Serotonergic Systems in the Regulation of Sexually Dimorphic Responses to Cocaine

By

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A dissertation submitted to the Graduate Faculty in Psychology (Biopsychology Subprogram) in partial fulfillment of the requirements of the degree of Doctor of Philosophy, The City University of New York

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This manuscript has been read and accepted for the Graduate Faculty in Psychology (Biopsychology Subprogram) in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

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Advisor: Professor Vanya Quinones-Jenab

Sex differences in cocaine abuse have been consistently reported in humans. Similarly, rats show sexually dimorphic neuorochemical and behavioral effects after cocaine administration. This research aims to determine whether serotonin (5-HT) plays a significant role in cocaine-induced behavioral sex differences found after cocaine administration. We hypothesize that 5-HT (at the level of release, reuptake, and/or receptor activation) mediate sexual dimorphisms seen after cocaine administration. To this end, male and female rats were pretreated (i.p.) with WAY 100635 (a 5-HT_{1A} antagonist; 0, 0.4, 0.8 & 1.6 mg/kg; 15 min pretreatment), GR 129735 (a 5-HT_{1B} receptor antagonist; 0, 5, 10, 15 mg/kg; 30 min pretreatment), or Fluoxetine (0, 5, 10, 15 mg/kg; 1 hour) followed by an i.p. injections of saline or cocaine (20mg/kg); behavioral responses were then measured. Further, we assessed activation of the 5-HT_{1A} receptor after saline or cocaine administration utilizing *in vitro* autoradiography of agonist stimulated [^{35}S]GTPγS in mesocorticolimbic and nigrostriatal pathways which are involved in cocaine behavioral hyperactivation. Finally, sexual dimorphisms of the activation of the endocrine system by the co-administration of cocaine and the 5-HT_{1A} antagonist WAY 100635 was explored via radioimmunoassay. Increases in cocaine-induced behaviors were found in both male and female rats however, females demonstrated heightened responses. Sexual dimorphisms

in the attenuation of cocaine-induced behaviors was found with administration of the 5-HT_{1A} antagonist WAY 100635 and the 5-HT_{1B} antagonist GR 127935. Furthermore, we found differential activation of the 5-HT_{1A} G-protein receptor which varied by sex and drug administered. Our findings suggest that intrinsic sex differences exist in the regulation of $5HT_{1A}$ and 5-HT_{1B} receptors and not by the serotonin transporter in cocaine-induced behaviors. Moreover, cocaine activated G-protein 5-HT_{1A} receptors is sexually dimorphic and suggests a principal role in the modulation of sexually dimorphic aspects of cocaine-induced behaviors. Decreased corticosterone levels were only found in females after WAY 100635 and saline. As suggested in previous literature a possible anxiolytic effect of this antagonist could clarify this finding. The understanding how the serotonergic system contributes to sex differences in cocaine-induced behavioral activation will allow for more efficient pharmacological treatment of male and female cocaine abusers.

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Table of Contents

List of Tables

Page

List of Figures

Page

Page

Chapter 1: Background

A. History of Cocaine Abuse:

Cocaine, considered by many as the most potent natural stimulant, is a principal alkaloid derivative extracted from the leaves of Erythroxylon coca shrub. It is found most abundantly in the northwestern parts of the Amazon, specifically Bolivia and Peru (1-3)}. Although the widespread increase of utilization of cocaine has only been seen in the past hundred years, cocaine use dates much further back in history. As early the 6th century AD many cultures, including ancient Indian's, provided descriptions of the substances origin and their belief in its' supernatural powers (1-3). The first emergence of the modern utilization of cocaine occurred in the mid-nineteenth century. Cocaine was used as a main ingredient in many tonics and elixirs, as well as, a very effective local anesthetic for surgeries. Cocaine was also used to treat a wide variety of illnesses, including but not limited to, asthma, digestive disorders, melancholy, toothaches and as a treatment for various kinds of substance addiction such as alcohol and morphine (1-3).

Increased popularity in the use of cocaine in everyday products as well as being used to cure a number of medical pathologies, resulted in an increase in its use. This rapid increase in the use of cocaine brought about concerns of side effects and likelihood of addiction. By the early twentieth century the American Medical Association being concerned with the increased utilization of cocaine and ultimately its' opportunity for abuse, began to place higher standards on its use (1-3). Due to increased discoveries of the adverse effects of cocaine, the federal government, through the Pure Food and Drug Act of 1906, required that all products and medicines containing cocaine be listed on its labels. This was then followed by the Harrison Narcotic Act of 1914 which halted the use of cocaine in common medications and required formal registration of those involved in the importation of the drug (1-3). Cocaine's huge potential for abuse and toxic effects classify it as a

schedule II narcotic and is limited in its use as a controlled substance (1-3). Because of its usefulness as an anesthetic and vasoconstrictor, cocaine is still readily used as a local anesthetic for eye, ear and throat surgeries (1-3).

B. Overview and Rationale:

Since the ban of its sale to the public, the use of cocaine has fluctuated. Cocaine use is not limited to one demographic, being used by all ethnicities, genders and ages. The incidence of cocaine use rose substantially throughout the 1970's, with a peak in the mid 1980's (1.7 million new users) (4). This peak declined in 1991 with an estimate of 0.7 million new users. However, since then cocaine use has been steadily increasing, reaching 5.9 million users aged 12 years or older in the United States in 2003 (4)

The average age of cocaine use is 20.8 years of age (4). However, differences in the use, rate of dependence and sensitivity has been shown to vary by age and sex. Recently, Chen et al., 2002 explored gender, age and race/ethnic differences in the prevalence of subjects past year cocaine dependence. They found that cocaine use and the criterion for cocaine dependence was higher in adolescent females than adolescent males. Further females were more likely to use cocaine at an earlier age and at a greater frequency when compared to adolescent males (5). Females report increased symptoms, which are associated with drug dependence such as "inability to cut down" and "need for larger quantities" (5). Women also administer cocaine by more addictive routes and progress to dependence more rapidly than men (6). This increased dependence in females is due to female adolescents demonstrating more frequent use, use higher quantities, and show a greater sensitivity to the effects of low doses of cocaine. In addition, women have reported feeling more

nervousness after cocaine use, take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria compared to males and have more severe drug use at intake (6,7).

Differences in the initial use of cocaine by males and females has also been seen and reported by the National Survey on Drug Use and Health (NSDUH). Approximately 600,000 of the estimated 2 million Americans who used cocaine in 2002 were women (4). In 2002, NSDUH reported men were more likely to report current illicit drug use and opportunity to try cocaine than women (10.3% vs. 6.4%) (4). In addition, since 1975, males have generally comprised the majority of cocaine initiates with 0.7 million new male users compared to 0.5 million new female users reported in 2002. Though these statistics seem to suggest males have a greater opportunity to try and initially use cocaine, researchers have found that they are not more likely than females to progress to intense use following initial use (8,9). Many believe that the development of dependence differs between men and women. For example, women enter treatment programs after fewer years of drug use than do men (5-7,10). In addition, females seem to show a more accelerated transition from a more casual and controlled kind of use to an uncontrolled "binge" patterns of use than men (8,10). Moreover, men and women differ in cues that spark drug cravings and subsequent relapse, with men being more influenced by internal cues and woman by external cues (5,7,11). In addition, generally any cues associated with cocaine produce cravings to a greater degree in women compared to men (10). This progression to intense use is paralleled with evidence that females, in both humans and animals, are much more sensitive to the effects of cocaine, suggesting that the progression to addiction is more rapid in females when compared to males (8,9,12-22). These sex-specific differences in cocaine use and behavior patterns suggest that the biological basis of addiction is sexually dimorphic and differentially regulated.

Chronic and acute cocaine administration has been shown to cause a multitude of short and long lasting changes to brain neurochemistry, neuroendocrine and behavioral systems (15,16,23-31). However, much more extensive research is needed to elucidate the exact mechanisms involved in these sexually dimorphic behaviors at both the neurochemical and behavioral levels. Understanding exact neurobiological mechanisms of cocaine's effects can be utilized in the proper management and subsequent treatment of cocaine dependence and addiction in both men and women. Moreover, with this knowledge of sex-specific differences treatments can be adapted for optimal effectiveness in both men and women.

C. The serotonin system, synthesis, receptors and location:

The serotonergic system is involved in a number of cognitive and behavioral functions including locomotion, grooming behavior, eating and drinking, pain, sexual activity, effects of drugs and alcohol, aggression, fear, sleep, classical conditioning and learning (32-34). Serotonergic pathologies include addiction, eating disorders, depression and suicidal behavior, anxiety, migraine headaches, as well as autism, obsessive compulsive disorders, Turret's syndrome, Huntington's disease and schizophrenia (34,35). Serotonin is integral in normal brain development being involved in neurogenesis and axonal branching during various stages of development (36).

i. Serotonin Synthesis:

Serotonin is formed by the hydroxylation or the addition of an hydroxyl group (-OH) to the amino acid L-tryptophan, by the rate limiting enzyme tryptophan hydroxylase forming 5-hydroxytryptophan (5-HTP) (35). Tryptophan, the substrate for this enzyme, is the rate limiting factor in the intact organism (35). Decarboxylation by the L-amino acid decarboxylase enzyme removing the carboxyl group (-COOH) from 5-hydroxytryptophan produces serotonin (35).

Serotonin is then metabolized by monoamine oxidase (MAO) to form 5-hydroxyindole acetic acid (5HIAA), the principle metabolite, which is actively transported across the blood brain barrier and out of the brain; See Fig. 1 (35). Serotonin exerts its complicated actions by binding to specific cell surface receptors, which have been classified into extensive groups. This grouping has been based on pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second messenger coupling (34,36,37).

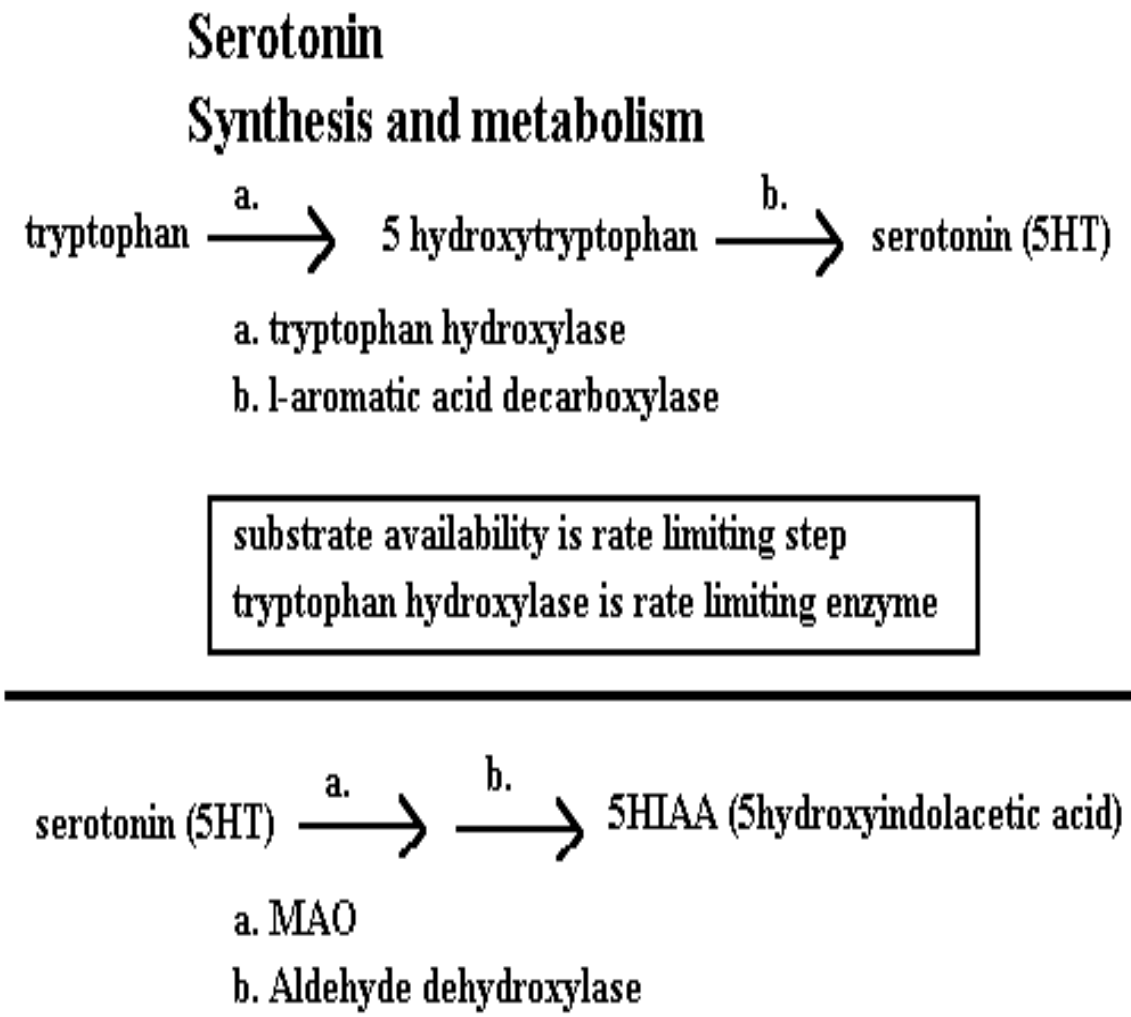


Figure 1: The synthesis of serotonin. Taken from Cell Science: http://www.cellscience.com/CCA.htm

ii. Serotonin Neurons Receptors and Location:

As summarized in Table 1, there are 14 known serotonin receptors and at least seven families of receptor populations and subpopulations (34,37). They include 5-HT_1 through 5-HT_7 and are involved in a plethora of behaviors (34,35,38,39). All are G-protein coupled with one exception; the 5-HT_3 receptor is a ligand-gated ion channel (34,40). The 5-HT_1 group is subdivided into at least 5 receptors named 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{1D,}$ 5-HT_{1E} and 5-HT_{1F} receptors (34,35,41). Most are inhibitory receptors, sharing coupling with Gαi/Gαo proteins to inhibit adenylyl cyclase (AC) and/or modulate other signaling pathways and ion channels (34,35,41). Like 5-HT_1 receptors 5-HT_2 receptors are distributed widely throughout the brain with the greatest density in the neocortex (32,34). The 5-HT_2 family has 3 receptor subtypes, 5-$HT2_A$, 5-$HT2_B$, and 5-$HT2_C$ receptors (32,34,41). 5-HT_2 receptors are coupled positively to phopholipase C and mobilize intracellular calcium (33,34). 5-HT_3 receptors are unique in the 5-HT family in that they are nonselective Na+/K+ ion channel receptors (32,34,41). 5-HT_3 receptors are found in the periphery and in the central nervous system (32,34,41). The 5-HT_4 stimulates adenylyl cyclase in the brain and in peripheral tissues and is found in high density in the nigrostriatal and mesolimbic systems of the brain and believed to have a facilitative role in cocaine-induced hyperlocomotion (32,33). Little is known at this time about the 5-HT_5, 5-HT_6 (involved in memory, retention of information and attention) and 5-HT_7 (involved in circadian function) receptors (32,33). 5-HT_5, 5-HT_6 and 5-HT_7 receptors are positively coupled to adenylyl cyclase and are excitatory (34,35,39). Much more research must be performed with receptors 5-HT4-7 to know more about the exact functioning of each.

Serotonergic neurons originating in the raphe nuclei possess high densities of somatodendritic autoreceptors, which regulate cell firing. In projection area's postsynaptic

receptors, autoreceptors and pre- and postsynaptic heteroreceptors have been found (32,32,33,33,39,42-48). Activation of 5-HT autoreceptors suppress or excite serotonergic activity; in turn causing a reduction or increase of 5-HT release from terminal areas and subsequent firing of 5-HT cells while heteroreceptors regulate other neurotransmitter systems (28,32,34,39-41,47,49-55).

Table: 1 Overview of all serotonin receptors, general location and function

Receptor Name	Receptor Location	Receptor Function
5-HT1A	Somatodendritically densely expressed in the dorsal and median raphe nuclei	Implicated in thermoregulation, hypotension, sleep, food intake, motor and sexual behavior, involved in depression and anxiety
	In projection areas, in limbic forebrain regions, including the hippocampus (CA1 and CA3), amygdala, lateral septum, and frontal cortex	Found presynaptically as an autoreceptor modulating 5-HT release. In projection area postsynaptically mediating inhibitory response of cocaine.
	Hypothalamus, basal ganglia, substantia nigra, cerebellum and NAc	Coupled to various effectors systems, including ion channels, adenylyl cyclase and/or kinases via negatively coupled G proteins (Gαi/Gαo proteins)
5-HT1B	Somatodendritically densely expressed in the dorsal and median raphe nuclei	Implicated in several physiological functions, behaviors, and psychiatric diseases including, locomotor activity, drug abuse reinforcement, aggressive behavior, anxiety, depression and migraine's
	In projection areas, in limbic forebrain regions, including the hippocampus, amygdala, and frontal cortex	Found both presynaptically and postsynaptically and constitute terminal autoreceptors regulating the release of 5-HT. Distributed throughout the brain in both serotonergic and non-serotonergic neurons where they act as auto- or heteroreceptors
	Hypothalamus, basal ganglia, caudate and putamen, substantia nigra, cerebellum and NAc	Negatively coupled to adenylyl cyclase by an intermediate G protein-type protein. Also includes mitogen-activated protein kinase (MAP-kinase) signaling system
	Superior layer of dorsal horn of the spinal cord	
5-HT1D	Globus pallidus Ventral pallidum Caudate-putamen Subthalamic nucleus Substantia nigra Optic tract	Implicated in anxiety, depression, and other neuropsychiatric disorders Act as presynaptic autoreceptor and heteroreceptors Inhibit adenylyl cyclase activity through coupling with Gαi/Gαo proteins. Regulate potassium and calcium channels.
5-HT1E	Cortical areas Caudate-putamen Amygdala Hippocampus	Migraines Negatively coupled to adenylyl cyclase
5-HT1F	Dorsal raphe nucleus Hippocampus Cerebral cortex Striatum Thalamus Hypothalamus	Migraines Negatively coupled to adenylyl cyclase, decreasing cAMP

5-HT2A	Forebrain regions, including the neocortex and entorhinal and pyriform cortex Striatum Limbic regions Basal ganglia	Implicated in appetite control, thermoregulation, sleep, cardiovascular function, muscle contraction, depression and the actions of classical hallucinogens Pharmacological and behavioral effects are mediated postsynaptically Are coupled positively to phospholipase C, and increase intracellular calcium.
5-HT2B	Cerebellum Lateral septum Hypothalamus Amygdala	Implicated in anxiety, feeding and grooming and rats Are coupled positively to phospholipase C, and increase intracellular calcium.
5-HT2C	Cortex Striatum Hippocampus Limbic system Basal ganglia	Implicated in hypoactivity, hypophagia, and anxiogenesis Postsynaptic Are coupled positively to phospholipase C, and increase intracellular calcium
5-HT3	Frontal cortex Hippocampus Amygdala Cerebral cortex VTA	Locomotion, anxiety-related behavior, reinforcement, and cognition Postsynaptic receptor. Only 5-HT receptor that is not G protein coupled. Is a ligand gated channel
5-HT4	Nigrostriatal and mesolimbic systems Hippocampus	Locomotor activity and reinforcement Positively coupled to adenylyl-cyclase and are excitatory
5-HT5	Cerebral cortex Hippocampus Cerebellum Amygdala	Involved in motor control, feeding, anxiety, and depression Positively coupled to adenylyl cyclase and are excitatory
5-HT6	Striatum NAc Hippocampus Cortex	Positively coupled to adenylyl cyclase and are excitatory
5-HT7	Human vascular smooth muscle cells Frontal cortical astrocytes	Positively coupled to adenylyl cyclase and are excitatory

iii. Serotonin Neurons Location:

All serotonin neurons originate from midbrain and brainstem raphe nuclei. Long ascending (originating specifically from the dorsal raphe nucleus and median raphe nucleus), as well as, descending (originating from the obscurus, magnus and pallidus raphe nuclei) projections exists. Ascending projections provide innervations to the hippocampus, striatum, amygdala, hypothalamus, thalamus, cerebellum, ventral tegmental area (VTA), substantia nigra, nucleus accumbens (NAc), and the prefrontal cortex (56,57). The median and dorsal raphe nuclei projections have been extensively examined and are found to project to both similar and distinct areas from each other; for instance showing no overlapping regions in the forebrain (58). The median raphe nucleus (MRN) innervates a number of areas including the dorsal raphe nucleus, hippocampus, hypothalamus, thalamus, septum and frontal cortex. The dorsal raphe nucleus (DRN) innervates the median raphe nucleus, hippocampus, hypothalamus, thalamus, ventral tegmental area, substantia nigra, striatum, amygdala and nucleus accumbens. The descending pathways originate in the caudal raphe nuclei and terminate in the dorsal horn of the spinal cord (which has an effect on the inhibition of pain transmission) and the ventral horn (regulation of motor neuron output); see Fig. 2 and 3 (35,58).

Interestingly, recently researchers have determined that 5-HT projections within the brain may not actually form direct synaptic contacts with target cells but are also functioning via volume or paracrine transmission (34,39,59,60). Therefore, a transmitter is released at the axons within the terminal areas and acts at receptors that are away from the release site, which in turn allows for transmission to a number of target cells (32). This large diffuse mechanism of release may be a major player in the complex, and multiple interactions of the serotonergic system (34).

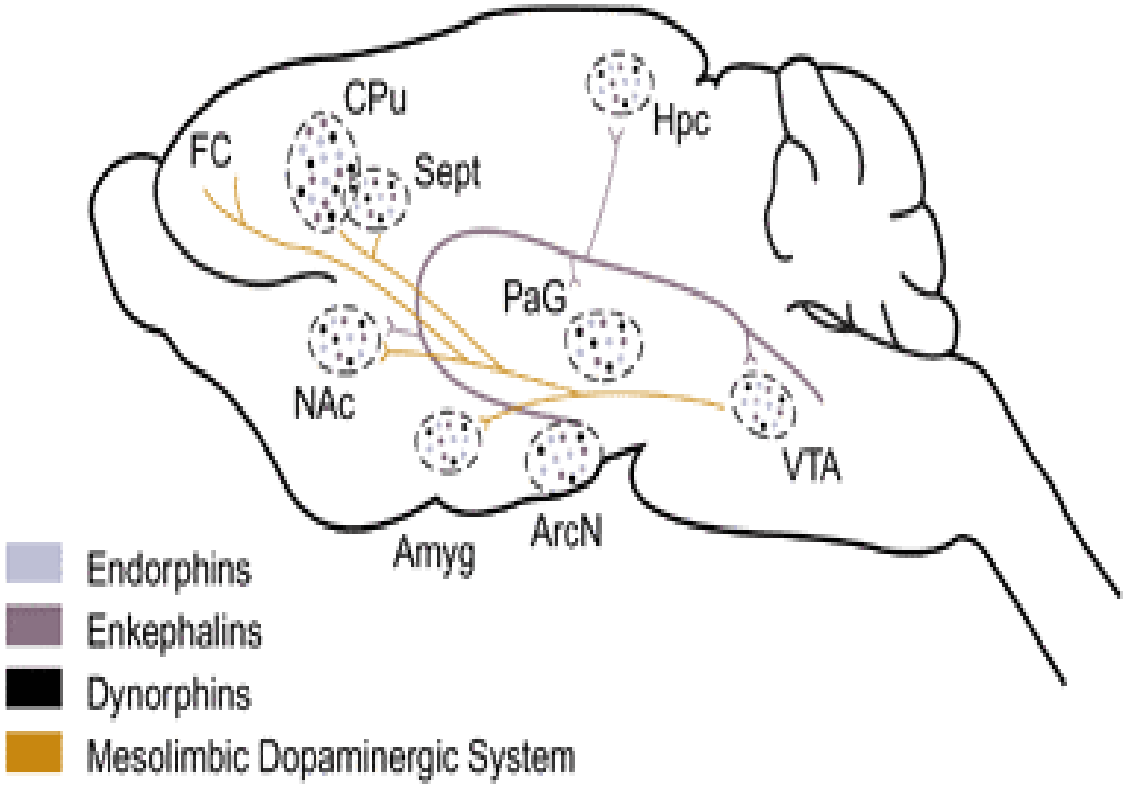


Figure 2: Serotonergic Pathway. Taken from Muller et al., 2006. Serotonergic system is found in the same areas as the mesolimbic dopaminergic system above. Abbreviations: Hpc, hippocampus; NAc, nucleus accumbens; FC, prefrontal cortex; Sept, Septum; VTA, ventral tegmental area; Amyg, amygdala; CPu, caudate putamen; ArcN, arcuate nucleus.

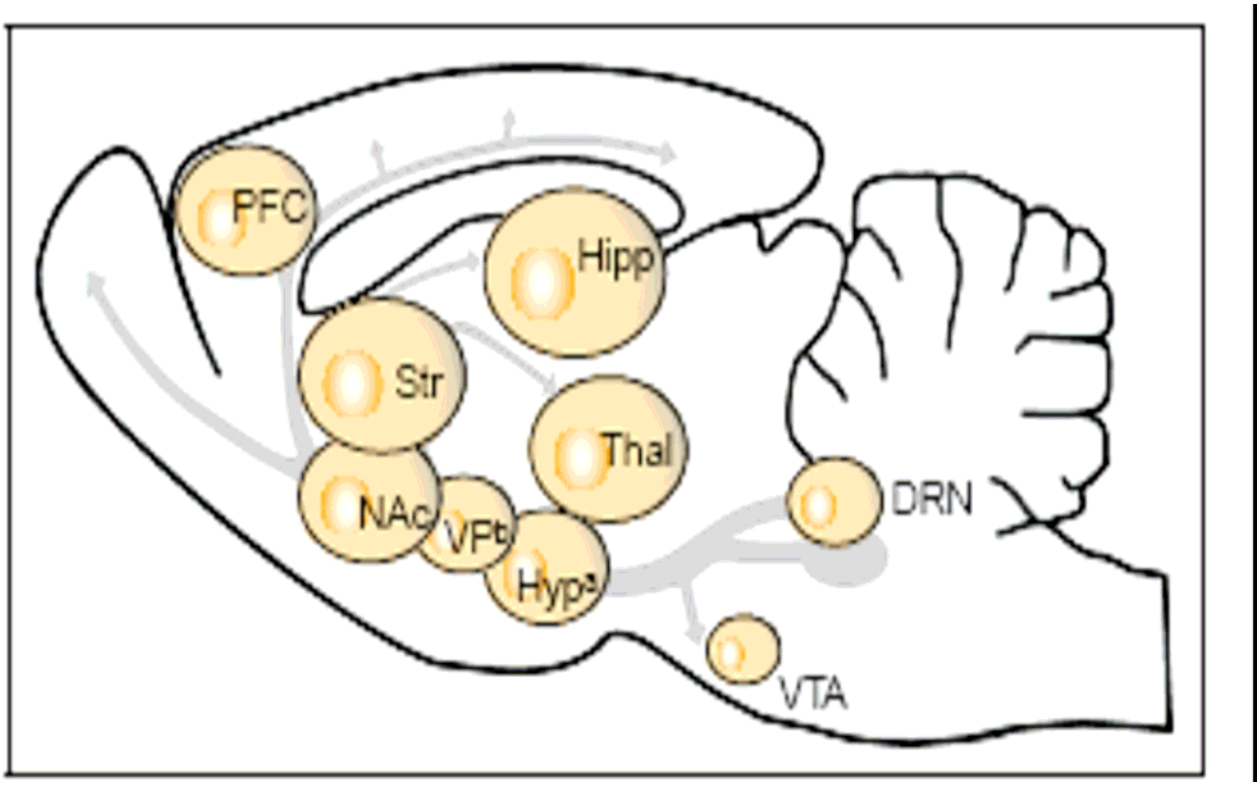


Figure 3: Serotonergic Pathway. Taken from Muller et al., 2006. Major ascending 5-HT projections are shown in grey. Abbreviations: DRN, dorsal raphe nucleus; Hipp, hippocampus; Hyp, hypothalamus; NAc, nucleus accumbens (C, core; S, shell); PFC, prefrontal cortex; SN, substantia nigra; Str, dorsal striatum; Thal, thalamus; VP, ventral pallidum; VTA, ventral tegmental area.

iv. 5-HT_{1A} Receptors:

5-HT_{1A} receptors are involved specifically in sexual behavior, mood, aggression, appetite control, thermoregulation, sleep-wake functions, cardiovascular function, motor activity, feeding, grooming, anxiety-related behaviors and depression (33-36). More importantly, 5-HT_{1A} receptors are increasingly implicated in the effects of alcohol and other abusive drugs including cocaine.

5-HT_{1A} receptors have been found most abundantly in the dorsal and median raphe nuclei and limbic forebrain regions, including the hippocampus (CA1 and CA3), amygdala, lateral septum, cingulate and entorhinal cortex and the frontal cortex (33-36,61). A medium density has been found in the amygdala and the hypothalamus and a low density has been reported in extrapyramidal areas such as the basal ganglia, substantia nigra, cerebellum and NAc (34,35,39,44). 5-HT_{1A} mRNA is found mainly in regions where 5-HT_{1A} binding sites are found. This suggests that these receptors are not transported away from their site of synthesis and targeted in the somatodendritic areas of neurons (41).

5-HT_{1A} receptors are somatodendritic presynaptic autoreceptors and postsynaptic receptors in terminal regions (33,62). Specifically, 5-HT_{1A} receptors are localized in the MRN and DRN as somatodendritic neurons which act as inhibitory autoreceptors at the level of the soma and dendrites of these neurons and inhibit firing as impulse-modulating autoreceptors (33,34,36,63). Postsynaptic receptors and heteroreceptors are found in projection neurons such as the neocortex, hippocampus and other limbic structures (33,34,62). Postsynaptic 5-HT_{1A} receptors may also play a role in the regulation of firing and serotonin release through neuronal feedback loops between projection neurons and raphe nuclei and has been demonstrated in the cortex and amygdala (64). However, evidence also suggests the existence of presynaptic heteroreceptors that not only regulate their own

release but may in fact regulate the release of other transmitters, such as dopamine; see Fig. 4 & 6 (34,36,47,61,65).

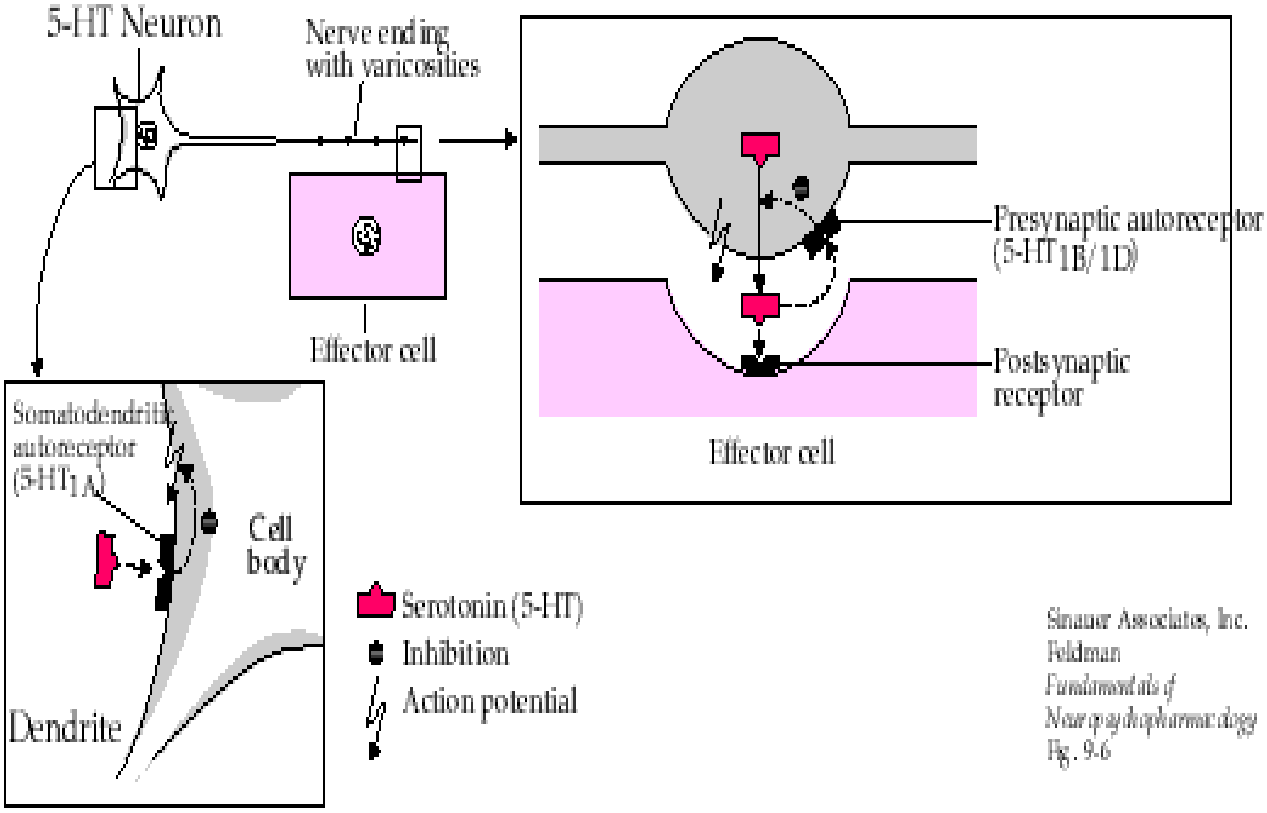


Figure 4: Somatodendritic and postsynaptic autoreceptors on serotonergic neurons. Taken from Feldmans' Fundamentals of Neuropsychopharmacology, 1999. www.chemistry.emory.edu/justice/seminar/5ht1.htm

v. 5-HT$_{1A}$ mechanism of action:

5-HT$_{1A}$ receptors are known to elicit diverse molecular and cellular responses. They are G-protein coupled receptors (GPCR) and are coupled to various effector systems, including activation of ion channels such as the opening of G-protein-gated inwardly rectifying potassium (GIRK) channels in the hippocampus and dorsal raphe neurons as well as inhibition of Ca2+ currents in several neuronal types (34,35,41,61). Most 5-HT$_{1A}$ receptors are negatively coupled to G proteins (Gαi/Gαo proteins) and inhibit adenylyl cyclase reducing the levels of cyclic (cAMP) in cells however; however stimulation of adenylyl cyclase has been seen (34-36,41,61,66). 5-HT$_{1A}$ receptors are mainly coupled to Gαo and Gαi in the hippocampus and frontal cortex and are coupled to Gαz in the hypothalamus (41). In addition, 5-HT$_{1A}$ modulates activation of phosphatidylinositol-specific phopholipase C (PI-PLC) turnover, protein kinase C, and ERK Map Kinases (41). It is suggested that differences in the coupling of 5-HT$_{1A}$ receptors to different G-proteins might account for regional differences in 5-HT$_{1A}$ receptor activation in differential brain areas and thus exert subsequent effects on behavior; See Fig. 5 (34,39,67)

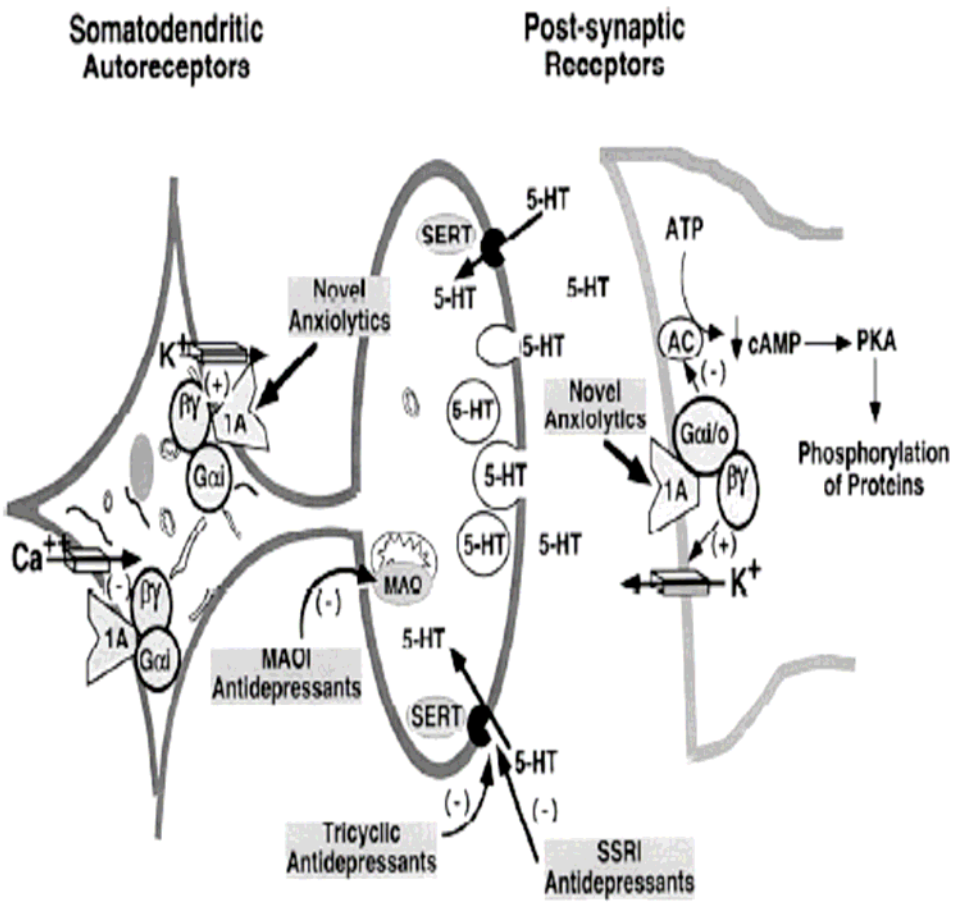


Figure. 5: Schematic showing the various effector systems of the 5-HT$_{1A}$ receptor. Taken from Hensler et al., 2003.

vi. 5-HT_{1B} Receptor:

5-HT_{1B} receptors are involved in a number of physiological functions, behaviors, and psychiatric diseases including, locomotor activity, drug abuse reinforcement, aggressive behavior, anxiety, depression and migraine's (48). The presence of 5-HT_{1B} receptors has only been demonstrated in the central nervous system of rodents (rats, mice and hamsters) (34,48,68). In humans, a homologue, the 5-HT_{1D} beta-receptor has been demonstrated (34,48,68).

5-HT_{1B} receptors are densely expressed in the raphe nuclei and in other motor centers including the basal ganglia, caudate and putamen, NAc, VTA and deep cerebellar nuclei (34,48,69-71). Other densely populated areas include cerebellum, amygdala, hippocampus, hypothalamus and superior layer of dorsal horn of the spinal cord, and are moderately expressed in the frontal cortex (33,34,40,48,61,71-73). 5-HT_{1B} mRNA has been found in the raphe nuclei, caudate putamen, amygdala, thalamus, CPu, NAc, CA1 pyramidal neurons, and projection zones of the subtantia nigra, VTA, globus pallidus and deep cerebellar nuclei (34,48,71). Unlike the 5-HT_{1A} receptor, numerous mismatches have been seen between receptor binding and mRNA expression suggesting that 5-HT_{1B} receptors are principally located at the nerve terminals (34,48,71).

5-HT_{1B} receptors are release and synthesis modulating autoreceptors on 5-HT terminals and as heteroreceptors to control the release of other neurotransmitters (74). These receptors are distributed throughout the brain in both serotonergic and non-serotonergic neurons, including but not limited to serotonergic, dopaminergic, GABAergic, acetylcholinergic and glutamanergic neurons where they act as auto- or heteroreceptors (37,48,71). 5-HT_{1B} receptors have been difficult to study due to the diverse nature of cellular localization and the lack of highly selective and potent agonists and antagonists; See Fig. 4 and 6 (71).

vii. 5-HT_{1B} mechanism of action:

5-HT_{1B} receptors are negatively coupled to adenylyl cyclase by an intermediate G protein-type protein Gαi or Gαo (34,48). The signal transduction mechanism mediating 5-HT_{1B} effects also includes the mitogen-activated protein kinase (MAP-kinase) signaling system; See Fig. 5 (34,48).

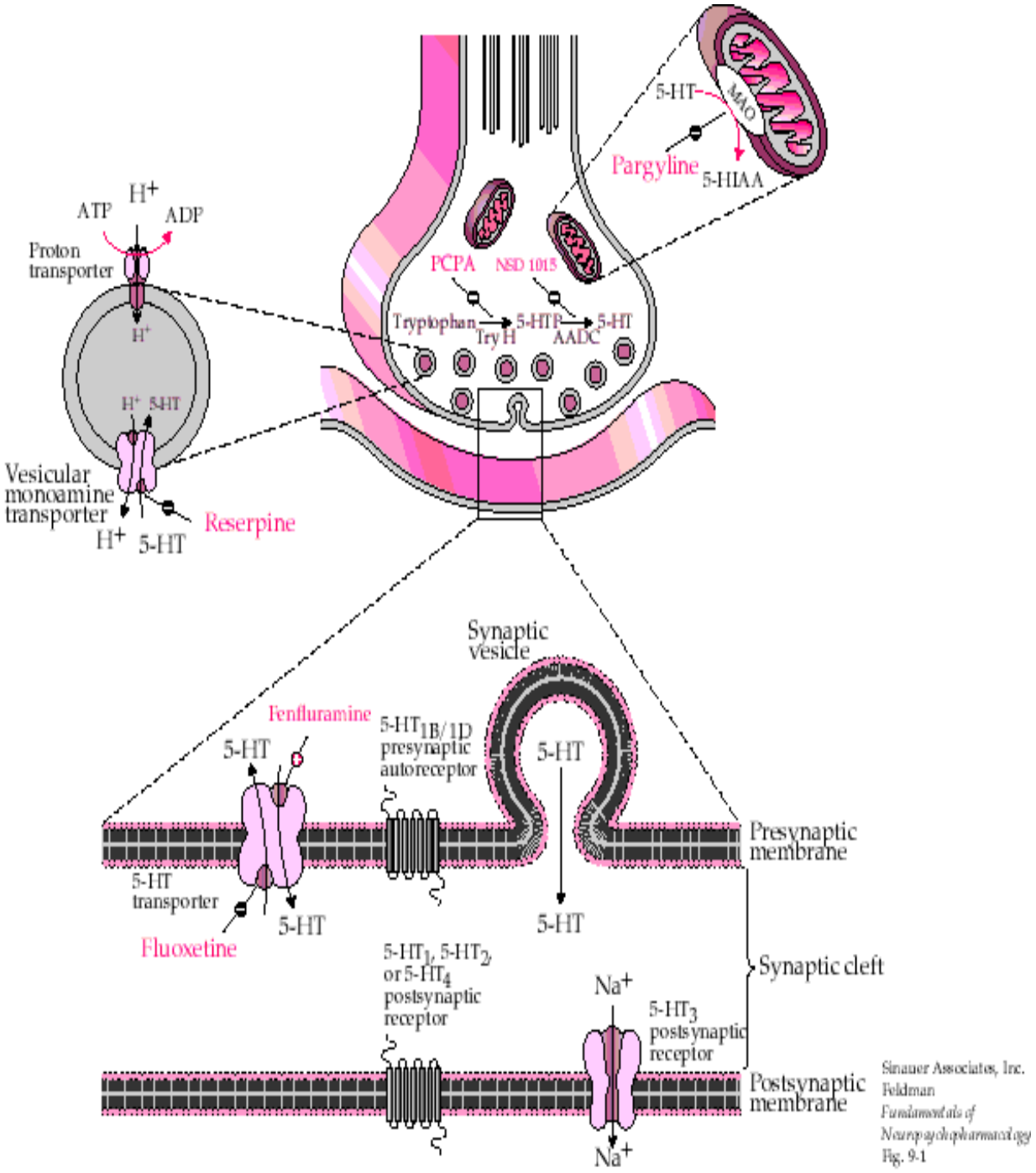


Figure 6: The Serotonergic Synapse. Illustration of the processes of serotonin (5-HT) synthesis and metabolism, presynaptic and vesicular 5-HT uptake, and vesicular 5-HT release. Pre- and postsynaptic 5-HT receptors and sites of action of serotonergic drugs are shown. Taken from: Feldman et al., 1999: Fundamentals of Neuropharmacology.

D. Mechanisms of Cocaine Action:

The mechanisms of cocaine's action have been extensively studied and well documented. Cocaine differentially binds to serotonin (SERT), dopamine (DAT) and norepinepherine (NAT) transporters causing a disruption of the normal reuptake of these transmitters into the presynaptic neurons; See Fig. 7 (24,25,65,75,76). This disruption causes an overall increase in synaptic concentration causing a change in overall functioning (i.e. increasing inhibition or excitation of neuronal firing and subsequent activation) (38).

Studies have demonstrated that acute cocaine administration causes an increase in overall extracellular dopamine (DA), norepinephrine (NE), and serotonin (5-HT) levels, which has been correlated to increases in extracellular cocaine concentration (28,32,38,54,55,65). In all these neurotransmitter systems, both *in vivo* and *in vitro* experiments have shown that reuptake blockade is caused by cocaine administration. This sequence of events results in many of the overt cocaine-induced behaviors seen in organisms (38,76-83). Due to the scope of this dissertation, DA and NE will only be briefly discussed.

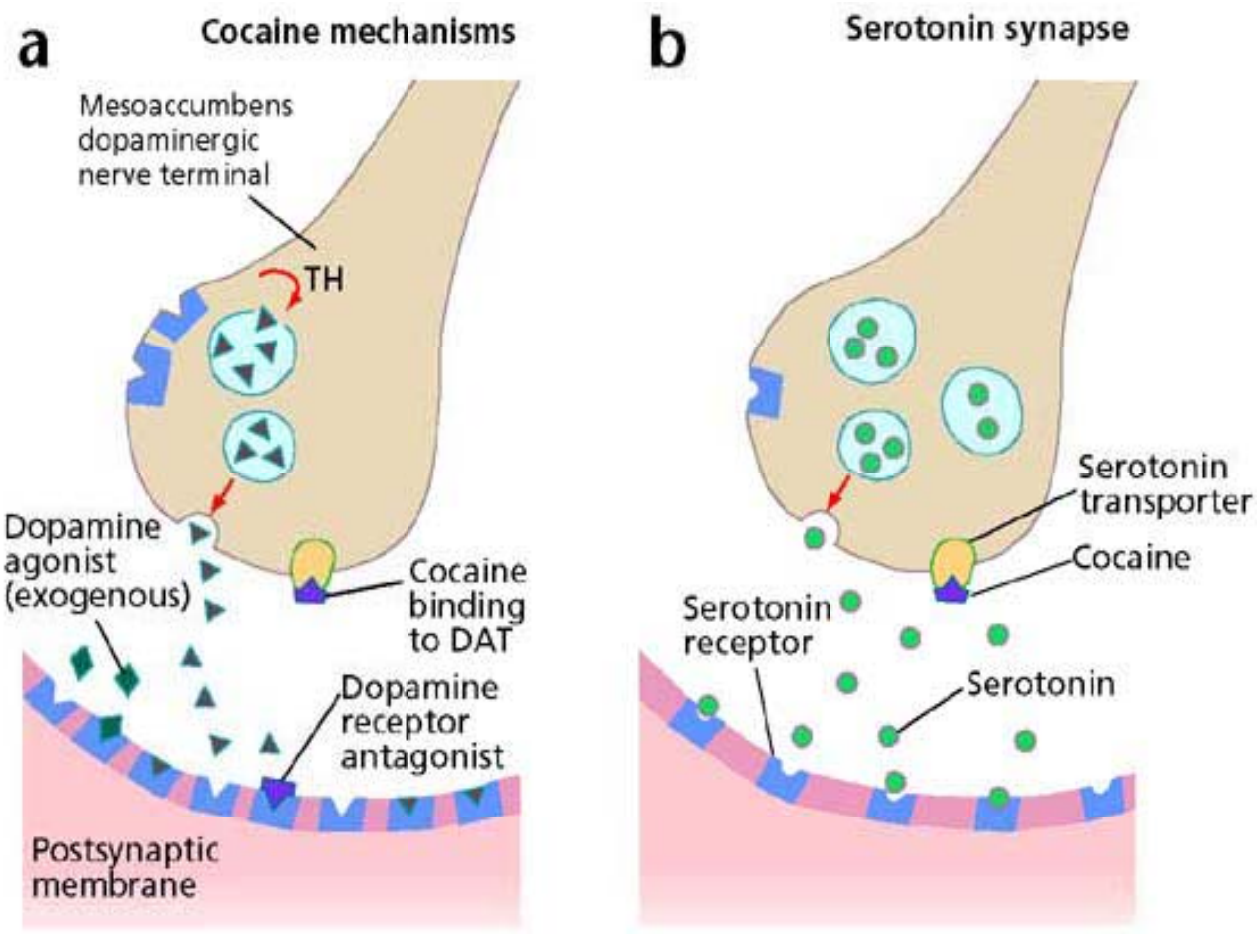


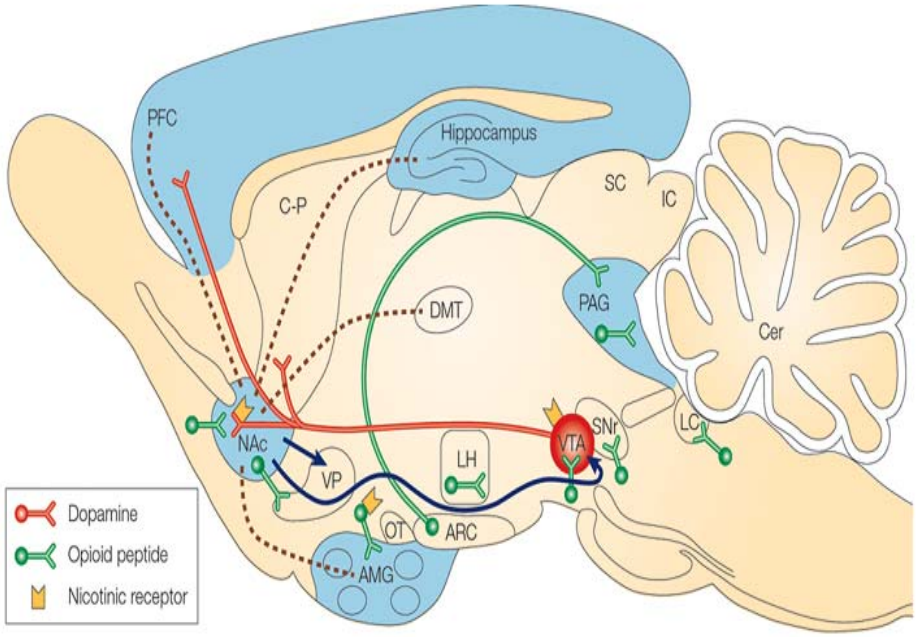
Figure 7: Dopamine and Serotonergic role in cocaine's mechanism of action. (a) Cocaine increases extracellular dopamine levels by binding to the DAT to inhibit dopamine reuptake. **(b)** Cocaine increases extracellular serotonin levels by binding to the SERT to inhibit dopamine reuptake. Taken from S. Barak Caine (1998).

E. Dopamine and Norepinephrine Systems and Their Interactions with Cocaine:

The mesolimbic dopamine and nigrostriatal pathways are believed, for the most part, to be the main mediators of behavioral effects of acute and chronic cocaine administration. The mesolimbic dopamine pathway includes dopaminergic cell bodies originating in the ventral tegmental area (VTA) projecting to a number of brain regions including the frontal cortex, the nucleus accumbens (NAc) with nigrostriatal projections to the caudate putamen (CPu); see Fig. 8 (13,32,38,76,84).

This potentiation of mesolimbic DA transmission is integral in the mediation of cocaine-induced locomotion, reward, discrimination and sensitization (38,76-80,82,84). The increase in dopamine in the NAc (mesoaccumbens pathway) has been regarded as one of the major neurochemical mediators of cocaine-induced locomotor behavioral effects (38,54,76,85). Both lesion and pharmacological manipulation have caused direct effects on all cocaine-induced behaviors previously discussed (28,32,33,38,40,54,55,86,87).

Cocaine also blocks the NE reuptake transporter in peripheral sympathetic nerve terminals (24,32,38,40,88). This increases the overall norepinephrine concentration in the synaptic cleft, which has been found to have major implications in catastrophic cardiovascular events, related to cocaine use (88). That the norepinephrine system is affected by cocaine is demonstrated by major effects on both the parasympathetic and sympathetic function (88).



(Nestler et al., 2001)

Figure 8: The reward circuit. Dopaminergic projections from the VTA terminating in the nucleus accumbens, caudate putaman, and the frontal cortex are of primary interest in drug abuse research. Taken from Nestler, E.J. (2001)

F. Cocaines action on the serotonergic system:

Among the monamines, cocaine is the most potent inhibitor of the impulse activity of 5-HT neurons when compared to either DA or NE neurons (89,90). In addition, cocaine has the highest affinity for the 5-HT transporter compared to either the DA or NE tranporter (33,91). Cocaine inhibits uptake of the 5-HT precursor tryptophan and the activity of tryptophan hydroxylase contributing to its ability to suppress 5-HT synthesis (89). Furthermore, cocaine blocks reuptake of 5-HT increasing the availability of 5-HT levels in the synaptic cleft (33).

G. Effects of Cocaine on Behavior:

Cocaine increases locomotor behavior in both animals and humans, in a dose-dependent manner (38,92). Microinjection of cocaine into the nucleus accumbens, anterior dorsal and ventro lateral striatum show dose-dependent increases in locomotor activation such as increased horizontal and vertical (rearing) motor activity (38,77,89). At lower doses cocaine-induces spontaneous behaviors such as locomotion and grooming and causes a reduction in appetite (38). With increasing doses of cocaine, stereotypical behavior, which includes sniffing, head bobs, head weaves, forepaw treading and more sluggish behavior are seen (78). In addition, cocaine produces both subjective and rewarding effects in both humans and animals. This is examined via self-reports in humans and conditioning and self-administration paradigms in rats (7,93)

H. Effects of Cocaine on the Serotonergic System:

Evidence has demonstrated that serotonergic mechanisms are directly and indirectly involved in the behavioral and rewarding effects of cocaine (35,38,77,78,80-82,85,92,94-96). Increases in 5-HT levels and alterations in neuronal firing from cocaine administration have been seen in a number

of brain areas (38). These increases have been correlated with alterations in cocaine-induced locomotor activity (34,77). The hippocampus–accumbens projection is believed to be a major pathway in cocaine-induced locomotor activation in rats (33,54,55).

The reduction of the synthesis of serotonin with selective neurotoxins or tryptophan hydroxylase inhibitors show a potentiation of cocaine-induced hyperlocomotion and causes the increase in the reinforcing effects of cocaine (35,81,83,96). This potentiation is due to the reduction of 5-HT in the brain, suggesting that the 5-HT system may act as an inhibitory mechanism on cocaine-induced hyperlocomotion (35,92,97,98).

Conversely, increasing the amount of 5-HT available in the brain, as with administration of 5-HT precursor tryptophan hydroxylase, causes attenuation of cocaine-induced hyperlocomotion and increased stereotypy (35,38,81,83). Moreover, increasing 5-HT levels with SSRI's or increasing dietary tryptophan reduces the self-administration of cocaine (99,100,100). Thus, suggesting that 5-HT is an inhibitory mechanism resulting in decreased hyperactivity when increased amounts of 5-HT are present (89,92,100). This in part is due to activation of auto regulatory feedback mechanisms, by the stimulation of impulse-modulating autoreceptors on 5-HT neurons, which accounts for cocaine-induced inhibitory responses; as well as, other 5-HT receptors being activated (48,89,100).

Single unit extracellular recordings have shown that intravenous cocaine administration causes a depression of spontaneous firing of 5-HT neurons, as well as, potentiating inhibitory effects in the DR nucleus of the rat (23). This decrease in firing is believed to be caused by the inhibition of 5-HT reuptake, which causes an increase in the synaptic concentration of serotonin, which in turn activates the somatodendritic autoreceptors which reduces neuronal firing (34,43,45).

In vivo microdialysis after systemic injections of cocaine demonstrates increases extracellular 5-HT, 5-HIAA, the serotonin metabolite, and 5-HIAA/5-HT concentration in a number of specific brain regions in male rats (23,26-28,32,33,52,54,55,65,75,87,96,101-103). Specifically, 5-HT increases are found in the raphe nucleus (dorsal raphe nucleus), striatum (caudate, putamen and NAc), VTA, hippocampus areas (specifically the hippocampus-accumbens projection), amygdala, thalamus and the frontal cortex (13,23,26-28,32,33,47,52,54,55,65,75,87,96,101-105). However, in females acute administration of cocaine causes decreases in serotonin (5-HT), and 5-hydroxyindole acetic acid (5-IAA), in the nucleus accumbens (13,104,105).

H. Role of 5-HT_{1A} and 5-HT_{1B} on Cocaine-Induced Behaviors:

i. 5-HT_{1A} Receptor:

For an overview, see Table 2 and 3. The 5-HT_{1A} receptor has been implicated directly in the effects of cocaine-induced behavioral effects (28,32,38,47,54,106). At the systemic level 5-HT_{1A} receptor activation with specific agonists causes the increase of cocaine-induced hyperlocomotion (38,47,51-53,53). The depletion of 5-HT in terminal regions is believed to be caused by agonists' ability to increase somatodendritic autoreceptor activation in the raphe nuclei causing the reduction in 5-HT in terminal regions (47,50-53). This reduction is caused by the suppression of impulse activity of 5-HT neurons (28,33,38,40,40,46,47,54,106). In the hippocampus and NAc a direct decrease of 5-HT concentration and 5-HIAA/5-HT ratios have been demonstrated after 8-OH-DPAT administrations (28,52-54).

Conflicting evidence has been found with local injection of 8-OH-DPAT, suggesting substantial differences between brain area and activation (33). In the ventral hippocampus 8-OH-DPAT induced 5-HT_{1A} receptor stimulation causes the suppression of cocaine-induced

hyperlocomotion, whereas stimulation in the ventral striatal region caused increases in hyperlocomotion (33,55). In addition, local activation of 5-HT_{1A} receptors in the NAc does not affect the increase in 5-HT level after cocaine injection (33,58,69).

Stimulation of 5-HT_{1A} receptors with specific agonists facilitates the establishment of locomotor sensitization while antagonists attenuate locomotor sensitization (52,107). Systemic application of 8-OH-DPAT and other agonists that stimulate 5-HT_{1A} receptors in the VTA or substantia nigra does not substitute for cocaine or mediate the discriminate stimulus properties of cocaine (33,63,100). However, stimulation of the 5-HT_{1A} receptor locally in specific brain areas by agonists increases self-administration and facilitates conditioned place preference (33,63,100). Others have found the opposite, with acute administration of 8-OHDPAT reducing response rates for cocaine self-administration (22,63,100).

Pharmacologic manipulation of 5-HT_{1A} receptors with N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cylhexanecarboxaminde maleate (WAY 100635), a 5-HT_{1A} antagonist, before cocaine administration, decreases overall cocaine-induced behavioral activity (28,40,51-55,65,107-109). In the hippocampus and nucleus accumbens, after cocaine administration, 5-HT_{1A} receptor antagonism with WAY 100635 inhibited cocaine-induced locomotor activity while increasing 5-HT concentrations (28,32,54). In addition, 5-HIAA/5-HT ratios are decreased after WAY 100635 administration (28,52-54). Microinjection of WAY 100635 into the dorsal raphe nucleus of female rats an increase in total locomotor activity and rearing is found with a decrease in head bobs (96). In contrast, microinjection of WAY 100635, into the median raphe nucleus, did not alter the stimulant effect of cocaine on locomotor activity; rears or head bobs; suggesting that 5-HT_{1A} effects are anatomically specific (96,110). No effects were found with systemic treatment of the antagonist in female rats.

Cocaine binds 5-HT transporter inhibiting the reuptake of 5-HT from the synaptic cleft. This yields an increase in 5-HT not only stimulating postsynaptic receptors but also activating inhibitory 5-HT_{1A} autoreceptors somatodendritically which causes a decrease in neuronal activity (32,33,38,40,47,55,65,89). 5-HT_{1A} receptor antagonists have been shown to prevent 5-HT_{1A} receptor mediated autoinhibition of serotonergic neuron firing (38,53,55). The use of WAY 100635 blocked 5-HT_{1A} receptors attenuated the inhibitory effect of somatodendritically released 5-HT on 5-HT neurons (32,33,38,40,47,55,65,89). The effect of local 5-HT reuptake blockade in terminal areas by cocaine combined with 5-HT_{1A} antagonism serves to potentiate extracellular 5-HT concentrations in terminal regions of 5-HT projections (32,33,38,40,47,55,65,89).

For instance, Flesinoxan, known to be a highly efficient agonist at the pre- and postsynaptic 5-HT_{1A} receptor sites, causes a dose-dependent inhibition of firing of DRN serotonergic neurons, which is eliminated by administration of WAY 100635 (111). S15535, an agonist and partial agonist (depending on dose) at pre- and postsynaptic 5-HT_{1A} receptor sites, also reduced DRN firing, which was reversed by WAY 100635 (111).

ii. 5-HT_{1B} Receptor:

For an overview, see Table 2 and 3. Evidence of the involvement of the 5-HT_{1B} receptors in cocaine-induced behavioral activities has been conflicting. Although there are a number of available ligands that target the 5-HT_{1B} receptor, they lack specific affinity and selectivity (40). However, a number of researchers have found that 5-HT_{1B} receptors do in fact play a role in the mediation of behavioral, reinforcing and subjective effects of cocaine (48).

There is an abundance of 5-HT_{1B} receptors in a number of motor areas of rats, suggesting a major role in cocaine-induced locomotor activity (48,73,89,92,99,100). Systemic or cerebral administration of agonists such as RU 24969, elicits robust cocaine-induced locomotor hyperactivity

in a dose-dependent manner which is reversed by 5-HT_{1B} antagonists such as GR 127935, or pindolol (7,38,48,89,99,100,112-114). In addition, 5HT_{1B} receptor antagonist GR 127935, decreases cocaine-induced locomotor hyperactivity; even, in some cases, almost totally blocking acute treatments of cocaine (1,48,114,115). Further, cocaine-induced hyperactivity with agonists is potentiated in 5-HT_{1B} KO mice (112,116). However, local stimulation of the 5-HT_{1B} receptor in the nucleus accumbens core using the 5-HT_{1B} agonist CP 93129 did not affect hyperlocomotion after cocaine administration. However, stimulation in the nucleus accumbens shell facilitated cocaine-induced hyperactivity (1,48,117-119). 5-HT_{1B} receptors located in the ventral tegmental area (VTA) were found to not be involved in the locomotor hyperactivity and sensitization to cocaine administration in male Wistar rats (1). Although 5-HT_{1B} receptors have no involvement in locomotor sensitization in rats, KO mice show a potentiation (112,116,119). Therefore agents reducing 5-HT levels enhance the locomotor response to cocaine and those increasing 5-HT decrease behavioral responses (40,48,53,70). This is demonstrated by the ability of the 5-HT_{1B} agonists such as CP 93129 to increase the synthesis of 5-HT upon activation (117).

A number of agonists and antagonist, which target the 5-HT1B receptor, have been found to affect cocaine reward. Specifically, RU 24969 can partially substitute for cocaine in drug-discrimination studies (63,100). Conversely, local stimulation in the VTA, NAc core and NAc shell does not substitute for cocaine in drug-discrimination studies (33). Self-administration is facilitated by pretreatment into the VTA with 5-HT_{1B} receptor agonists CP 94253 and RU 24969 in rats, while it is blocked by the selective antagonist GR 127935 in rats (48,63,100,120-122). However, potentiation of self-administration is seen in KO mice (112). Both pharmacological and transgenic experimentation have found a facilitory role of the 5-HT_{1B} receptor in conditioned place preference (33,123).

iii. 5-HT Transporter (SERT):

As described previously the major mechanism for removing serotonin from the synaptic cleft is reuptake by the serotonin transporter (SERT) (24,91,124-126). Selective serotonin reuptake inhibitors (SSRI's) target the 5-HT transporter and block the reuptake of 5-HT. SSRI's increase 5-HT in a number of brain regions including the raphe nuclei and forebrain areas and play a major role in the expression of cocaine's behavioral effects (100,127).

Both systemic and local application of the SSRI's fluoxetine and/or fluvoxamine have been found to increase 5-HT levels and enhance acute cocaine-induced locomotor activity in rats (65,75,89,96,100,125,128). In most instances this induced 5-HT reuptake blockade caused a potentiation in cocaine-induced locomotor behaviors (38,75,96,128)}. However, Reith et al., 1991 demonstrated that specific reuptake inhibitors, that increase extracellular 5-HT available within serotonergic cells, did not attenuate cocaine-induced hyperlocomotion (96,109,128).

The SSRI's such as citalopram, fluoxetine, fluvoxamine, and sertraline do not completely mimic the stimulus effects of cocaine but do have some effects on the discriminative stimulus and subjective properties of cocaine in both humans and rodents (38,89,100,129,130). Fluoxetine or sertraline can shift the dose-effect curve of cocaine to the left demonstrating an enhancement of the stimulus properties of cocaine in rats by the decrease in self-administration (63,89,100,131,132).

Table 2. The role of 5-HT receptors in the behavioral effects of systemic administration of cocaine. Taken from Muller et al., 2006.

Cocaine-induced behavior	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_3	5-HT_4	5-HT_6
Hyperlocomotion	↑	↑[a]	↑	--	↓	↑	↑	--
Eating suppression	--	NI	NI	NI	NI	NI	NI	NI
Grooming suppression	--	NI	NI	NI	NI	↑	NI	NI
Aggression	↓	NI	NI	NI	NI	↑	NI	NI
Substitution	--	Partial	--	NI	--	Partial	NI	--
Discriminative stimulus:	--	↑	↑	NI	↓	--	NI	--
Sensitization:								
establishment	↑	--[c]	--	--	--	--	NI	NI
expression	↑	--	↑	--	--	↑	NI	NI
Place preference:								
establishment	--	↑	NI	NI	NI	↑[c]	NI	NI
expression	↑	NI	NI	NI	NI	--	NI	NI
Self-administration:	↓	↑[c]	--	NI	↓	--	NI	--
SAR by cue	--	↓	↑	NI	↓	--	NI	NI
SAR by cocaine	↑	↓	↑	NI	↓	NI	NI	NI

a = Receptor contributions assigned to a specific cocaine-addiction-related behavior indicate the results of most studies. Species are laboratory rats, mice, hamsters and monkeys.

b = Abbreviations: NI, not investigated; SAR, reinstatement of self-administration; ↓ = facilitation; ↑ = attenuation; -- = no contribution.

c = Different findings have been obtained using transgenic mice

Table 3: Effect of 5-HT compounds on motor activity elicited by cocaine: Adapted from Cunningham and Callahan 1994 and revised.

5-HT Compound	Effect on Cocaine-Induced Motor Activity
Reuptake Inhibitor	
Fluoxetine	NC Hyperactivity; ↑ Hyperactivity
Fluvoxamine	↑ Hyperactivity
Paroxetine	NC Hyperactivity
Sertraline	NC Hyperactivity
Synthesis inhibitor	
PCPA	↑ Hyperactivity
Precursor	
5-HTP	↓ Hyperactivity
5-HT$_{1A}$ agonists	
OHDPAT	↕ Hyperactivity; Most ↑
5-HT$_{1A}$ antagonist	
WAY 100635	↕ Hyperactivity; Most ↓
5-HT$_{1B}$ agonists	
RU 24969	↕ Hyperactivity; Most ↑
CP94253	
5-HT$_{1B}$ antagonist	
GR 127935	↕ Hyperactivity; Most ↓
Pindolol	
Propranol	

I. Sex Differences:

i. Sex Differences in the Serotonergic System:

Differences in serotonergic neuroanatomy, neurochemistry and behavior of male and female humans and rats have been found. Females have increased serotonin synthesis and serotonin metabolites in a number of brain regions; summarized in Table 4 (133,134). Female rats exhibit 5-HT mediated syndrome at lower doses of the 5-HT precursor L-tryptophan than males (96,135). In addition, females' exhibit increased sensitivity to the anxiolytic effects of 5-HT_{1A} agonists compared to males (135,136). Females demonstrate a more rapid attenuation of hypothermic and adrenocortical responses to repeated 5-HT_{1A} stimulation, decreased prolactin response to 5-HT, and increased cortisol (133,136). Females also have a greater number of serotonergic fibers in the sexually dimorphic nucleus-preoptic area (POA). Using positron emission tomography (PET), researchers have found that women have a smaller rate of serotonin synthesis. In addition, 5-HT synthesis is reduced about four times more than it is in men following tryptophan depletion (62). In humans, cerebrospinal fluid 5-HIAA is increased in selected syndromes in women; however, whole brain serotonin synthesis is decreased in women when compared to men (137).

However, in rats, females appear to have an overall increase of regional concentrations of serotonin in the brain (133,138). Synthesis and levels of 5-HT and 5-HIAA are higher in females in the brainstem, limbic forebrain and the cortex in rats (62,138). In addition, 5-HIAA levels and 5-HIAA/5-HT ratios were significantly higher in females in the hypothalamus/preoptic area, limbic forebrain, hippocampus, striatum and cortex (133,138). Tryptophan concentrations were significantly higher in females in the brainstem, striatum and cortex than males (62,138,139). Furthermore, inhibition with L-amino acid decarboxylase produced a more profound accumulation of 5-hydroxytryptophan (5-HTP) in females (138).

Table 4: Sexual Dimorphisms in the serotonergic system; Adapted from Rubinow et. al, 1998

Measure	**Sex Difference**	**Brain Region**	**Citation**
Sexual dimorphisms in Rats			
5-HT, 5-HIAA levels	F > M	Whole brain, brainstem limbic forebrain, anterior hypothalamus	Carlsson et al.,1985;1988
Stimulated 5-HT and serotonin syndrome (after MAOI and L-tryptophan)	F > M	Whole brain	Carlsson et al., 1985; Biegon et al., 1979
5-HT synthesis	F > M	Forebrain, hippocampus, whole brain	Haleem et al., 1990; Rosecrans et al., 1979; Vaccari et al., 1977; Carlsson et al., 1985
5-HT synapse/receptor distribution	F ≠ M	MPN	Simerly et al., 1984; Zhang et al. 1999
5-HT_{1A} receptor binding	F ≠ M	Hippocampus	Zhang et al., 1999
5-HT_{1A} receptor mRNA expression	F < M	Hypothalamus, amygdala, hippocampus	Zhang et al., 1999
Response to 8-OH-DPAT			
↓ 5-HT synthesis ↑ corticosterone ↓ temperature ↑ prolactin ↓ 5-HIAA/5-HT ratio Hyperphagia	F > M F < M	Hippocampus Hypothalamus	Zhang et al., 1999 Haleem et al. 1989; Carlsson et al., 1985; Matsuda et al., 1991; Carlsson and Eriksson 1986; Maswood et al., 1995; Ebenezer and Tite, 1997; Salamanca and Uphouse, 1992; Uphouse et al., 1991
Sexual dimorphisms in Humans			
5-HT synapse/receptor	F > M	Anterior cingulate, dorsal raphe, hippocampus, amygdala; medial PFC, orbital PFC	Parsey et al., 2002, 2005; Fischette et. al., 1983
5-HT, 5-HIAA synthesis 5-HIAA/5-HT ratios	F > M F < M F ≠ M	Brainstem, limbic forebrain, cortex, hypothalamus, hippocampus, striatum	Parsey et al., 1999; Nishizawa et al., 1997 Arato et al., 1991

ii. Sex Differences in the Behavioral and Subjective Effects of Cocaine:

Sex differences exist in the pattern of cocaine abuse, dependence, and addiction in humans. Cocaine dependence has been found to be higher in adolescent women than men (8,9). Females are also more likely to use cocaine at an earlier age, take less time to become addicted, and enter treatment at an earlier (8,9,93,140). Women also consume cocaine by more addictive routes and progress to dependence more rapidly than men (93). This evidence suggests that the pattern of cocaine use, and onset of addiction, is more rapid and more intense in females than males (7,22). Therefore, the incidence of the lifelong progression and degree of dependence to cocaine is higher in females when compared to males (7,22,93). In humans a number of behavioral and subjective effects of cocaine exist differentially in males and females. Women experience increased nervousness following intranasal administration; they take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria and have a longer duration of the 'high' associated with cocaine compared to males (7,93). Furthermore, Robbins et al., 1999 found that cocaine cues induce more drug cravings in females compared to male addicts.

In rodents sex differences in cocaine-induced total locomotor, ambulatory, rearing and stereotypical behaviors are seen; females showing higher levels compared to males (13,15,17,20,94,141-145). Females show a much more robust hyperactivity and increased exaggeration of cocaine-induced behaviors after initial cocaine administration (13,146). Females also acquire cocaine discrimination at a faster rate and acquire cocaine self-administration more quickly, have higher rates of self-administration and are more sensitive in the reinstatement of self-administration of cocaine (18-20,94). Russo et al, 2003 found that cocaine conditioned place preference (CPP) occurred after a shorter number of pairings, at lower doses of cocaine, compared to male rats. Festa 2003, demonstrated that after a single cocaine administration, female rats not only

have greater locomotor activity than male rats they also have more prolonged and robust locomotor activity (13). Sex differences exist in sensitization; female rats show higher levels of sensitization to repeated cocaine administration, as well as being sensitized with a lower dose of cocaine compared to males (5,87,144).

Acute administration of cocaine in female rats cause decreases in serotonin (5-HT), and 5-hydroxyindole acetic acid (5-IAA), in the nucleus accumbens (NAc) whereas increases of 5-HT is seen in males in the NAc (13,31). Perrotti et al., 2000 found that in female Fischer rats, the co-administration of progesterone and cocaine resulted in higher levels of 5-HT and 5-HIAA, in the medial prefrontal cortex (mPFC), when compared to other cocaine, OVX and hormone-treated rats. However, no difference in turnover ratios (5-HIAA/5-HT) was observed. This is consistent with studies with male rats with increases of serotonin synthesis and turnover in the medial prefrontal cortex which is also correlated with locomotor activity (51,142,149-151). These sex differences have been demonstrated to be caused by some mechanism other than metabolism differences, as levels of plasma cocaine have been found to be the same in both males and females (13,147). Others have found no effect of cocaine on serotonin levels compared to saline controls (148).

iii. Sex differences in 5-HT_{1A} receptors:

In male rats 5-HT_{1A} receptor mRNA is higher in the hypothalamus amygdala, and the hippocampus when compared to females (152). However, no significant differences in the distribution of 5-HT_{1A} receptor binding sites were found. In KO mice however, a sex differences was found with females having lower binding than male rats (153). Human females, however, have greater 5-HT_{1A} receptor binding when compared to males (62). Specifically, both *in vitro* and *in vivo* methods have demonstrated females have higher binding in the dorsal raphe, amygdala, anterior cingulated, medial prefrontal cortex and orbital prefrontal cortex (62,135). Moreover, 8-OH-DPAT,

causes twice as much decrease of 5-HT synthesis rates in the hippocampus of females (-64%) than in male rats (-32%) (62). Specifically, females demonstrate an increased behavioral response to the application of the serotonin 5-HT_{1A} agonist 8-OH-DPAT, with decreases in 5-HT synthesis in the hippocampus (133-135). Sex differences were also observed in 5-HT_{1A} receptor expression, and in the serotonergic activity of the hippocampus and hypothalamus before and after a forced swim test paradigm (154). Specifically, that hypothalamic 5-HT_{1A} mRNA levels were decreased in female rats while in male rats' hippocampal 5-HT_{1A} mRNA levels were increased (154).

iv. Sex differences in 5-HT_{1B} receptors:

Studies have addressed sex differences in the 5-HT_{1B} receptor. In 5-HT_{1B} receptor knockout mice microdialysis studies confirmed significantly higher baseline levels of hippocampal 5-HT in female mice compared to males (153). In addition, both male and female 5-HT_{1B} receptor knockout mice demonstrated greater dialysis responses to fluoxetine as well (153). Differences in receptor density have yet to be explored.

v. Sex differences in SERT:

It is suggested that higher 5-HT synthesis in women may account for lower 5-HT transporter availability (62,135). However, higher diencephalon 5-HT transporter availability was observed in healthy women and women are repeatedly found to have higher 5-HT transporters when compared to men (135,155-157). Using single photon emission computed tomography (SPECT), imaging [123I] beta-CIT uptake was higher in the striatum (10%), diencephalon (15%), and brainstem (15%) in females when compared to males (135).

J. Neuroendocrine Effects of Cocaine:

Cocaine causes dramatic effects in the functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Cocaine rapidly triggers the release of corticotrophin-releasing hormone (CRH), which in turn, stimulates the rise in plasma adreno-corticotropic hormone (ACTH) (143,158). Acute chronic cocaine administration has shown dramatic increases in plasma levels of ACTH and corticosterone in rats and cortisol in human males and females (30,159). A sex difference exists in this effect with female rats having a greater increase in ACTH and corticosterone serum levels after acute cocaine exposure compared to males (143,160). For example, using a sensitization paradigm, Chin et al., 2002, found that corticosterone serum levels were greater in female rats that received a single injection compared to those that received injections for 14 days and received a challenge dose. Furthermore, Russo et al., 2003 utilizing a CPP paradigm which used i.p. injections of saline or cocaine (0, 5, 10, & 20 mg/kg) paired with a specific environmental stimuli (i.e. black or white chamber) found that after acute cocaine administration females had greater ACTH and corticosterone plasma levels compared to males (148). Specifically, dose-dependent increases of corticosterone plasma levels correlate with increasing doses of cocaine (21,148). In addition, plasma corticosterone has been shown to influence acquisition maintenance and reinstatement of cocaine self-administration (159,161,162). Interestingly, ovariectomy decreases cocaine-induced ACTH and corticosterone levels in females, whereas castration has no effect (30). Thus, the enhanced HPA axis effects of cocaine in female rats could contribute to enhanced behavioral reactivity seen in female rats compared to males (143,145).

Unlike research examining cocaine's effects on the HPA axis, cocaine's effects on the hypothalamic-pituitary gonadal (HPG) axis has been much more inconsistent. This may be due to the complex differences in the reproductive systems of males and females; and the complexity of

cycles' present in female mammals. However, cocaine has been shown to affect HPG activation at all levels of its feedback loop. Increased LH and FSH have been found in both men and women after cocaine administration (147,163,163). It has been hypothesized that these LH and FSH modulations are due to cocaine's direct effect on GnRH neurons or hypothalamic neurotransmitter modulation of GnRH release (30). In contrast, evidence in rats has shown increased LH, but not FSH, in female rats after cocaine administration (164).

Cocaine increases plasma levels of progesterone in rats (145,165). Plasma progesterone levels were significantly higher in male rats after binge pattern cocaine administration compared to saline-treated animals, which was not seen following a single injection (5). Compared to males, females showed increased progesterone levels following both binge administration, as well as, a single injection (5,13,165,166).

K. Hormones and the Serotonergic system

Evidence suggests that sexual dimorphisms seen in male and female rats could be attributed to interactions among serotonergic and hormonal systems (167). For instance, presynaptically, progesterone receptors have been localized within 5-HT cell bodies (167,168). Moreover, estrogen and progesterone receptors are present on raphe interneurons (168). mRNA levels for the tryptophan hydroxylase, SERT and the 5-HT_{1A} somatodendritic autoreceptor have all shown effects of hormonal treatments (169,170).

Specifically, it has been hypothesized that ovarian steroids, estrogen (E) and progesterone (P), may modulate the function of the serotonergic (5-HT) system by modulating neuronal firing activity (171). Differences in firing between male and female rats within the dorsal raphe nuclei during normal male and female E and P fluctuations were found. The average firing activity of 5-

HT neurons was significantly higher in males (41%) compared to freely cycling and overiectomized females (171). Firing rates of neurons in the dorsal raphe nucleus was correlated with progesterone levels, with higher levels of progesterone causing increases in firing (171).

However, progesterone has been shown to decrease the extracellular levels of 5-HT and its turnover rate in different regions of the hypothalamus and the midbrain central gray, following E priming (172,173). Moreover, estrogen treatments have been shown to decrease the serotonin reuptake transporter (SERT) mRNA levels in monkeys with increases seen in rats (157,170). Estrogen and progesterone have been found to upregulate 5-HT transporter expression and modulate adaptations in serotonergic receptors in response to antidepressant treatment (155,169,170). In addition, hippocampal levels of 5-HT have been found to be consistently higher in female rats during both diestrus and proestrus than in age-matched males (152). Ovariectomized females decreased overall levels of 5-HT, as well as, increased 5-HT turnover ratios compared with intact females in the VTA but not in the NAc (148). Researchers suggest that gonadal hormones may differentially modulate 5-HT cell bodies in the VTA and raphe nucleus. Gonadectomy in males significantly increases 5-HT_{1A} messenger RNA content in the cortex hypothalamus, hippocampus, amygdala and dorsal raphe (152).

L. 5-HT_{1A} and the Endocrine system

At the level of the paraventricular nucleus, serotonin is released from dorsal raphe' nerve terminals acting on 5-HT_{1A} receptors. This causes an increase in adrenocorticorophin hormone (ACTH) and thereafter coriticosterone release (174). Neuroendocrine studies in rats have found that 5-HT_{1A} agonists, such as 8-OH-DPAT or buspirone show dose-dependent increased elevations of plasma ACTH and corticosteroids which can be blocked by 5-HT_{1A} antagonists (such as pindolol or

WAY 100635) (34,52,175,176). WAY 100635 administration alone has been shown to reduce levels of corticosterone in male Wistar rats when given alone (28,54). Others have found that WAY 100635 blocks 8-OH-DPAT-induced elevation of plasma ACTH but does not effect corticosterone alone (109).

Acute application of low doses of corticosterone attenuates 5-HT_{1A} receptor function in CA1 subfields of the rat hippocampus with higher concentrations enhancing 5-HT_{1A} hippocampal function (177). 5-HT_{1A} receptors, either on serotonergic nerve terminals or postsynaptic neurons are found to be down regulated in response to chronic corticosterone treatment (28,53,54). However, acute *in vitro* treatment with corticosterone has been found to attenuate 5-HT_{1A} autoinhibition in the DRN (178). Conversely, Fairchild et al., 2003 found no effect on 5-HT_{1A} receptor function at maximal doses of corticosterone. Finally, administration with dexamethasone, which was suggested to be a more potent glucocorticoid than corticosterone, showed no effect.

III. Significance and Specific Aims:

As discussed, a number of sex differences have been found in cocaine's behavioral and neurochemical effects. Mechanisms underlying these sex differences are poorly understood. Recently, Festa et. al (2004), demonstrated that although there are sex differences in the dopaminergic system after cocaine administration, these differences were not as robust as expected. In addition, dopamine knockout mice have been found to continue to self-administer cocaine with only double knockouts of both the dopamine (DAT) and serotonergic (SERT) transporters showing diminished effects. The serotonergic (5-HT) system has recently been implicated in the modulation of cocaine-induced activity in rats. For example, increases in serotonergic activity after cocaine administration have been reported, giving rise to behaviors, such as, increased locomotion. Particularly, the 5-HT_{1A}, 5-HT_{1B} receptors and the serotonin transporter have been implicated in the modulation of various neurochemical and behavioral cocaine effects. Therefore, this research aims to determine whether 5-HT plays a significant role in the sex differences in cocaine-induced behavioral effects. We hypothesize that 5-HT (at the level of release, reuptake, and/or receptor level activation) mediates sexual dimorphisms seen after cocaine administration. Understanding how the serotonergic system contributes to sex differences in cocaine-induced behavioral activation will allow for more efficient treatment of male and female cocaine abusers. To test these postulates, the following aims were proposed:

AIMS:

Specific Aim 1: We hypothesize that sex differences in cocaine-induced motor behavior responses are mediated by serotonin receptor activation. To this end, fluoxetine (a general serotonin reuptake inhibitor), 5-HT_{1A} and 5-HT_{1B} antagonists were co-administered with cocaine or saline prior to behavioral assessment of male and female rats.

Specific Aim 2: We hypothesize that sex differences seen in cocaine-induced behaviors are based on activational differences in serotonergic second messenger systems. Serotonin receptors were measured via functional autoradiography basally, as well as, after acute cocaine administration.

Specific Aim 3: We hypothesize that the co-administration of cocaine and 5-HT_{1A} and 5-HT_{1B} antagonists will have differential effects on endocrine activity. Progesterone and corticosterone levels in male and female rats were measured by radioimmunoassay.

Chapter 2: Sex Differences in 5-HT$_{1A}$ and 5-HT$_{1B}$ Serotonin Receptor Effects on Acute Cocaine Behavioral Responses

1. Introduction

Sex differences exist in the pattern of cocaine abuse, dependence, and addiction in humans (8,9,93,140,7,22). Cocaine dependence has been found to be higher in adolescent women than men (8,9). Females are also more likely to use cocaine at an earlier age, take less time to become addicted, and enter treatment at an earlier (8,9,93,140). Women also consume cocaine by more addictive routes and progress to dependence more rapidly than men (93). This evidence suggests that the pattern of cocaine use, and on-set of addiction, is more rapid and more intense in females than males (7,22). Therefore, the incidence of the lifelong progression and degree of dependence to cocaine is higher in females when compared to males (7,22,93). In humans a number of behavioral and subjective effects of cocaine exist differentially in males and females. Women experience increased nervousness following intranasal administration; they take longer to feel the subjective effects of cocaine, report less euphoria and dysphoria and have a longer duration of the 'high' associated with cocaine compared to males (7,93). Furthermore, Robbins et al., 1999 found that cocaine cues induce more drug cravings in females compared to male addicts. Females also have a greater number of serotonergic fibers in the sexually dimorphic nucleus-preoptic area (POA) (62). Using positron emission tomography (PET), researchers have found that women have a smaller rate of serotonin synthesis (62). In addition, 5-HT synthesis is reduced about four times more than it is in men following tryptophan depletion (62). In humans, cerebrospinal fluid 5-HIAA is increased in selected syndromes in women; however, whole brain serotonin synthesis is decreased in women when compared to men (137).

In rodents sex differences in cocaine-induced total locomotor, ambulatory, rearing and stereotypical behaviors are seen; females showing higher levels compared to males (13,15,17,20,94,141-145). Females show a much more robust hyperactivity and increased exaggeration of cocaine-induced behaviors after initial cocaine administration (13,146). Females also acquire cocaine discrimination at a faster rate, self-administer cocaine more quickly and are more sensitive in the reinstatement of self-administration (18-20,94). Russo et al, 2003 found that in females cocaine conditioned place preference (CPP) occurred after a shorter amount of pairings and at lower doses of cocaine than male rats. Festa et al., 2003 demonstrated that after single cocaine administration, female rats not only have greater locomotor activity than male rats but also have more prolonged and robust locomotor activity than males (13). In addition, female rats exhibit 5-HT mediated syndrome at lower doses of the 5-HT precursor L-tryptophan than males (96,135). These dimorphic effects may be due to dimorphisms seen in the levels of 5-HT and their metabolites with females having overall increased levels of 5-HT and 5-HIAA compared to males (133).

Increasing evidence suggest that serotonergic mechanisms are directly and indirectly involved in the locomotor behavioral effects of cocaine (5,35,38,65,77,78,80,82,85,95,166,182). Specifically, in male rats, both the 5-HT_{1A} and 5-HT_{1B} receptors have been implicated in a number of cocaine-induced behavioral responses including cocaine-induced locomotor hyperactivity. For instance, inhibition of 5-HT_{1A} with N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cylhexanecarboxaminde maleate (WAY 100635) a 5-HT_{1A} antagonist, dose-dependently attenuated behavioral effects of cocaine (28,32,51-55,65,107-109,183). Conversely, after 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) administration, a 5-HT_{1A} receptor agonist, increased behavioral activity is seen (1,51-53,107,108). Additionally, 5-HT_{1B} antagonists, such as 2'methyl-4'-(5-methyl [1, 2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4methodoxy-3-(4-methyl-piperazin-1-yl)-

phenyl]-amide (GR 127935) decreases cocaine-induced locomotor hyperactivity dose-dependently (38,99,112,115,116,118,119). Moreover, administration of the 5-HT_{1B} agonists, such as 5-methoxy-3-1,2,3,6-tetrahydro-4-pyridinyl-1H-indole (RU 24969), dose-dependently increased locomotor activity (29,38,87,118,119).

Although it is accepted that there is a fundamental role of 5-HT in cocaine-induced behavioral effects, the role of sex differences in serotonin receptor modulation in cocaine-induced behaviors, has yet to be elucidated. The aim of this research is to test the hypotheses that sex differences in the activation of 5-HT_{1A} and 5-HT_{1B} receptors contribute to the sexually dimorphic patterns seen in cocaine-induced hyperactivity. Specifically, pharmacological antagonism of these receptors will have a direct attenuation of sexually dimorphic cocaine-induced hyperlocomotor activity.

2. Methods

2.1 Animals

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed for one week prior to experimental manipulations in standard plastic cages (20 x 20 x 41 cm^3). Rats had free access to standard lab chow and water *ad libitum* and maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.). Rats were handled and weighed for 3 days prior to experimental manipulations. Three separate cohorts of animals were used for behavioral studies with an n of 8 per group. In female rats repeated vaginal lavage attenuates cocaine-induced activity, and establishes a place preference and increased dopamine release in the striatum (184). Thus females were placed into experimental groups randomly without regard to estrous cycle stage. All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH

publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Drugs

Cocaine hydrochloride, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-ycylhexanecarboxaminde maleate (WAY 100635), and 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-corboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR 127935) were purchased from Sigma Chemical Co. (St. Louis, MO).

2.3 Drug treatment and experimental paradigm

Cocaine, WAY 100635, and GR 127935 solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneally. All injections were performed in the home cage. After 15 minutes pre-treatment with WAY 100635 (0, 0.4, 0.8, or 1.6 mg/kg) or 30 minutes with GR 127935 (0, 5, 10, or 15 mg/kg), rats were injected with (20 mg/kg) of cocaine or saline. This cocaine dose was previously demonstrated to produce significant increases in locomotion and robust sex differences in cocaine-stimulated motor activity, without reaching either a minimal or maximal effect in either sex (13). Antagonist doses and pretreatment schedules were chosen based on previous reports in male rats, which demonstrated an inhibition of locomotor behavioral responses to cocaine (7,47,50-53,99,185,186).

2.4 Behavioral Measurement

Total locomotor, ambulatory and rearing activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described (160). All behavioral activities were recorded for one-hour post-drug treatment. Total locomotor

activity represented the sum of counts in the horizontal frame. Ambulatory activity represented the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Rearing activity represented total counts of vertical motion.

2.5 Statistical Analysis

Total locomotor, ambulatory, and rearing data were summed for each subject and presented as mean ± standard error of the mean (SEM). A three-way analyses of variance (ANOVAs) were used to determine the effects of antagonist administration, cocaine, and sex on cocaine-induced behavioral responses: ANTAGONIST DOSES (vehicle, WAY 100635, or GR 127935) x DRUG CONDITION (cocaine or saline) x SEX (male vs. female). Within sex, two-way ANOVAs were used to determine the effects of antagonist dose on cocaine-induced behavior: antagonist dose (vehicle, WAY 100635, or GR 127935) x cocaine (cocaine or saline). When significance was obtained, Fisher LSD test was used. To account for these baseline effects, delta value was calculated; the difference between cocaine effects minus their respective saline behavioral responses. A p-value of less than 0.05 was considered significant in all statistical analysis.

3. Results

3.1 Sex Differences in the effects of cocaine-induced behavioral activity using WAY 100635

As shown in Figure 9-11, a significant main effect of sex was obtained [total locomotor: $F(1, 112) = 42.89$, $p = 0.0001$; ambulatory: $F(1, 110) = 23.02$, $p = 0.0001$; rearing: $F(1, 114) = 41.23$, $p = 0.0001$]; where overall female rats had higher behavioral activity than male rats ($p < 0.05$ for all comparisons). A significant main effect of drug was also observed [total locomotor: $F(1, 112) = 312.00$, $p = 0.0001$; ambulatory: $F(1, 110) = 165.83$, $p = 0.0001$; rearing: $F(1, 114) = 415.69$, $p =$

0.0001]; overall, cocaine increased all three behavioral responses ($p < 0.05$ for all comparisons). Furthermore, a significant drug and sex interaction was observed in all three behaviors [total locomotor: $F(1,112) = 16.21$, $p = 0.0001$; ambulatory: $F(1, 110) = 10.4209$, $p = 0.0016$; rearing: $F(1, 114) = 24.27$, $p = 0.0001$]; where cocaine-treated, female rats had higher behavioral counts than male rats ($p < 0.05$ for all responses). In both male and female rats saline-treated rats, WAY 100635 did not alter any of the behavioral responses [total locomotor: $F(3, 57) = 2.7052$, $p = 0.0537$; ambulatory: $F(3, 56) = 0.2816$, $p = 0.8384$; rearing: $F(3, 60) = 1.2294$, $p = 0.3064$]. However, a significant drug and antagonist interaction was obtained in all three behaviors [total locomotor: $F(3,112) = 6.81$, $p = 0.0002$; ambulatory: $F(3, 110) = 6.71$, $p = 0.0003$; rearing: $F(3, 114) = 5.24$, $p = 0.0019$]; where antagonist attenuated cocaine-induced behavioral responses compared to vehicle in males and females respectively ($p < 0.05$ for all responses).

In female rats, co-administration of cocaine and WAY 100635 decreased total locomotor, ambulatory and rearing responses [total locomotor: $F(3, 55) = 4.57$, $p = 0.0062$; ambulatory: $F(3, 56) = 5.22$, $p = 0.0029$; rearing: $F(3, 56) = 2.94$, $p = 0.0408$]; all tested doses decreased total locomotor and ambulatory when compared to vehicle-treated rats. However, the 0.4 mg/kg dose produced the most attenuated cocaine-induced total locomotor and ambulatory responses when compared to other doses tested [total locomotor: $p = 0.0016$; ambulatory: $p = 0.0001$; rearing: $p = 0.0127$]. However, only the 0.4 mg/kg dose produced a significant attenuation of rearing activity when compared to vehicle-treated cocaine rats [rearing: $p = 0.0127$].

In male rats, co-administration of cocaine and WAY 100635 decreased total locomotor and rearing behavior [total locomotor: $F(3, 57) = 2.8274$, $p = 0.0465$; rearing: $F(3, 58) = 3.51$, $p<0.0205$]; all doses of WAY 100635 produced a decrease in behavioral activity when compared to vehicle-treated cocaine rats. Similar to females, the 0.4 mg/kg dose produced the most attenuated

behavioral responses when compared to other doses tested [total locomotor: $p = 0.0000$; rearing: = $p = 0.0000$]. However, unlike female rats, in male rats WAY 100635 failed to significantly attenuate cocaine-induced ambulatory activity [ambulatory: $p = (0.0671)$.

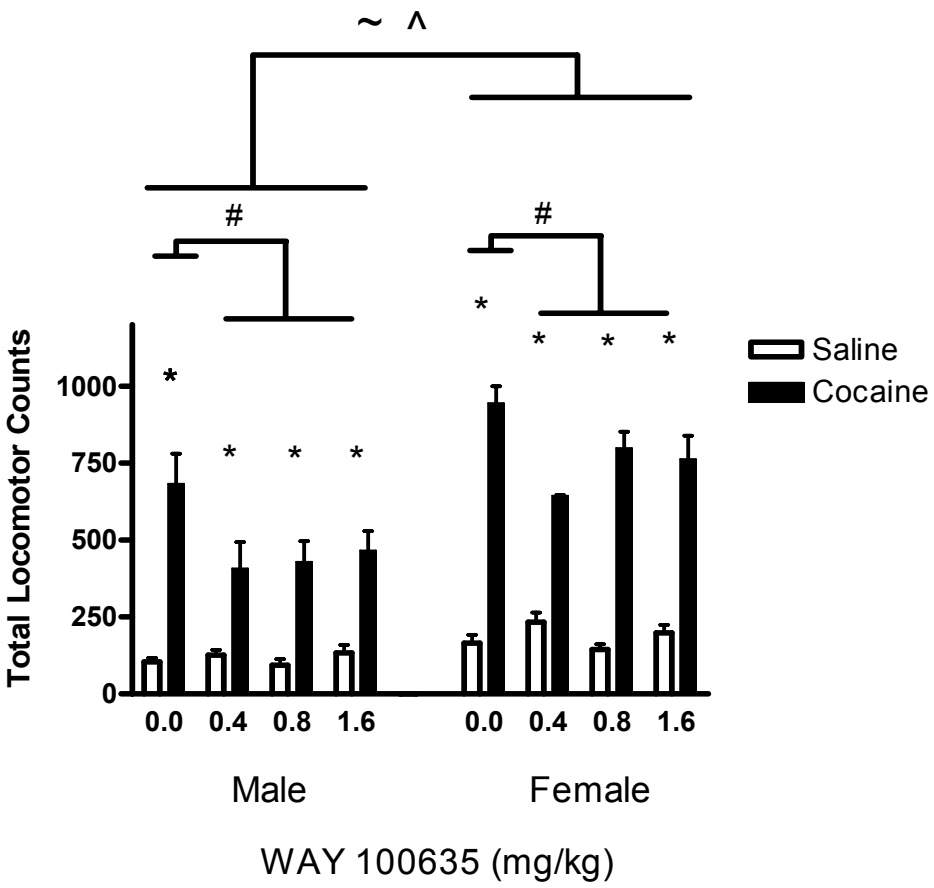


Figure 9: Sex, antagonist dose and drug affects total locomotor activity. Mean total locomotor counts in male and female rats after single i.p. injection the 5-HT_{1A} antagonist WAY 100635 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of total locomotor counts for male and female rats at varying dose of antagonist and drug administration. ~ Denotes a main effect of sex. * Denotes a significant main effect of cocaine as compared to respective saline treated groups ($p<0.05$). ^ Denotes a significant effect of sex and drug with increased locomotor behavior in females compared to males after cocaine administration ($p<0.05$). # Denotes a significant effect of drug and antagonist with attenuation of locomotor behavior found with cocaine and antagonist compared to respective saline and vehicle treated groups ($p<0.05$). ($n = 8$).

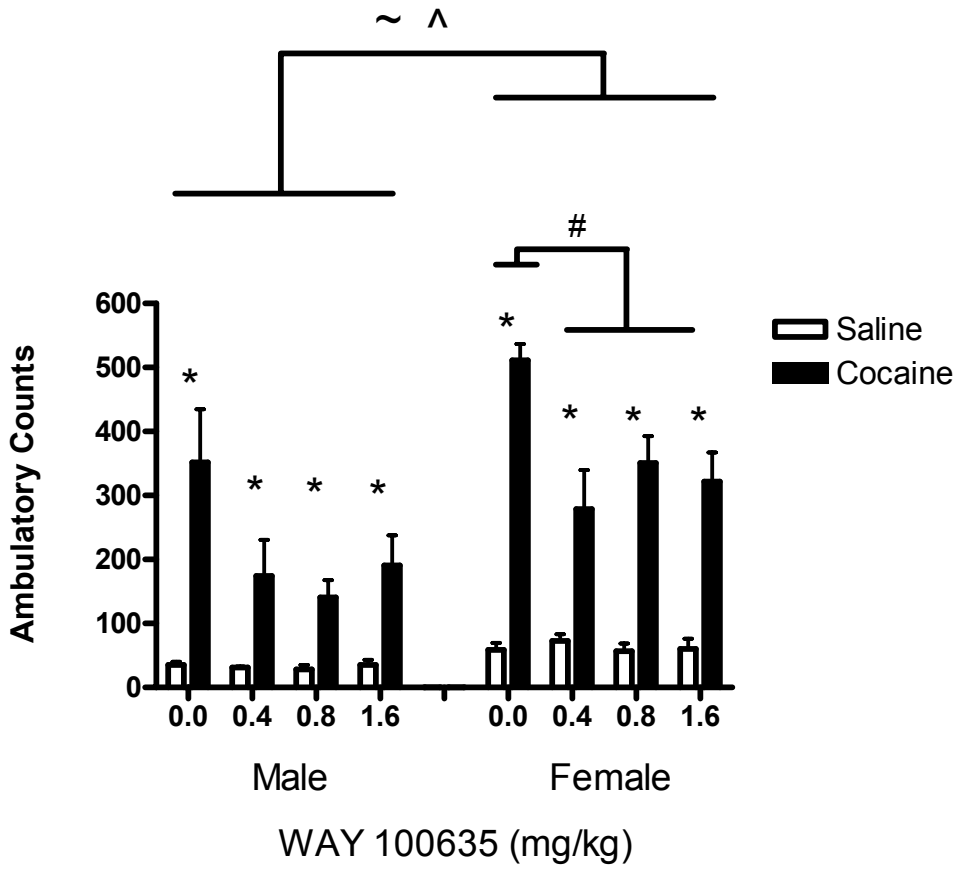


Figure 10: Sex, antagonist dose and drug affects ambulatory activity. Mean ambulatory counts in male and female rats after single i.p. injection of the 5-HT_{1A} antagonist WAY 100635 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of ambulatory counts for male and female rats at varying dose of antagonist and drug administration. ~ Denotes a main effect of sex. * Denotes a significant main effect of cocaine as compared to respective saline treated groups ($p<0.05$). ^ Denotes a significant effect of sex and drug with increased ambulatory behavior in females compared to males after cocaine administration ($p<0.05$). # Denotes a significant effect of drug and antagonist with attenuation of ambulatory behavior found with cocaine and antagonist compared to respective saline and vehicle treated groups ($p<0.05$). ($n = 8$).

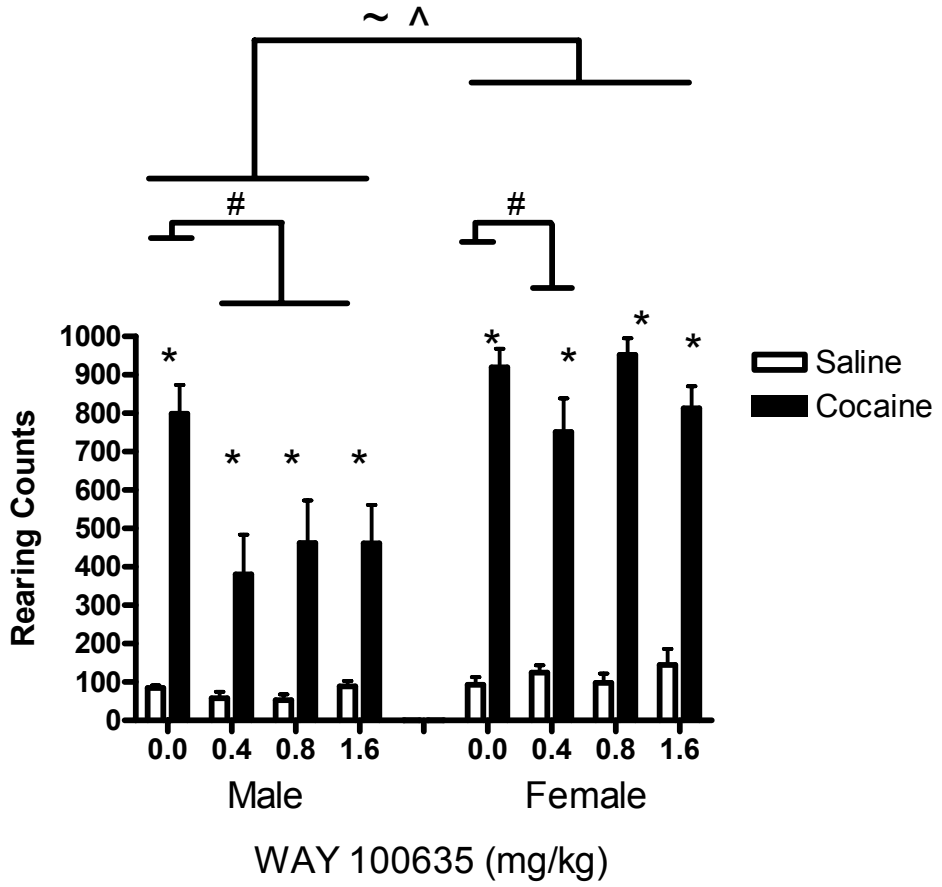


Figure 11: Sex, dose and drug affects rearing activity. Mean rearing counts in male and female rats after single i.p. injection of the 5-HT_{1A} antagonist WAY 100635 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of rearing counts for male and female rats at varying dose of antagonist and drug administration. ~ Denotes a main effect of sex. * Denotes a significant main effect of cocaine as compared to respective saline treated groups ($p<0.05$). ^ Denotes a significant effect of sex and drug with increased rearing behavior in females compared to males after cocaine administration ($p<0.05$). # Denotes a significant effect of drug and antagonist with attenuation of rearing behavior found with cocaine and antagonist compared to respective saline and vehicle treated groups ($p<0.05$). ($n = 8$).

3.2 Sex Differences in the Blockade of Cocaine-Induced Behavioral activity using a 5-HT$_{1B}$ Antagonist:

As shown in Figure 12-14, similar to WAY 100635, a significant main effect of sex was obtained in saline-treated controls regardless of behavioral activity [total locomotor: $F(1, 120) = 24.33$, $p = 0.0000$; ambulatory: $F(1, 118) = 6.65$, $p = 0.0111$; rearing: $F(1, 122) = 84.15$, $p=0.0000$]; female rats had overall higher behavioral activity than male rats ($p<0.05$ for all comparisons). A significant main effect of drug was also observed [total locomotor: $F(1, 120) = 357.33$, $p = 0.0000$; ambulatory: $F(1, 118) = 204.20$, $p = 0.0000$; rearing: $F(1, 122) = 555.84$, $p = 0.0000$]; overall, cocaine increased all three behavioral responses ($p < 0.05$ for all comparisons). Furthermore, a significant interaction was observed between sex and drug in total locomotor behavior and rearing behaviors [total locomotor: $F(1,120) = 8.391$, $p = 0.0044$; ambulatory: $F(1, 118) = 3.53$, $p = 0.0626$; Fig XX); rearing: $F(1, 122) = 52.04$, $p = 0.0000$]; cocaine-treated females rats had higher behavioral counts than male rats ($p < 0.05$ for all responses).

In both sexes, GR 127935 altered all three behavioral baseline responses [total locomotor: $F(3,62) = 6.8719$, $p = 0.0004$; ambulatory: $F(3,59) = 4.67870$, $p = 0.0053$; rearing: $F(3,63) = 7.3528$, $p = 0.0002$]. To account for these baseline effects, data was analyzed as the difference of cocaine effect minus their respective saline activity. As shown in Fig 15-17, in all three behaviors a significant effect of antagonist was observed [total locomotor: $F(3, 58) = 3.2283$, $p = 0.0288$; ambulatory: $F(3, 59) = 4.0612$, $p = 0.0108$; rearing: $F(3, 59) = 4.9291$, $p = 0.0040$]; the 15 mg/kg GR 127935 dose attenuated behavioral responses compared to vehicle treatment ($p < 0.05$ for all responses). In female rats only a significant effect of antagonist was observed for ambulatory behaviors [ambulatory: $F(3, 31) = 4.7364$, $p = 0.0078$]; 10 and 15 mg/kg doses were significantly lower than vehicle treatment [ambulatory: $p = 0.0441$, $p = 0.0078$]. In male rats, only a significant

effect of the antagonist was observed in rearing behaviors [rearing: $F(3, 27) = 4.1516$, $p = 0.0152$]; 15 mg/kg of GR 127935 significantly attenuated rearing activity when compared to vehicle treatment [rearing: $p = 0.0038$].

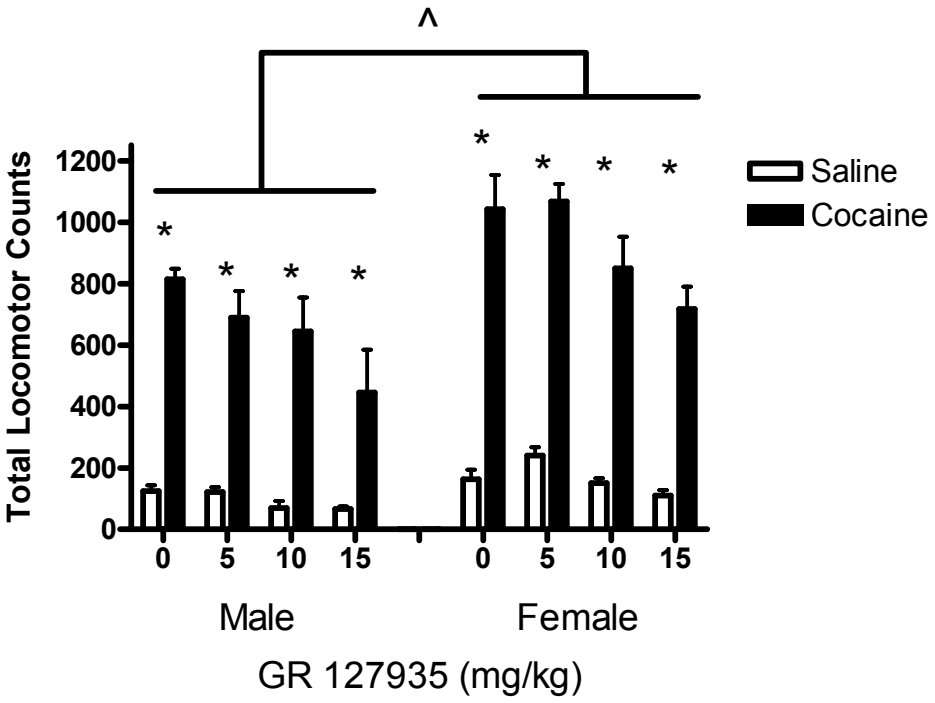


Figure 12: Sex, antagonist dose and drug affects total locomotor activity. Mean total locomotor counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of total locomotor counts for male and female rats at varying dose of antagonist and drug administration. ^ Denotes a significant main effect of sex; regardless of manipulation females had higher total locomotor counts compared to males ($p<0.05$). * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) ($n = 8$).

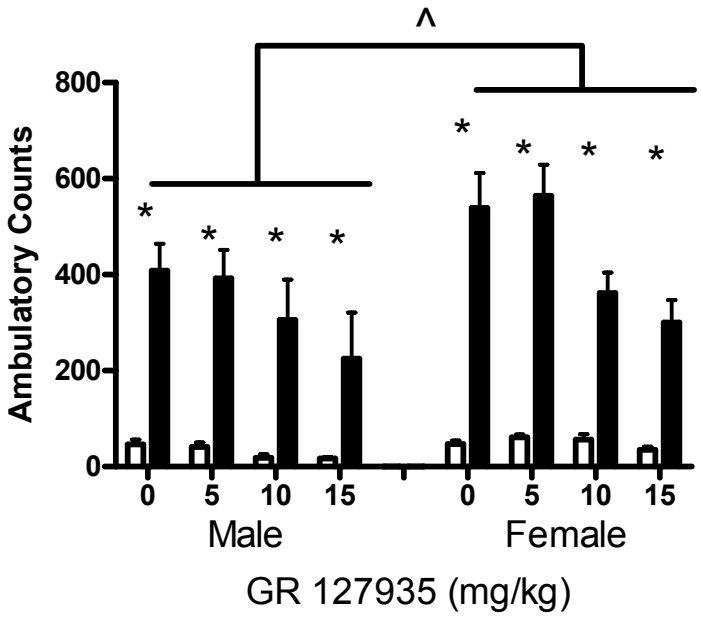


Figure 13: Sex, antagonist dose and drug affects ambulatory activity. Mean ambulatory counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of ambulatory counts for male and female rats at varying dose of antagonist and drug administration. ^ Denotes a significant main effect of sex; regardless of manipulation females had higher ambulatory counts compared to males ($p<0.05$). * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) ($n = 8$).

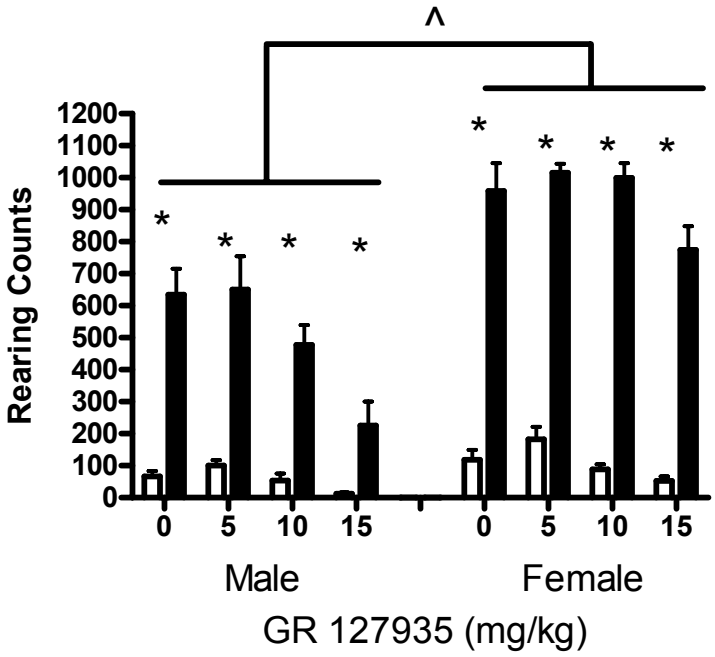


Figure 14: Sex, antagonist dose and drug affects rearing activity. Mean rearing counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. Shown as the mean of rearing counts for male and female rats at varying dose of antagonist and drug administration. ^ Denotes a significant main effect of sex; regardless of manipulation females had higher rearing counts compared to males ($p<0.05$). * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) ($n = 8$).

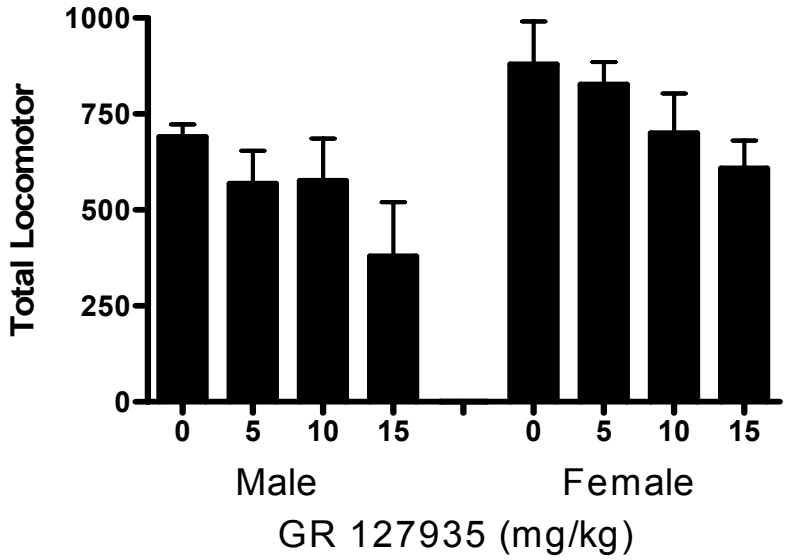


Figure 15: Sex, antagonist dose and drug affects total locomotor activity. Delta value of mean total locomotor counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. No significant effects were found.

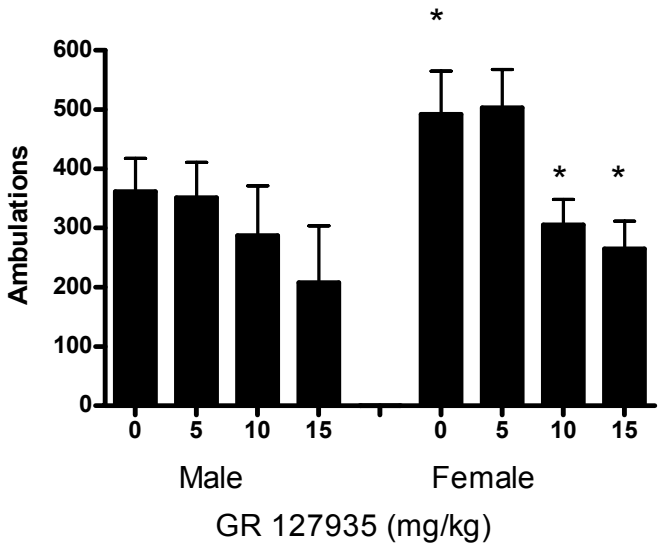


Figure 16: Sex, antagonist dose and drug affects ambulatory activity. Delta value of mean ambulatory counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. * Denotes a significant drug effect.

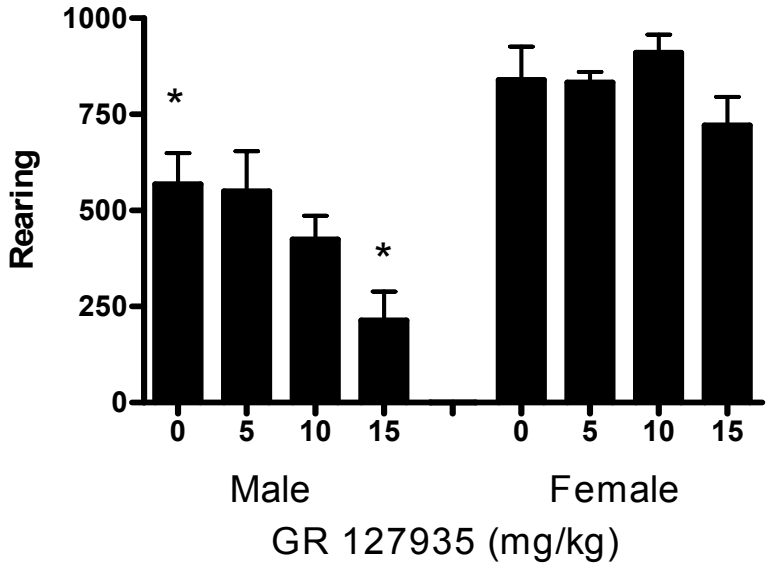


Figure 17: Sex, antagonist dose and drug affects rearing activity. Delta value of mean rearing counts in male and female rats after single i.p. injection of the 5-HT_{1B} antagonist GR 127935 followed by a single i.p. injection of cocaine (20 mg/kg) or saline. * Denotes a significant drug effect.

4. Discussion

The present study determined the efficacy of 5-HT_{1A} and 5-HT_{1B} receptor antagonist administration on cocaine-induced hyperlocomotor activity in male and female rats. As previously reported, 5-HT_{1A} and 5-HT_{1B} receptors mediate several cocaine-induced behavioral responses in male rats (33,38,53). We extend these observations by demonstrating that similar to male rats, both the 5-HT_{1A} and 5-HT_{1B} receptors are involved in the modulation of cocaine-induced behavioral responses in female rats. Moreover, female rats showed more robust cocaine-induced increases in total locomotor, ambulatory and rearing behaviors compared to males. This is consistent with previously published observations (5,13,15,17,18,20,94,141,144,145,148,160,166,181).

At the behavioral level, we found a number of cocaine-induced behavioral sex differences. Overall, cocaine-treated females demonstrated higher total locomotor, ambulatory and rearing behavior compared to males in all experimental observations. Similar to previous studies we demonstrated that higher doses of cocaine are required in male rats to achieve responses similar to those of female rats. This supports the increased sensitivity to cocaine in females as compared to males. Studies have shown that these behaviors are dose and sex dependent following acute cocaine injections. However, previous studies have defined the sex related dose decency of cocaine. A dose of 20 mg/kg was selected for our studies, since it is neither a maximal nor a minimal dose for either male or female rats.

5-HT_{1A} receptors have been found to have a critical role in cocaine-induced locomotor activity (28,32,33,40,47,50-55,110). In both male and female rats, WAY 100635 did not alter baseline activity. This indicates that the behavioral responses reported herein suggests a specific involvement of WAY 100635 and the 5-HT_{1A} receptors activation in cocaine-induced hyperactivational responses (28,32,33,40,47,50-55,110). In line with previous research, WAY

100635 produced an attenuation of cocaine-induced hyperlocomotor activity in male rats (28,32,33,40,47,50-55,107,109,110). We broaden these findings, demonstrating the blockade of the 5-HT_{1A} receptor attenuates cocaine-induced locomotor activity in female rats in a similar manner. This is inconsistent with previous reports, in which systemic administration of WAY 100635 did not affect cocaine-induced locomotor activity and number of rears in female Wistar rats, however head bobs were increased (110). This discrepancy however, could be attributed to strain differences, dose used, and route of drug administration. However, when WAY 100635 was micro-injected into the DRN, an increase in total locomotor activity and rearing was found (96). Interestingly, they found no effect when microinjected into the MRN (96). This suggests a specific involvement of the 5-HT_{1A} receptor in cocaine-induced behavioral activation in the dorsal raphe nucleus. Differences in a number of behavioral studies of the serotonergic system have found conflicting results with systemic versus local application (such as micro-injection) in cocaine-induced behavioral activation (28,32,33,40,47,50-55,110).

Sex differences were found in the ability of WAY 100635 to attenuate cocaine-induced behaviors. For instance, in males WAY 100635 was not effective in the reduction of cocaine-induced ambulatory behaviors but was effective in all other behaviors examined. In addition, only the 0.4 mg/kg dose was effective in reducing rearing behaviors in females but was effective at all other doses in all other behaviors. This was not seen in males, with all doses attenuating all significant effects in cocaine-induced behaviors. Consistent with previous reports, 0.4 mg/kg of WAY 100635 was sufficient in selectively blocking the effects of cocaine-induced hyperlocomotion in male rats (52, 205). In fact, in both male and female rats 0.4 mg/kg was the optimal dose for attenuating cocaine-induced hyperlocomotion. Previously, it has been revealed that lower concentrations of agonists have a stronger affinity for presynaptic 5-HT_{1A} autoreceptors in the raphe

nuclei and therefore, are considerably more sensitive to pharmacological manipulation than postsynaptic 5-HT_{1A} receptors located in projection terminal regions (28,32,33,40,47,50-55,205). Higher doses seem to target both autoreceptors and postsynaptic receptors similarly (47). Though it has not been specifically determined in antagonists, it is likely that lower concentrations are specifically targeting these receptors due to presynaptic autoreceptor sensitivity and higher receptor reserve (28,32,33,40,50,55,205,47). When comparing systemic and local manipulation of cocaine or other 5-HT agonists, differential effects are seen (47). This suggests that the activation of somatodendritic autoreceptors is necessary for increased levels 5-HT found in projection areas after cocaine administration. Local application in projection areas with cocaine or agonists and thus specific activation of postsynaptic receptors in some cases, does not cause this same increase. The importance of 5-HT_{1A} presynaptic autoreceptors in cocaine-induced behavioral effects are further evidenced by the fact that agonist-induced increases in 5-HT levels are not blocked by antagonists with local application in projection areas as well. Therefore, cocaine-induced 5-HT increases seen in projection areas such as the frontal cortex, nucleus accumbens and hippocampus, are subsequently caused by the modulation of 5-HT_{1A} somatodendritic autoreceptors. In addition, it has been suggested that 5-HT release is modulated by presynaptic 5-HT_{1A} receptors with dopamine and other neurotransmitters being modulated by postsynaptic 5-HT_{1A} receptors and 5-HT_{1A} heteroreceptors (28,32,33,40,47,50-55,205). Furthermore, WAY 100635 blocks 5-HT_{1A} receptor agonist-induced increase in 5-HT release but does not block agonist-induced increases of DA or NA release in the rat frontal cortex, nucleus accumbens and hippocampus (28,32,33,40,47,50-55,205). To further this dose theory, researchers have found that the effects of 5-HT_{1A} receptor agonists on 5-HT release were blocked by a low dose of WAY 100635 while a high dose of WAY 100635 was required to attenuate 5-HT_{1A} receptor agonist-induced increase in DA release (28,32,33,40,47,50-55,205). This

evidence suggests that WAY 100635 has a preferential action at somatodendritic 5-HT_{1A} receptors in the attenuation of cocaine-induced hyperlocomotor activity in male and female rats.

In addition, females are known to have heightened levels of serotonin in the brain. Furthermore, cocaine is known to decrease serotonin levels in female rats and increase them in males. Interestingly, WAY 100635 at the optimal dose of 0.4 mg/kg in both male and female rats was equally sufficient in attenuating cocaine-induced hyperlocomotor activation. This evidence suggests that in both male and female rats, WAY 100635 at this dose targets presynaptic receptors in somatodendritic regions of the serotonergic system with equal efficacy and is effective in the attenuation of cocaine-induced behavioral activity of male and female rats. Females therefore, do not require a higher dose of WAY 100635 to cause similar attenuation of cocaine-induced hyperlocomotion seen in males, as might be expected.

Carey et al., 2001 suggests that cocaine causes 5-HT increases in a number of brain regions in the somatodendritic regions of 5-HT neurons, which increases 5-HT_{1A} somatodendritic autoreceptor activation, in turn inhibiting 5-HT neuronal activity (47,50-53,187). Therefore, blocking the 5-HT_{1A} somatodendritic autoreceptors with WAY 100635 blocks the normal increase of inhibition normally caused by increased somatodendritic 5-HT after cocaine administration (65). Therefore, the cocaine-induced increase in extracellular 5-HT in terminal regions is potentiated. Overall, it is accepted that when cocaine-induced 5-HT levels are potentiated, cocaine-induced locomotor activation is attenuated (35,38,48,81,83,89,99,100).

The activation of 5-HT_{1B} receptor is directly involved in the mediation of cocaine-induced hyperactivity in both male and female rats. Unlike WAY 100635, GR 127935 produced a baseline effect which is inconsistent with most reports (1,99,115,118,119,122,186,188). After controlling for baseline effects, similar to previous work, we found that 5-HT_{1B} receptor activation mediates

cocaine-induced hyperlocomotor activity in male rat (38,99,119). Specifically, we found that antagonism of the 5-HT_{1B} receptor with GR 127935 attenuates cocaine-induced hyperlocomotor activity in male rat and female rats (38,99,119). To our knowledge this is the first report demonstrating that in female rats 5-HT_{1B} also plays a direct role in cocaine-induced hyperlocomotor activity.

Sex differences were observed in the efficacy of GR 127935 to inhibit cocaine-induced activity. This sexually dimorphic antagonism was behaviorally specific. Analysis by sex revealed no reduction in either sex in total locomotor behaviors. A significant reduction in ambulatory activity due to GR 127935 treatment was found in females (10 and 15 mg/kg dose attenuated when compared to saline) however, no significant effects were found in males. Conversely, in rearing behaviors, a significant reduction in cocaine-induced hyperlocomotor behavior was seen only in male rats (showing a reduction only with 15 mg/kg dose). Locomotor behaviors have been suggested to be postsynaptically mediated by 5-HT_{1B} receptors (34). As described in Chapter 1, 5-HT_{1B} receptors are found more abundantly in projection areas of the serotonergic pathway which includes a wealth of receptors in motor control centers (34,48,71). Thus sex differences in the efficacy of GR 127935 to attenuate hyperlocomotor responses after cocaine administration consequently implies a sex difference in postsynaptic 5-HT_{1B} receptors in the regulation of cocaine-induced hyperlocomotion.

Presynaptically, 5-HT_{1A} receptors are more abundantly found as presynaptic autoreceptors, whereas on post-synaptic nerve terminals they can modulate activity in non-serotonergic neurons (47,189). Conversely, 5-HT_{1B} receptors are found more abundantly in post-synaptic nerve terminals. Furthermore, it is known that 5-HT_{1A} somatodendritic autoreceptors are more sensitive to pharmacological stimulation compared to lower sensitivity in postsynaptic receptors (44,69).

Therefore, it is feasible that the sexually dimorphic activation of 5-HT_{1A} presynaptic autoreceptors may contribute to the differential effects demonstrated in male and female rats. This in turn alters the efficacy and activation of known postsynaptic autoreceptors (such as 5-HT_{1A}, 5-HT_{1B} or other receptor families). Future research should be directed towards determining the underlying basis of these sexually dimorphic aspects of cocaine-induced hyperlocomotion. Specifically, dimorphisms found in the levels of serotonin and its metabolites as well as dimorphisms found in cocaine-induced hyperlocomotion after pharmacological manipulation could suggest that differences in receptor number could be subject and thus explored. Evidence on 5-HT_{1A} receptor number has only been explored in juvenile rats in which no differences were found. However, they did find a dimorphism of mRNA expression with females having lower levels of mRNA compared to males. Ultimately, this suggests that there could possibly be sex differences in receptor number expressed in adult rats and should thus be explored. Our findings suggest that the sexual dimorphisms, seen in cocaine-induced hyperlocomotion, may involve differential release of 5-HT and the subsequent activation of presynaptic and postsynaptic receptors, autoreceptors and heteroreceptors also may be sexually dimorphic.

Chapter 3: The effects of cocaine on sex differences in the activation of the 5-HT$_{1A}$ receptors using in vitro autoradiography of receptor-activated G proteins by agonist-stimulated guanylyl 5' [γ-[^{35}S] thio]-triphosphate binding.

1. Introduction

Sex differences in serotonergic neuroanatomy, neurochemistry and behavior in both humans and rats have been found. Female rats have increased serotonin synthesis and serotonin metabolites in a number of brain regions when compared to males (133,134,190). Specifically, in the mesocorticolimbic system (which plays a major role in motor and reward responses to cocaine) sexual dimorphic patterns in the serotonergic (5-HT) system have been found (13,87,100,128,191). It is known that 5-HT$_{1A}$ receptors found in the mesocorticolimbic system modulate cocaine-induced behaviors. As shown in Chapter 2, both male and female rats showed an attenuation of cocaine-induced hyperlocomotion in response to co-administration of cocaine and 5-HT$_{1A}$ antagonists; the opposite effects being seen with agonists. In male rats increases in extracellular levels of 5-HT, 5-HIAA (a serotonin metabolite) and the 5-HIAA/5-HT turnover ratio after cocaine administration have been shown in the prefrontal cortex, nucleus accumbens and hippocampus (13,23,26-28,32,47,50-54,65,75,86,87,102-105,110,192). However, in the nucleus accumbens of females, cocaine decreases of 5-HT and 5-HIAA levels, in the nucleus accumbens (NAc) have been found (13,104). These sexually dimorphic transmitter responses to cocaine administration are known however, molecular and cellular mechanisms involved in these responses is unknown.

5-HT$_{1A}$ receptors are G-protein coupled receptors (GPCR) and are coupled to various effectors systems, including activation of ion channels such as the opening of G-protein-gated inwardly rectifying potassium (GIRK) channels in the hippocampus as well as inhibition of Ca2+ currents in several neuronal types (34-36,39,41). It is well established that somatodendritic 5-HT$_{1A}$

receptors cause neuronal hyperpolarization through G-protein coupled opening of K^+ channels and consequently 5-HT neuronal firing is reduced (46). In addition, postsynaptic 5-HT_{1A} receptors mediate via G-proteins causing neuronal hyperpolarization and inhibition of adenylyl cyclase (34-36,39,41,66). It is suggested that differences in the coupling of 5-HT_{1A} receptors to different G-proteins might account for regional differences in 5-HT_{1A} receptor activation in differential brain areas and thus subsequent effects on a plethora of behaviors (34-36,39,41,66). Chronic cocaine administration is known to cause both the increase and decrease of protein-coupled receptor signaling in several brain regions (84). Specifically, it has been demonstrated that chronic cocaine treatment increases and decreases both monoamine and non-monoamine receptor signal transduction within the mesocorticolimbic system in male rats (193-198).

Because of the high receptor levels found in mesocorticolimbic areas and the dimorphisms seen in 5-HT levels, we chose specific areas within this pathway to examine 5-HT_{1A} receptor activation after cocaine administration. The aim of this study is to determine if sexually dimorphic patterns in the activation of 5-HT_{1A} receptors, in the mesocorticolimbic and nigrostriatal regions contribute to the known sexually dimorphic behavioral responses found in response to cocaine administration (34-36,39,41,66,199,200). To this end functional activity of 5-HT_{1A} receptors was measured using *in vitro* autoradiography of receptor-activated G proteins by agonist-stimulated guanylyl 5'[γ-[^{35}S]thio]-triphosphate ([^{35}S]GTPγS) binding (66,201). This assay is known for determining the functionality of the G-protein after pharmacological manipulation (66,201). The first step in the signaling cascade following activation of G-protein coupled receptors is an exchange of GDP for GTP on the α-subunit of the interacting G-protein (See figure 18). Agonist-induced incorporation of guanosine 5'-O ([^{35}S]GTPγS) triphosphate ([^{35}S]GTPγS), a non-hydrolysable

analogue of GTP, provides a great way to measure the amount of the receptor coupling to G-protein and thus receptor activation (66,201).

2. Methods

2.1 Animals

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed for one week prior to experimental manipulations in standard plastic cages (20 x 20 x 41 cm^3). Rats had free access to standard lab chow and water *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.). Rats were handled and weighed for 3 days prior to experimental manipulations. Three separate cohorts of animals were used for behavioral studies with an n of 4 per group. In female rats repeated vaginal lavage attenuates cocaine-induced activity, and establishes a place preference and increased dopamine release in the striatum (184). Thus, females were placed randomly into experimental groups without regard to estrous cycle stage (184). All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Drugs

Cocaine hydrochloride, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-2-pyridinyl-ycylhexanecarboxaminde maleate (WAY 100635) were purchased from Sigma-Aldrich (St. Louis, MO). Non-labeled guanosine 5' – (γ-thio) triphosphate (GTPγS), GDP and adenosine deaminase were purchased from Sigma-Aldrich. Labeled [^{35}S] GTPγS (1393 Ci/mmol; 1 Ci = 37 GBq) was

purchased from NEN-Perkin Elmer, Life Science (Boston, MA, USA). Hyperfilm-βmax was purchased from Amersham. All other reagent grade chemicals were obtained from Sigma-Aldrich.

2.3 Drug treatment and experimental paradigm

Cocaine and WAY 100635 were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneal. All injections were performed in the home cage. After 15 minutes pre-treatment with WAY 100635 (0, 0.4, 0.8, or 1.6 mg/kg) rats were injected with cocaine (20 mg/kg) or saline. This cocaine dose was previously demonstrated to produce significant increases in locomotion and robust sex differences in cocaine-stimulated motor activity without reaching a maximal effect in either sex (13). Antagonist doses and pretreatment schedule were chosen based on previous reports in male rats, which demonstrated an inhibition of locomotor behavioral responses to cocaine (52,53).

2.4 Brain Tissue Dissections

Following decapitation, brains were rapidly removed, frozen in methylbutane (-40˚C for 30 seconds), and stored at -80˚C. Coronal sections (20 μm) thaw-mounted onto Superfrost Plus Gold glass slides (Fischer, Pittsburgh, PA). Frontal cortex (Interaural: 13. 70 mm/Bregma: 4:70 mm); Nucleus Accumbens (Interaural: 11.70 mm/Bregma: 2.70 mm); Caudate putamen (Interaural: 10.60 mm/Bregma: 1.60 mm); Hippocampus and Amygdala (Interaural: 5.40 mm/Bregma: -3.60 mm). The slides were stored at -80˚C until used.

2.5 Agonist-Stimulated GTP [γ-^{35}S] Binding Autoradiography.

As previously described by Sims et al., (1995) and Febo et al., (2003). Slides were incubated at 25°C for 10 minutes in incubation buffer [50 mM Tris HCl, 3 mM $MgCl^2$, 0.2 mM EGTA, 100 mM NAcl at a pH of 7.7]. Sections were then incubated with 2mM GDP for 30 minutes, followed by an incubation in assay buffer at room temperature for 2 hours [0.1 nM GTP[γ-35S] (1393 Ci/mmol; 1 Ci = 37 GBq), 2 mM GDP, 0.2 mM DTT]. Receptor stimulation was determined by adding agonist to the assay buffer (0.3 μM 8-OH-DPAT). Basal activity was assessed with GDP in the absence of agonist. Nonspecific binding was assessed in the presence of 10 μM unlabeled GTP. All sections were then rinsed twice for 3 minutes in ice cold Tris-buffer [50mM Tris HCl (pH 7.0 at 25°C), 0.2 mM DTT]. Slides were then dipped in ice-cold deionized water, dried rapidly under a stream of cool air, dried overnight, and exposed to Hyperfilm-βmax for 24 hrs. The agonist-stimulated activity was calculated by subtracting the optical density of basal sections from that of agonist-stimulated sections. Results were expressed as percentage to basal binding.

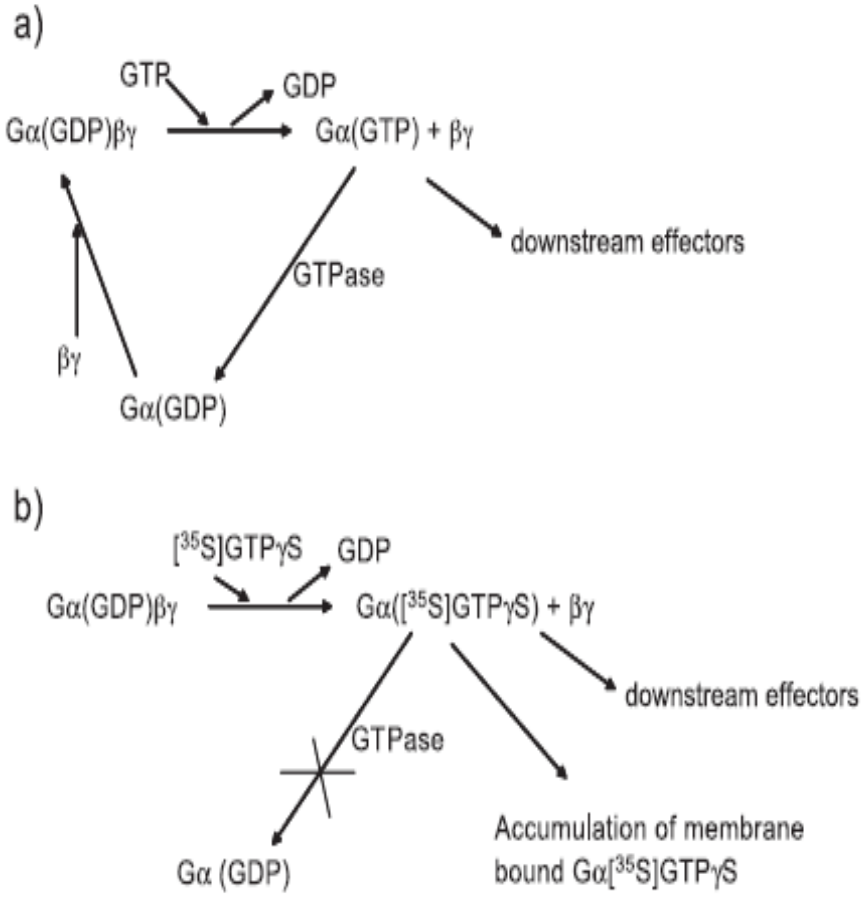


Figure 18: Concept of *in vitro* autoradiography of receptor-activated G proteins by agonist-stimulated guanylyl 5'[γ-[^{35}S]thio]-triphosphate ([^{35}S]GTPγS) binding (66,201).
a) Agonist binding to receptor causes the exchange of GTP for GDP on the α subunit of the G protein; Gα-GTP and Gβγ subunits activate cellular effectors. The GTPase activity of the Gα subunit hydrolyses GTP to GDP, Gα and Gβγ subunits re-associate and the system is turned off.
b) In the presence of [^{35}S]GTPγS, exchange of [^{35}S]GTPγS for GDP occurs, but the GTPase activity of the Gα subunit is unable to hydrolyze Gα-bound [^{35}S]GTPγS, which accumulates during the assay period and the radioactive buildup can be visualized by exposure to film.

2.6 Statistical analysis:

t-test analyses were used to determine statistically significant differences between cocaine versus saline-treated animals. A two-way ANOVA was used to interactions between drug (saline or cocaine) x sex (male vs. female). Fisher LSD post hoc tests were used for multiple comparisons when appropriate. A p-value of less than 0.05 was considered significant for all statistical analysis.

3. Results

3.1 Sex Differences in the 5-HT_{1A} Binding

As shown in Table 4 and Figures 19-22, cocaine significantly increased 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in most brain areas studied. Of interest, a reduction of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding was observed in the amygdala of both male and female rats. Furthermore, a reduction of binding was also observed in the shell of the nucleus accumbens of females and in the hippocampus (CA1 and CA3) of males.

In the dorsal and rostral frontal cortex of both males and females a significant main effect of drug was obtained in the [dorsal frontal cortex: F (1, 12) = 0.0068, p = 0.0024; rostral frontal cortex: F (1, 12) = 13.2866, p = 0.0034; Fig. 16]; cocaine increased activation of both males and females compared to saline; see Fig. 19. As shown in Figure 20, in the dorsal medial caudate putamen of both male and female rats a significant drug effect was also found [F(1, 12) = 4.9211, p = 0.046]; cocaine treatment increased 5-HT_{1A} agonist-stimulated [^{35}S]GTPγS binding when compared to saline-treated groups.

A significant main effect of sex was observed in the nucleus accumbens core [F (1, 12) = 0.0085, p = 0.0292]; males regardless of drug treatment had higher 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding that female rats; see figure 21 (p = 0.0294). A significant drug effect was also found

in the core of the nucleus accumbens [F (1, 12) = 0.0074, p = 0.0394]. In males, cocaine increased 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding, while it decreased binding levels in females (p = 0.0395). Therefore, differential activation of the 5-HT_{1A} receptor was sex and brain region specific.

Table 5: t-test for drug effect in 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding

Brain Area	Male	Female
Dorsal Frontal Cortex (FCxD)	t(14) = -6.9620, p = 0.0000	t(14) = -7.0772, p = 0.0000
Rostral Frontal Cortex (FCxR)	t(14) = -7.1840, p = 0.0000	t(14) = -7.2109, p = 0.0000
Ventral Frontal Cortex (FCxV)	t(14) = -7.6793, p = 0.0000	t(14) = -7.7491, p = 0.0000
Dorsal Medial CPu (CPuDM)	t(14) = -7.6879, p = 0.0000	t(14) = -7.7308, p = 0.0000
Dorsal Lateral CPu (CPuDL)	t(14) = -7.6879, p = 0.0000	t(14) = -7.7491, p = 0.0000
Nucleus Accumbens Core (NAcC)	t(14) = -7.6788, p = 0.0000	t(14) = -7.9321, p = 0.0000
Nucleus Accumbens Shell (NAcS)	t(14) = -7.7207, p = 0.0000	t(14) = -7.8172, p = 0.0000
Hippocampus CA1 (CA1)	t(14) = -7.2990, p = 0.0000	t(14) = -7.3663, p = 0.0000
Hippocampus CA3 (CA3)	t(14) = -7.5317, p = 0.0000	t(14) = -7.6430, p = 0.0000
Amygdala	t(14) = -7.6907, p = 0.0000	t(14) = -7.6659, p = 0.0000

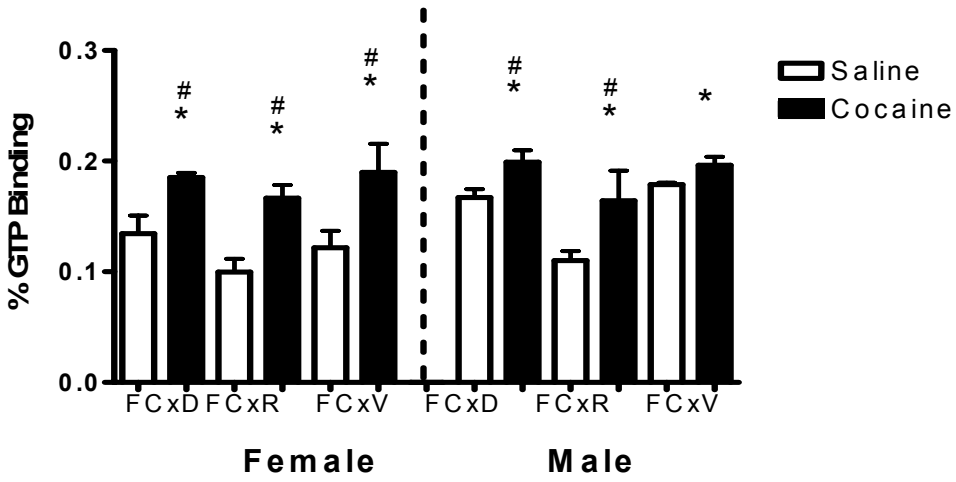


Figure 19: Drug affects 5-HT_{1A} receptor activity in male and female rats. Percent of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in the dorsal (FCxD), rostral (FCxR) and ventral (FCxV) frontal cortex. Percent of [^{35}S] GTPγS binding was determined by the subtraction of the optical density of basal sections from its respective agonist-stimulated sections. Cocaine caused an increase in binding in both male and female rats compared to saline ($p < 0.05$). * Denotes a significant drug effect; analysis by t-test. # Denotes a significant drug effect; analysis by ANOVA.

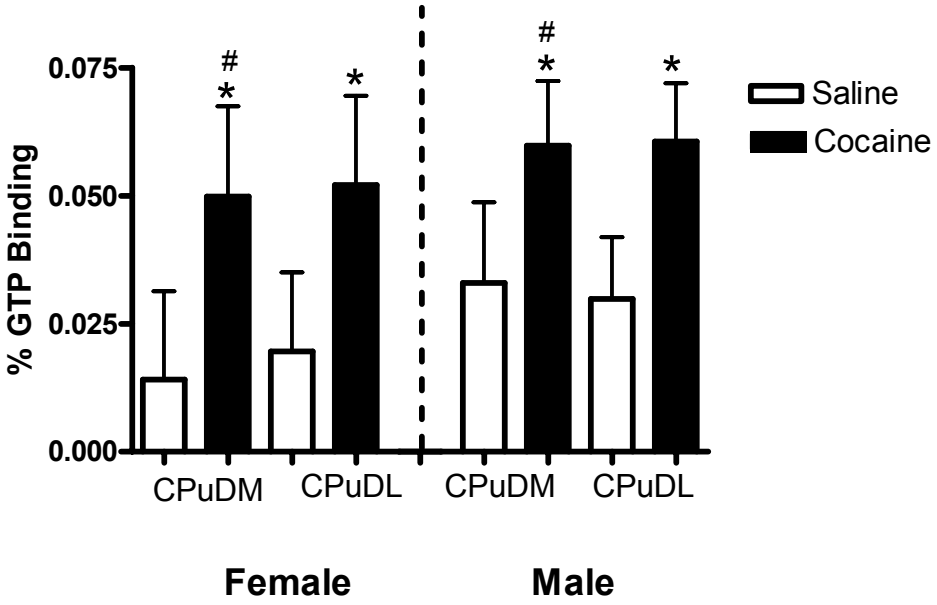


Figure 20: Drug affects 5-HT_{1A} receptor activity in male and female rats. Percent of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in the dorsal medial (CPuDM) and dorsal lateral (CPuDL) CPu cortex. Percent of [^{35}S] GTPγS binding was determined by the subtraction of the optical density of basal sections from its respective agonist-stimulated sections. Cocaine caused an increase in binding in both male and female rats compared to saline ($p < 0.05$). Denotes a significant drug effect; analysis by t-test. # Denotes a significant drug effect; analysis by ANOVA.

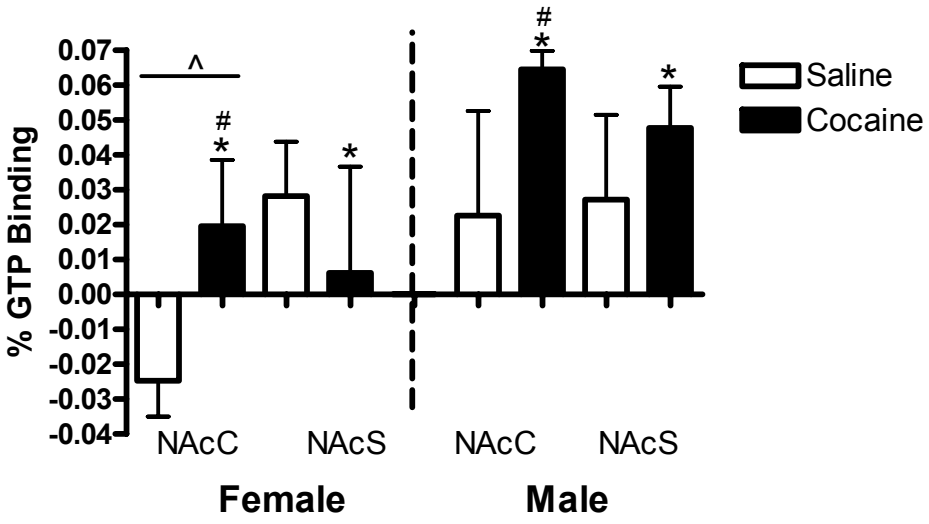


Figure 21: Drug affects 5-HT_{1A} receptor activity in male and female rats. Percent of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in the nucleus accumbens core (NAcC) and nucleus accumbens shell (NAcS). Percent of [^{35}S] GTPγS binding was determined by the subtraction of the optical density of basal sections from its respective agonist-stimulated sections. Cocaine caused an increase in binding in both male and female rats compared to saline in the nucleus accumbens core and in males in the nucleus accumbens shell ($p < 0.05$). A decrease was found females in the nucleus accumbens shell. ^ Denotes a sex effect. * Denotes a significant drug effect; analysis by t-test. # Denotes a significant drug effect; analysis by ANOVA.

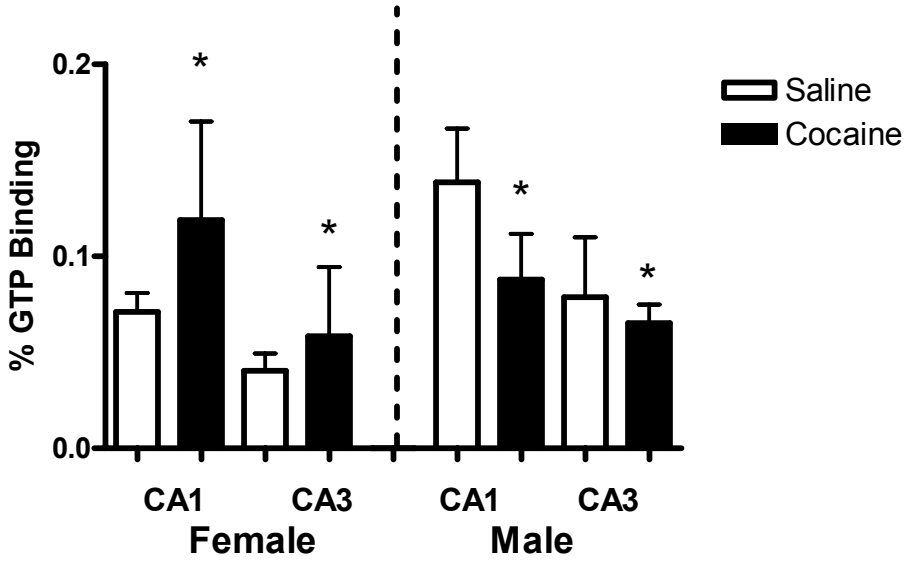


Figure 22: Drug affects 5-HT_{1A} receptor activity in male and female rats. Percent of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in the hippocampus CA1 and hippocampus CA3. Percent of [^{35}S] GTPγS binding was determined by the subtraction of the optical density of basal sections from its respective agonist-stimulated sections. Cocaine caused an increase in binding in both male and female rats compared to saline ($p < 0.05$). * Denotes a significant drug effect; analysis by t-test.

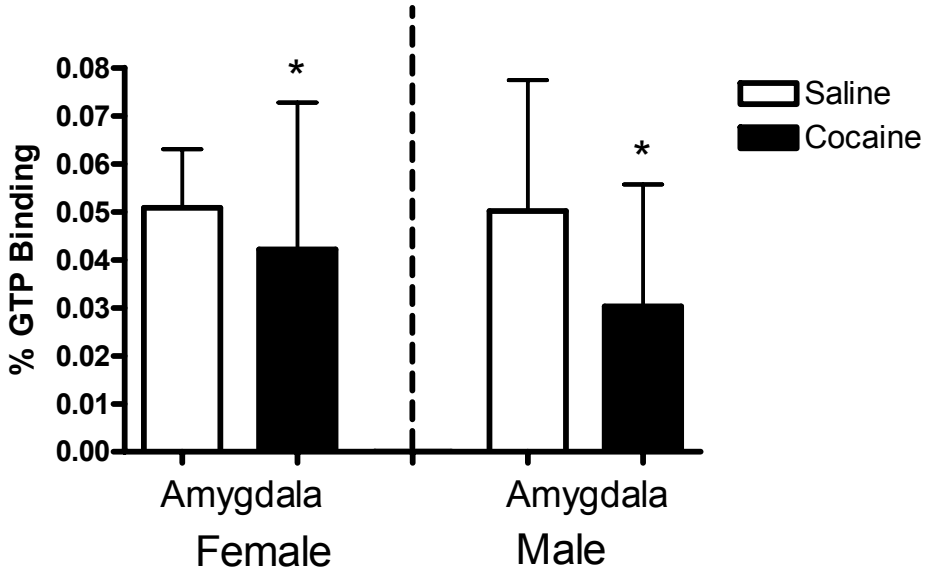


Figure 23: Drug affects 5-HT_{1A} receptor activity in male and female rats. Percent of 5-HT_{1A} agonist-stimulated [^{35}S] GTPγS binding in the amygdala. Percent of [^{35}S] GTPγS binding was determined by the subtraction of the optical density of basal sections from its respective agonist-stimulated sections. Cocaine caused a decrease in binding in both male and female rats compared to saline ($p < 0.05$). * Denotes a significant drug effect; analysis by t-test.

4. Discussion

Autoradiography has demonstrated a high density of 5-HT_{1A} receptors in dorsal and median raphe nuclei, as well as, limbic forebrain regions of rats, including the hippocampus (CA1 and CA3), amygdala, and frontal cortex with lower densities found in the nucleus accumbens (33-36,39,69). Similar to previous work we found specific activation of 5-HT_{1A} receptors using 5-HT_{1A} receptor mediated [^{35}S]GTPγS binding in the frontal cortex and hippocampus (66,201). We also found specific activation of 5-HT_{1A} receptors in the nucleus accumbens, amygdala and caudate putamen, which to our knowledge has not been previously examined. Therefore, we found a direct relationship between the known neuroanatomical location of 5-HT_{1A} receptors in male rats with the functional activation of 5-HT_{1A} receptors in response to cocaine administration in male and female rats presently. Moreover, to the best of our knowledge, this is the first study demonstrating 5-HT_{1A} receptor-stimulated [^{35}S] GTPγS binding altered by acute cocaine administration. Notably, this is the first study addressing sex differences in the activation of 5-HT_{1A} receptors to cocaine administration.

8-OH-DPAT-stimulated [^{35}S] GTPγS binding measures the ability of the agonist 8-OH-DPAT to stimulate the exchange of GTPγS for GDP and is thus a direct assessment of receptor activation of G-proteins (66,201). Cocaine caused activational effects of the 5-HT_{1A} receptor in both male and female rats (66,201). With cocaine administration 8-OH-DPAT's ability to stimulate the exchange of GTPγS for GDP was increased; an indication that the activation of G-proteins by the receptor was increased because of agonist binding (66,201). Conversely, in a number of areas this ability to stimulate the exchange of GTPγS for GDP is decreased, an indication that the activation of G-proteins by the receptor is reduced (66,201). A reduced capacity of the 5-HT_{1A} receptor to activate G-proteins may be due to regulatory processes (e.g. phosphorylation) at the level of the G-

protein in addition to a number of complex modulatory activational processes of this receptor (201). Specifically, we found increases in functional [^{35}S]GTPγS binding and thus activation of 5-HT$_{1A}$ receptors in the dorsal, rostral and ventral areas of the frontal cortex, dorsal medial and dorsal lateral areas of the caudate putamen and the core area of the nucleus accumbens. Decreases of [^{35}S] GTPγS binding was found in the amygdala in both male and female rats. Interestingly, sexually dimorphic differences were found in the shell area of the nucleus accumbens with males having an increase and females having a decrease in [^{35}S] GTPγS binding. Sex differences were also found in and CA1 and CA3 areas of the hippocampus, with increases in [^{35}S]GTPγS binding in females in both areas and decreases in [^{35}S]GTPγS binding in males.

In the frontal cortex of both male and female rats, cocaine increased agonist-stimulated [^{35}S] GTPγS binding compared to saline-treated animals. This is consistent with previous reports which found increases of 5-HT in the pre-frontal cortex of male rats (13,51,105). In addition, others have found that co-administration of progesterone and cocaine in overiectomized female rats, which "mimics" male endocrine levels, resulted in higher levels of 5-HT in the prefrontal cortex (105). Thus, the increase of [^{35}S]GTPγS binding after stimulation with 5-HT$_{1A}$ agonist stimulated G-protein activation may be a direct result of an increase of 5-HT release from the dorsal raphe nucleus due to cocaine administration (13,51,105). It has been suggested that 5-HT$_{1A}$ postsynaptic receptors have pro-serotonergic effects while somatodendritic receptors are inhibitory. Therefore, in males we could assume that the increases in 5-HT levels in projection areas are causing activational effects upon 5-HT$_{1A}$ receptors, which cause the enhancement of cocaine-induced behavioral activation. However, increases of activity in female rats was found suggesting that increased activation is modulating the decreases in 5-HT levels found in response to cocaine administration. This

dimorphic modulation could be found in regulation of activation, which is further down the second messenger signal cascade in female rats and should be the focus of future examination (13, 51,105).

In the caudate putamen, an increase in [^{35}S] GTPγS binding after cocaine administration was found in both males and females. This is in contradiction to previous work in which no change in the levels of 5-HT after cocaine administration was found (50,52,52). However, μ opioid receptor and D1 dopamine receptor agonists have shown increases in [^{35}S]GTPγS binding in the caudate putamen of males (193-197,202). Finally, in the amygdala a decrease in 5-HT$_{1A}$ activation was found in both male and female rats. This is consistent with reports of chronic WF-23 administration in which a reduction of [^{35}S]GTPγS binding was observed (193,194,203,204). WF-23 is a potent tropane analog which like cocaine blocks dopamine, serotonin, and norepinephrine transporters with high affinity *in vitro* and is shown to substitute for cocaine and maintain cocaine responding in self-administration paradigms in both rodents (193,194,203,204).

In the core of the nucleus accumbens of both males and females increases in [^{35}S] GTPγS binding after cocaine administration was found. However, a sex difference in the activation of 5-HT$_{1A}$ receptors in the nucleus accumbens shell was observed. In the shell of the nucleus, accumbens males had an increase of 5-HT$_{1A}$ binding after cocaine administration compared to saline. This is consistent with earlier findings of increased 5-HT levels in this area after cocaine administration in the NAc in male rats (32,40,53). Further, it has been shown that co-administration of WAY 100635 and cocaine decreased cocaine-induced behaviors while increasing cocaine-induced levels in nucleus accumbens. This could be reversed by agonist treatment in male rats (32,40). Our data suggests that increased levels of 5-HT after systemic injections are regulated by the activation of 5-HT$_{1A}$ receptors in the nucleus accumbens. Females had a reduction in 5-HT$_{1A}$ agonist-stimulated [^{35}S] GTPγS binding levels after cocaine administration. This is in line with previous evidence of female rats

showing decreased 5-HT, and 5-HIAA, in the nucleus accumbens (NAc) after cocaine administration (13,104). In addition, repeated cocaine administration in OVX and OVX-EB rats demonstrated a decreases of [^{35}S]GTPγS binding in the nucleus accumbens in OVX-EB and increases in OVX female rats (200). Additionally, other agonists which target GABA, DA as well as 5-HT$_{1A}$ receptors show decreases in [^{35}S]GTPγS binding in the nucleus accumbens of female but not male rats (193-197,200). Taken together, these results suggest a sexually dimorphic response within the nucleus accumbens in cocaine-induced behavioral activation.

It has been previously demonstrated that females have stronger reward activation to cocaine than male rats (21,148). Since the shell of the nucleus accumbens has been postulated to be an important area in reward ,the sexually dimorphic regulation of nucleus accumbens [^{35}S]GTPγS binding may contribute to the known sexually dimorphic aspects of cocaine reward (206). In addition, microinjections of 5-HT$_{1B}$ legends into the shell, but not core, dose-dependently attenuated the psychostimulant-induced locomotor activity and thus, as previously discussed, could also be linked with cocaine-induced effects of 5-HT$_{1A}$ receptors (118). Therefore, sexually dimorphic aspects of cocaine-induced locomotor activation are found in the shell of the nucleus accumbens.

Sex differences in [^{35}S] GTPγS activation of hippocampal levels were also found. Increases of 5-HT$_{1A}$ [^{35}S] GTPγS activation were seen in both the CA1 and CA3 regions of females while in males a significant decrease in activation was seen in response to cocaine administration. This is inconsistent with previous findings of increased 5-HT levels after cocaine administration in the hippocampus of male rats (47). Increases of GABA$_B$ receptor-stimulated [^{35}S] GTPγS binding was increased by 25% in the hippocampus after chronic administration of buspirone treatment (197). To the best of our knowledge, this is the first time 5-HT$_{1A}$ [^{35}S] GTPγS binding has been examined in

female rats. Our results, consistent with previous reports in male rats, show an involvement of 5-HT_{1A} receptor activation in the hippocampus of female rats.

Hippocampus-accumbens projection and its serotonergic innervations play a prominent role in the regulation of psychostimulant related locomotor activation (47). Thus, we postulate that sexual dimorphisms exist in differential brain activation of 5-HT_{1A} receptors in response to cocaine administration in these areas. In parallel to our behavioral research and the known neuroanatomical abundance found in these areas, we would suggest that our results demonstrate a direct involvement in the regulation of cocaine-induced behavioral activities within the hippocampus-accumbens pathway. Moreover, the differential effects found in these areas imply the location of sexual dimorphisms in the rewarding aspect seen in cocaine-administration and other reward paradigms. Russo et al., 2003 demonstrated sexually dimorphic responses in the development and acquisition of reward. They hypothesized that intrinsic differences in the processes underlying learning and memory may be involved. Serotonin has also been implicated in the processes involved in learning and memory (207). Thus, the differential activation of 5-HT_{1A} receptors in the hippocampus may underlie sexual dimorphic responses in cocaine-induced reward and memory.

Overall, we found that the 5-HT_{1A} receptor activity is directly affected by cocaine administration in a number of brain areas involved in cocaine-induced behaviors. However, this is to the best of knowledge the first time looking at acute administration of cocaine's effect on 5-HT_{1A} receptor activation with [^{35}S] GTPγS. Previous work has been done with male rats with chronic administration of cocaine. In this study, the fact that we determined activation of only one hour may cause limitations as actual effects at this level may not be physiologically possible and thus a time course should be determined. However, taken together with the previous study we provide evidence of **s**exually dimorphic cocaine-induced activation of the 5-HT_{1A} receptor. Determining if there are

sex differences in 5-HT_{1A} receptor number is directly related to effects of this differential activation as discrepancies between receptor number and specific activation of G-proteins has been found previously.

Chapter 4: Sex differences in the blockade of the serotonin transporter using the reuptake inhibitor fluoxetine

1. Introduction

It is known that cocaine binds with high affinity to the serotonin (SERT) transporter causing the inhibition of presynaptic reuptake of serotonin (24,25,65,76). This disruption causes an overall increase in synaptic concentration subsequently causing a change in neuronal firing (38). SERT is involved in the regulation of various cocaine-induced behaviors including locomotor hyperactivity and reward (96,128). For instance, it has been shown that only when mice are double knockouts of both the DAT and SERT is the elimination of cocaine reward seen with the elimination of conditioned place preference (112,116,208-210).

Studies with SSRI's have shown conflicting evidence in cocaine-induced behavioral responses. 5-HT reuptake blockade with the administration of a number of SSRI's including fluoxetine and fluvoxamine, have been found to increase 5-HT levels and enhance acute cocaine locomotor activity in male rats (38,75,83,96,128). However, others have found SSRI's which increase extracellular 5-HT available within serotonergic cells, but did not potentiate cocaine-induced hyperlocomotion and still others found no effect (38,75,83,96,128). In addition, SSRI's have not only been implicated in locomotor activity of rats but also are implicated in the rewarding properties of cocaine (89,100,109,129,130). For instance, SSRI's can enhance the stimulant effects of cocaine by reducing the propensity to self-administer cocaine (89,100,109,129,130). Although a clear picture is emerging demonstrating a fundamental role of 5-HT in cocaine-induced behavioral effects, the role of sex differences in 5-HT activity and serotonin reuptake modulation of cocaine-induced behaviors is yet to be determined. The aim of this research is to test the hypotheses that sex

differences in the activation of the serotonin transporter contribute to sexually dimorphic patterns seen in cocaine-induced hyperactivity.

2. Methods

2.1 Animals

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed for one week prior to experimental manipulations in standard plastic cages (20 x 20 x 41 cm^3). Rats had free access to standard lab chow and water *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.). Rats were handled and weighed for 3 days prior to experimental manipulations. Three separate cohorts of animals were used for behavioral studies with an n of 8 per group. Since repeated vaginal lavage attenuates cocaine-induced activity, abolishes estrous cycle effects, and establishes a place preference in female rats and increased dopamine release in the striatum (184). Females were placed into groups at random without regard to estrous cycle (184). All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Drugs

Cocaine hydrochloride and fluoxetine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.3 Drug treatment and experimental paradigm

Cocaine and fluoxetine were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneally. All injections were performed in the home cage. After 1 hour pre-treatment with fluoxetine (0, 1, 5, 10 mg/kg), rats were injected with (20 mg/kg) of cocaine or saline. This cocaine dose was previously demonstrated to produce significant increases in locomotion and robust sex differences in cocaine-stimulated motor activity, without reaching a maximal effect in either sex (13). Fluoxetine doses and pretreatment schedule were chosen based on previous reports in male rats, which demonstrated an inhibition of locomotor behavioral responses to cocaine (38,100)**.**

2.4 Behavioral Measurement

Total locomotor, ambulatory and rearing activities were monitored in the home cages with a Photobeam Activity System from San Diego Instruments (San Diego, CA) as previously described (160). All behavioral activities were recorded for one-hour post-drug treatment. Total locomotor activity represented the sum of counts in the horizontal frame. Ambulatory activity represented the number of counts produced by two consecutive photobeam interruptions in the horizontal frame. Rearing activity represented total counts of vertical motion.

2.5 Statistical Analysis

Total locomotor, ambulatory, and rearing data were summed for each subject and presented as mean ± standard error of the mean (SEM). Three-way analyses of variance (ANOVAs) were used to determine the effects of antagonist administration, cocaine, and sex on cocaine-induced behavioral responses: SSRI DOSES (vehicle or fluoxetine) x DRUG CONDITION (cocaine or saline) x SEX

(male or female). Within sex, two-way ANOVAs were used to determine the effects of SSRI dose on cocaine-induced behavior: antagonist dose (vehicle or fluoxetine) x cocaine (cocaine or saline). When significance was obtained, Fisher LSD test was used. To account for these baseline effects, data was analyzed as the difference of cocaine effect minus their respective saline activity. A p-value of less than 0.05 was considered significant in all statistical analysis.

3. Results

3.1 Sex Differences in the Blockade of the Serotonin Transporter using the Serotonin Reuptake Inhibitor Fluoxetine

As shown in Figure 24-26, a significant main effect of drug was obtained for drug [total locomotor: $F(1, 96) = 94.1542$, $p=0.0000$; ambulatory: $F(1, 98) = 59.8902$, $p=0.0000$; rearing: $F(1, 98) = 130.5588$, $p=0.0000$]; overall, cocaine increased all three behavioral responses ($p<0.05$ for all comparisons).

In both sexes, Fig 24-26, fluoxetine altered behavioral baseline of total locomotor and rearing behaviors [total locomotor: $F(3, 48) = 3.4628$, $p = 0.0233$; rearing: $F(3, 48) = 9.31144$, $p = 0.0002$]. To account for these baseline effects, data was analyzed as the difference of cocaine effect minus their respective saline activity. As shown in Fig 27-29, after correcting for the baseline effect, fluoxetine did not significantly alter any behavioral responses.

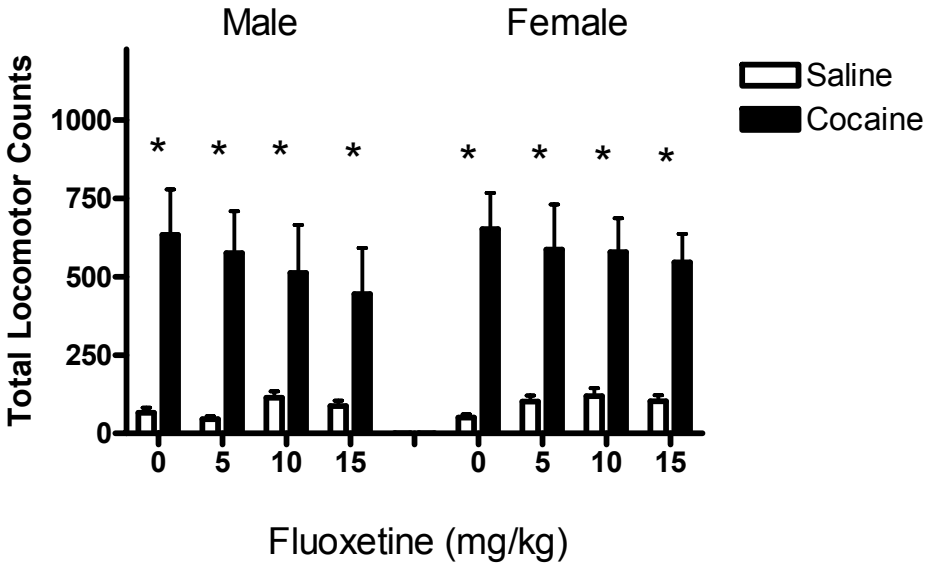


Figure 24: Drug does not affect total locomotor activity. Mean total locomotor counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine, followed by a single i.p. injection of cocaine (20 mg/kg) or saline. * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) (n=8).

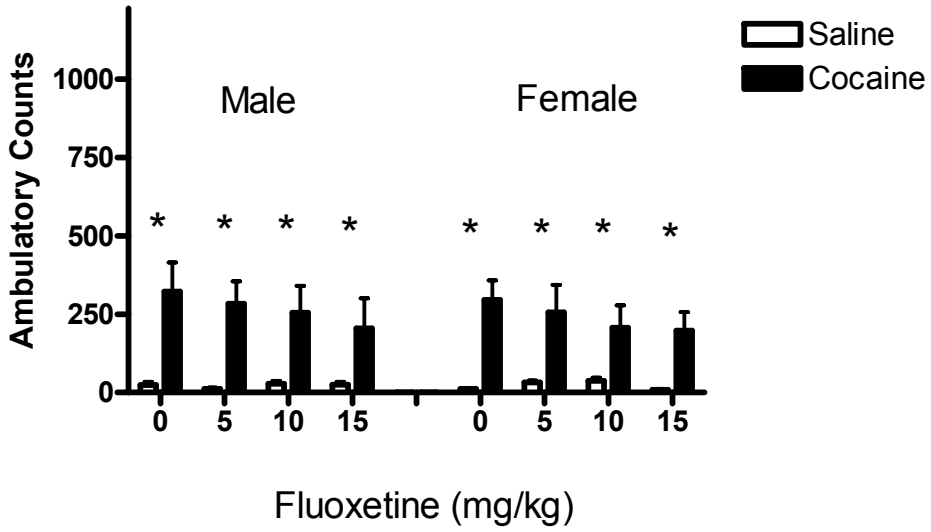


Figure 25: Drug does not affect ambulatory activity. Mean ambulatory counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine, followed by a single i.p. injection of cocaine (20 mg/kg) or saline. * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) (n=8).

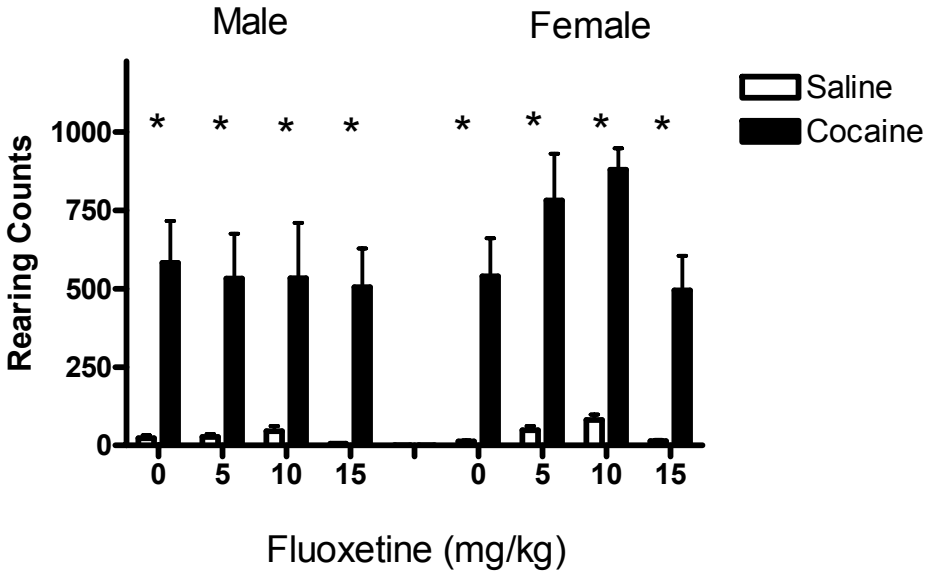


Figure 26: Drug does not affect rearing activity. Mean rearing counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine, followed by a single i.p. injection of cocaine (20 mg/kg) or saline. * Denotes a significant effect of cocaine as compared to respective saline treated groups ($p<0.05$) (n=8).

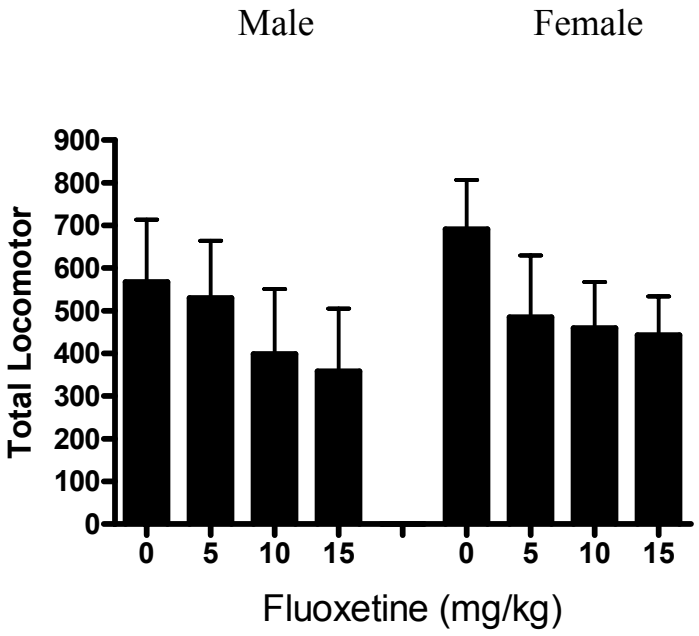


Figure 27: Drug does not affect locomotor activity. Delta value of mean total locomotor counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine followed by a single i.p. injection of cocaine (20 mg/kg) or saline.

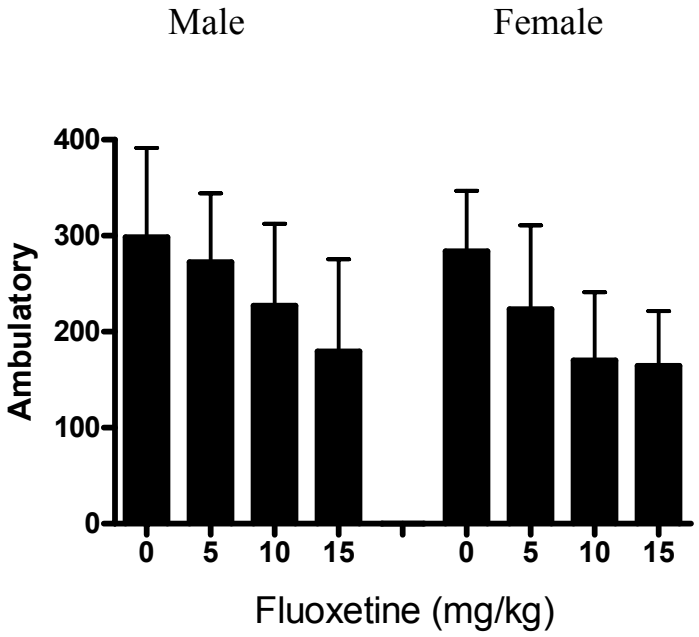


Figure 28: Drug does not affect ambulatory activity. Delta value of mean ambulatory counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine followed by a single i.p. injection of cocaine (20 mg/kg) or saline.

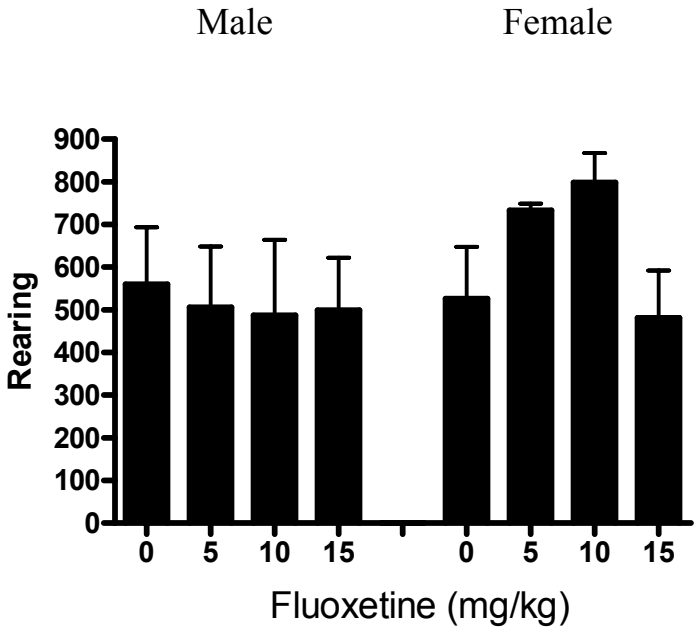


Figure 29: Drug does not affect rearing activity. Delta value of mean rearing counts in male and female rats after single i.p. injection of the serotonin reuptake inhibitor Fluoxetine followed by a single i.p. injection of cocaine (20 mg/kg) or saline.

4. Discussion

Contrary to our hypothesis, a significant effect was only found for drug administration; with cocaine showing a robust increase compared to saline in all conditions and each sex examined. We found that fluoxetine failed to alter cocaine-induced hyperlocomotor activity. This is consistent with previous research which found that fluoxetine failed to alter the locomotor activity induced by cocaine in male and female C57BL/6BJ mice (75). Moreover, although cocaine conditioned place preference (CPP) development was increased by fluoxetine in male rats, no locomotor effects were seen (211). Specifically, no change in the number of entries into the two conditioning compartments was observed suggesting that locomotor activity was unaffected by fluoxetine (211). However, the bulk of literature with SSRI's fluoxetine show potentiations of cocaine-induced locomotor activity (96,100,128,212). Similar to previous reports, though not significant, in females an increase in rearing behavior was observed.

Reith et al., 1991 suggested that a lack of effect in their study may have been due to the stimulation of autoreceptors, inhibiting the 5-HT release and suppressing the activity of serotonergic neurons (75). For instance, fluoxetine increases extracellular 5-HT levels in forebrain regions; therefore it could be suggested that 5-HT in the synaptic cleft reduces 5-HT release via stimulation of presynaptic autoreceptors (96). Previous evidence suggests that the blockade of inhibitory autoreceptors cause the increase of 5-HT levels and subsequent attenuation of cocaine-induced hyperlocomotor behavior. This however, was opposite to the previous findings and therefore presently we would conclude like previous research that the serotonergic transporter is not involved in sexually dimorphic aspects of cocaine-induced behavioral activation. However, similar behavioral trends were found in both male and female rats similarly in the current study and should be taken into consideration for future studies.

Our results suggest that SERT is not involved in the sexual dimorphisms seen in cocaine-induced locomotor activity. It is known that SERT is involved in the rewarding aspects of cocaine. Therefore, we might have had more robust results if we had looked at other behaviors exploring reward aspects of cocaine rather than locomotor behavior alone. In addition, the activation of autoreceptors and the vast number of receptors that may be involved in the effects of the co-administration of fluoxetine and cocaine make conclusions of specific effects of manipulation of SERT difficult. Therefore, more specific isolation of the transporter, such as with the use of conditional knockouts, should be explored to elucidate sex differences in the manipulation of the serotonergic transporter in cocaine-induced behavioral responses.

Chapter 5: Effects of WAY 100635, cocaine and sex differences seen in activation of the endocrine system of Fischer rats

Sexual dimorphisms exist in the activation of the HPA axis after cocaine administration. Specifically, females have greater ACTH and corticosterone serum levels after acute and chronic cocaine administration than male rats (809, 2412, 2860, 1551, 1765, 151, 12). Ovariectomy decreases cocaine-induced ACTH and corticosterone levels in females, whereas castration had no effect on males suggesting a role of gonadal hormones in this effect (30). Thus, the enhanced effect of cocaine administration on the HPA axis in female rats could contribute to enhanced behavioral reactivity that is seen in female rats (145). Cocaine also has substantial effects on the HPG axis and gonadal hormones. For instance, cocaine increases plasma levels of progesterone in rats in a dose-dependent manner (145,213,214). In addition, researchers have found progesterone plasma levels to be significantly higher in cocaine treated intact female rats compared to saline controls (145,160,184,213,215). Further, progesterone treatment has been found to affect cocaine-induced increases in serotonin levels in the medial prefrontal cortex (216).

At the level of the paraventricular nucleus, serotonin is released from dorsal raphe' nerve terminals acting on 5-HT_{1A} receptors which augment adrenocorticorophin hormone (ACTH) and thereafter coriticosterone release (217). Neuroendocrine studies in rats have found that 5-HT_{1A} agonists such as 8-OH-DPAT, buspirone, isapirone and gepirone have dose-dependent increases in levels of plasma oxytocin, prolactin, ACTH and corticosteroids (34,50,109,176, 133, 136). These neuroendocrine responses are blocked by 5-HT_{1A} antagonists, such as pindolol or WAY 100635 (34,50,109,176). In addition, females demonstrate a more rapid attenuation of hypothermic and adrenocortical responses to repeated 5-HT_{1A} stimulation (133,136). Therefore, we utilized WAY 100635, a 5-HT_{1A} receptor antagonist to determine the effects of this receptor in cocaine-induced

corticosterone and progesterone level effects. The aim of this research is to test the hypotheses that sex differences exist in interaction between 5-HT_{1A} receptors and the HPA and HPG systems.

2. Methods

2.1 Animals

Eight-week-old male and female Fischer rats purchased from Charles River (Kingston, NY) were individually housed for one week prior to experimental manipulations in standard plastic cages (20 x 20 x 41 cm^3). Rats had free access to standard lab chow and water *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 9:00 a.m.). Rats were handled and weighed for 3 days prior to experimental manipulations. Three separate cohorts of animals were used for behavioral studies with an n of 8 per group. Since repeated vaginal lavage attenuates cocaine-induced activity, abolishes estrous cycle effects, and establishes a place preference in female rats and increased dopamine release in the striatum (184). Females were placed into groups at random without regard to estrous cycle (184). All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 865-112, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2 Drugs

Cocaine hydrochloride, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-ycylhexanecarboxaminde maleate (WAY 100635), and 2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-corboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.3 Drug treatment and experimental paradigm

Cocaine and WAY 100635 solutions were prepared daily by dissolution in physiological saline (0.9%) and injected intra-peritoneal. All injections were performed in the home cage. After 15 minutes pre-treatment with WAY 100635 (0, 0.4, 0.8, or 1.6 mg/kg), rats were injected with (20 mg/kg) of cocaine or saline. This cocaine dose was previously demonstrated to produce significant increases in locomotion and robust sex differences in cocaine-stimulated motor activity, without reaching a maximal effect in either sex (13). Antagonist doses and pretreatment schedules were chosen based on previous reports in male rats, which demonstrated an inhibition of locomotor behavioral responses to cocaine (47,50-53).

2.4 Serum Levels for Corticosterone and Progesterone

One hour after cocaine or saline administration, the rats were sacrificed by decapitation, following a brief exposure (20 seconds) to CO_2. Trunk blood was collected and centrifuged at 3,000 RPM for 15 minutes at 4°C. Serum was then collected and stored at -80°C. Serum was analyzed with Coat-A-Count radioimmunoassay kits for corticosterone and progesterone (National Diagnostic, San Diego, CA). Intra-assay coefficients of variation were less than 10.0% ± 1.0%. Serum levels corticosterone and progesterone are expressed as ng/mL.

2.6 Statistical Analysis

A three-way analyses of variance (ANOVAs) were used to determine the effects of antagonist administration, cocaine, and sex hormone levels: ANTAGONIST DOSE (vehicle, 0.4, 0.8, 1.6) x DRUG (cocaine or saline) x SEX (male vs. female). Within sex, two-way ANOVAs were used to determine the effects of antagonist dose on cocaine-induced behavior: ANTAGONIST

DOSE (vehicle, WAY 100635,) x DRUG (cocaine or saline). A Fisher LSD post hoc analysis test was used when appropriate. A p-value of <0.05 was considered significant in all statistical analysis.

3. Results:

3.1 Effects of WAY 100635 and Cocaine on Corticosterone Levels

As shown in figure 30, a main sex effect was observed [$F(1, 106) = 14.6976$, $p = 0.0002$]; females had overall higher levels of corticosterone levels ($p < 0.05$ for all comparisons). A drug X antagonist interaction was also observed [$F(3, 106) = 3.21638$, $p = 0.0258$]; a 1.6 mg/kg WAY 100635 dose induced a significant decrease in corticosterone levels as compared to rats treated with a 0.4 mg/kg dose ($p= 0.0083$). Furthermore, among all animals who received a 1.6 mg/kg dose, saline-treated rats exhibited significantly lower levels of corticosterone than those treated with cocaine ($p = 0.0107$).

Shown in figure 30, a within sex analysis revealed that in males a drug effect failed to reach significance ($F(1, 53) = 3.4838$, $p = 0.0675$. In females, a drug X antagonist effect was seen [$F(3, 53) = 3.0330$, $p = 0.03715$]. Saline treated female rats had lower corticosterone serum levels than rats treated with WAY at the dose of 0.4 mg/kg or 0.8 mg/kg dose ($p = 0.0364$). In addition, saline-treated female rats had lower corticosterone levels at the 1.6 mg/kg dose when compared to the 0.4 mg/kg dose ($p = 0.01716$). Finally, in females there was a significant drug-induced potentiation of corticosterone levels associated with the co-administration of cocaine and 1.6 mg/kg dose of antagonist, an effect which was not observed in male rats ($p = 0.0369$).

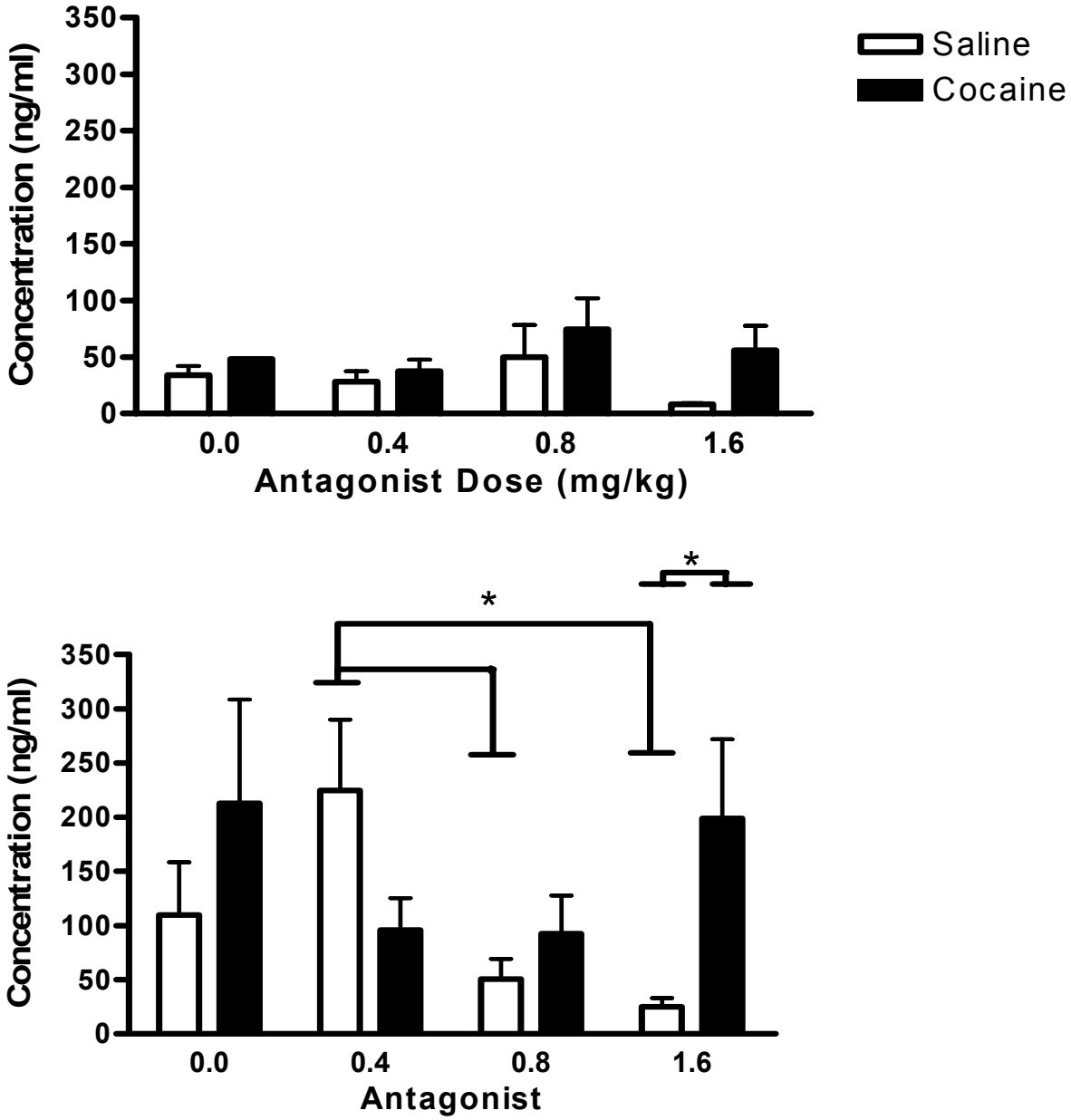


Figure 30: Sex, antagonist dose and drug affect corticosterone serum levels in A. males but not B. females. In females administered saline the 5-HT_{1A} antagonist decreased corticosterone levels with increasing doses. In addition at the highest dose cocaine differed from saline in females. * Represents a significant drug by antagonist interaction.

3.2 Effects of WAY 100635 and Cocaine on Progesterone Levels

As shown in figure 31, when progesterone serum levels were analyzed, a main sex effect was observed [$F(1, 77) = 49.4092$, $p = 0.0000$; female rats had overall higher levels of progesterone than male rats. A main drug effect was observed [$F(1, 77) = 9.1609$, $p = 0.0033$]; cocaine increased progesterone serum levels when compared to saline treatment in both male and female rats. Finally, a sex X drug significant interaction was also observed [$F(1, 77) = 9.1327$, $p = 0.0034$]; in female rats, a cocaine-induced increase in progesterone levels was observed.

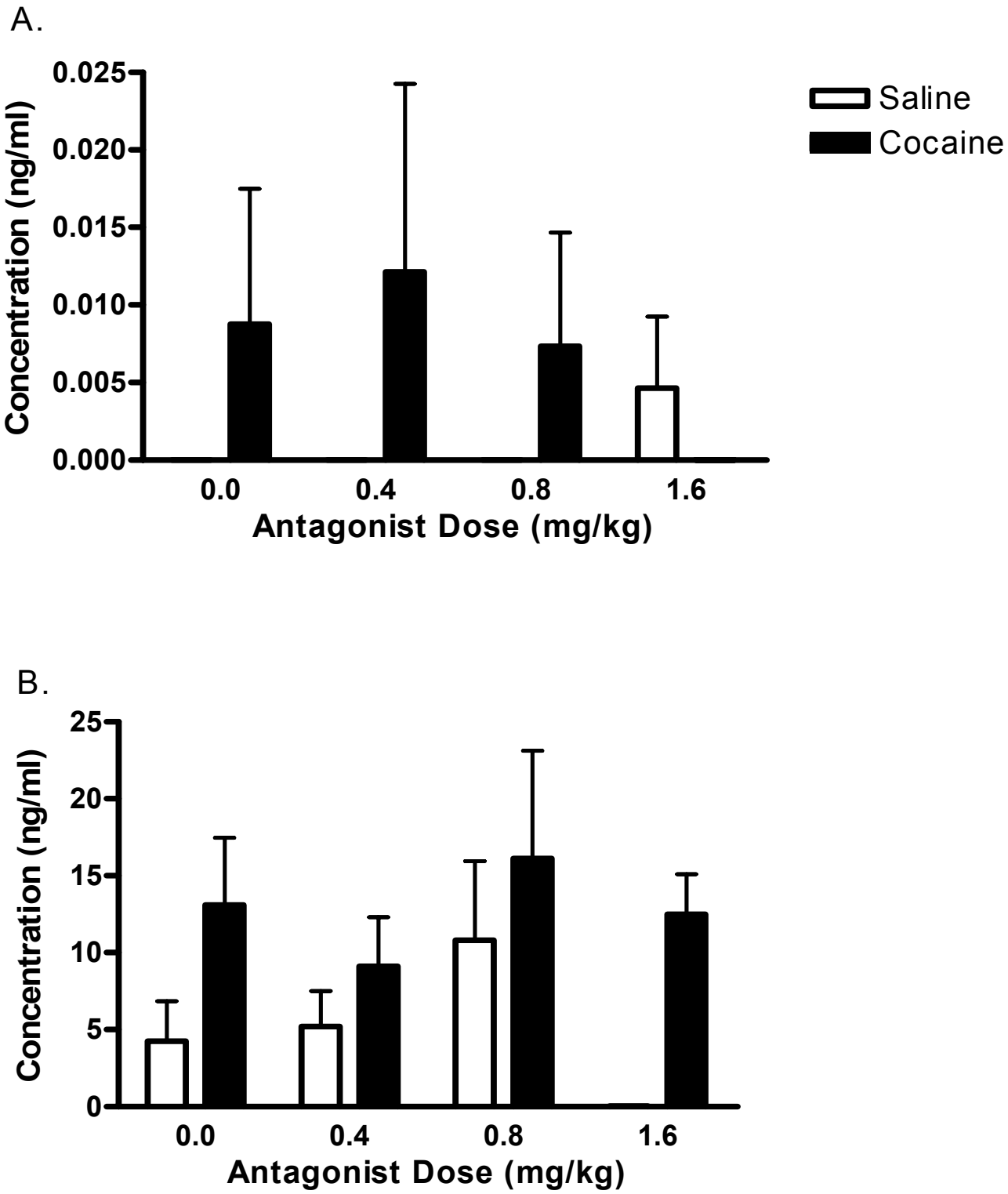


Figure 31: Sex, antagonist dose and drug do not affect progesterone serum levels in A. males but not B. females.

4. Discussion

Consistent with previous findings we found an increase of both progesterone and corticosterone with cocaine administration (30,94,143,160,165,180,218). Previous research has shown that WAY 100635 reduces corticosterone levels in male Wistar rats when given alone while others have found no significant changes of WAY 100635 administration (28,54). However, the effects of the co-administration of cocaine and WAY 100635 have been inconsistent (28, 54). Therefore, we assessed the effects of WAY 100635 on cocaine-induced increases of progesterone and corticosterone in male and female rats.

We did not find any significant cocaine-induced effects of corticosterone levels after WAY 100635 administration in male rats. Conversely, female rats had a reduction of cocaine-induced corticosterone levels after 0.4 mg/kg dose of WAY 100635 administration compared to vehicle treatment; however it failed to reach significance ($p = 0.0859$). There was a cocaine-induced increase with the highest dose of WAY 100635 in females. In addition, in females, there was a reduction of corticosterone levels in saline treated animals with increasing doses of WAY 100635. Similar to previous studies in males, WAY 100635 when given alone seemed to cause an anxiolytic effect decreasing stress hormones and stress related behaviors (30,94,143,160,165,180,218).

A number of studies have shown effects of 5-HT_{1A} regulation of the HPA axis. For instance, hypothalamic 5-HT_{1A} receptors are coupled to G-proteins which stimulate both oxytocin and corticosterone release (219). In addition, WAY 100635 is known to block 8-OH-DPAT-induced elevation of plasma ACTH and corticosterone secretion (109,152). Further, ACTH release evoked by microinfusion of 5-HT over the PVN is blocked by pretreatment with the 5-HT_{1A} antagonist pindolol (27). Further, acute *in vitro* treatment of corticosterone has been found to attenuate 5-HT_{1A} autoinhibition in the DRN (178). There is evidence that corticosteroids modulate the postsynaptic 5-

HT_{1A} receptor function (220). Interestingly, 5-HT_{1A} receptors, both pre and postsynaptic neurons are downregulated to chronic corticosterone treatment (177). A chronic level of corticosterone attenuates 5-HT_{1A} receptor function in the hippocampus which is dissimilar to that seen in acute treatments (177). However, Fairchild et al., 2003 found no effect on 5-HT_{1A} receptor function at maximal doses and furthermore application with dexamethasone, which is a more potent glucocorticoid agonist than corticosterone, they found no effect.

To our knowledge, this is the first experiment examining 5-HT_{1A} manipulation of progesterone in male and female rats. No significant effects were found in progesterone levels. However, in both males and females, consistent with previous studies, an increase in progesterone serum levels after cocaine administration was demonstrated (104,145,165,180). Therefore, we conclude that progesterone is not directly involved in the mediation of or by 5-HT_{1A} receptors after cocaine administration.

Our results suggest that a sex difference in the activation of corticosterone is differentially regulated by the 5-HT_{1A} antagonist WAY 100635 basally. This could suggest that progesterone and corticosterone are not directly involved in the modulation of sexually dimorphic aspects of cocaine-induced hyperlocomotor activity after antagonist administration. However, similar to previous evidence, a significant reduction of basal levels of corticosterone was found after the administration of WAY 100635 in both male and female rats. To the best of our knowledge this is the first time this has been explored. This suggests that WAY 100635 may be involved in anxiolytic properties and other behavioral effects related to stress rather than cocaine-induced effects. In addition, it is suggested that this effect on anxiety may be sexually dimorphic in nature. For instance, female rats exhibit increased sensitivity to the anxiolytic effects of 5-HT_{1A} agonists compared to males (135,136). Therefore, looking at the effects of WAY 100635 on cocaine-induced anxiety during

withdrawal could be interesting to explore. We conclude that WAY 100635 does not have a significant effect on cocaine-induced endocrine effects. However, pharmacological manipulation of the 5-HT_{1A} receptor in the treatment of anxiety, which is currently being explored, is appealing.

Chapter 6: Conclusions

It has been reported previously, that the serotonergic system is involved in the regulation of cocaine-induced locomotor activity. Many have suggested that sexually dimorphic behavioral responses to cocaine administration are due to sex differences in basal and cocaine-induced serotonin release, re-uptake and receptor levels. However, the exact receptors and mechanisms involved in these dimorphic responses have yet to be elucidated. Through our work, we conclude that the 5-HT_{1A} and 5-HT_{1B} receptor manipulation causes sexually dimorphic effects in cocaine-induced hyperlocomotion. Interestingly, we found that the serotonin transporter is not involved in this sexually dimorphic behavior. Our fluoxetine evidence may provide further evidence of the involvement of 5-HT_{1A} and 5-HT_{1B} receptors in sexually dimorphic responses of cocaine-induced hyperlocomotion. Our neurobiological results demonstrate that activity of 5-HT_{1A} receptors is directly involved in sexually dimorphic cocaine-induced hyperlocomotion. Therefore, sexually dimorphic effects of 5-HT levels found after cocaine administration may have a direct effect on the subsequent activation of 5-HT_{1A} receptors, which may also be sexually dimorphic. Finally, we found a modest affect of cocaine administration on the HPA and HPG axes. Therefore, although cocaine is exerting an effect on the endocrine system, these effects may not be as robustly involved in sexually dimorphic aspects of cocaine-induced behaviors. Taken as a whole, we find support for our hypothesis that the serotonergic system directly influences and/or regulates sex differences found in cocaine-induced locomotor behaviors.

5-HT_{1A} and 5-HT_{1B} receptors:

At the behavioral level, we found a number of cocaine-induced behavioral sex differences. Overall, cocaine-treated females demonstrated higher total locomotor, ambulation and rearing

behavior compared to males in all experimental observations. Similar to previous studies this demonstrates that higher doses of cocaine are required in male rats to achieve responses similar to those of female rats, demonstrating an increased sensitivity to cocaine in females as compared to male rats. Although previous studies have shown that these behaviors are dose and sex dependent, after acute cocaine injections we utilized a general 20 mg/kg dose of cocaine. Though findings were more robust in females results suggest that a comparable 20 mg/kg dose of cocaine given to both male and female rats is optimal when determining sex differences in cocaine-induced locomotor hyperactivity in male rats.

As previously reported, we found that serotonin 5-HT_{1A} and 5-HT_{1B} mediate cocaine-induced behavioral hyperlocomotor responses in rats. We extended these reported observations by demonstrating that in female rats, similar to male rats, both 5-HT_{1A} and 5-HT_{1B} receptors are involved in the modulation of cocaine-induced behavioral responses. We found that in both male and female rats WAY 100635 did not alter general activity. This suggests that WAY 100635 acts specifically in the regulation of 5-HT_{1A} receptor cocaine-induced effects in male and female rats and but baseline behaviors. Moreover, we found that WAY 100635 caused an attenuation of cocaine-induced hyperlocomotion in response to cocaine administration in both male and female rats. Differentially, WAY 100635's ability to attenuate cocaine-induced behaviors differs by specificity of motor activation of the behavior. Specifically, in males WAY 100635 was not effective in the reduction of cocaine-induced ambulatory behaviors but was effective in all other behaviors examined. In addition, in females, WAY 100635 was effective in reducing behaviors in total locomotor and ambulatory behaviors in all doses. However, only the 0.4 mg/kg dose was effective in reducing rearing behavior in females. This was not seen in males with all doses producing an attenuation of cocaine-induced behaviors in rearing behavior.

Consistent with previous reports, 0.4 mg/kg of WAY 100635 was sufficient in selectively blocking the effects of cocaine-induced behaviors in male rats (52, 205). In fact, in both male and female rats, 0.4 mg/kg was the optimal dose for attenuating cocaine-induced hyperlocomotion. Previously, it has been shown that lower concentrations of agonists have a stronger affinity for the presynaptic 5-HT_{1A} autoreceptors in the raphe nuclei and therefore, are considerably more sensitive to pharmacological manipulation than postsynaptic 5-HT_{1A} receptors located in projection terminal regions (28,32,33,40,47,50-55,205). Higher doses seem to target both autoreceptors and postsynaptic receptors similarly (47). Though it has not been specifically determined in antagonists, it is possible that lower concentrations are specifically targeting these receptors due to presynaptic autoreceptor sensitivity. When comparing systemic and local manipulation of cocaine or other 5-HT agonists, differential effects are seen. This suggests that activation of somatodendritic autoreceptors is necessary for the increased amounts of 5-HT in projection areas found in response to cocaine administration. Local application in projection areas with cocaine or agonists and thus specific activation of postsynaptic receptors in some cases, does not cause this same increase. The importance of 5-HT_{1A} autoreceptors in cocaine-induced behavioral effects are further evidenced by the fact that agonist-induced increases in 5-HT are not blocked by antagonists with local application in projection areas. This suggests that 5-HT increases seen in projection areas such as the frontal cortex, nucleus accumbens and hippocampus are subsequently affected by the activation of somatodendritic autoreceptors. In addition, it has been suggested that 5-HT release is modulated by presynaptic 5-HT_{1A} receptors with dopamine and other neurotransmitters being modulated by postsynaptic 5-HT_{1A} receptors and 5-HT_{1A} heteroreceptors. However, WAY 100635 blocks 5-HT_{1A} receptor agonist-induced increase in 5-HT release but does not block agonist-induced increases of DA or NA release in the rat hippocampus, frontal cortex and nucleus accumbens. To further this

dose theory, researchers have found that the effects of 5-HT_{1A} receptor agonists on 5-HT release were blocked by a low dose of WAY 100635 while a high dose of WAY 100635 was required to attenuate 5-HT_{1A} receptor agonist-induced increase in DA release. This evidence suggests that WAY 100635 has a preferential action at somatodendritic 5-HT_{1A} receptors in male and female rats. As demonstrated previously, females are known to have heightened levels of serotonin in the brain. Therefore, it is interesting that the combination of higher 5-HT increases plus a possible increase of 5-HT somatodendritically from cocaine administration, WAY 100635's 0.4 mg/kg dose was also optimal in the reduction of cocaine-induced locomotor activity in both male and female rats in the same manner. This suggests that in both male and female rats, WAY 100635 at this dose targets presynaptic receptors in somatodendritic regions of the serotonergic system with equal efficacy and is effective in the attenuation of cocaine-induced behavioral activity of male and female rats.

Therefore, the mechanisms involved in the behavioral effects of the 5-HT_{1A} receptor in cocaine-induced behavioral activation in male and female rats can be predicted. Cocaine binds to the 5-HT transporter inhibiting the reuptake of 5-HT from the synaptic cleft. This yields an increase in 5-HT not only stimulating postsynaptic receptors but also activating inhibitory 5-HT_{1A} autoreceptors somatodendritically, which causes a decrease in neuronal activity and 5-HT release. The use of WAY 100635 blocks 5-HT_{1A} receptors that attenuates the inhibitory effect of somatodendritically released 5-HT on 5-HT neurons. The effect of local 5-HT reuptake blockade in terminal areas by cocaine combined with 5-HT_{1A} antagonism serves to potentiate extracellular 5-HT concentrations in terminal regions of 5-HT projections. Such projections have been found in the prefrontal cortex, nucleus accumbens and hippocampus.

Unlike WAY 100635, GR 127935 produced a baseline effect. This suggests that the 5-HT_{1B} receptor is not only specifically involved in the regulation of cocaine-induced mechanisms and

behavioral activation. The compound GR 127935 is not highly selective in its regulation and could activation of a number of other serotonin receptors and other neurotransmitter systems. While it has been suggested that GR 127935 is a partial agonist, our effects indicate that it works as an antagonist at the 5-HT_{1B} receptors in cocaine-induced behavioral activation after elimination of the baseline effect. We found the 5-$HT_{1B,}$ receptor antagonist GR 127935 attenuates cocaine-induced hyperlocomotor activity in male and female rats. Sex differences were observed in the efficacy of GR 127935 to inhibit cocaine-induced activity. This sexual dimorphic antagonism was behaviorally specific and varied by dose. Although, analysis by sex revealed no reduction in either sex in total locomotor behaviors, significance of the reduction in females was found only in ambulatory activity (10 and 15 mg/kg dose attenuated when compared to saline). In males, however, no significant effects of GR 127935 treatment were found in ambulatory behaviors. Conversely, in rearing behaviors, significant reductions in cocaine-induced hyperlocomotor behavior were seen in male rats (showing a reduction only with 15 mg/kg dose); which was not seen in females. Locomotor behavior is believed to be postsynaptically mediated. As described in Chapter 1, 5-HT_{1B} receptors are found more abundantly in projection areas of the serotonergic pathway including a wealth of receptors in motor control centers (48). Throughout the brain, 5-HT_{1B} receptors are located both presynaptically and postsynaptically in 5-HT projections. They are known to be abundantly found as terminal autoreceptors where they inhibit the release of serotonin from nerve terminals. Thus, sex differences in the efficacy of GR 127935 to attenuate cocaine-induced behavioral responses implicate differential regulation of 5-HT_{1B} postsynaptic autoreceptors in male and female rats. In addition to altering neurotransmission, terminally they also interact with several other transmitter systems in various areas of the brain, making it difficult to pinpoint the exact mechanism being activated in cocaine-induced locomotor effects. Stimulation of postsynaptic 5-HT_{1B} receptors alter

the activity of dopamine, acetylcholine, GABA, noradrenaline, and glutamate, and thus could be involved in the sexually dimorphic aspects of cocaine-induced behavioral responses seen.

5-HT_{1B} autoreceptors are known to mediate the feedback control of 5-HT release in the rat brain, a mechanism that is alleged to prevent overstimulation of postsynaptic receptors. Therefore, cocaine causes an increase in 5-HT in terminal areas by blocking its reuptake. However, the increased activation of postsynaptic autoreceptor feedback reduces this 5-HT increase. By blocking postsynaptic autoreceptors with GR 127935, the negative feedback is eliminated and 5-HT increases are seen which is known to subsequently have a reduced effect on overt cocaine-induced behavioral activation. 5-HT_{1B} receptors are also known to be heteroreceptors. The nucleus accumbens and VTA are heavily innervated by both serotonergic and dopaminergic projections. In addition, cocaine is known to cause substantial increases of 5-HT and DA in these areas. It is known that 5-HT_{1B} receptors in these areas act as inhibitory heteroreceptors in axon terminals of GABAergic nucleus accumbens neurons that project to the VTA. Therefore, it is suggested that the activation of 5-HT_{1B} receptors on GABAergic projections to the NAc and VTA inhibit local GABA release. This causes VTA neurons to be released from their inhibition and to the disinhibition of mesolimbic DA cell bodies. Agonists reduce extracellular GABA levels in the VTA, leading to greater post-cocaine deficits in the GABA levels in the VTA. This suggests that 5-HT agonists induce a disinhibition of the mesoaccumbens DA projection, thus enhancing the effects of DA reuptake blockade by cocaine in the nucleus accumbens. It is also suggested that agonists potentiate cocaine-induced increases in nucleus accumbens DA levels by activating 5-HT_{1B} heteroreceptors that regulate the activity of the hippocampal-accumbens glutamate projections. Therefore, antagonist may have the opposite effects seen with the co-administration of agonists and cocaine on cocaine-induced effects.

SERT:

The serotonin transporter has been suggested to be integral in the effects of cocaine. Researchers have found that manipulations with SSRI's cause an increase in locomotor hyperactivity in both male and female rats. However, we did not find any significant effects in cocaine-induced behavioral activation in male or female rats using the SSRI fluoxetine. There are a number of reasons why we may not have found similar effects of fluoxetine presently. Firstly, to the best of our knowledge this was the first time in which fluoxetine was administered to male and female Fischer rats, suggesting that the activation of serotonin reuptake is unlike those seen in other strains. Secondly, SSRI's are known to have a robust effect on the rewarding aspects of cocaine via self-administration and CPP paradigms. Previous evidence with cocaine-induced behavioral effects has been more modest and contradictory. Therefore, we might have found more of an involvement of the transporter if we had examined more rewarding aspects of cocaine-induced behavior. Thirdly, we used a higher dose of cocaine and used systemic injection of fluoxetine rather than local application in specific brain areas. In addition, it has been suggested that fluoxetine is known to cause a more modest increase compared to precursors or other SSRI's, and may not induce a sufficiently robust increase of 5-HT to alter behavioral effects. Therefore a more potent SSRI may have revealed more robust findings. Finally, though fluoxetine is known to cause an increase in extracellular 5-HT levels in terminal regions it may indirectly stimulate 5-HT_{1A} somatodendritic autoreceptors and reduce neuronal firing, suggesting that the SSRI was "self-limiting" (117, 75). Therefore, conditional knockouts could be utilized to properly isolate the specific effects of the serotonin transporter in cocaine-induced hyperlocomotor behavior. In addition research has determined that both SSRIs and cocaine bind with high affinity to SERT. Therefore, SSRIs would have little or no further impact upon 5-HT than that induced by cocaine. Previous researchers have

suggested certain drug treatments, which alter the balance between dopamine and serotonin such as cocaine, may be the cause of specific cocaine-induced effects. For instance, cocaine may cause more dopaminergic effects if serotonergic effects are diminished or more serotonergic if dopaminergic effects are potentiated. The modulation of this balance is believed to directly affect cocaine-induced behaviors and neurochemical activation. Therefore, pharmacological manipulation of either the 5-HT_{1A} or 5-HT_{1B} receptors could effect this balance while drugs that directly block SERT such as Fluoxetine may not effect this shift in balance toward 5-HT because further increases in 5-HT transport beyond that caused by cocaine would be inconsequential. Overall, our results suggest that the 5-HT transporter does not modulate cocaine-induced locomotor hyperactivity in male and female Fischer rats.

Activation of 5-HT_{1A} receptors:

The serotonergic system is an extremely complex system. Cocaine-induced effects have been found at many levels of normal functioning including release, reuptake and in juveniles, binding. Previously, we determined sex differences in pharmacological manipulation of both the 5-HT_{1A} and 5-HT_{1B} receptors and thus concluding that the activation of these receptors may be involved in this sexually dimorphic response. To substantiate our belief that 5-HT_{1A} receptors were involved in the reduction of cocaine-induced behavioral hyperlocomotion, we examined the activation the G-protein linked receptor. It is known that differential and sometimes conflicting result can occur due to route of administration utilized. In this study we used systemic administration to confirm the effects seen in overt sexual differences in cocaine-induced behavioral responses were due to the direct activation 5-HT_{1A} neurons. We used 5-HT_{1A} receptor mediated [^{35}S]GTPγS binding to determine if there were consistencies between behavioral activation and 5-

HT_{1A} activation. We selected areas known by autoradiographic means to have a high number of receptors or have been implicated in the cocaine-induced effects of 5-HT levels. Specifically, autoradiography demonstrates high levels of 5-HT_{1A} receptors in dorsal and median raphe nuclei, limbic forebrain regions of rats, including the hippocampus (CA1 and CA3), amygdala, and frontal cortex with a lower density found in the NAc (33-36,39,69). It is known that the density of a given receptor does not directly correlate with its activation within specific brain areas. We found that in both male and female rats a number of brain areas show differential activation of 5-HT_{1A} receptors in response to cocaine administration. Similar to previous work we found specific functional activity using 5-HT_{1A} receptor mediated [^{35}S]GTPγS binding in the frontal cortex and hippocampus (66,201). We also found specific activation of 5-HT_{1A} receptors in the nucleus accumbens, amygdala and caudate putamen, which has not been previously examined. Therefore, we found a direct relationship between the known neuroanatomical location of 5-HT_{1A} receptors with the functional activation of signal transduction of 5-HT_{1A} receptors in response to cocaine administration in male and female rats. Though G-protein-coupled receptor responses to chronic agonist or drug exposure have done previously, this is the first time that the systemic effects of cocaine on 5-HT_{1A} receptors in have been analyzed in both male and female Fischer rats. Moreover, to the best of our knowledge this is the first study demonstrating 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding is altered by systemic acute cocaine administration. Notably, this is the first study addressing sex differences in the activation of 5-HT_{1A} receptors to cocaine administration.

8-OH-DPAT-stimulated [^{35}S]GTPγS binding measures the ability of the agonist 8-OH-DPAT to stimulate the exchange of GTPγS for GDP; thus a direct assessment of receptor activation of G-proteins. Cocaine caused an activational effect in both male and female rats. Significant effects of cocaine-induced activation of the 5-HT_{1A} receptor were found in a number of brain regions that have

not been previously explored. With cocaine administration in a number of brain areas, 8-OH-DPAT's ability to stimulate the exchange of GTPγS for GDP was increased; an indication that the activation of G-proteins by the receptor was increased as a result of agonist binding. Conversely, in a number of areas this ability to stimulate the exchange of GTPγS for GDP is decreased, an indication that the activation of G-protein's by the receptor is reduced as a result of in ability of agonist binding. A reduced capacity of the 5-HT_{1A} receptor to activate G-protein may be due to regulatory processes (e.g. phosphorylation) at the level of the G-protein in addition to a number of complex modulatory activational processes of this receptor (201). Specifically, we found increases in functional [^{35}S]GTPγS binding and thus activation of 5-HT_{1A} receptors in the dorsal, rostral and ventral areas of the frontal cortex, dorsal medial and dorsal lateral areas of the caudate putamen and the core area of the nucleus accumbens. Decreases of [^{35}S]GTPγS binding was found in the amygdala in both male and female rats. Interestingly, sex differences were seen in the shell area of the nucleus accumbens with males having increases and females having a decrease in [^{35}S]GTPγS binding. Sex differences were also found in and CA1 and CA3 areas of the hippocampus, with increases in [^{35}S]GTPγS binding in females in both areas and decreases in [^{35}S]GTPγS binding in males.

In the frontal cortex of both male and female rats, cocaine increased agonist-stimulated [^{35}S]GTPγS binding compared to saline-treated animals. This is consistent with previous reports of increases of 5-HT in the pre-frontal cortex of male rats (13,51,105). In addition, others have found that co-administration of progesterone and cocaine in overiectomized female rats, which "mimics" male endocrine levels, resulted in higher levels of 5-HT in the prefrontal cortex (105). Thus, the increase of [^{35}S]GTPγS binding after stimulation with 5-HT_{1A} agonist stimulated G-protein activation may be a direct result of an increase of 5-HT release from the dorsal raphe nucleus due to

cocaine administration. However, increases of activity in female rats suggests that though decreases in 5-HT levels are found in response to cocaine administration their activation could suggest an involvement of 5-HT_{1A} receptors in this reduction. In particular we found increases in 5-HT_{1A} activity in both male and female rats in the prefrontal cortex.

In the caudate putamen an increase in [^{35}S]GTPγS binding after cocaine administration was found in both males and females. This is inconsistent with previous work in which no change in the levels of 5-HT after cocaine administration was found (50,52,52). However, μ opioid receptor and D1 receptor agonists have shown increases in [^{35}S]GTPγS binding in the caudate putamen of males (193-197,202). This same increase was found in the caudate putamen, suggesting that in both male and females that 5-HT_{1A} activation modulate cocaine-induced behavioral locomotor activities seen in male and female rats. WF-23 is a potent tropane analog which like cocaine blocks dopamine, serotonin, and norepinephrine transporters with high affinity *in vitro* and is shown to substitute for cocaine and maintain cocaine responding in self-administration paradigms in both rodents (193,194,203,204). Opposite effects have been found with chronic WF-23 administration, causing reductions in this area in 5-HT_{1A}, D2, alpha2-agrenergic [^{35}S]GTPγS binding.

In the amygdala a decrease in 5-HT_{1A} activation was found in both male and female rats. This is consistent with chronic WF-23 with reductions in this area of 5-HT_{1A}, D2, alpha2-agrenergic binding. In the amygdala, known more for its ability to regulate emotions a decrease in 5-HT_{1A} receptor activation was seen in both male and female rats, implying a reduction in activation of these receptors are found after cocaine administration.

In the core of the nucleus accumbens of both males and females increases in [^{35}S]GTPγS binding after cocaine administration was found. However, a sex difference in the activation of 5-HT_{1A} receptors in the nucleus accumbens shell was observed. In the shell of the nucleus accumbens,

males had an increase of 5-HT_{1A} binding after cocaine administration compared to saline. This is consistent with findings of increased 5-HT levels after cocaine administration in the NAc in male rats. Further, they found that co-administration of WAY 100635 and cocaine decreased cocaine-induced behaviors while increasing cocaine-induced levels in nucleus accumbens, which could be reversed by agonist treatment in male rats. Our data suggests that increased levels of 5-HT after systemic injections are regulated by the activation of 5-HT_{1A} receptors in the nucleus accumbens. This is in line with the finding that overall, pharmacological manipulation at the 5-HT_{1A} somatodendritic autoreceptor inhibits 5-HT neuronal activity whereas activation of 5-HT_{1A} postsynaptic receptors may have pro-serotonergic effects (205). Females had a reduction in 5-HT_{1A} agonist-stimulated [^{35}S]GTPγS binding levels to cocaine administration. Consistent previous reports female rats showed decreased 5-HT, and 5-HIAA, in the nucleus accumbens (NAc) after cocaine administration (13,104). Additionally, other agonists which target GABA, DA as well as 5-HT_{1A} receptors show decreases in [^{35}S]GTPγS binding in the nucleus accumbens of female and not male rats (193-197,200). Taken together these results suggest a sexual dimorphic response within the nucleus accumbens in cocaine-induced behavioral activation. It has been previously demonstrated that female have stronger reward activation to cocaine than male rats (21,148). Since the shell of the nucleus accumbens has been postulated to be an important area in reward ,the sexually dimorphic regulation of nucleus accumbens [^{35}S]GTPγS binding may contribute to the known sexually dimorphic aspects of cocaine reward (206). In addition, microinjections of 5-HT_{1B} ligands into the shell, but not core, dose-dependently attenuated the psychostimulant-induced locomotor activity and thus as previously discussed could also be linked with cocaine-induced effects of 5-HT_{1A} receptors (118). Therefore, sexually dimorphic aspects of cocaine-induced locomotor activation are found in the shell of the nucleus accumbens.

Sex differences in [^{35}S]GTPγS activation of hippocampal levels were also found. Increases of 5-HT_{1A} [^{35}S]GTPγS activation were seen in both the CA1 and CA3 regions of females while in males a significant decrease in activation was seen in response to cocaine administration. Using chronic WF-23 they found significant reductions in this area in 5-HT_{1A}, D2 and alpha2-agrenergic binding. In addition, they found that after a single injection of WF-23 a significant reduction of 5-HT_{1A} activity was found. This is inconsistent with previous findings of increased 5-HT levels after cocaine administration in hippocampus of male rats (47).

Overall, we found that the 5-HT_{1A} receptor activity is directly affected by cocaine administration in a number of brain areas involved in cocaine-induced behaviors. In addition, this activation is sexually dimorphic in rats. Determining if there are sex differences in 5-HT_{1A} receptor number should thus be an important direction for future research to determine if receptor number is directly related to effects of this differential activation.

Hormones:

The serotonergic system and the HPA systems are believed to have reciprocal regulatory function in the feedback management of an organism's response to stress. Consistent with previous reports, we found sex differences in basal levels of corticosterone and cocaine-induced corticosterone levels and hence HPA activity, females having increased levels in each. Consistent with previous findings in our lab, no significant differences in drug-induced corticosterone modulation were found in male rats. This may suggest an interaction in male rats of gonadal hormones blunting the effects of corticosterone level increase. Therefore, the regulation of corticosterone associated with cocaine administration is sexually dimorphic. In line with previous evidence, the enhanced HPA response to cocaine could suggest sexually dimorphic aspects of

cocaine-induced behaviors seen when administered cocaine. Researchers have found that hippocampal 5-HT_{1A} receptors are under tonic inhibitory control of corticosterone. Increased corticosterone is believed to cause a dampening of this inhibitory feedback mechanism at the level of the hippocampus. Activation of parvocellular cells in a medial subregion within the hypothalamic paraventricular nucleus mediates the secretion of ACTH. This is a specific receptor/G-protein or G-protein/effector coupling at the levels of both paraventricular nucleus and pituitary gland. Researchers have suggested that these sex differences could exist further in the pathway of CRF release. For instance, they could be caused by increased production and release to CRF, enhanced responsivity of the pituitary of CRF, enhanced ACTH production at the pituitary or enhanced adrenocortical response.

Furthermore, in general agreement with previous research, though not statistically significant, in line with previous research, we found an apparent cocaine-induced increase of plasma levels of progesterone following acute cocaine administration in male and female rats. However, as suggested above, gonadal hormones may be the modulator of this corticosterone effect of cocaine administration, given that ovariectomy leads to decreases of ACTH and corticosterone levels, with no changes being seen in males following castration.

Summary:

Our findings suggest that the serotonergic system is paramount in sexual dimorphic cocaine-induced hyperlocomotor behaviors. The 5-HT_{1A} and 5-HT_{1B} receptors play a crucial role in these sexual dimorphic behaviors. Moreover, sexual dimorphic specific activation of the G-protein coupled 5-HT_{1A} receptor is found after cocaine administration suggesting a specific involvement in cocaine-induced behavioral dimorphisms seen in male and female rats (See Table 6 for summary of

all findings). Specifically, WAY 100635 has been shown to block cocaine-induced locomotor behaviors while increasing 5-HT levels in the nucleus accumbens and hippocampus with no changes found in dopaminergic levels. Therefore, we could suggest that cocaine-induced increases in 5-HT but not DA concentration is under inhibitory control of 5-HT_{1A} receptors. Muller et al., 2002, suggests a possible neuronal mechanism involved in the antagonism of cocaine-induced effects on behavior. Cocaine is known to cause an increase in 5-HT and Acetylcholine (ACh) levels in the hippocampus. The increased 5-HT leads to a stronger activation of inhibitory 5-HT_{1A} receptors at GABAergic interneurons. This increased ACh concentration, mediated by stimulation of 5-HT_{1A} receptors in the cholinergic terminals, inhibits GABA release by the interneurons. This leads to a disinhibition of the principal cells of the hippocampus causing increased cholinergic activity, which further increases excitability of the principal cells. Although initially hyperpolarizing, prolonged serotonergic activation has been shown to depolarize principal cells. Increased activity in hippocampal principal cells is associated with an activation of the hippocampal accumbens glutamanergic projection, which results in an increase of locomotor activity. If you pre-administer WAY 100635 before cocaine treatment the 5-HT concentration in the hippocampus is increased compared to the normal cocaine treatment alone. Therefore, antagonism of high-density hippocampal 5-HT_{1A}-receptors by WAY 100635 may reduce 5-HT induced inhibition of GABAergic interneurons, and, thus, allow stronger inhibitory influence on principal cells. The potentiated 5-HT concentration and the 5-HT_{1A} receptor antagonism causes increased suppression of cholinergic influence on hippocampal cells. Therefore, causing the reduction of activity of the hippocampus-accumbens projection and the NAc glutamate release, this subsequently causes a decrease in locomotor activation.

However, any definitive conclusion such as this can not be determined as multiple interactional effects between a multitude of transmitters in a number of brain regions has been found. For instance, infusion of serotonin into the nucleus accumbens and VTA cause increases of dopamine levels and subsequent locomotor activation. This evidence suggests that 5-HT_{1B} receptor heteroreceptors may be involved. 5-HT_{1B} receptors act as inhibitory heteroreceptors in axon terminals of GABAergic nucleus accumbens neurons that project to the VTA causing the inhibition of GABA release. VTA neurons are released from their inhibition and therefore cause the disinhibition of mesolimbic DA cell bodies after cocaine administration. It is also suggested that potentiation of cocaine-induced increases in nucleus accumbens DA levels are due to activation of 5-HT_{1B} heteroreceptors that regulate the activity of the hippocampal-accumbens glutamate projections. Therefore administration of GR 127935 effects normal inhibition of GABA release and does not release DA neurons from their inhibition from GABAergic neurons thus showing an attenuation of normal cocaine-induced behavioral activation.

Previous reports demonstrate that the administration of WAY 100635 has electrophysiological effects on 5-HT stimulation of 5-HT_{1A} receptors at both the pre and postsynaptic receptors. Specifically, that hyperpolarization occurs in both the dorsal raphe nucleus area and hippocampus after 5-HT_{1A} stimulation. However, this effect is completely prevented by WAY 100635. To substantiate our belief that 5-HT_{1A} receptors were specifically involved in the reduction of cocaine-induced behavioral hyperlocomotion in a number of brain area's known to be involved in cocaine-induced hyperlocomotor activity we examined the activation of G-protein 5-HT_{1A} receptors in male and female rats. This is first experiment to examine cocaine-induced activity of 5-HT_{1A} receptors using 5-HT_{1A} [^{35}S]GTPγS binding in female rats. Interestingly, sex differences were found in the nucleus accumbens shell and not the core. Additionally, biochemical results

demonstrate that in response to cocaine administration in males, levels of 5-HT and its metabolite are increased while in females it is decreased in this area. Therefore, our evidence suggests that the sexually dimorphic activation and inactivation of the 5-HT_{1A} receptor in response to cocaine administration is area specific and correlates specifically with dimorphisms seen both biochemically and behaviorally. In females one would suggest that 5-HT_{1A} decreased activation in response to cocaine administration may be the basis of reductions of 5-HT levels found in females after cocaine administration. However, at this time we do not have a plausible explanation of the exact mechanism causing this finding and remains to be determined. It could be suggested however, that due to basal increases of 5-HT levels in female rats compared to males, that a compensatory mechanism could exist in female rats due to overstimulation of the receptors due to cocaine specific increases of 5-HT. In addition, differences in firing rates of 5-HT neurons in the dorsal raphe nucleus have been found with male rats have higher spontaneous firing rates and lower GABAergic tonic inhibition of 5-HT firing than those of female rats. Therefore these differences could be subject in the effects found herein.

Though somewhat conflicting, when exploring the dopamine literature the nucleus accumbens shell has been involved with more rewarding aspects of cocaines effects and the core being involved more in the regulation of cocaine-induced hyperlocomotor activity. Similarly conflicting, serotonergic evidence suggests that the involvement of the 5-HT_{1B} heteroreceptor interactions could show the same pattern of activation and subsequent cocaine-induced behaviors between the core and the shell. However, recently they have found that microinjection of cocaine into the nucleus accumbens shell but not core contributed to increased cocaine-induced behavioral activation, which was found to be linked to the 5-HT_{1B} receptor. Therefore, it could be suggested that these areas of the nucleus accumbens are functionally distinct in behavioral activation of

locomotor and rewarding responses to cocaine and herein suggests that the 5-HT_{1A} receptor may be also be involved. In addition, local injection of cocaine in the nucleus accumbens does not cause an increase in 5-HT like systemic injections. Therefore, we could assume that both increases in levels of neurotransmitter and subsequent activation of receptors in the NAc is not occurring within this structure but may be regulated in the areas that project to them such as the VTA.

The Hippocampus-accumbens projection and its serotonergic innervations play a prominent role in the regulation of psychostimulant related locomotor activation (47). A sexual dimorphic activation was found in both the CA1 and CA3 areas of the hippocampus in male and female rats' demonstrating that the regulation of 5-HT_{1A} receptor activation is sexually dimorphic. Furthermore our data suggests that this activation is sexually dimorphic and could thus contribute to the known sexually dimorphic behavioral hyperlocomotion seen in response to cocaine administration. This evidence parallels our behavioral research and the known neuroanatomical receptor abundance found in these areas. Therefore, adding weight to the theory that the serotonin hippocampal-accumbens pathway is involved in the modulation of cocaine-induced locomotor activity. Moreover, females demonstrate an increased behavioral response to the application of the serotonin 5-HT_{1A} agonist 8-OH-DPAT, with decreases in 5-HT synthesis in the hippocampus. 8-OH-DPAT, causes twice as much decrease of 5-HT synthesis rates in the hippocampus of females (-64%) than in male rats (-32%). Therefore, if you suggest that cocaine is acting upon these receptors in an agonistic way then these differences could suggest that increased activation in the hippocampus is causing a decrease in synthesis in females, which is seen in areas connected to the hippocampus such as the nucleus accumbens. However, the specific explanation for the inactivation in males is yet to be determined.

Though the exact mechanisms, which regulate sexual dimorphisms seen in cocaine-induced behavioral effects, are not known past and present research has led to the assumption that the dopaminergic and serotonergic systems as well as a number of other systems are by some means involved in these effects. However, previous reports of sex differences found in the dopaminergic system compliment our findings here. This research compliments the needed information to properly assess sexually dimorphic aspects of cocaine-induced behavioral activation at all levels of neurobiological and neurochemical transmission; See figure 32.

Implications:

Drug addiction is a prevalent problem in the United States today. There are known sex differences in all aspects of addiction. Understanding exact neurobiological mechanisms of cocaine's effects would improve treatment of cocaine dependence and addiction in both men and women. The current research indicates possible mechanisms in which to target for therapy in cocaine addiction. Our findings suggest that the 5-HT_{1A} and 5-HT_{1B} receptors are sexually dimorphic in their regulation of cocaine-induced behavioral locomotor activities. This evidence, along with the implied mechanisms may be useful in applying specific pharmacological manipulations that specifically target 5-HT_{1A} and 5-HT_{1B} receptors in men and women. Knowing the exact mechanisms involved sexually dimorphic responses to cocaine could help to implement proper therapeutic treatment in male and females appropriately.

Limitations:

The involvement of the serotonergic system in cocaine addiction has been difficult to isolate due to its extreme complexity. This is due to the multitude of receptors that can be activated, the

diversity of the mechanisms of activation of those receptors as well as the abundance of these receptors throughout the entire central nervous system. In addition, the serotonergic system is diverse in its interactions, with an array of additional neurotransmitter and hormonal systems making the venture of determining exact mechanisms of cocaine addiction complex. The large number of possible combination of variables becomes even more complex and differs with each cocaine-induced aspect of behavior examined. For instance, different mechanisms of the serotonergic system have been found in the modulation of a multitude of cocaine-induced behaviors, including but not limited to, locomotor behaviors, reward (which activate different receptors depending on paradigm being used), sensitization, tolerance, and withdrawal. Additionally, the various aspects of cocaine-induced behaviors vary by sex, species, strain, and the paradigm employed. Even when researchers are looking at one aspect of cocaine addiction i.e. locomotor behavior, utilizing photobeam versus the utilization of another apparatus such as an open field paradigm could create confounds (i.e. stress increases, increased ability of exploratory behaviors). Furthermore, there are profound differences of systemic and local application of administration. This would suggest that local application is optimal in pinpointing exact mechanisms involved in a specific aspect of cocaine addition. We used systemic injections; therefore, in addition to activation that we found with both 5-HT_{1A} and 5-HT_{1B} other mechanisms are involved and would be the focus of future research.

Some limitations identified in this research and must be considered. Our experimental manipulations were based on research which was completed with male rats. Many of our findings in female rats were derived via the use of novel experimental procedures, not previously performed with females. Evidence has shown different effects in males versus females, therefore any conclusion is limited by the experimental techniques that are currently available. To our knowledge this research is the first to explore these specific effects in females. Future research should expand

upon our findings and determine a specific dose response curve to increase the validity of interpretation of specific antagonist effects on cocaine-induced effects in females. This would allow for further determination of exact involvement of the serotonergic system in cocaine-induced effects in females and would allow for direct comparison with previous male data allowing further interpretation of the exact mechanisms involved in cocaine-induced sexual dimorphisms.

Moreover, a number of sex differences have been found not related to cocaine's effects. For instance, compounds that specifically target inhibitory somatodendritic autoreceptors such as WAY100635 demonstrate anxiolytic effects in rats. There are known sex differences in the rate of anxiety. Females having increased level of anxiety-induced reactions in both human and rodent models compared to males. Therefore, differential effects may have been caused by sexual dimorphisms found in the activation of anxiety effects and not cocaine-induced effects. WAY 100635 has been known to cause anxiolytic effects. The inability of WAY 100635 to cause such robust reduction of cocaine-induced rearing activity in females could suggest that dimorphisms seen in the increased effect of cocaine-induced anxiety as well as increased rearing behavior compared to males.

In addition, a number of studies that we used for comparison were chronic cocaine administration paradigms. It is known that chronic and acute cocaine, though similar in many ways, cause differential effects and may involve more aspects of cocaine behaviours such as tolerance or sensitization. In addition, laboratories conducting similar research use different age and strains of rats. In addition, unlike other research we did not use a pre-administration of an agonist to stimulate 5-HT in addition to our specific manipulations. Though more robust findings may have been with the administration of an agonist prior to cocaine administration, we demonstrate that it is not necessary in determining the sexually dimorphic aspects of cocaine-induced hyperlocomotor

activation. Finally, other aspects of behavioural research could be confounding. For instance, light cycles, food and water availability, handling procedures, and variation in handling procedures, injection paradigms and not to mention individual animal variation.

Finally, examination of the activation of G-proteins is extremely complex thus making it difficult to make specific conclusion based on 5-HT_{1A} receptor activation alone. For instance, different cell types could respond differently to the same stimulus, based on their anatomical location, physiological state and functions. More specific the complexity of cellular responses of 5-HT receptors are influenced by a number of variables including numbers of receptors present, subcellular localizations of the receptors, differential sensitivities to various ligands, differential rates of receptor synthesis, internalization, degradation and desensitization, receptor reserve, different levels of spontaneous receptor activity, co-stimulation with other receptor types, as well as non-G-protein-mediated signals (Raymond et al., 1999). In addition, stimulation of the receptor and subsequent signal transduction can occur in the presence or absence of changes in receptor level. Cross-talk among various receptor systems and G-proteins make any definitive conclusions extremely difficult since known activation alone can not give you information of location or timing of expression within the signaling cascade after cocaine-administration. Finally, there is multiple factors naturally occurring within any organism that you can not control. For instance, at all times a single receptor subtype typically occurs among a multitude of neurotransmitter, neuromodulatory, and hormonal stimulation leading to complex and dynamic cellular environment (Raymond et al., 1999). In addition, the application of any drug is going to differ in its effects among individual organisms depending on the physiological state of the cell (degree of activity) and state of organism at the time the drug is administered. Therefore, researchers suggest that the only way to truly explore direct activation of the 5-HT_{1A} receptor alone would be through a transfected method into a

cellular model which previously lacked that or related receptors or conditional knockouts for the 5-HT_{1A} receptor.

Though these issues are to be considered, the effects of the sexual dimorphic aspects of cocaine found presently are stronger than the possible limitations discussed. However, many areas of this research require elucidation and is subject to much further research. One thing that can be concluded however, is that no lone transmitter system or brain regions is specifically involved in the aspects discussed here. Rather interactional and additive relationships exist among them and ultimately cause the effects demonstrated in the present and previous research; See figure 33.

A.) Cocaine-induced behaviors after administration of specific antagonist compared to vehicle; male and female rats

Antagonist	Total Locomotor Male	Total Locomotor Female	Ambulatory Male	Ambulatory Female	Rearing Male	Rearing Female
WAY 100635	**Decreased Activation ↓**	**Decreased Activation ↓**	No Change	**Decreased Activation ↓**	**Decreased Activation ↓**	**Decreased Activation ↓**
GR 129735	No Change	No Change	No Change	**Decreased Activation ↓**	**Decreased Activation ↓**	No Change
Fluoxetine	No Change	No Change	No Change	No Change	No Change	No Change

B.) 5-HT_{1A} agonist-stimulated [^{35}S]GTPγS binding levels to cocaine administration

Brain Area	Male	Female
Dorsal frontal cortex	Increased Activation ↑	Increased Activation ↑
Rostral frontal cortex	Increased Activation ↑	Increased Activation ↑
Ventral frontal cortex	Increased Activation ↑	Increased Activation ↑
NAc Core	Increased Activation ↑	Increased Activation ↑
NAc Shell	**Increased Activation ↑**	**Decreased Activation ↓**
CA1 Hippocampus	**Decreased Activation ↓**	**Increased Activation ↑**
CA3 Hippocampus	**Decreased Activation ↓**	**Increased Activation ↑**
Amygdala	Decreased Activation ↓	Decreased Activation ↓

Figure 32: Summary of all results.
A.) Cocaine-induced behaviors after administration of specific antagonist compared to vehicle; male and female rats
B.) 5-HT_{1A} agonist-stimulated [^{35}S]GTPγS binding levels to cocaine administration

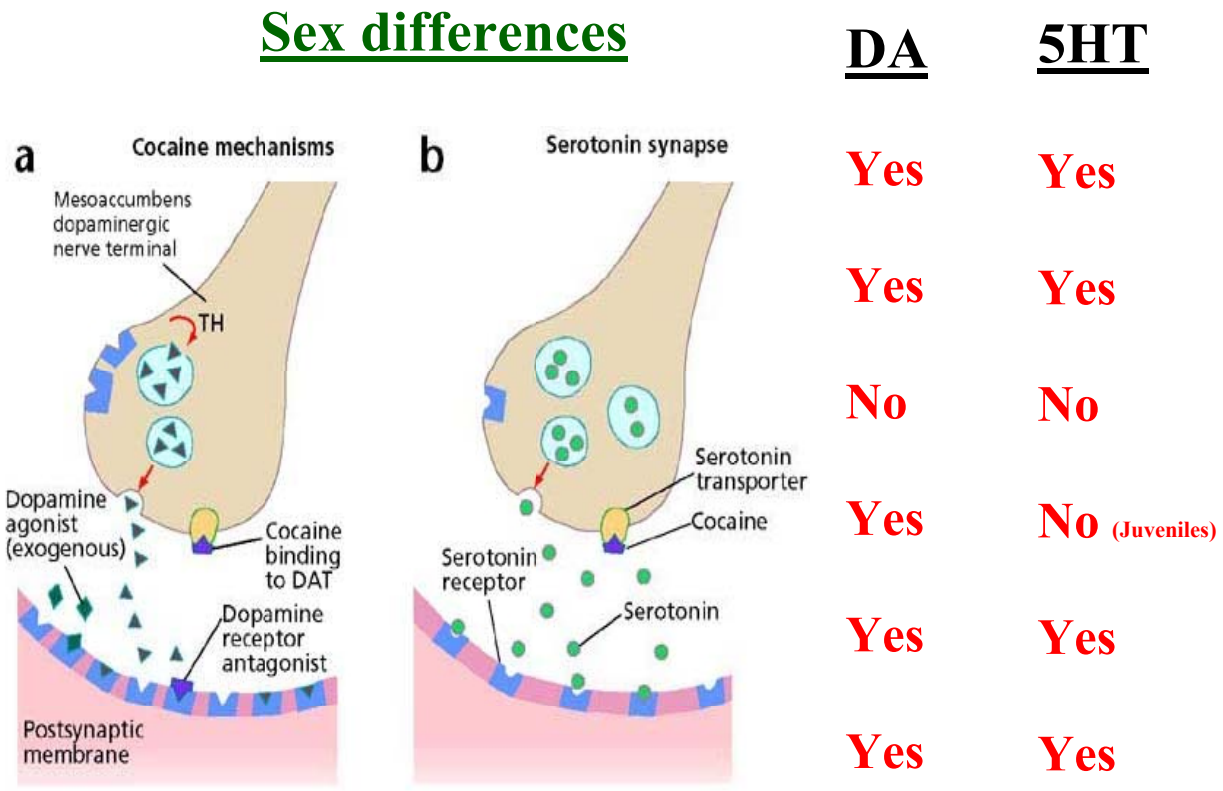


Figure 33: Known cocaine-induced sex differences in the dopamine and serotonergic system. A.) Dopamine B.) Serotonin

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