6B), although there was a trend ($p<.07$) toward higher frequencies of combined intromissions and mounts by the TP group compared to controls.

Social Aggression Test With a Non-Receptive Female

In the social aggression test using a non-receptive female there were no significant differences in any of the sexual or aggression measures between any of the groups (Table 3). The TP + PCPA and the PCPA alone groups did not significantly differ from controls in any of the sexual or aggression measures for this test. The TP alone group also did not differ from controls in any measure. Following withdrawal from the combination of TP and PCPA all measures remained at control levels.

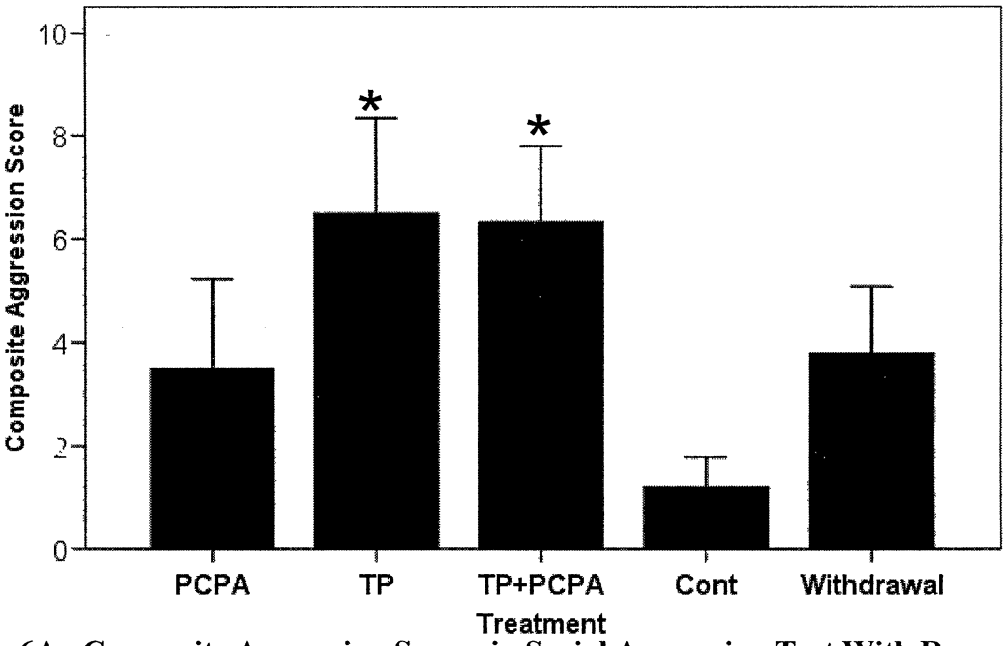


Figure 6A. Composite Aggression Scores in Social Aggression Test With Receptive Female. Mean (± SEM) composite aggression score for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p<.05$) difference relative to controls.

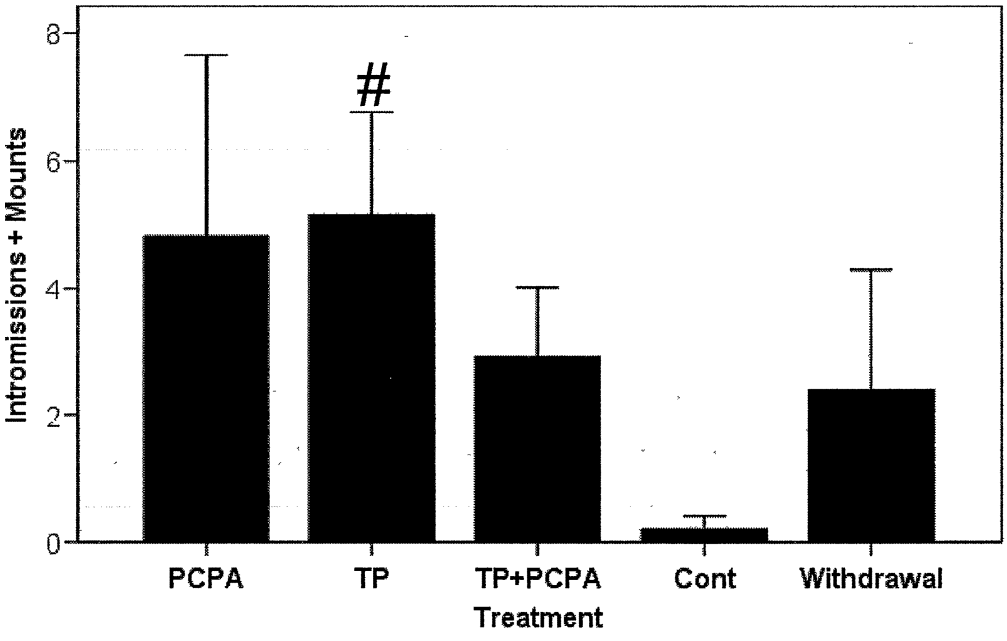


Figure 6B. Intromissions + Mounts in Social Aggression Test With Receptive Female. Mean (± SEM) number of combined mounts and intromissions for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).
# Indicates a ($p<.07$) difference relative to controls

Table 3. Social Aggression Test With Non-Receptive Female. Shown are the mean number of mounts, intromissions, dominance mounts threats, boxing interactions, dominance postures, attacks, lateral kicks and the composite aggression score followed by the SEM. N's are provided in parenthesis.

	PCPA	TP	TP + PCPA	Cont
Mounts	1.8 ± 1.5 (6)	1.8 ± .8 (6)	2 ± .8 (12)	0 ± 0 (5)
Intromissions	1.5 ± .8 (6)	.7 ± .7 (6)	.8 ± .5 (12)	0 ± 0 (5)
Dominance Mounts	2.8 ± 1.4 (6)	1.0 ± .4 (6)	2.2 ± .9 (12)	.4 ± .4 (5)
Boxing	± .5 (6)	1.7 ± .8 (6)	.3 ± .3 (12)	0 ± 0 (5)
Dominance Postures	.7 ± .2 (6)	.5 ± .3 (6)	.3 ± .2 (12)	.6 ± .2 (5)
Lateral Kicks	1.8 ± 1.0 (6)	3.0 ± 1.6 (6)	1.7 ± .5 (12)	.2 ± .2 (5)
Threats	.2 ± .2 (6)	.2 ± .2 (6)	.5 ± .3 (12)	0 ± 0 (5)
Attacks	0 ± 0 (6)	.3 ± .3 (6)	.3 ± .2 (12)	.4 ± .2 (5)
Composite Aggression Score	6.0 ± 2.0 (6)	6.7 ± 2.8 (6)	5.2 ± 1.1 (12)	1.6 ± .9 (5)

Nose-Poke Test

During the nose poke test nose poke frequency, nose poke duration, latency to nose poke and freezing duration were recorded. No significant differences in nose poke frequency, or freezing behavior were found between any of the treatment groups compared to controls, as shown in Table 4. However, there was a significant difference found in the latency to nose poke. The PCPA group exhibited a significantly ($p<.05$) longer latency to nose poke relative to controls, as shown in Figure 7A. The PCPA group also showed a non-significant increase in freezing behavior compared to controls, as shown in Figure 7B. Following withdrawal from the combination of TP and PCPA all nose poke test measures remained at control levels.

Table 4. Nose-Poke Test. Shown are the mean nose poke frequency and nose poke duration for each group. The table also shows the SEM. N's for each treatment group are provided in parenthesis.

	PCPA	TP	TP + PCPA	Cont	Withdrawal
Nose Poke Frequency	3.0 ± 1.1 (6)	6.7 ± 2.2 (6)	6.9 ± 1.4 (12)	3.6 ± .4 (5)	5.8 ± 1.9 (5)
Nose Poke Duration	2.0 ± .6 (6)	4.2 ± .7 (6)	5.3 ± 1.2 (12)	2.2 ± .4 (5)	0 ± 0 (5)

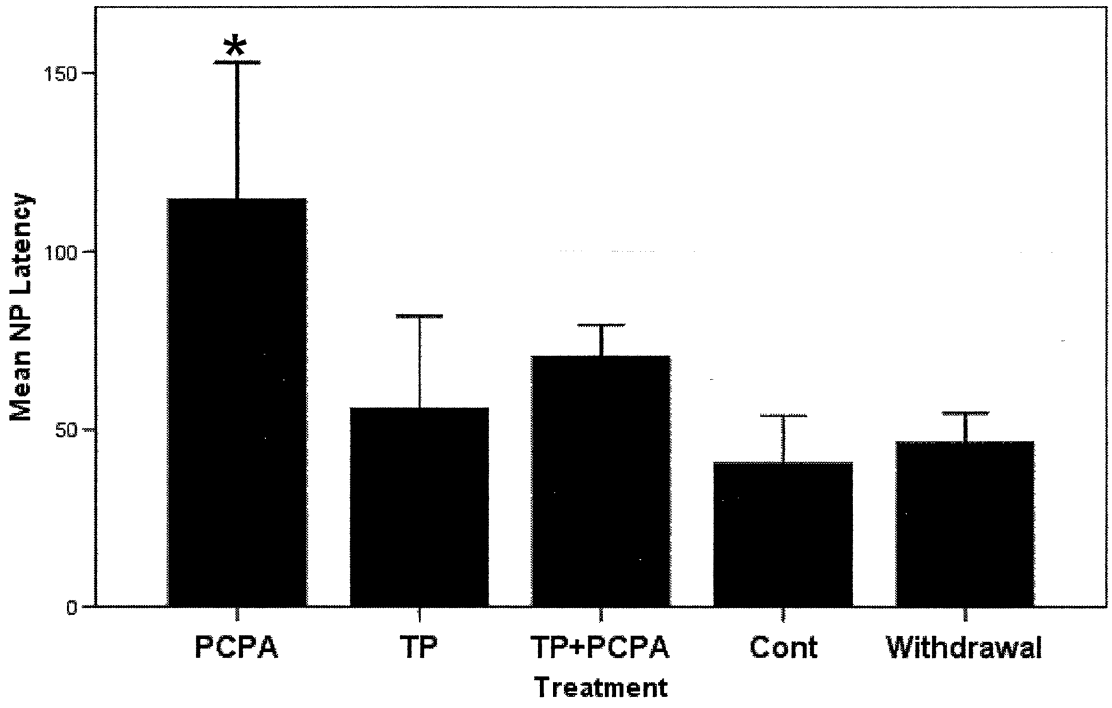


Figure 7A. Nose Poke Latency in Nose-Poke Test. Mean (± SEM) latency, in seconds, to nose poke for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).

* Indicates a significant ($p<.05$) difference relative to controls.

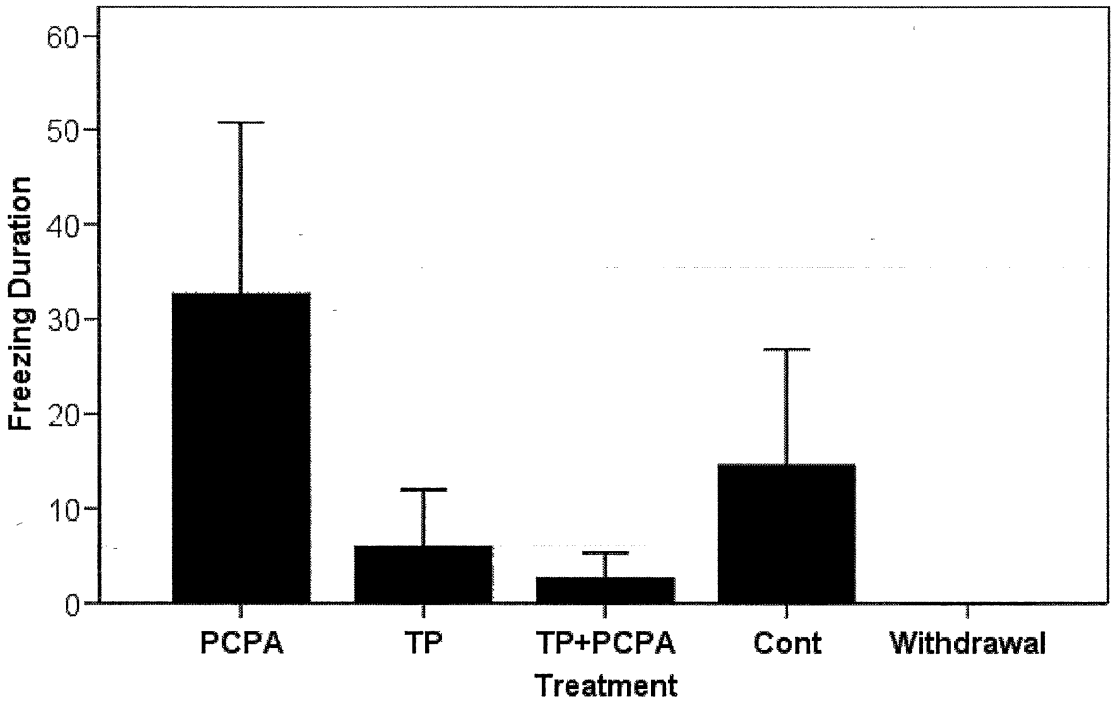


Figure 7B. Freezing Duration in Nose-Poke Test. Mean (± SEM) freezing duration, in seconds, for each of the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=12), Cont (n=5), Withdrawal (n=5).

Biochemical Data

5-HT Analysis

HPLC revealed 5-HT concentrations in the cortex, striatum, hypothalamus, brainstem and hippocampus were significantly ($p<.01$) depleted in both the PCPA alone and the TP + PCPA groups compared to controls, as shown in Figures 8A-8E. PCPA alone reduced 5-HT 55.1% - 78.7% compared to controls, as seen in Table 5A. TP+PCPA reduced 5-HT concentrations 62.5%-88.2% compared to controls. 5-HT concentrations in the cortex were significantly ($p<.01$) greater in the TP alone group compared to controls, as shown in Figure 8A. The TP group did not significantly differ from the control group in any other brain region analyzed. Following withdrawal from the combination of TP + PCPA there were no significant differences in 5-HT concentrations compared to controls.

Tryptophan Analysis

TP + PCPA was found to significantly reduce tryptophan concentrations 31.1% - 66.1% in the cortex, striatum, hypothalamus, brainstem and hippocampus compared to controls, as shown in Figures 9A – 9E and Table 5B. The administration of PCPA alone significantly ($p<.01$) lowered tryptophan concentrations by 20.5% - 65.5% in the cortex, hypothalamus, brainstem and hippocampus, relative to controls (Table 5B). In the striatum PCPA only lowered tryptophan concentrations by 2.1%. Relative to controls, TP significantly ($p<.01$) reduced tryptophan concentrations, 19.3% to 27.9%, in all brain regions except for the brainstem, where TP increased tryptophan concentrations by 42.5% (Table 5B). Following withdrawal from the combination of TP + PCPA, tryptophan concentrations returned to control levels.

5-HIAA Analysis

HPLC revealed 5-HIAA concentrations in the cortex, striatum, hypothalamus, brainstem and hippocampus were significantly ($p<.01$) depleted in both the PCPA alone and the TP + PCPA groups compared to controls, as shown in Figures 10A – 10E. TP +PCPA depleted 5-HIAA concentrations 66.1% - 97.4%, while PCPA alone depleted 5-HIAA concentrations 65.1 – 95.1% relative to controls, as seen in Table 5C. TP alone was found to significantly deplete 5-HIAA concentrations, relative to controls, in the hypothalamus 39.2% (Table 5C). 5-HIAA concentrations in the TP group did not significantly differ from control levels in any other brain region analyzed. Following withdrawal from the combination of TP in combination with PCPA, 5-HIAA concentrations in the hippocampus were still significantly ($p<.05$) lower than controls. In all other brain regions the withdrawal group had 5-HIAA levels that were not significantly different from controls, but significantly higher than those from the TP + PCPA group.

5-HT Turnover Analysis

The results from analysis of serotonin metabolic turnover (5-HIAA/5-HT ratio) in the cortex, striatum, hypothalamus, brainstem, and hippocampus are provided in Figures 11A-11E. TP + PCPA significantly ($p<.01$) reduced 5-HT turnover in the striatum and hippocampus. PCPA alone significantly ($p<.05$) reduced turnover in the striatum, hypothalamus, and the hippocampus. TP alone significantly ($p<.01$) reduced turnover in the cortex and the hypothalamus. Following withdrawal from the combination of TP and PCPA there were no significant differences in turnover compared to controls.

Figure 8A. Cortex.

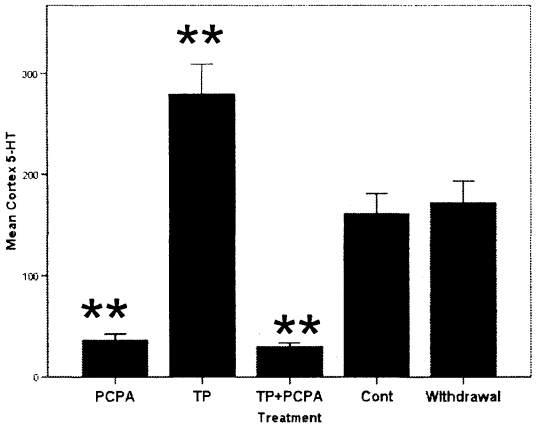


Figure 8B. Hypothalamus.

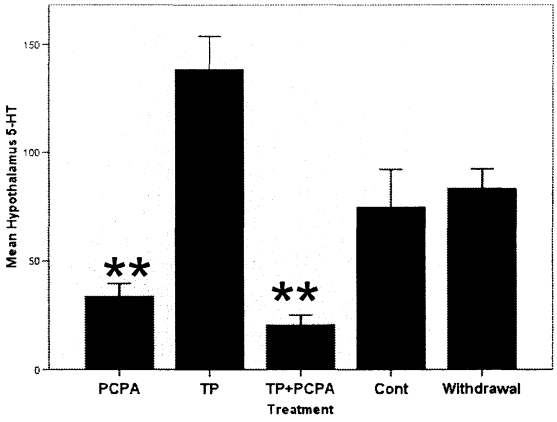


Figure 8C. Brain Stem.

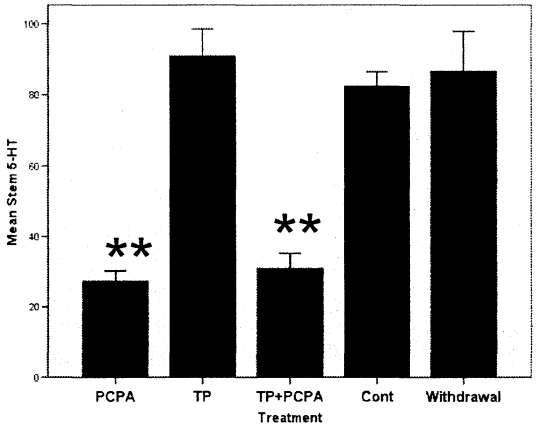


Figure 8D. Hippocampus.

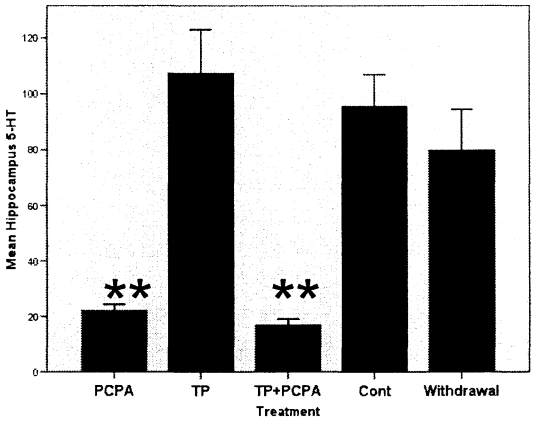


Figure 8E. Striatum.

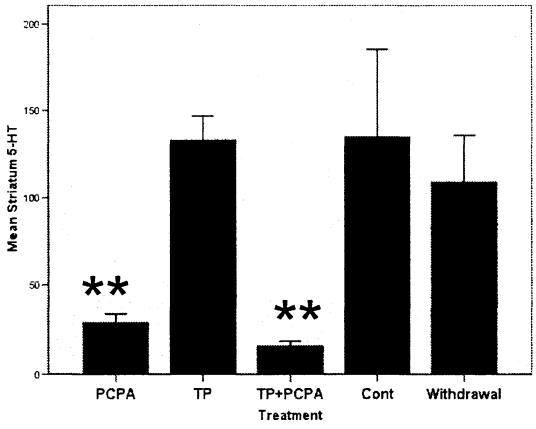


Figure 8 A-E. 5-HT Figures. Mean (± SEM) 5-HT concentration (ng/g) for the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=6), Cont (n=5), Withdrawal (n=5).
** Indicates a significant ($p<.01$) difference relative to controls.

Figure 9A. Cortex.

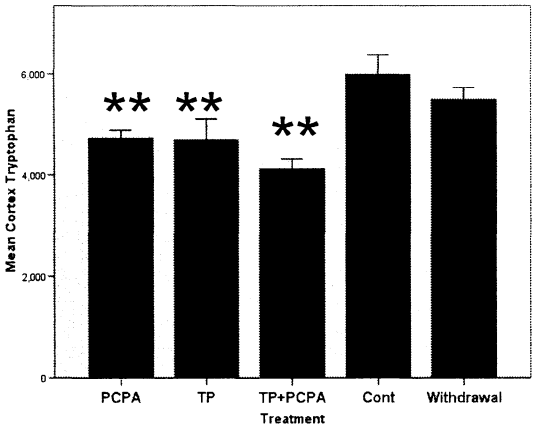


Figure 9B. Hypothalamus

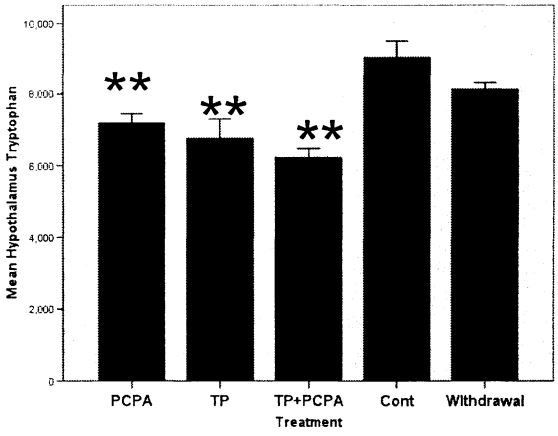


Figure 9C. Brain Stem.

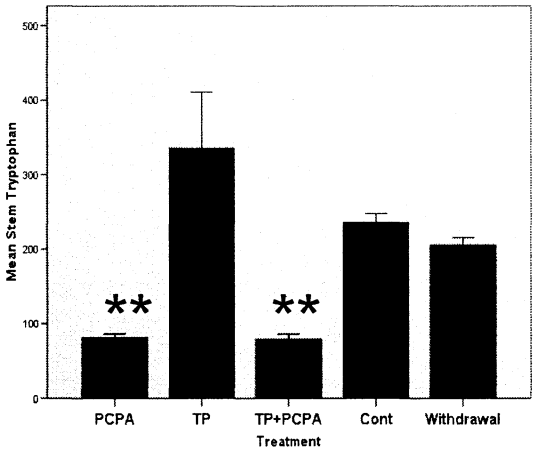


Figure 9D. Hippocampus.

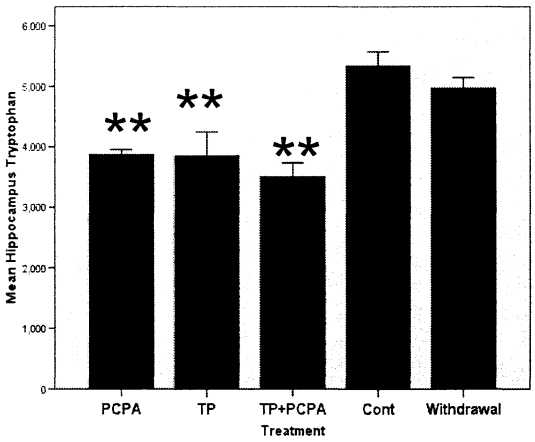


Figure 9E. Striatum.

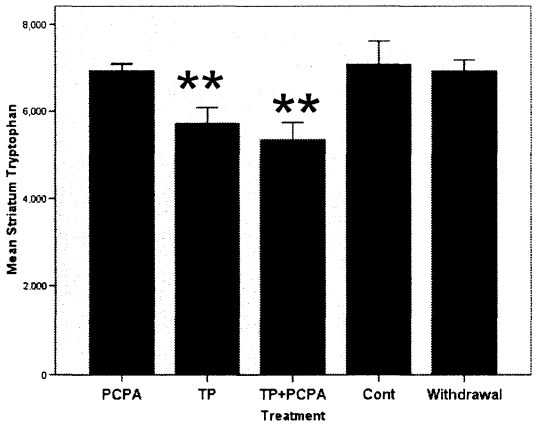


Figure 9 A-E. Tryptophan Figures. Mean (± SEM) tryptophan concentration (ng/g) for the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=6), Cont (n=5), Withdrawal (n=5).
** Indicates a significant ($p<.01$) difference relative to controls.

Figure 10A. Cortex.

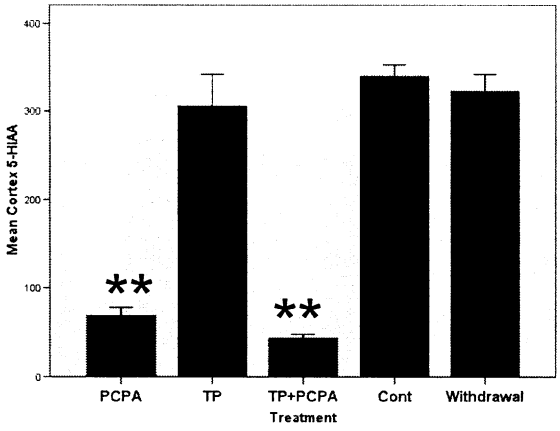


Figure 10B. Hypothalamus.

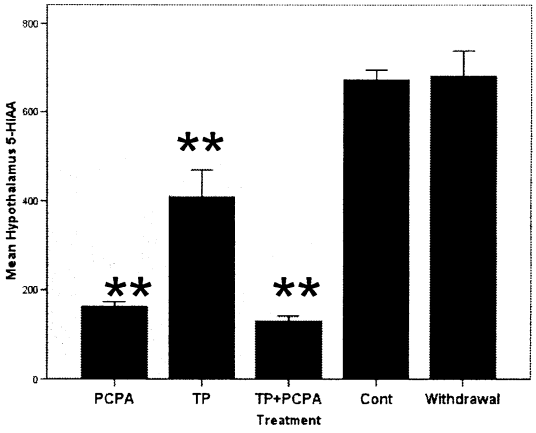


Figure 10C. Brain Stem.

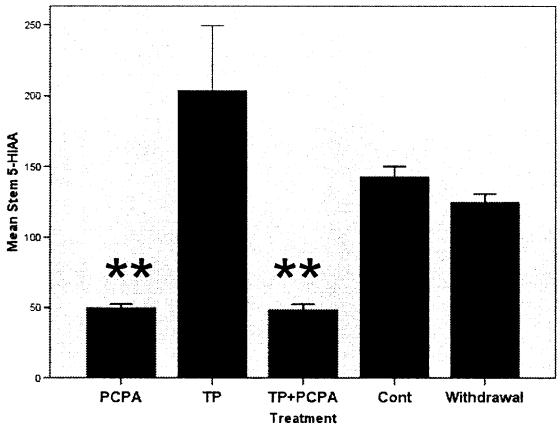


Figure 10D. Hippocampus.

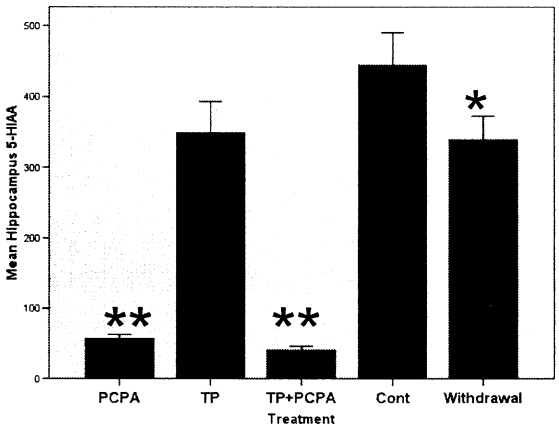


Figure 10E. Striatum.

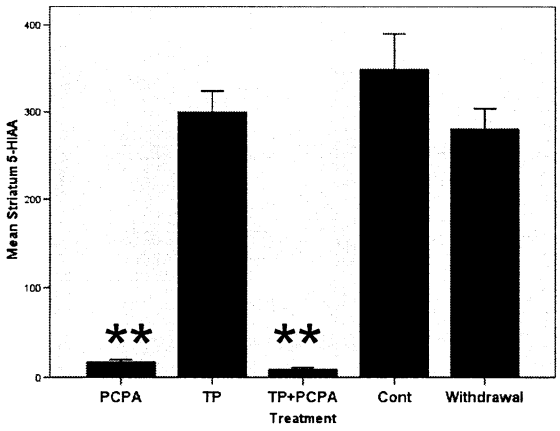


Figure 10 A-E. 5-HIAA Figures. Mean (± SEM) 5-HIAA concentration (ng/g) for the five treatment groups: PCPA (n=6), TP (n=6), TP +PCPA (n=6), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p<.05$)
** Indicates a significant ($p<.01$) difference relative to controls.

Figure 11A. Cortex.

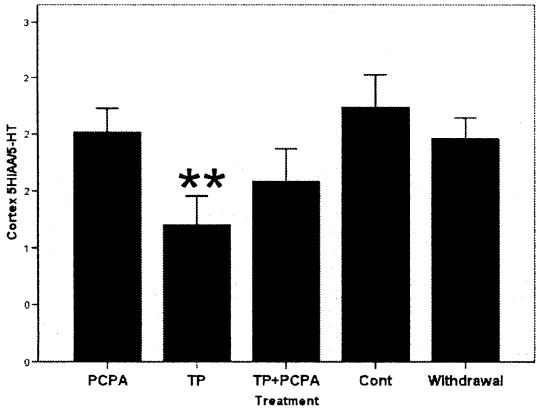


Figure 11B. Hypothalamus.

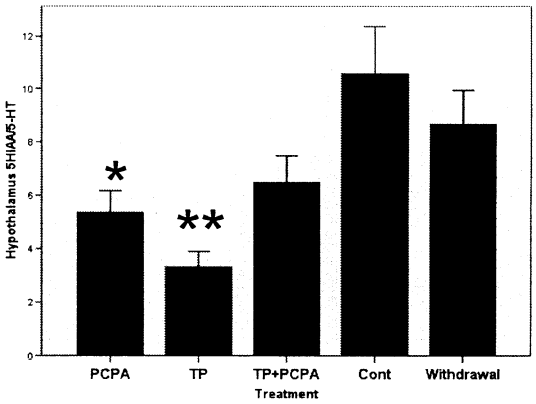


Figure 11C Brain Stem.

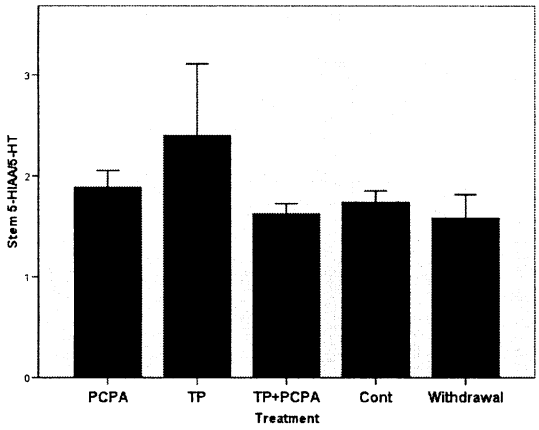


Figure 11D. Hippocampus.

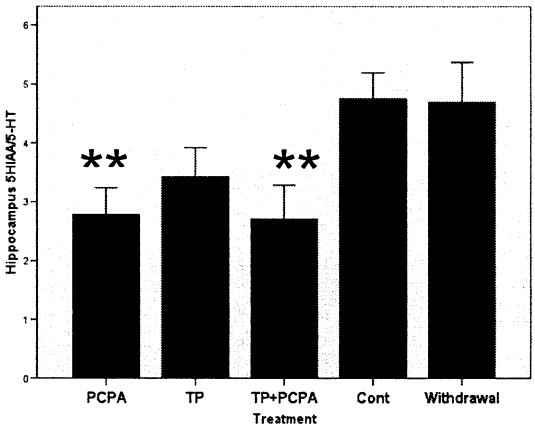


Figure 11E. Striatum

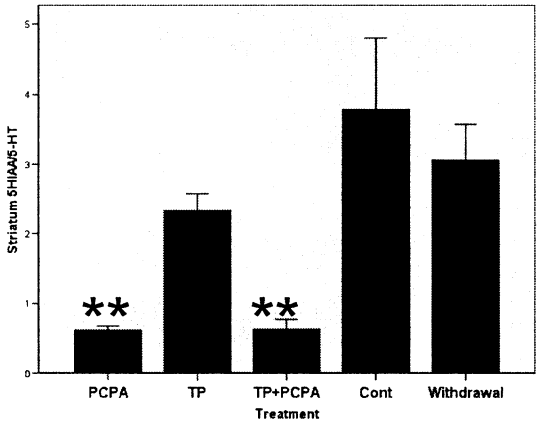


Figure 11 A-E. 5-HT Turnover Figures. Mean (± SEM) 5-HIAA/5-HT metabolic turnover for the five treatment groups: PCPA (n=6), TP (n=6), TP + PCPA (n=6), Cont (n=5), Withdrawal (n=5).
* Indicates a significant ($p<.05$) difference relative to controls.
** Indicates a significant ($p<.01$) difference relative to controls.

Table 5A – 5C. Depletion Percentages. Values are expressed as percent deletion relative to controls for the four treatment groups: PCPA (n=6), TP (n=6), TP + PCPA (n=6), Withdrawal (n=5).
* Indicates a significant ($p<.05$) difference from controls.
** Indicates a significant ($p<.01$) difference from controls.

	PCPA	TP	TP + PCPA	Withdrawal
Cortex	77.6%**	+73.0%**	81.53%**	+6.6%
Striatum	78.7%**	1.6%	88.2%**	19.4%
Hippocampus	76.9%**	+12.5%	82.3%**	16.4%
Stem	66.9%**	+10.2%	62.5%**	+5.1%
Hypothalamus	55.1%**	+84.5%	72.5%**	+11.3%

Table 5A

Tryptophan Depletion Percentages Relative to Controls

	PCPA	TP	TP + PCPA	Withdrawal
Cortex	20.9%**	21.5%**	31.2%**	8.32%
Striatum	2.1%	19.3%**	24.7%**	2.16%
Hippocampus	27.5%**	27.9%**	34.5%**	6.9%
Stem	65.1%**	+42.5%	66.1%**	12.6%
Hypothalamus	20.5%**	25.2%**	31.1%**	10.1%

Table 5B

5-HIAA Depletion Percentages Relative to Controls

	PCPA	TP	TP + PCPA	Withdrawal
Cortex	79.7%**	9.9%	87.2%**	4.9%
Striatum	95.1%**	14.1%	97.4%**	19.6%
Hippocampus	87.1%**	21.4%	90.8%**	23.6%*
Stem	65.1%**	+42.5%	66.1%**	12.6%
Hypothalamus	75.8%**	39.2%**	80.6%**	+1.3%

Table 5C

CHAPTER VIII

DISCUSSION

The current study provides a comprehensive behavioral and neurochemical assessment of both short and long term effects of AAS alone and in combination with low 5-HT in adolescence.

Previously, it was reported that chronic administration of a 100 mg/kg dose of PCPA alone and in combination with AAS induces significant depletion of 5-HT and 5-HIAA levels in adolescent male Long Evans Rats (Keleta, 2007). The current study reports that administration of a significantly lower dose of PCPA (50 mg/kg) alone and in combination with chronic exposure to AAS throughout adolescence produces significant, but less dramatic depletion of central 5-HT and 5-HIAA in the hypothalamus, hippocampus, striatum, frontal cortex, and brain stem. This is a very small dose compared to previous studies examining the behavioral effects of PCPA (Matte & Turnow, 1978; Vergnes, et al, 1986). However, in the present study, the chronic administration of 50 mg/kg of PCPA administered to adolescent male rats was enough to produce significant differences in locomotor activity and aggression compared to controls. In accordance with previous studies significant decreases in tryptophan and 5-HT/5HIAA turnover were also found (Keleta et al., 2007).

Recent literature has shown that 5-HT and its metabolites are affected by exposure to AAS during adolescence (Grimes & Melloni, 2004; Keleta et al., 2007). The

current study found that the effects of TP on 5-HT was minimal in most brain regions, although TP did significantly increase 5-HT levels in the frontal cortex. This increase in 5-HT in the frontal cortex is consistent with previous findings (Keleta et al., 2007). A decrease of 5-HIAA in the hypothalamus was also associated with AAS exposure. TP treatment also elicited a decreased in 5-HT turnover and tryptophan levels compared to controls in several brain regions.

Consistent with previous findings the present study found that locomotor activity was significantly attenuated by the administration of PCPA (Dringenberg et al., 1995; Genot et al., 1984; Matte and Turnow, 1978). Dringenberg et al. (1995), found a dose dependant decrease in locomotor activity following acute administration of PCPA (150-1000 mg/kg). Chronic administration of 100mg/kg of PCPA throughout the adolescent period in male Long-Evans rats has also been shown to attenuate locomotor activity (Keleta et al., 2007). The present study found similar results using a lower dose of PCPA (50 mg/kg), and also found a decrease in locomotor activity relative to controls in the group receiving PCPA in combination with TP. Consistent with previous findings, AAS alone did not have an effect on locomotor activity in male rats (Keleta et al., 2007; Salvador et al., 1994). Therefore, since locomotor activity was regulated by the administration of PCPA it is believed that locomotor activity is primarily modulated by brain 5-HT levels. Keleta et al. (2007) suggested that the reason for the decrease in locomotor activity by animals given PCPA may be that low levels central 5-HT enhance freezing behavior. Bolles (1970) referred to freezing behavior as a "species specific defense mechanism." This freezing behavior is exhibited in a potentially threatening situation and could be categorized as an anti-predator defensive behavior (Blanchard et.

al 1998). A recent study found that rats given PCPA exhibited an exaggeration in fear behavior (Hughes & Keele, 2006). This could lead to the increase in freezing of the PCPA-treated males in the open field test and thereby decrease the animal's locomotor activity. Kulikov et al. (1990) suggested that locomotor activity is a manifestation of adaptive behavior to new environments. Enhancement of central 5-HT turnover has been shown to contribute to the adaptation to stress (Masuda et al., 1993). Perhaps one of the roles of 5-HT and its metabolites is in controlling the adaptability of an organism.

Kulikov (1990) also suggested that animals with low locomotor activity in an open field test also spent less time orienting to a new environment and exhibited more emotional reactivity to a novel environment. The lack of exploratory behavior can have very negative effects on an organism, including the inability to gain access to scarce food sources. The nose poke test found that even in the presence of a rewarding stimulus, animals receiving PCPA alone exhibited a non-significant increase in freezing behavior and a significantly higher latency to nose poke than control animals. Once again this behavior test provides further evidence that in a novel environment animals receiving PCPA react more fearfully to their surroundings and were more reluctant to explore. This freezing behavior could be very debilitating to an organism that lives in a competitive environment, as rats typically do. This increase in fearful response to the novel environment can also be seen as a learned association. Species generally have biological predispositions which shape learned associations (Seligman & Meyer, 1970). Another interesting effect obtained in the nose poke test was with the group receiving the combination of TP and PCPA. TP seemed to attenuate the low 5-HT effect on nose poke latency. In the presence of a rewarding stimulus, TP perhaps enhanced arousal in the

animal and it was able to overcome the fear of the unfamiliar environment and pursue the reward at levels comparable to controls.

In accordance with previous research, PCPA elicited a decrease in mount, intromission, and ejaculatory latencies (McIntosh & Barfield 1984). All groups exhibited a decrease in mount, intromission and ejaculatory latencies. Groups receiving PCPA alone and PCPA in combination with TP exhibited a decrease in mount latency that was significantly lower than the control group. This nonsignificant decrease in sexual behavior latencies by sexually experienced males corresponds with previous research (Farrell and McGinnis, 2003; Feinberg et. al., 1997; Keleta, 2007; Wesson and McGinnis, 2006). This decrease in the latency of sexual behavior measures could be viewed as an increase in sexual motivation. Therefore, since the effects of the three treatment groups were so similar, perhaps low 5-HT and TP both play a similar role in the display of increased sexual motivation within an organism.

Consistent with previous studies, AAS exposure did not elicit an increase in aggression using the resident-intruder paradigm without provocation (Keleta et al., 2007). Smaller opponent animals did not elicit an increase in aggression for the group receiving TP either before or after provocation. In accordance with previous literature this suggests that AAS does not inhibit the animals from discriminating between a threatening and non-threatening opponent (Breuer and McGinnis, 2001). The animals receiving AAS did not need to exhibit aggression because there was no uncertainty about which animal was dominant in this social setting. However, animals treated with AAS alone and physically provoked, exhibited a significant increase in the number of dominance mounts toward the smaller opponents when compared to the number of dominance mounts in the aggression

test with no provocation. Therefore, AAS did induce heightened reactivity following physical provocation, thus supporting the hypothesis that AASs sensitize animals to their surroundings and lower the threshold to respond to provocation with aggression (McGinnis, 2004). In the same aggression test low 5-HT induced by PCPA administration alone produced a significant increase in aggression without provocation and heightened levels of aggression in the group receiving both PCPA and AAS. Taken together these results indicate that low 5-HT may induce heightened aggression even against non-threatening opponents. This corresponds with results from animal studies in which PCPA induced mouse-killing behavior in rats (Miczek et al., 1975), as well as human studies in which low levels of 5-HIAA were related to socially maladaptive aggression (Higley et al., 1998; Linnoila & Virkkunen, 1992).

In the final behavior test experimental animals were placed in the home cage of a male and female paired for two weeks prior to the test. The female was sexually receptive in one test and not receptive in the other. This test can be said to measure social aggression, which Albert (1980) defined as, "aggression for the purpose of establishing, altering, or maintaining a dominance hierarchy" (p. 358). This could also be categorized as competitive aggression. No differences between groups were found when the experimental animals were placed in the cage with a previously paired male opponent and a non-receptive female. However, differences between groups were found when animals were placed in a cage with an opponent and a sexually receptive female. AAS alone, as well as in combination with PCPA, significantly increased aggression towards the similar sized male opponent in this threatening situation. This is consistent with results that have shown that testosterone increases social and competitive aggression

(Albert et al., 1986; Albert et al., 1989). This heightened aggressive response observed by animals receiving TP alone and in combination with PCPA was only seen when a sexually receptive female was present. Vaginal secretions, which are strongest just before sexual receptivity, have been shown to elicit increased intermale aggression (Ciacciao et al., 1979), and therefore could be the reason why the non-receptive female elicited an less intermale aggression than the sexually receptive one. Therefore, this could possibly be seen as competitive aggression in which the males are fighting in order to establish dominance before mating ensues. This increase in aggression relative to controls was not seen in the group receiving PCPA alone. Perhaps this is due to exaggerated freezing in response to a novel or potentially threatening environment, as seen in the locomotor activity test.

Taken together these aggression tests provide further evidence that AAS exposure does not induce unprovoked aggression. It also provides evidence that AAS primarily facilitates aggression for the purpose of dominance. When dominance does not need to be shown and no provocation is present, as in the case of the aggression test without provocation using the prepubertal opponent, AAS will not induce an increase in aggression. This supports the hypothesis that AAS induces aggression by lowering the threshold to elicit aggression, which in this case is physical provocation. Low 5-HT seems to be related to inappropriate aggression, and exaggerated freezing behavior in male rats. It is also interesting to note that in the present study adolescent animals with low levels of 5-HT displayed similar aggressive behaviors, i.e. more aggression toward smaller opponents, as adolescent animals exposed to social subjugation throughout

adolescence (Delville et. al, 1998), perhaps implying that 5-HT is lowered through social subjugation.

5-HT and tryptophan levels in the withdrawal group, receiving TP + PCPA, all returned to control levels after 14 days of withdrawal. The only significant difference from controls remaining was in 5-HIAA levels which were lower in the hippocampus. This suggests that PCPA did not have a toxic effect on 5-HT levels even when administered chronically in adolescence animals. Not only did 5-HT levels return to control levels, but all of the behaviors that were significantly different from controls in the TP + PCPA group were no longer significant following withdrawal. However, aggression in the presence of physical or environmental provocation remained higher in the withdrawal group than that of control animals, thus suggesting that there might have been some long term effects of chronic 5-HT depletion combined with AAS abuse.

In conclusion, chronic exposure to AAS during adolescence increases the reactivity in social situations in which the dominance hierarchy is not established. This reactivity may also occur when the animal is challenged or provoked. AAS was also shown to produce significant effects on brain 5-HT, 5-HIAA, Tryptophan and 5-HT/5HIAA turnover in certain brain areas. These neurochemical and behavioral changes induced by AAS abuse during adolescence were attenuated following withdrawal. When AAS administration during adolescence is combined with dramatically low 5-HT levels, induced by PCPA, inappropriate aggression, decreases in locomotor activity and exaggerated freezing behaviors are often exhibited in addition to the AAS induced aggressive reactivity to provocation. However, even these dramatically low 5-HT levels and behavioral effects return to control levels following withdrawal from TP + PCPA.

Although the behavioral effects were no longer significantly different from controls following withdrawal from TP + PCPA, many of the behavioral tests measuring the reactivity of the animal to provoking stimuli remained elevated following withdrawal. This suggests that AAS abuse during adolescence could produce lasting behavioral effects. This heightened aggressive reactivity following withdrawal of TP + PCPA could either be in the process of returning to control levels or fixed at a level above control animals. A longer withdrawal period would gain further knowledge into whether or not this aggressive reactivity would completely return to control levels. Certain contextual cues may be sufficient in producing an exaggerated aggressive response following an even longer withdrawal period. However, even if aggressive reactivity is shown to completely return to control levels following a longer withdrawal period, other damaging effects of AAS abuse, such as liver, kidney and heart damage, may be present and irreversible. Also, given that adolescence is a time in which individuals make decisions that could affect the rest of their lives, cognitive disturbances underlying this over-reactivity could interfere with their education and other long-term goals. It is also important to realize that poor decision making during adolescence may continue to negatively affect someone for the rest of their life. Aggressively over-reacting in non-threatening situations does not only pose a threat to the adolescent individuals abusing AAS, but it also affects those around them. With a growing concern for the safety of children in educational environments, AAS should be seen as a major threat against this safety. We must attempt to make parents, children and educational administrators aware of the effects of AAS abuse during adolescence so that these possibly disastrous effects may be averted.

There are still many questions regarding how AASs induce an increase in aggression and in what environments this aggression is most prevalent. Perhaps 5-HT related chemicals are a mechanism by which AASs induce increased aggression. However, as seen in the current study low 5-HT and AAS induce aggression in different environments. In vivo techniques could be used to help determine differences in 5-HT levels before, during and following aggressive encounters in different environments. One interesting brain area I would look for changes would be in the amygdala, since it controls outbursts of behavior and it was found in the present study that aggressive reactivity is associated with AAS abuse. Future research could also be directed into the understanding of which 5-HT receptor subtypes AAS might effect. Through a better understanding of the exact mechanisms by which AAS induce heightened aggression, and the environments which elicit this response, we will be able to more accurately assess the long term effects of AAS induced aggression.

I would also be interested in the effects of AAS abuse by females. Very little research has examined the effect of AAS abuse by females. With a growing popularity in women's sports it would be interesting to see how AAS abuse could effect not only the professional woman athlete but the developing adolescent woman. Perhaps AAS abuse by women during adolescence would prove to be particularly debilitating.

Another area that lacks much research is in the manner in which AAS are taken. Exactly, what different AASs are people mixing together and in what order are they taking these cocktails? With this information we could simulate AAS abuse more accurately using animals and therefore provide information on the effects of AAS exposure during adolescence.

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VITA

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