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COCAINE DEPENDENCE: THE ROLE OF SEROTONIN GENES IN

Lorena Maili

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COCAINE DEPENDENCE: THE ROLE OF SEROTONIN GENES IN
ATTENTIONAL BIAS AND IMPULSIVITY

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A

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Houston, Texas

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Animal studies have shown that behavioral responses to cocaine-related cues are altered by serotonergic medications. The effects of pharmacological agents on serotonin receptors 2a (5-HT_{2A}) and 2c (5-HT_{2C}), have yielded results suggesting that selective 5-HT_{2A} antagonists and 5-HT_{2C} agonists promote the disruption of cocaine-associated memories. One measure of cocaine related cues in humans is attentional bias, in which cocaine dependent individuals show greater response latency for cocaine related words than neutral words. Data from our laboratory shows that cocaine dependent subjects have altered attentional bias compared to controls.

The purpose of this thesis was to investigate the role of the serotonin system in attentional bias and impulsivity in cocaine dependent individuals. We focused on the serotonin transporter, serotonin receptors 2A and 2C and tryptophan hydroxylase 1 and 2 (TPH1 and TPH2). We predicted that attentional bias and impulsivity would be higher in cocaine dependent individuals who had lower serotonin function.

In the current study, we found a significant association between *TPH2* genotype and attentional bias for the second block of the cocaine Stroop task. There was also a significant association between average attentional bias and HTTLPR genotype in the cocaine dependent individuals. The HT2C receptor genotype and attentional bias in our study sample also showed a significant difference. We did not find a significant difference between the serotonin 2A receptor variants or the *TPH1* variants and attentional bias in the cocaine dependent group.

In conclusion, the current study suggests that serotonergic medications should be utilized as pharmacotherapeutic treatment for cocaine addiction. Our results indicate that TPH2, the serotonin transporter and 2C receptor should be targeted in such a way as to modulate both, leading to increased synaptic serotonin function.

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1- INTRODUCTION

The definition and public opinion of substance addiction has changed in the last few decades. What was once viewed as a problem of will, is now known to be a disease. Several systems are thought to be dysfunctional in drug addiction, including inhibitory, learning, memory and hormonal systems. As a society, we have come to understand that addiction is an important issue facing an alarming number of individuals (Leshner 1997). Severe negative consequences of drug abuse and addiction also include indirect consequences such as infectious diseases that spread among drug users, crime and violence.

Many of the changes that result from drug use persist even after the individual ceases drug use. These changes might be genetic, molecular or cellular, and in turn lead to functional and behavioral changes. When drug-using individuals want to abstain from drugs, the major challenges for quitting are likely due to alterations in brain activity, receptor sensitization/desensitization and gene regulation.

Despite the efforts of clinicians and researchers to find an effective way to rescue an individual from drug dependence, treatment has not been established. Although early pharmacotherapeutic endeavors focused on reversing the illicit drug's primary effects, recently researchers are working on elucidating the neurobiological adaptations that take place after chronic abuse (Koob and Volkow 2010). Information on the behavioral, genetic and epigenetic changes that result from drug use, is increasingly being utilized in the development of treatment.

Addiction is a disorder which begins with the acute use of a drug and progresses to chronic drug-seeking. The term addiction refers to the loss of control over drug use despite unwanted consequences (O'Brien, Volkow et al. 2006). Drug addiction develops in several stages; initiation of drug use, intermittent to regular use, and, finally, addiction and relapse (Kreek, Nielsen et al. 2005). Features of addiction are the development of dependence to the drug, which creates a physiological need of the drug for the individual to function properly, the development of tolerance, manifested as a need for larger doses of the drug to attain the same effect, the development of withdrawal, symptoms that

occur when the drug is discontinued, and relapse, which occurs when after a period of abstinence, the drug is used again by the drug dependent individual.

The term drug dependence refers to the physiological adaptation to repeated administration of a drug, and occurs with many drugs, but is also the term used to clinically diagnose an individual with a substance use disorder (O'Brien, Volkow et al. 2006). For the purposes of this thesis, the term drug dependent will be used to signify both clinical diagnosis using the DSM-IV and the manifestation of the characteristics of addiction. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) depicts drug dependence to contain three categories: preoccupation/anticipation, binge/ intoxication, and withdrawal/ negative affect (1994).

A maladaptive pattern of substance use leading to clinically significant impairment or distress as manifested by three (or more) of the following, occurring at any time in the same 12-month period:

1. tolerance, as defined by either of the following:
 - a. a need for markedly increased amounts of the substance to achieve intoxication or desired effect
 - b. markedly diminished effect with continued use of the same amount of the substance
2. withdrawal, as manifested by either of the following:
 - a. the characteristic withdrawal syndrome for the substance
 - b. the same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms
3. the substance is often taken in larger amounts or over a longer period than was intended
4. there is a persistent desire or unsuccessful efforts to cut down or control substance use
5. a great deal of time is spent in activities necessary to obtain the substance (e.g. visiting multiple doctors or driving long distances), use the substance (e.g. chain-smoking), or recover from its effects
6. important social, occupational, or recreational activities are given up or reduced because of substance use
7. the substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption)

Table 1: *Criteria for Drug Dependence According to the DSM-IV, people are classified as dependent when they show three or more of the following symptoms within one year*

Drug dependence can also be thought of in terms of changes in neuroadaptive or neuroendocrine homeostasis. After a drug is chronically administered, adaptive processes to counteract the drug's effects take place in the central nervous system. During withdrawal, these functional neuroadaptive changes are still in place and may produce the symptoms experienced during abstinence (such as anxiety, mood dysregulation, and somatic abnormalities) and lead to relapse (Koob, Sanna et al. 1998). For example, rats exposed to cocaine for 12 hours experience reductions in release of dopamine and serotonin in the nucleus accumbens (Weiss, Markou et al. 1992; Weiss, Paulus et al. 1992). These changes can persist for different periods of time, depending on the length of administration (abuse).

On the other hand, the conditioning theory of drug dependence hypothesizes that in individuals who are cocaine dependent, stimuli which have been conditioned with drug use can alone produce the desire for cocaine, which can lead to relapse (Weiss, Ciccocioppo et al. 2001). The conditioning theory implies that once the individual is exposed to drug-related stimuli in the environment, relapse often follows due to the pairing of the rewarding actions of the drug with the previously neutral stimulus. A third approach in conceptualizing drug dependence is by depicting it in terms of impulsivity and compulsivity (Koob and Volkow 2010). Impulsivity is defined behaviorally as

a predisposition toward rapid, unplanned reactions to internal and external stimuli without regard for the negative consequences of these reactions to themselves or others (Moeller, Dougherty et al. 2001).

Compulsivity is defined as

elements of behavior that result in perseveration in responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations (Koob and Volkow 2010) .

Koob and Volkow hypothesize that impulsivity is important in the beginning stages of drug addiction: during this time reinforcement drives drug seeking and taking. Both impulsivity and compulsivity, on the other hand, are important in the later stages: negative reinforcement, in this case to avoid an aversive state without the drug on board,

drives the same behavior in a compulsive automatic way by this point (Koob and Volkow 2010). Additionally, Kreek & Nielsen have proposed that impulsivity is important in the earlier stages of drug addiction (Kreek, Nielsen et al. 2005).

In a 2008 US survey, over 700,000 individuals used cocaine for the first time, which averages to 2,000 initiates per day, with the average age at first use being 19.8 years (SAMHSA 2008). The National Survey on Drug Use and Health revealed that 5.3 million Americans aged 12 and older had abused cocaine and 1.1 million had abused crack at least once in 2008 (SAMHSA 2008). Substance abuse has been identified as the nation's number one health problem, resulting in more deaths, illnesses, disabilities, and cost to society than any other preventable disease (Horgan, Skwara et al. 2001). Drugs of abuse disrupt physiological systems, contributing to drug addiction and relapse. However, we are only recently beginning to understand the molecular changes that take place during the development of addiction and during withdrawal (Kreek, Bart et al. 2005).

1.1 – *NEUROSCIENCE OF COCAINE*

Since its synthesis from the coca plant in 1884, cocaine has been medically used as an anesthetic, anorectic, cardiovascular stimulant, etc. Recreationally used cocaine can be found in three common forms: cocaine hydrochloride salt (powder) or freebase and crack rocks which can be smoked (Goldstein, DesLauriers et al. 2009). Cocaine exerts its primary effects by blocking neurotransmitter uptake by the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) transporters (Uhl, Hall et al. 2002). Cocaine can also exert effects on ligand- and voltage-gated channels by blocking these as well (Uhl, Hall et al. 2002). Cocaine non-competitively blocks the uptake of these monoamines by binding to a site on the transporter that is different from the substrate binding site (McElvain and Schenk 1992). Acute administration of cocaine leads to an increased level of serotonin in the medial prefrontal cortex and the hypothalamus (Yang, Bao et al. 1992). In the dorsal raphe, acute cocaine also suppresses the spontaneous activity of serotonin neurons while decreasing the synthesis of serotonin in the striatum, nucleus accumbens, and medial prefrontal cortex (Galloway 1990).

Chronic doses of cocaine can increase the number of serotonin uptake sites in prefrontal cortex and dorsal raphe regions. This in turn can alter the ability of cocaine to inhibit the serotonergic activity in dorsal raphe neurons (Cunningham, Paris et al. 1992). A study by Egan et al studied the resulting changes of chronic cocaine treatment on levels of serotonin (5-HT) and levels of one of its metabolites, 5-hydroxyindoleacetic acid (5-HIAA) levels in cortex during periods of withdrawal in rats (Egan, Wing et al. 1994). Repeated cocaine injections produced a long-term decrease in 5-HT levels in frontal cortex regions. There was a relationship between withdrawal time and 5-HT levels, with longer withdrawal times producing a greater reduction in 5-HT neurotransmission, “suggesting a progressive reduction” (Egan, Wing et al. 1994). When cocaine is injected into the ventral tegmental area (VTA), dopamine, norepinephrine and serotonin increase in a concentration-dependent fashion (Chen and Reith 1994).

Cocaine affects several anatomical areas in the brain, including the ventral tegmental area, which is important for producing the reinforcing properties of cocaine. The VTA receives inputs from the 5-HT system and is innervated by norepinephrine (NE)-containing neurons. Repeated drug use leads to processes that reverse the resulting effects of the drug. When an individual abstains from the drug, the compensatory processes that have developed are still active and may be responsible for the negative cognitive experience during withdrawal (Winstanley 2007).

1.2 – ATTENTIONAL BIAS

Drugs of abuse are salient stimuli that lead to a strong association between drug cues and the positive (euphoric) effects of the drug. Substance dependent individuals react to these cues when they appear in the environment. For example, when alcohol abusers come into contact with something that is reminiscent of an alcoholic beverage (for example, seeing a drink or smelling the ethanol) they react to the cue and experience physiological arousal and a state termed “craving” (Carter and Tiffany 1999). Craving has been defined as a psychological state where the individual feels an obsessive urge to repeat drug administration and recapture drug-induced euphoria (Copersino, Serper et al.

2004). The expectancies experienced during craving are thought to influence drug-seeking behaviors.

Robinson and Berridge synthesized a model where drug-related stimuli attain incentive-motivational properties, which in turn changes the way an individual reacts to those stimuli. In this model, when an individual repeatedly administers a substance of abuse, a neurophysiological response takes place that in turn leads to sensitization with each subsequent administration. Cravings for the substance develop after the substance has acquired strong motivational properties and obtaining the substance becomes a priority (Robinson and Berridge 1993). These incentive-motivational properties take place as a result of classical conditioning. When the drug user is administering the drug, stimuli or cues in the environment, after one or several pairings with drug use, begin to elicit components of the original response that the drug caused. The stimulus from then on

grabs attention, becomes attractive and ‘wanted’ and thus guides behavior to the incentive (Robinson and Berridge 1993), p. 261).

Franken extended this model by suggesting when drug-cues are encountered, they become the focus of attention. This increases craving and attention-grabbing properties of the stimulus until the substance is acquired and used (Franken 2003).

Attentional orienting is the

selective biasing of information processing towards specific attributes of events based on changing motivation, volition, or expectation (Lepsien and Nobre 2006).

It is hypothesized that a network in the parietal-frontal axis handles information from early stages of perceptual analysis using top-down processing (Lepsien and Nobre 2006). There are two types of implicit indirect measures of cognitive function used by researchers: ones that measure a bias in attention towards stimuli that are related to alcohol or drugs and ones that measure implicit memory associations (Wiers, Bartholow et al. 2007). Implicit measures provide an automatic measurement of the construct the researcher is measuring, which may capture the implicit processes responsible for the behaviors in the natural environment (Wiers, Bartholow et al. 2007). One task that

measures the extent to which drug related cues direct attention in drug users, or attentional bias for a drug stimulus, is the Drug Stroop task (Cox, Fadardi et al. 2006).

The original Stroop task was constructed to study interference in verbal naming and the associated attentional processes (Stroop 1935). The person performing is shown cards with words that spell out colors in incongruent ink: for example the word “yellow” in green ink or the word “brown” in red ink. In addition to the color words there are also rows of Xs which serve as the control stimuli. Stroop and subsequent investigators found that it takes subjects longer to name the ink color of the color words than the meaningless rows of Xs, and this effect is also observed with words that are related to colors such as “sky” and “grass” (Stroop 1935), also reviewed in (Williams, Mathews et al. 1996). Williams et al suggest that if the word stimulus semantically activates processing, then interference in color naming is likely to take place (Williams, Mathews et al. 1996).

Different variations of the Stroop task are widely used to measure attentional bias to words or pictures associated with concerns relevant to a subject’s clinical condition (Cox, Fadardi et al. 2006). The term bias is used to signify a tendency for an individual to allocate more attention to certain stimuli that have become salient (for example drug-related stimuli). Craving and treatment outcome are also associated with attentional bias in drug users (Copersino, Serper et al. 2004; Carpenter, Schreiber et al. 2006; Marissen, Franken et al. 2006). The Stroop task, therefore, measures the interference these drug stimuli cause on cognitive commands/processes (Vadhan, Carpenter et al. 2007). It requires participants to focus on the color of the words or the edge color of pictures while inhibiting responses to any other feature that word or picture (Cox, Fadardi et al. 2006).

This Stroop task has been used to measure the attentional bias of the cocaine-dependent subjects to stimuli associated with cocaine use. Using two versions of the Cocaine Stroop task, Hester and colleagues found that reaction time of cocaine-dependent subjects to name the color of cocaine-related words was significantly slower than that of neutral words (Hester, Dixon et al. 2006). For one version of the task, which uses pictures that are related to cocaine use, evocative (snakes, erotica, mutilated body parts, etc), or neutral, cocaine users found the drug-related pictures as distracting as the evocative pictures. Vadhan and colleagues reported that a group of treatment-seeking, cocaine-dependent subjects showed greater interference by cocaine-related words than

nontreatment seeking subjects (Vadhan, Carpenter et al. 2007). In a study by Copersino et al, three groups of subjects performed the cocaine-word Stroop task: cocaine dependent patients, patients with schizophrenia and cocaine dependent patients with comorbid schizophrenia (Copersino, Serper et al. 2004). There was a significant difference between reaction time for cocaine words compared to neutral words only in the cocaine patient group.

The above studies suggest that cocaine-related stimuli acquired incentive properties in these subjects. In an addiction treatment study, attentional bias to the cocaine-related stimuli in the cocaine-dependent subjects was associated with worse treatment outcome (Carpenter, Schreiber et al. 2006). Attentional bias was also associated with cocaine craving severity ratings (Copersino, Serper et al. 2004). Taken together, these studies suggest the experimental utility of investigating attentional bias in cocaine-dependent subjects using the cocaine Stroop task. The Stroop task is thought to detect an alteration in attentional processing in which drug-related cues are more salient and therefore capture attention better.

1.3 – *IMPULSIVITY*

Recent models of addiction include the idea that drug users have elevated impulsivity and poor inhibitory control due to compromised executive function, which is any higher order brain function, usually processed by the frontal lobe (Field and Cox 2008). Dysfunction in the prefrontal cortex might result in an inability to inhibit impulses to react to increased incentive salience of conditioned drug cues (Dawe, Gullo et al. 2004). Impulsivity has also been classified as an endophenotype or vulnerability trait for stimulant dependence (Ersche, Turton et al. 2010).

Impulsive behavior is comprised of several independent dimensions, but some commonalties include behavioral disinhibition, “intolerance of delay to rewards,” quick decision making without consideration of consequences, hyperactivity and poor attentional ability (Winstanley 2007). Moeller has suggested that impulsivity incorporates multiple distinct psychological concepts (Moeller, Barratt et al. 2001).

The Barratt Impulsiveness Scale version 11 (BIS-11) is a widely-used questionnaire for measuring impulsivity. However, multiple paradigms to measure impulsive behavior in both human and non-human subjects are used by investigators. They can be divided into two categories: those measuring decision making and those measuring impulsive action (Winstanley 2007).

In addition to the attentional bias for cocaine-related stimuli, cocaine-dependent subjects also show other cognitive deficits related to dysfunction of prefrontal cortex such as increased trait impulsivity and impaired inhibitory control. Several studies have reported that cocaine dependent subjects have significantly higher scores on the BIS-11, compared to healthy controls (Moeller, Barratt et al. 2004; Moeller, Hasan et al. 2005).

Individuals with substance dependence show a variety of impulse control deficits. Brain imaging studies have shown that in individuals who have recently stopped using drugs, the orbitofrontal cortex is less active (Volkow, Wang et al. 2005). This change in cortical activity may be related to the increased levels of impulsivity seen in drug users (Volkow, Wang et al. 2005). Abstinent drug using individuals also perform poorly on gambling tasks, and made premature-like responses on the immediate and delayed memory task (IMT/DMT) (Moeller, Hasan et al. 2005).

Cocaine-dependent subjects with high impulsivity and poor inhibitory control may have difficulty inhibiting their response to cocaine-related stimuli. This relates to treatment. In a study by Moeller et al, self-report impulsivity, measured by the Barratt Impulsiveness Scale version 11 (BIS-11), was correlated with severity of cocaine use and severity of cocaine withdrawal symptoms. The motor impulsivity subscale exhibited the highest effect. Also, subjects with high impulsivity at baseline, were more likely to drop out of treatment (Moeller, Dougherty et al. 2001).

1.4- SEROTONIN

Serotonin was discovered 60 years ago and is one of the biogenic amine neurotransmitters. One of the major neurotransmitters of the nervous systems, serotonin is responsible for several regulatory functions such as mood states, food intake, and impulsive aggression. In addition to its involvement in the central nervous system,

serotonin also plays a role in cardiovascular, pulmonary, gastrointestinal, and genitourinary systems (Kandel 2000). Serotonergic neurons modulate the activity of cortical and subcortical neurons in several ways by activating both inhibitory and excitatory receptor subtypes (Kandel 2000).

Cell bodies of serotonergic neurons are concentrated in and around the raphe nuclei of the brain stem. The raphe is composed of three nuclei: the dorsal, median, and the raphe magnus pallidus. Fibers from neurons in the dorsal raphe innervate dopaminergic neurons of the substantia nigra and the ventral tegmental area, influencing the levels of dopamine in these regions. Projections from the median raphe go through the stria terminalis and fornix to reach the amygdala and hippocampus. Projections also travel to cortex, superior colliculi and cerebellum. Serotonin-containing neurons are also found in the hypothalamus, especially at the suprachiasmatic nucleus—an area that regulates circadian rhythms which include the sleep-wake cycle. Along with the activity of noradrenergic neurons, the activity of serotonergic neurons fluctuates with sleep and wakefulness (Nolte 1999). Cells from the caudal raphe nuclei project to the brainstem and spinal cord (Nolte 1999). The diverse connectivity of serotonergic neurons allows this neurotransmitter system to exert effects on a wide range of anatomical regions.

1.4 – *SEROTONIN AND CUE REACTIVITY*

Rodent studies have shown that behavioral responses to cocaine-related cues are altered by serotonergic medications (Fletcher, Grottick et al. 2002). Cunningham et al have investigated the effects of pharmacological agents on serotonin receptors 2A (5-HT_{2A}) and 2C (5-HT_{2C}), finding that selective 5-HT_{2A} antagonists and 5-HT_{2C} agonists promote the disruption of cocaine-associated memories (Nic Dhonnchadha, Fox et al. 2009).

One measure of responsivity to cocaine related cues in humans is attentional bias, in which cocaine dependent individuals show greater response latency for cocaine related words than neutral words. Data from our laboratory and other literature shows that cocaine dependent subjects have altered attentional bias compared to controls and this is in agreement with other findings (Hester, Dixon et al. 2006; Liu, Lane et al. 2011). Since

serotonergic medications alter behavioral responses to cocaine related stimuli in rodents, it is possible that polymorphisms which were found to be associated with alterations in the serotonergic system could also affect behavioral responses to cocaine related cues in humans.

Serotonin synthesis involves several steps and is highly regulated. Serotonin and the essential amino acid tryptophan from which it is derived, are both indoles (aromatic compounds), with a five member ring that has nitrogen joined to a benzene (Lodish 2008). Two enzymes are needed to synthesize serotonin: tryptophan hydroxylase, an oxidase similar to tyrosine hydroxylase and aromatic amino acid decarboxylase (AAAD) (Kandel 2000). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme responsible for the synthesis of 5-HT. After tryptophan (an essential amino acid) is hydroxylated, 5-hydroxytryptophan decarboxylase catalyzes the final step to result in serotonin, or 5-hydroxytryptamine (5-HT) (Nolte 1999).

There are 14 known subtypes of serotonin receptors, classified into seven families. The 5-HT₃ receptor is ionotropic, while the rest are metabotropic. The metabotropic receptors are G-protein-coupled receptors (GPCRs) and have seven transmembrane domains. The GPCRs are categorized into four groups based on their main second messenger system: the 5-HT₁-family of receptors uses G_{ai}/G_{ao} proteins while the 5-HT₂ family couples G_{aq} proteins. The 5-HT₄, 5-HT₆, and 5-HT₇ receptors use G_s proteins and the 5-HT₅ receptors are unknown (Raymond, Mukhin et al. 2001).

The limbic system is dense in 5-HT_{1A} receptors, with the auto-receptors being localized in the cell bodies and dendrites of the dorsal and median raphe nuclei (Riad, Garcia et al. 2000; Lanfumey and Hamon 2004). In the hippocampal area, these receptors are found post-synaptically and are negatively coupled to adenylyl cyclase (AC) via G_{ai} and/or G_{ao} proteins (De Vivo and Maayani 1986; Weiss, Sebben et al. 1986). 5-HT_{1A} receptors inhibit neuronal firing through activation of G-protein-gated inwardly rectifying potassium (GIRK) currents and inhibition of Ca²⁺ channels (Oleskevich 1995; Sodickson and Bean 1998).

For the purpose of this thesis, I will focus on the 5-HT_{2A} and 5-HT_{2C} receptors. The 5-HT_{2A} receptors are abundant in prefrontal cortex (Miner, Backstrom et al. 2003). It is the site of action of many illicit and pharmacological substances, for example, LSD and

atypical anti-psychotics (Aghajanian and Marek 1999). These receptors have been characterized by immunohistochemical techniques to the apical dendrite shafts, proximal to the pyramidal cell soma (Jakab and Goldman-Rakic 1998).

The HT_{2C} receptors are densely expressed in regions implicated in anxiety, mood, drug-induced hallucinogenesis, reward, neuroendocrine regulation and appetite (Roth, Berry et al. 1998). They couple to the G_q family of G proteins, activating phospholipase C to hydrolyze phosphoinositide and produce two second messengers, diacylglycerol and inositol triphosphate (Roth, Berry et al. 1998). Inositol triphosphate mobilizes calcium from intracellular stores, increasing the intracellular calcium concentration while diacylglycerol activates protein kinase C. A study that administered the 5-HT_{2C} agonist m-chlorophenylpiperazine (m-CPP) showed that compared to healthy volunteers, patients with alcoholism, anxiety disorders or affective disorders had differences in hormonal and psychological responses after administration of m-CPP (Charney, Woods et al. 1987; Joseph-Vanderpool, Jacobsen et al. 1993).

The goal of this thesis is to determine whether attentional bias to cocaine related stimuli in cocaine dependent subjects is associated with functional polymorphisms of the serotonin transporter promoter polymorphic region (*5-HTTLPR*) of the *serotonin transporter* gene (*SLC6A4*), which has been shown to affect serotonin transporter expression (Hu, Lipsky et al. 2006). Additionally, we will examine the association between attentional bias and two serotonin receptor polymorphisms, one in the 5-HT_{2A} and the other in the 5-HT_{2C} receptors. The functional 5-HT_{2A} T102C polymorphism (rs6311) has been reported to modulate transcriptional activity and the 5-HT_{2C} Cys23Ser polymorphism (rs6318) has been reported to alter receptor function (Poleskaya and Sokolov 2002; Okada, Northup et al. 2004)

Serotonin transporter *SLC6A4* gene:

The serotonin transporter (5-HTT), encoded by the *SLC6A4* gene and located at 17q11.2, codes for a central regulator of serotonin turnover, transporting serotonin from the synaptic cleft into the presynaptic neuron; terminating serotonin's action. A 20-23 base pair repeat polymorphism in the *SLC6A4* promoter (termed *5-HTTLPR*) has been

shown to affect transcriptional activity of this gene. The short or “S” allele with 14 repeats was shown to have lowered transcriptional activity than that the long or “L” allele with 16 repeats (Heils, Teufel et al. 1996; Lesch, Bengel et al. 1996; Heils, Mossner et al. 1997). This repeat polymorphism was found to be associated with anxiety-related traits and depression (Lesch, Bengel et al. 1996). Recently, a single nucleotide polymorphism (SNP) (A/G) rs25531, was identified in the repeat sequence of the L-allele that subdivided the polymorphism further into the L_A and L_G alleles (Hu, Lipsky et al. 2006). The L_G and S alleles were found to have similar low expression levels. This allows the genotypes to be classified as having high (L_A/L_A), or low (L_A/L_G, S/L_G, L_G/L_G, S/S) transcriptional efficacy. The S_A allele is very rare and was not present in our study sample (Wendland, Martin et al. 2006). More than 20 studies have examined the relationship of *SLC6A4* variants and response to antidepressant treatment [reviewed in (Horstmann and Binder 2009)]. A meta-analysis of 1400 subjects of different ethnicities from 15 studies reported diminished response to antidepressant therapy in S-allele carriers (Serretti, Kato et al. 2007). A more recent meta-analysis found evidence that 5-HTTLPR S allele moderates the relationship between stress exposure and development of clinical depression (Karg, Burmeister et al. 2011). Chronic treatment with cocaine *in vitro* causes enhanced surface expression of the serotonin transporter in cells, while post-mortem studies have revealed an up-regulation of 5-HTT by cocaine (Little, Kirkman et al. 1993; Kittler, Lau et al. 2010).

Even though the internal SNP was identified some time ago, and shown to affect the functionality of the 5-HTT, many subsequent studies only genotype for the bi-allelic version of the 5-HTTLPR. In the present study, we analyzed our data both ways.

Serotonin receptor 2A *HTR2A* gene:

The 5-HT_{2A} receptor is encoded by the 5-*HTR_{2A}* gene located at 13q14.2. Several common SNPs in the 5-*HTR_{2A}* gene have been identified and studied: rs6311 (-1438A/G), and rs6312 (-783A/G) in the 5' upstream region, rs6313 (102T/C, Ser34Ser) and rs6314 (1354C/T, His452Tyr) in the coding region, and rs7997012 in the second intron of 5-*HTR_{2A}*. The variants rs6311 (-1438A/G) and rs6313 (102T/C) are reported to

be in linkage disequilibrium and rs6311 (-1438A/G) is located upstream of two alternative promoters for *HTR2A* (Peacock, Warren et al. 1993; Spurlock, Heils et al. 1998). Allele specific expression analysis has shown that the T allele of rs6313 (102T/C) was expressed at a higher level than the C allele in post-mortem schizophrenic brain tissues (Polesskaya and Sokolov 2002). The same type of analysis found that the 102C allele was expressed at lower levels than the 102T allele in brain tissue, but not in lymphocytes (Fukuda, Koga et al. 2006). Other studies have not found any differences in allele specific expression for the 102T and 102C alleles (Bray, Buckland et al. 2004; De Luca, Likhodi et al. 2007).

It has been reported that the *HTR2A* gene is imprinted, and is expressed only from the maternally derived allele (Kato, Shimizu et al. 1996; Kato, Ikawa et al. 1998). Another study however reported monoallelic expression of *HTR2A* in brain tissue in only 4 of 18 subjects (Bunzel, Blumcke et al. 1998). Both the -1438A/G and the 102C/T variants create CpG dinucleotide sites, which, have been reported to be methylated at high levels and *HTR2A* expression was positively correlated with C-methylation of the -1438 CpG site (Polesskaya, Aston et al. 2006). The -1438A/G variant has been shown to be associated with response to treatment with citalopram for major depressive disorder in a Korean population (Choi, Kang et al. 2005).

Serotonin receptor 2C *HTR2C* gene:

The serotonin receptor 2C is encoded by the *HTR2C* gene located on the X chromosome. A polymorphism at position 23 leads to a cysteine to serine change (Lappalainen, Zhang et al. 1995). The substituted amino acid is on the extracellular N-terminus of the 5-HT_{2C} receptor. Cysteine is involved in a disulfide bridge formed within G-protein coupled receptors (GPCRs). The cysteine at position 23 is the only cysteine residue in the *HTR2C* gene. It is hypothesized that the serine substitution disrupts disulfite bond formation within these receptors (Okada, Northup et al. 2004). Also, the N-terminal region of the 5-HT_{2C} receptor has an extra hydrophobic region which may be disrupted by the substitution and may affect the receptor's function (Hurley, Bloem et al.

1999). Because this receptor is located on the X-chromosome, there's the possibility for chimeric expression in females due to X-inactivation.

Okada and colleagues investigated whether Cys23Ser affects ligand affinity, transduction and constitutive activity of the 5-HT_{2C} receptor. The two alleles were compared after expression in COS-7 (kidney) cells. Their results demonstrated that this variant is functional. The serine substitution led to a constitutively more active receptor. While the results suggested that there may not be a difference between the two alleles when the receptor is uncoupled and inactive and the variant may not cause a change in affinity for G proteins or G protein activity when serotonin is present in excess, it can activate G_q to a higher degree in the absence of 5-HT, suggesting that it is constitutively more active (Okada, Northup et al. 2004). Constitutive activity is related to receptor desensitization, meaning constitutively active 5-HT_{2C} receptors are thought to be constitutively desensitized (Westphal, Backstrom et al. 1995).

Tryptophan Hydroxylase 1 and 2 TPH1 and TPH2 genes

Tryptophan hydroxylase is the rate limiting enzyme in serotonin synthesis (Koe and Weissman 1966; Jequier, Lovenberg et al. 1967). There are two forms of tryptophan hydroxylase, tryptophan hydroxylase (TPH1) which is a peripheral subtype (McKinney, Knappskog et al. 2005) and tryptophan hydroxylase 2 (TPH2) which is the neuronal subtype (Walther and Bader 2003). *TPH1* and *TPH2* are highly homologous and share key structural sequences for enzyme activity. Both *TPH1* and *TPH2* are expressed in the human central nervous system, including anatomical regions such as the cortex, hypothalamus, thalamus, hippocampus, amygdala, cerebellum and raphe nuclei (Zill, Buttner et al. 2007; Saetre, Lundmark et al. 2010). *TPH1* was identified and characterized first, and is found in the enterochromaffin cells of the gut and in the pineal gland (Zhang et al, 2006). *TPH2* is expressed predominately in the cells in the raphe nuclei and in peripheral enteric neurons in the gut (Cote, Thevenot et al. 2003; Walther, Peter et al. 2003).

The gene that encodes TPH1 is located on chromosome 11 in region 11p15.3-p14 (Craig, Boularand et al. 1991). Many variants have been identified in the gene, with

several associations found relating *TPH1* and alcoholism, nicotine dependence, antisocial behavior and heroin addiction (Nielsen, Goldman et al. 1994; New, Gelernter et al. 1998; New, Gelernter et al. 1998; Nielsen, Barral et al. 2008), reviewed in (Yuferov, Levran et al. 2010).

Variants in the *TPH2* gene were studied and identified by Nielsen and colleagues (Nielsen, Barral et al. 2008). The researchers resequenced the *TPH2* gene and identified both new and previously identified variants in individuals with addictive diseases and healthy volunteers. They looked for associations using the *TPH2* variants and the previously well-studied *TPH1* rs1799913 variant. There was a significant interaction between the *TPH1* variant and *TPH2* rs4290270 variant in heroin addicted Hispanic individuals. Based on these findings, in the present study we focused on these two polymorphisms of the two *TPH* genes.

2 – HYPOTHESIZED MODEL

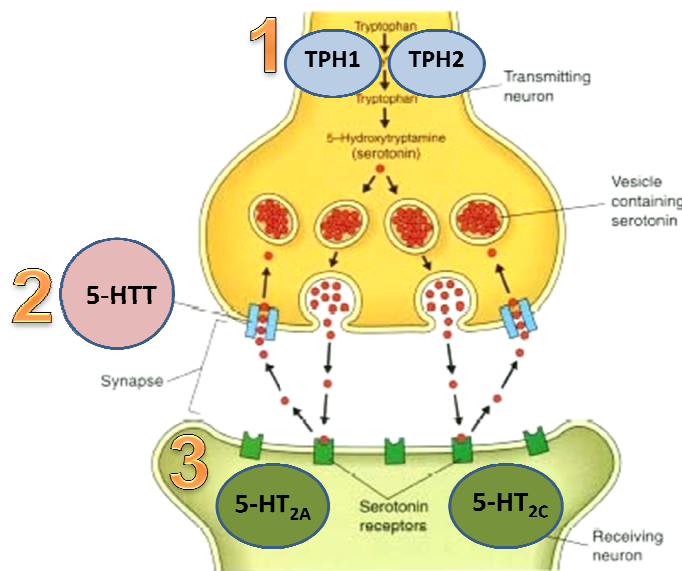


Figure 1: Proposed model for the role of the serotonergic system in attentional bias and impulsivity. A) The different steps in the serotonin pathway are depicted by synthesis (1), removal of neurotransmitter from the synapse (2) and continuation of neurotransmission by the receptors in the post-synaptic or receiving neuron (3).

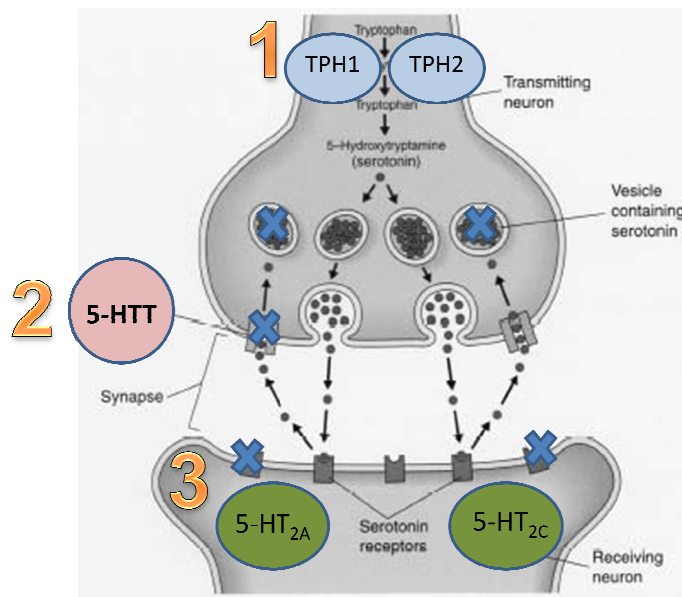


Figure 2: Proposed model after chronic cocaine exposure. A decrease in production of serotonin is hypothesized through reduced expression of the TPH1 and TPH2 genes (1), increased 5-HTT activity, removing released serotonin from the synapse at a higher rate (2), and changes in receptor function such as sensitization or desensitization for the 5-HT_{2A} and 5-HT_{2C} receptors (3).

There are four major processes that regulate serotonin system activity: 1) synthesis of the molecule which involves tryptophan hydroxylase 2) removal of the neurotransmitter by several key players, the serotonin transporter and breakdown by monoamine oxidase and L-aromatic amino acid decarboxylase, and 3) perpetuation of neuronal signaling through various serotonin receptors. The serotonergic system is a primary target of cocaine. After cocaine binds the serotonin transporter, the homeostasis of the system is perturbed, leading to changes that bring the neurotransmitter function back to baseline. There is evidence for crosstalk between different stages of serotonin function and transmission. For example, the serotonin 1A receptors are involved in feedback regulation of the serotonin system (Popova, Naumenko et al. 2010). These receptors are expressed both presynaptically and postsynaptically and are able to change the firing rate of the neuron, and therefore serotonin release (Barnes and Sharp 1999; Popova, Naumenko et al. 2010). Acute treatment with a 5-HT_{1A} agonist was shown to cause desensitization of 5-HT_{2A} receptors (Carrasco, Van de Kar et al. 2007). In another experiment, when rats were treated with a 5-HT_{1A} receptor agonist, a reduction in 5HT_{1A} mRNA levels, 5-HT_{2A} mRNA levels in frontal cortex and hippocampus, and *TPH2* mRNA levels in the midbrain (Popova, Naumenko et al. 2010). Perturbations in the serotonin system are also able to cause changes in other neurotransmitter systems.

We hypothesize that chronic cocaine exposure leads to reduced function of the serotonin transporter, 5-HT_{2A} and 5-HT_{2C} receptors and TPH1 and TPH2 in cocaine dependent individuals. We expect individuals who carry these variants in these genes that lead to an even further reduction in serotonin levels at the synapse (for example, individuals with the less receptors or more transporter expressed in their system) will be the ones that show more attentional bias for cocaine-cues and higher impulsivity (see specific aims below).

3 - SPECIFIC AIMS

Specific Aim 1: *To examine the relationship between genotype of the serotonin transporter insertion/deletion polymorphism (5-HTTLPR) and attentional bias in cocaine users and healthy controls.* We hypothesize that cocaine users will show more attentional bias to cocaine related stimuli and that this higher attentional bias will be associated with the long (higher expressing, L) form of the serotonin transporter, which has been shown to reduce serotonin levels at the synapse. We expect cocaine users that are homozygous for the higher activity (L) form of the transporter to display a higher attentional bias in the cue reactivity task.

Subaim 1a: *To examine the relationship between genotype of the serotonin transporter insertion/deletion polymorphism and impulsivity in cocaine users and healthy controls.*

Specific Aim 2: *To examine the relationship between HTR2A and HTR2C polymorphisms and attentional bias in cocaine dependent subjects.* Pharmacological agents for the 5-HT_{2A} and 5-HT_{2C} receptors have been shown to differentially affect cocaine-cue related memories, specifically 5-HT_{2A} receptor antagonists and 5-HT_{2C} receptor agonists reduce responses to cocaine cues in rats (Liu and Cunningham 2006; Nic Dhonnchadha, Fox et al. 2009). Therefore, we will genotype cocaine-dependent subjects for *HTR2A* and *HTR2C* receptor polymorphisms. We hypothesize that the T allele of the *HTR2A* polymorphism (rs6313), which leads to increased receptor expression, and the Ser allele of the *HTR2C* polymorphism (rs6318), which leads to less transcriptional activity, will both be associated with increased attentional bias.

Subaim 2a: *To examine the relationship between HTR2A and HTR2C polymorphisms and impulsivity in cocaine dependent subjects.*

4- METHODS

4.1 SUBJECTS

Fifty cocaine dependent and 20 healthy volunteer human participants were recruited through local newspaper advertisements in Houston. The cocaine dependent subjects were recruited using the Treatment Research Clinic of the Center for Neurobehavioral Research on Addictions within the UT Health Science Center. After an initial phone-interview screening, interested subjects had a first intake appointment where they signed an informed consent form, received a medical evaluation, completed various self-report neuropsychiatric questionnaires and had blood samples collected.

Inclusion criteria for cocaine dependent subjects were: age of at least 18 years, and meeting current DSM-IV criteria for cocaine dependence. If the subjects had current DSM-IV Axis I disorder other than substance abuse/dependence, a current diagnosis of other substance dependence besides cocaine, any serious non-psychiatric medical illness requiring ongoing medical treatment or which could affect the central nervous system, concomitant use of prescription medications that could affect the central nervous system, or active suicidal ideation, they were excluded from the study. Subjects were not excluded if they met criteria for alcohol or marijuana abuse.

For control subjects inclusion criteria were age of at least 18 years, and not meeting current or past DSM-IV criteria for any Axis 1 disorder. Exclusion criteria for control subjects included the same criteria as the cocaine subjects as well as any current or past substance abuse or dependence, any non-psychiatric or medical illness needing medical treatment or which might affect the nervous system, and a positive HIV test. Subjects were also excluded if they were experiencing suicidal ideation or scored greater than 15 on the Hamilton Depression and Anxiety Scale (Hamilton 1960). For female subjects, a positive pregnancy test or breast feeding were also exclusion criteria.

4.2 – BEHAVIORAL TASK



Figure 3: The Cocaine Stroop Task. Depicted above is a representational diagram of words in different colored ink. There were 4 categories of words presented to subjects in the task: neutral environmental, neutral furniture, neutral salient and cocaine-related.

The Cocaine Stroop task was designed to measure attentional bias to cocaine-related stimuli (Hester, Dixon et al. 2006; Vadhan, Carpenter et al. 2007). In this task, the participant is presented with words printed in color, and asked to name the color of each stimulus by pressing mouse buttons covered by colored stickers. The words (bold Arial font in size 18) appeared in red, blue or green font color against a black background. The stimulus words were cocaine-related (for example “Crack” “Rocks,” “High,” “Dealer,” etc) while others were neutral words (for example “Sofa,” “Oven,” “Cabinet,” “Window,” etc).

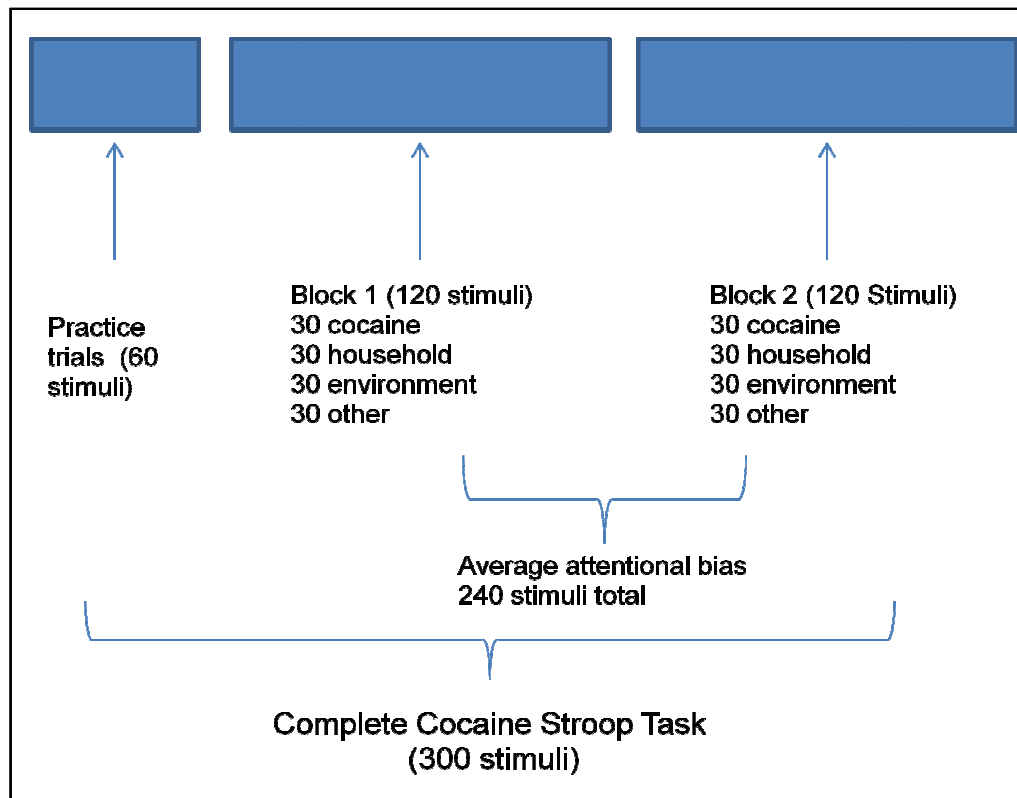


Figure 4: Diagram of stimulus presentation in the Cocaine Stroop task.

The participant was instructed to ignore the meaning of the words, and concentrate only on naming the color in which the word is written as quickly and as accurately as possible. A short beep sound was made for incorrect responses. Each subject practiced for 60 trials. Subsequently, four blocks of words were presented in two parts for the task. After the practice trials, one block of stimulus (cocaine) words, and three blocks of the neutral words were presented and this was repeated in random order. Attentional bias was scored as the mean cocaine reaction time minus the mean control reaction time. The stimulus was displayed on the screen until the subject responded or until 1800 milliseconds (ms) passed. A blank screen was presented for 500 ms and this was the inter-trial interval.

4.3 – IMPULSIVITY MEASURE

Subjects completed the Barratt Impulsiveness Scale. (BIS-11), the most widely used self-report measure for impulsivity (Patton, Stanford et al. 1995). The BIS-11 is a 30 item questionnaire which has been used in several previous studies on impulsivity and aggression (Allen et al. 1998b; Cherek et al. 1997). Each item in the questionnaire is rated from 1 to 4, and 3 subscales were determined by factor analysis: motor, attentional and nonplanning impulsiveness (Patton, Stanford et al. 1995; Stanford, Anderson et al. 2009). Items in the questionnaire include statements such as “I plan tasks carefully”, “I get easily bored when solving thought problems”, “I change residences”, “I am restless at the theatre or lectures”, etc. Subjects are instructed to answer quickly and honestly and rate each statement by marking one of the following options: Rarely/ Never, Occassionally, Often and Almost Always/ Always.

4.4- DNA SAMPLE COLLECTION

Blood samples were collected by a phlebotomist into an 8 ml EDTA tube. The tube was placed in a centrifuge and centrifuged at ~1500-2000 X g for 10-15 min at room temperature. Using a transfer pipet, the plasma, buffy coat, and red blood cell layers were removed and placed in separate cryostorage tubes and banked at -80 degrees. DNA was isolated from the buffy coat layer of blood samples using the Qiagen PureGene kit (Valencia, CA). After purification, DNA quality was measured using a NanoDrop (Wilmington, Delaware). Some samples were run on E-gels to make sure there was no degradation of the isolated DNA.

4.5 – GENOTYPING THE 5-HTT

We performed fragment analyses for the two insertion/deletions that are commonly found in the serotonin transporter *SLC6A4* gene: *5-HTTLPR* and *STIn2*. The promoter polymorphism *5-HTTLPR* was genotyped for the repeat polymorphism and the internal SNP rs25531, to determine the triallelic L_A, L_G and S serotonin transporter promoter alleles. The high expressing L_A allele was designated L' and the low expressing L_G and S alleles were grouped as S'.

Primers were designed using Vector NTI (Invitrogen, Carlsbad, CA) and ordered from the Midland Certified Reagent Company (Midland, Texas). The primers used were 5' GCAACCTCCCAGCAACTCCCTGTA 3' and 5' GAGGTGCAGGGGGATGCTGGAA 3' (Hu, Oroszi et al. 2005). The 5-HTTLPR was amplified using polymerase chain reaction (PCR). The PCR mix was comprised of 18.9 μ L H₂O, 2.5 μ L Invitrogen High Fidelity Buffer, 2.5 μ L of 10 mM dNTP mix, 1 μ L of 50 mM MgSO₄, 0.5 μ L of 25 μ M concentration of both the forward and reverse primers, 100 ng DNA and 0.1 μ L of Invitrogen Platinum High Fidelity Taq Polymerase. The total volume for each sample was 25 μ L. The sequence was: 1 cycle: 94°C @ 1 min; 30 cycles: 94°C @ 30 sec; 60°C @ 30 sec, 72°C @ 30 sec; and 1 cycle: 72°C @ 5 min.

To differentiate between the two forms of the L allele, 5 μ L of the PCR product was subsequently digested with *HpaII* restriction enzyme (New England Biolabs, Ipswich, MA), which cleaves the PCR product at rs25531 when there is a G allele present. All digested PCR product samples were run on precast polyacrylamide gels (Bio-Rad, Hercules, CA) in 1 % Tris/Borate/EDTA (TBE) buffer solution (Invitrogen) at 150 volts for 1 hour, stained with ethidium bromide and imaged with UV light using the Gel DockIt imager (UVP, Upland, CA). Two people confirmed the genotypes depicted on the gels.

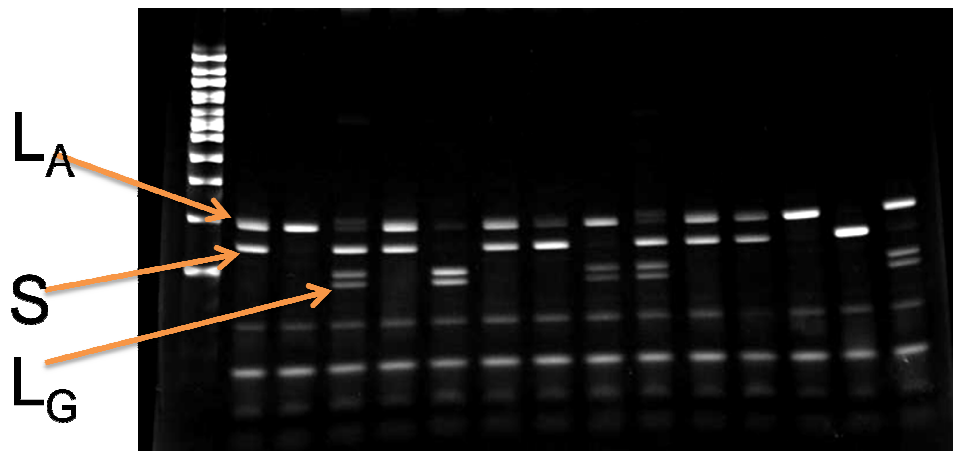


Figure 5: Genotyping method for 5-HTTLPR. After PCR amplification, each sample was digested with *HpaII* enzyme, which cut if there was a G present in the amplified gene product. If the G allele was present, this led to two small bands on the gel, as depicted above.

4.6 - Genotyping *TPH1* and *TPH2*

Genotypes were determined by a 5'-exonuclease fluorescence assay (TaqMan) using pre-designed TaqMan (Applied Biosystems, Foster City, CA) primer-probe sets (Assay ID C_2645661_10 for *TPH1* variant rs1799913 and C_26385365_10 for *TPH2* variant rs4290270) and analyzed on an ABI Prism 7900 (Applied Biosystems, Foster City, CA) as previously described (Whitcombe, Brownie et al. 1998; Ranade, Chang et al. 2001). PCR cycling was performed by incubation at 50°C for 2 min and at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 62°C for 1 min. Upon completion of PCR cycling, genotype analysis was performed on the ABI Prism_ 7900 sequence detection system using SDS 2.2 software (Applied Biosystems, Foster City, CA). All genotype analyses were performed during a time when the clinical status of the subjects was still blinded. TaqMan assays were performed in duplicate. We did not observe any inconsistencies between duplicate runs.

4.7- Genotyping the *HTR2A* and the *HTR2C* receptor variants

Genotypes for *HTR2A* and *HTR2C* were also determined by a 5'-exonuclease fluorescence assay (TaqMan) using pre-designed TaqMan (Applied Biosystems, Foster City, CA) primer-probe sets two variants in the *HTR2A* gene: rs6311 (Applied Biosystems, Assay ID C_3042197_1_) and rs6318 (Assay ID C_2270166_10) on an ABI Prism 7900 (Applied Biosystems, Foster City, CA). PCR cycling was performed by incubation at 50°C for 2 min and at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 62°C for 1 min. Genotype of the PCR products was analyzed using the ABI Prism 7900 sequence detection system using SDS 2.2 software (Applied Biosystems, Foster City, CA). All genotype analyses were performed during a time when the clinical status of the subjects was still blinded. Again, assays were performed in duplicate and we observed no inconsistent results between duplicate plates.

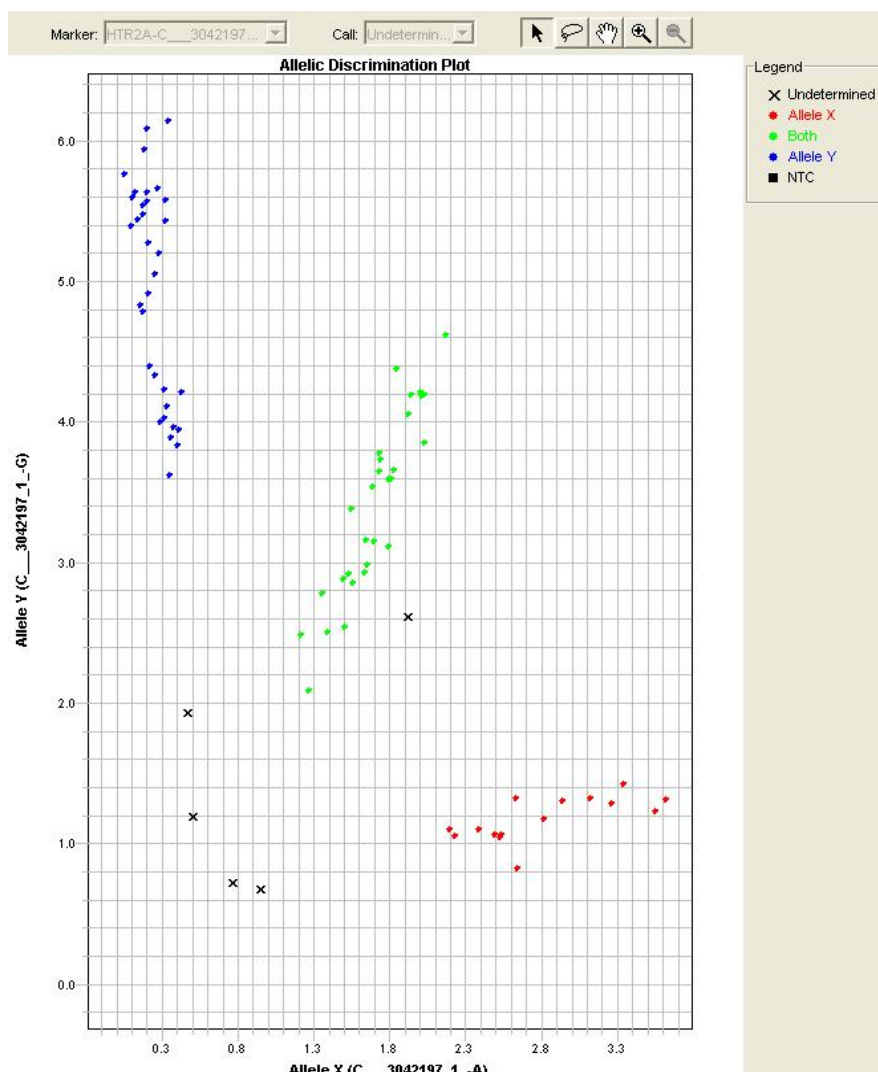


Figure 6: Genotyping method for TaqMan assays. After PCR amplification, each sample was analyzed using an ABI fluorescence reader. The allelic discrimination plot above shows the homozygotes for the first allele close to the X-axis (red) and the homozygotes for the second allele in the Y-axis (blue). The heterozygotes are depicted in the middle and the black X's are negative control samples.

4.8 - Statistical analysis

All variants were tested in the control group for Hardy Weinberg equilibrium. A p value of less than 0.05 was considered significant. The data was analyzed using several grouping methods. First we focused on only African American subjects, or all individuals combined. For both these large groups we examined cocaine users and control subjects together, only the cocaine users or only the control subjects. Within these groups, we either

did not control for any other variables, controlled for age and gender or controlled for age, gender and diagnosis.

Allelic and genotypic analyses were performed for the *HTR_{2A}*, *TPH1* and *TPH2* genes. For the *5-HTTLPR*, we analyzed genotype and allele frequencies and also grouped genotype analysis for LL vs. S carriers. In addition to this, we also analyzed the *5-HTTLPR* data as others have done in the past, without accounting for the internal SNP (simply L or S, ignoring that L_G is transcriptionally similar to S) in order to determine if there was a difference between the methods.

For the *HTR_{2C}*, the analysis was different because this gene is on the X-chromosome. We analyzed the *HTR_{2C}* data assuming men are homozygotes (looked at both genotypic and allelic information), focusing on the presence of the C allele, or focusing on the presence of the G allele.

Analysis of variance, Pearson Chi Square tests and Fischer's Exact tests were performed for genotype and allele frequency data for all genes with regard to group (cocaine or control.) We tested for Hardy-Weinberg Equilibrium in the control groups. None of the groups violated Hardy-Weinberg Equilibrium for any of the genes. The sample was analyzed as both ethnically heterogeneous and ethnically homogenous groups. African Americans comprised the largest group within the sample and were therefore analyzed separately. One way ANOVA tests were performed to determine significance of the association between genotypes or allele frequencies and attentional bias. We used a two way ANOVA test to examine if there were interactions between variables.

Power analyses was performed using the G*Program. Calculations were performed using the *post-hoc* option in the G*Power Program.

5– Results

All genes studied did not violate Hardy-Weinberg equilibrium in the control groups. The largest ethnic group in our sample was African Americans. To avoid bias due to population stratification, we analyzed the African American cocaine dependent subjects separately.

In our study sample, there was a significant difference in age and gender ratio between the cocaine dependent and healthy control groups, with the cocaine dependent group being significantly older ($p < 0.001$) and having significantly fewer women than the control group ($p = 0.003$) (refer to Table 1 below).

	Control	Cocaine
Age *	31 ± 9	46 ± 8
Gender**	10 F	8 F
	10 M	42 M
Ethnicity	16 African	34 African
	American	American
	2 Asian	13 Caucasian
	1 Caucasian	2 Hispanic
	1 Hispanic	1 Other

Table 2: Demographical Information and differences between groups. Age was significantly different between control and cocaine groups (* $t = 7.07$, $p < 0.001$), as well as the male to female ratio (** $\chi^2 = 8.65$, $p = 0.003$).

Our study aimed to determine which cocaine dependent individuals exhibited the highest attentional bias in the cocaine Stroop task. We analyzed the behavioral data by grouping all cocaine subjects and comparing the average score for the whole task and comparing it to control subjects. Overall, cocaine dependent individuals had significantly higher attentional bias scores than control subjects ($p = 0.01$). When we

analyzed the Cocaine Stroop task by blocks, both groups of subjects displayed higher attentional bias scores for the first block of the task (first 120 stimuli) than the second block. The scores between both blocks were significantly correlated (Pearson correlation = 0.40, $p < 0.001$) however, and the direction (cocaine dependent group had higher scores) was consistent between blocks. For the first block of the task, the cocaine dependent subjects scored significantly higher ($p = 0.003$ all ethnicities; $p = 0.01$ AA subjects only). The difference in attentional bias between cocaine and control groups was not significant for the second block (see Figure 7 below).

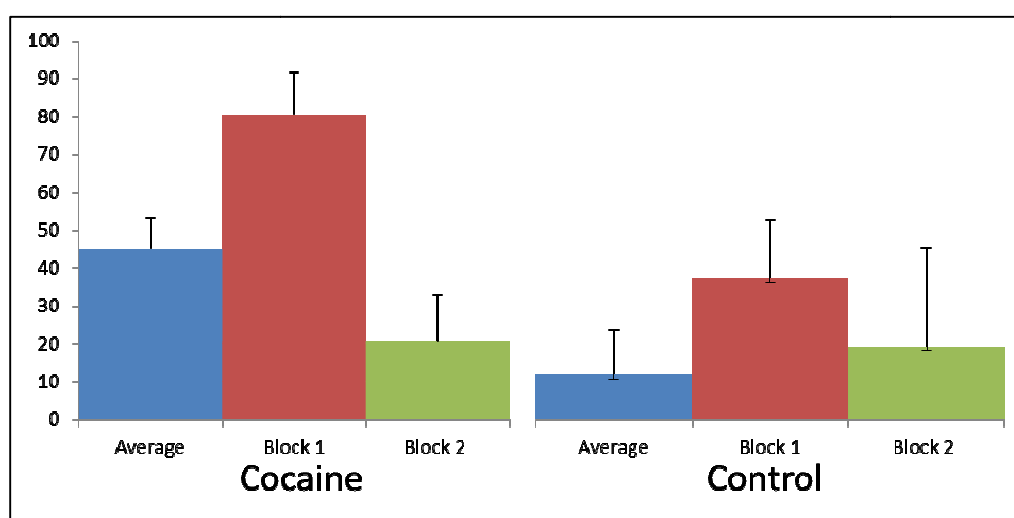


Figure 7: Stroop Task Results: cocaine dependent subjects had more attentional bias than controls. There was a decrease in attentional bias for both groups in the second block of the task (green bar) of the Cocaine Stroop Task.

We administered the BIS-11 to subjects to see if higher impulsivity was associated with serotonergic gene variants. We hypothesized that higher impulsivity would be observed in the cocaine dependent subject, a finding that has been consistently reported in the literature. Cocaine dependent subjects had higher self-report impulsivity in the total scores of the BIS-11 compared to non-drug using controls ($p < 0.001$). For the entire group of subjects, cocaine-dependent subjects also displayed higher self-report impulsivity on the attentional impulsivity ($p < 0.001$), motor impulsivity ($p = 0.003$) and nonplanning impulsivity ($p < 0.001$) subscale scores of the BIS-11. In the ethnically homogeneous group of African American

subjects only, BIS-11 total ($p < 0.001$) attentional ($p = 0.002$) and nonplanning ($p < 0.001$) were significantly higher, but groups were not significantly different in the motor subscale scores, although this was still a trend ($p = 0.06$).

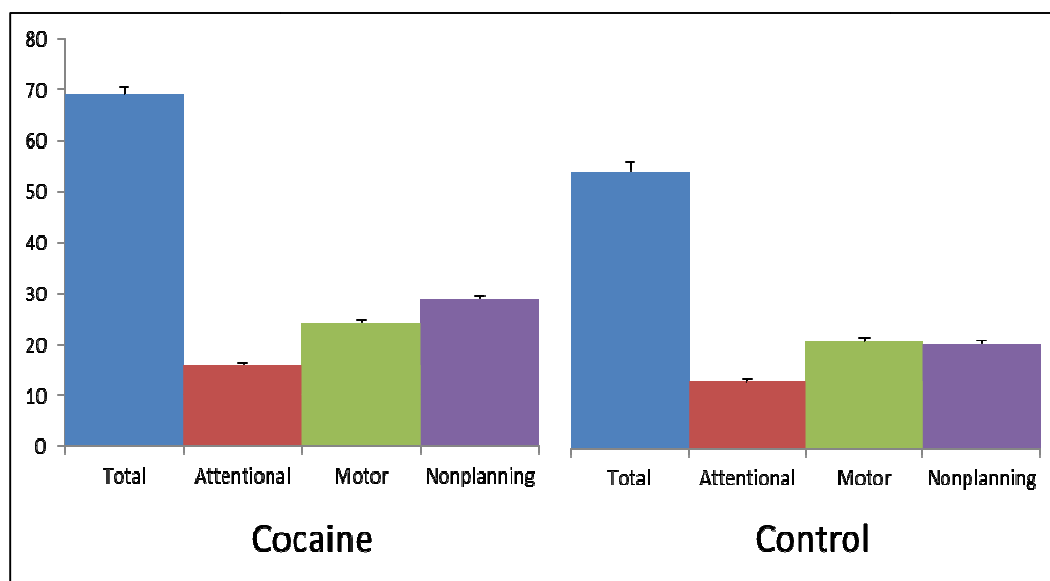


Figure 8: BIS-11 Results: Cocaine dependent subjects scored higher on the BIS-11 than controls in both the BIS total and subscale scores.

We observed different genetic associations for different blocks of the Cocaine Stroop task. For the average attentional bias score (both blocks of the task combined) we observed an association with *HTR2C*, for scores of the first block of the task we observed an effect when examining genotype groups for *5-HTTLPR*, and for the second block an association with *TPH2* was found.

We hypothesized that variants associated with lower serotonin function would be associated with higher attentional bias and impulsivity in cocaine dependent individuals. In the ethnically homogenous sample of African American cocaine dependent individuals, there were several significant findings. We analyzed the data for 5-HT_{2C} receptor by controlling for gender, and counting males twice (they have only one allele and this is similar to homozygous females). There was a significant difference between individuals with C and G 5-HT_{2C} receptor alleles in

average attentional bias for the Stroop task, and subjects with the C allele had higher attentional bias ($p = 0.02$)(See figure 8).

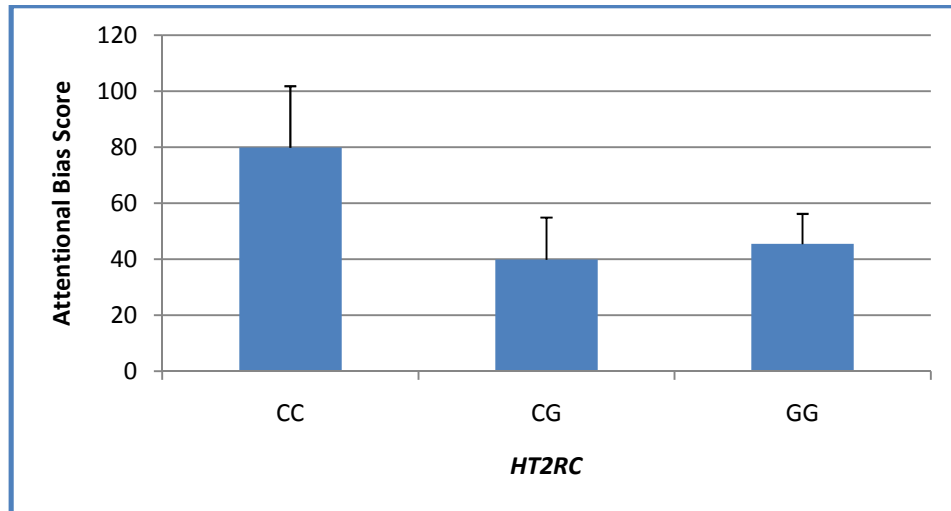


Figure 9: *The 5-HT_{2C} Variant and attentional bias: The C allele for the HTR2C gene was associated with higher average attentional bias in cocaine dependent subjects.*

To study the relationship between serotonin transporter function and attentional bias, we analyzed genotype and allele groups for the 5-HTTLPR variant and Stroop Task performance scores. For the two different allele groups (see figure below), we divided subjects into two genotype groups: the L' group which included the L_A/L_A genotype and the S' group which was comprised of the L_A/ L_G, L_A/ S, L_G/L_G, L_G/S and S/S genotypes. The latter group is also referred to as the S-carriers, because these individuals are likely to have low transporter function. There was a significant difference in attentional bias for the first block of the task between 5-HTTLPR L' and S' genotypes, with the S' genotype exhibiting higher attentional bias ($p = 0.04$).

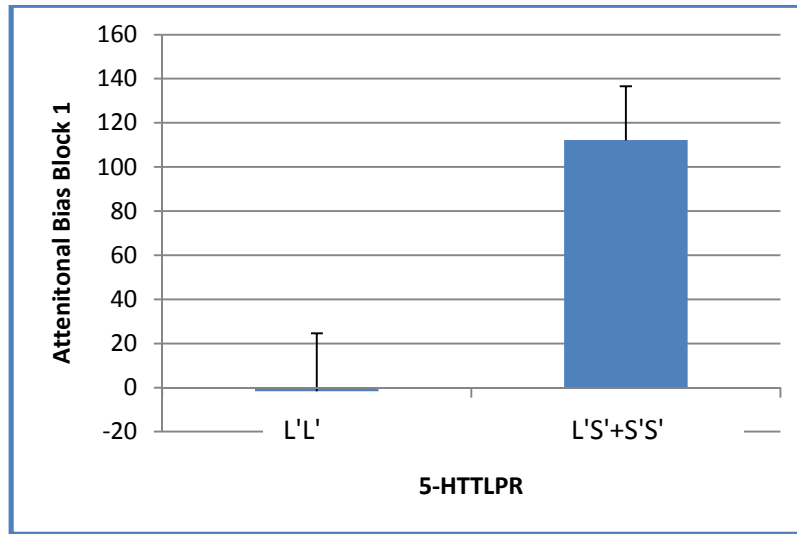


Figure 10: 5-HTTLPR genotype groups and attentional bias: Cocaine dependent individuals who were S-carriers for the 5-HTTLPR displayed higher attentional bias scores for the first block for the first block of the Cocaine Stroop Task.

Tryptophan hydroxylase plays an important role in serotonin synthesis, as the rate limiting enzyme. For this reason, we hypothesized that tryptophan hydroxylase variants might be associated with higher attentional bias and impulsivity. The variant for *TPH2* that we chose to study is SNP rs4390270, which has shown association with addiction-related disorders in the literature. A significant difference between *TPH2* genotype groups was observed for the second block of the Cocaine Stroop Task. We observed a significant difference between both *TPH2* genotypes and alleles in attentional bias scores ($p = 0.03$ and $p = 0.01$, respectively) with the TT genotype displaying the highest attentional bias for cocaine-related words.

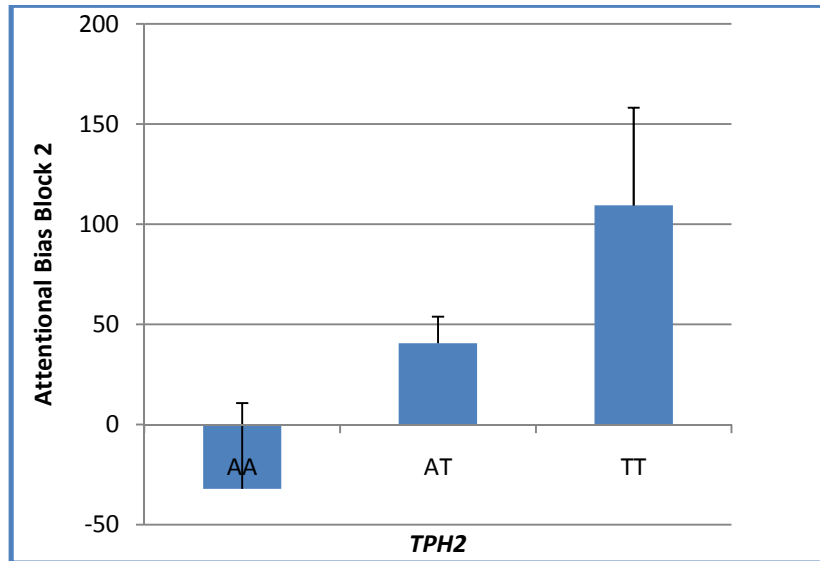


Figure 11: The TPH2 Variant and attentional bias: For the second block of the Cocaine Stroop Task, cocaine dependent individuals with the TT genotype displayed the highest attentional bias scores.

We also hypothesized that there would be a relationship between serotonin transporter variants and self-report impulsivity scores in cocaine dependent individuals. We analyzed BIS-11 scores for different genotype and allele groups of the 5-HTTLPR. In African Americans, there was a significant difference between 5-HTTLPR genotypes in scores for BIS total and attentional impulsivity ($p=0.03$, $p=0.02$ respectively). Unlike the results for attentional bias, it was the L' genotype that showed association with higher attentional impulsivity.

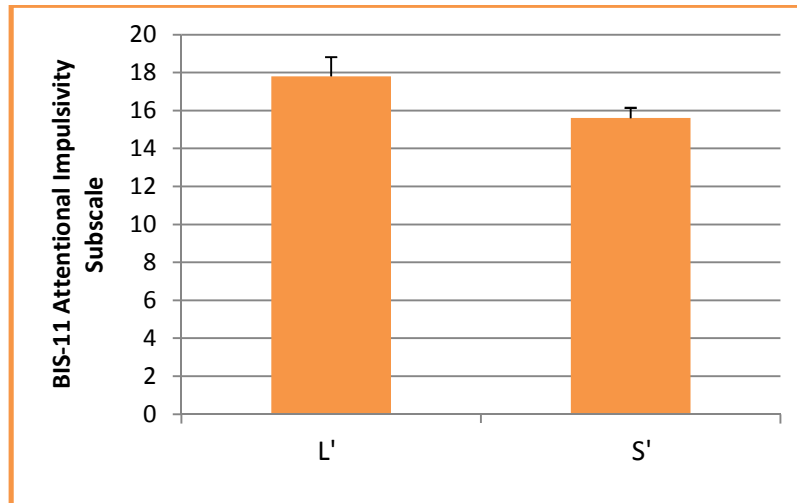


Figure 12: 5-HTTLPR genotype groups and impulsivity: Cocaine dependent users who were classified to be in the L genotype group displayed higher BIS-11 attentional impulsivity scores than the individuals in the S genotype group.

We did not theorize on different relationships between 5-HTTLPR genotype groups and impulsivity scores for cocaine users versus healthy controls. In our study sample, however, we did observe such a converse relationship. For the mixed ethnicity group, there was a significant interaction between 5-HTTLPR genotype and BIS attentional impulsivity ($p < 0.05$) indicative of a gene environment interaction. No significant effects were observed in either the mixed or African American control group. The power for the sample was calculated to be 0.58 for a one way ANOVA.

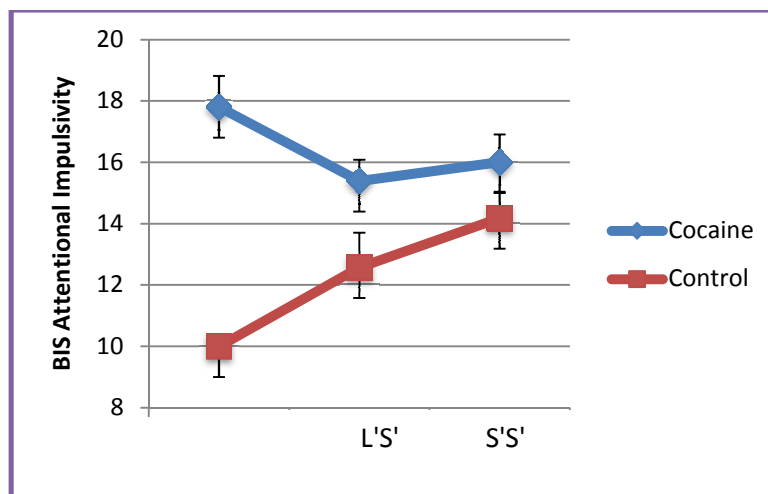


Figure 13: 5- HTTLPR genotypes and Impulsivity: An interaction effect was observed for genotype groups and Attentional Impulsivity subscale scores of the BIS-11 in cocaine versus control subjects.

6 – DISCUSSION

The purpose of this study was to investigate the role of variants in genes of the serotonin system in attentional bias and impulsivity in cocaine dependent individuals. We focused on several genes in the serotonin system, including the serotonin transporter, those coding for the serotonin receptors 2A and 2C and tryptophan hydroxylase 1 and 2. We predicted that attentional bias and impulsivity would be higher in cocaine dependent individuals who had lower serotonin function.

Serotonin Receptor 2C

There was a significant difference between 5-HT_{2C} receptor genotypes in attentional bias in our study sample. In preclinical studies, 5-HT_{2C} agonists reduce responses to cocaine cues in rats (Nic Dhonnchadha, Fox et al. 2009). Variants in the *HTR2C* gene have been associated with attention deficit hyperactivity disorder, schizophrenia, bipolar disorder, obesity in psychiatric patients, and other psychiatric disorders (Berg, Clarke et al. 2008; Drago and Serretti 2009). These data suggest that the 2C receptor may be involved in not only psychiatric disorders, but also impulse control. In the present study we genotyped subjects for the rs6318 Cys23Ser variant of the *HTR2C* gene, which has been shown to be associated with eating disorders and unipolar and bipolar affective disorders, reviewed in (Drago and Serretti 2009). Interestingly, this variant has also been associated with co-morbid bipolar disorder and alcoholism (Yasseen, Kennedy et al. 2010). Also, a study looking at monoamine metabolite concentrations in a male population that included alcoholic violent offenders and controls, found that the polymorphism contributes to variation of CSF 3-Methoxy-4-hydroxyphenylglycol (MHPG), a norepinephrine metabolite, with the Ser variant leading to higher levels of the metabolite, and the authors suggested that the Ser variant is less responsive to released serotonin (Lappalainen, Long et al. 1999). When cells are treated with inverse 5-HT_{2C} agonists, the Ser variant displays different desensitization responses than the Cys variant (Okada, Northup et al. 2004).

Our findings were that the Ser variant leads to higher average attentional bias in the cocaine dependent group, and this is in not in agreement with the preclinical literature (Filip and Cunningham 2002; Liu and Cunningham 2006). Perhaps, like Lappalainen et al suggested, the cocaine dependent individuals with the Ser variant are less responsive to released serotonin, due to different desensitization properties of the receptors. Also, it is possible that in cocaine dependent individuals, an increase in serotonin may take place after cocaine exposure and the desensitization properties of the receptor might come into play. Therefore, when serotonin is increased in the synapse, it doesn't have the agonist effects that have been associated with reduction of cue reactivity in animal experiments.

Serotonin Transporter

The current study yielded several significant results when we analyzed the 5-*HTTLPR* data. Due to inconsistency in the literature in genotyping 5-*HTTLPR*, we performed analysis by considering the internal SNP that influences the 5-*HTTLPR*'s function (triallelic method, (Hu, Lipsky et al. 2006) and not considering it (biallelic method). For the triallelic analysis, there was a significant difference between 5-*HTTLPR* genotypes in average attentional bias in cocaine dependent individuals (mixed ethnicity group). In the ethnically homogenous African American group of cocaine dependent individuals, when we analyzed attentional bias scores for the first block of the Stroop task there was a significant difference between 5-*HTTLPR* genotypes when individuals were grouped for the presence of the lower functioning variant (L'L' vs L'S' + S'S'), as has commonly been done in previous literature (Patkar, Berrettini et al. 2002). For the biallelic analysis, significant effects were observed, although these were all in the control group. Additionally, there was a significant difference between 5-*HTTLPR* genotypes in BIS total and attentional impulsivity subscale scores.

The serotonin transporter is important in that it controls synaptic levels of serotonin by pumping serotonin molecules back into the presynaptic neuron after they have been released, and essentially refreshing the synapse after action potential activity. The functional variant that we studied has been associated with both behavioral and psychiatric disorders, although findings have been inconsistent. The transporter is

expressed in cortical and limbic areas that process emotional components of behavior (Lesch, Bengel et al. 1996). For this reason, the serotonin transporter is also thought to act on emotional regulation, impulsivity and anxiety as a function of specific contexts (Courtet, Jollant et al. 2005). S-carriers, individuals possessing at least one S allele, have also displayed more anxiety-related traits compared to LL individuals in a large study of healthy volunteers (Lesch, Bengel et al. 1996). S carriers have also been reported to score lower for coping ability on an emotional appraisal questionnaire, and the investigators have classified S carriers as being cognitively vulnerable (Szily, Bowen et al. 2008). A study examining the role of *5-HTTLPR* genotype and the influence of framing in decision making found a significant association between the SS genotype and greater amygdala activation while making decisions inside a functional magnetic resonance imaging (fMRI) scanner (Roiser, de Martino et al. 2009).

Transporter genotype has also been associated with acquisition of fear conditioning, and S-carriers show better acquisition (Garpenstrand, Annas et al. 2001). In healthy individuals, carriers of the S allele have been reported to perform better on the Wisconsin Card Sorting Test, a measure of executive function, compared to LL individuals (Borg, Henningsson et al. 2009). Although previous studies have yielded inconsistent findings regarding cognitive performance and the *5-HTTLPR*, it may be possible that cocaine dependent individuals who are S carriers experience greater limbic activation when they encounter cocaine-related cues and this process contributes to greater attentional bias on the cocaine Stroop task.

Studies investigating the role of the *5-HTTLPR* in drug dependence have been contradictory and inconsistent. In a study examining serotonin transporter variants in several populations of different geographic origins, there was no association between *5-HTTLPR* alleles and alcohol dependence (Gelernter, Kranzler et al. 1997). Kremer et al 2005 found the L allele to be more abundant in smokers (Kremer, Bachner-Melman et al. 2005). Mannelli et al. examined the serotonin transporter gene in African American cocaine and alcohol-dependent individuals and found that people who carried the S allele had greater severity of alcohol use at admission and showed less improvement after treatment at follow-up (Mannelli, Patkar et al. 2005). Enoch et al found that the low activity form (S allele) was more common in a group of African American patients with

addiction compared to controls (Enoch, Gorodetsky et al. 2010) Another study reported that the LL genotype was higher in cocaine-dependent individuals, suggesting the long form of the transporter to be an addiction vulnerability variant. This difference was not significant when they limited their group to African Americans only, however (Patkar, Berrettini et al. 2001). Interestingly, one group investigating alcoholism, found that the L allele is associated with increased craving for alcohol at the beginning of alcohol withdrawal (Bleich, Bonsch et al. 2007). These last reports are in agreement with our findings.

We observed a difference in genotype frequencies of the *5-HTTLPR* between cocaine dependent individuals and control subjects when analyzing the total group, which was ethnically heterogeneous. When we restricted the group to those of African American ethnicity, this genotypic difference was not significant, but the allele frequency was different between the two groups. The L allele was more abundant in the cocaine dependent individuals.

The purpose of this study was to investigate the relationship between *5-HTTLPR* genotype and behavior in cocaine dependent users. The hypothesis was that there is a gene-environment interaction: cocaine dependent users with the L allele display the most attentional bias and impulsivity. We found that in cocaine dependent individuals, the SS individuals showed the highest attentional bias, while in the healthy control group the SS individuals showed the lowest attentional bias. Although we expected the attentional bias scores to be close to zero for the control subjects, this was not the case. Within this group there were subjects that had scores that resembled those in the cocaine group and subjects who had negative scores (indicating they were biased towards the neutral words). This could be due to experimental error or the controls in our sample were not honest about past cocaine use. These findings need to be replicated, due to the small sample size of our study and uneven genotype groups.

Tryptophan Hydroxylase 2

In the current study, we found a significant difference in attentional bias for the second block of the cocaine Stroop task between *TPH2* SNP rs4290270 genotypes.

Individuals with the TT genotype displayed the highest attentional bias scores. The *TPH2* gene has been reported to be critical in controlling serotonin levels in the brain (Dahl, Cubells et al. 2006). It has been the focus of many studies of psychiatric and addictive disorders. Zill et al reported an association between *TPH2* variants and major depression (Zill, Baghai et al. 2004). For the SNP we studied, although not many researchers have investigated its functional role, one study has identified that it may have functional effects and another has implicated that the T allele is associated with higher function (Lim, Pinsonneault et al. 2007; Roche and McKeon 2009).

More studies need to be performed to evaluate rs4290270's functional role before we can confidently theorize on the SNP's influence. However, if the studies mentioned above are accurate, our data indicate that perhaps higher *TPH2* function, which may lead to higher levels of serotonin being produced, is negatively affecting attentional bias in the cocaine dependent subjects. A recent publication reported altered levels of serotonin metabolites in cocaine dependent individuals compared to healthy controls (Patkar, Rozen et al. 2009). Because the *TPH2* variant we genotyped was associated with the largest effect in attentional bias in our sample of cocaine dependent individuals, we hypothesize that this gene may be a critical component in the alterations in tryptophan metabolism and this is in agreement with the data observed by Patkar et al. Even though the genotype associated with attentional bias in cocaine dependence may be producing more *TPH2* enzyme, which in turn would increase serotonin production, this modulation in the system may change the synaptic dynamics by leading to modifications in pre and post-synaptic receptors that may lead to more attentional bias. This variant could be creating functional differences by splicing, altered binding to enhancers and repressors, etc.

Studies that have focused on other *TPH2* variants that cause functional effects on the gene product, have found an association with depression (Zhang, Gainetdinov et al. 2005). *TPH2* genetic variants have been associated with affective disorders and bipolar disorder, as well (Harvey, Shink et al. 2004; De Luca, Likhodi et al. 2005). These results may be relevant to the present study because a high rate of mood disorders in individuals who use cocaine (abuse or dependent) has been reported (Rounsaville 2004). Reduced function in the *TPH2* enzyme may be a common feature of the pathophysiology of mood

disorders, impulsivity and cocaine addiction. In genetic vulnerability studies, however, *TPH2* has not always yielded positive findings. Dahl et al studied six variants in the *TPH2* gene that were in linkage disequilibrium with the more commonly studied variants (specifically rs7963717, rs1386495, rs4760816, rs1007023, rs1386486 and rs1872824) and did not find an association with the cocaine-dependent phenotype for an ethnically similar (African American) group of cocaine dependent individuals (Dahl, Cubells et al. 2006).

We did not observe an effect of the *TPH2* variants on self-reported impulsivity, and this is not consistent with other studies that have reported an association between *TPH2* gene variants and impulsivity. Stoltenberg et al. found an association between the TT genotype in variant rs1366483 and a longer response inhibition time in the Stop Task in a healthy male population (Stoltenberg, Glass et al. 2006). Another group found an association between the TT genotype of a *TPH2* -703 G/T polymorphism and errors on an executive control attention task (Reuter, Ott et al. 2007). Perhaps behavioral measures of impulsivity are more sensitive than self report scores, or the cocaine dependence made the difference between previously reported results and our sample's outcome.

Serotonin Receptor 2A

We found a trend between the 5-HT_{2A} receptor TT genotype and increased attentional bias in the cocaine dependent group. These individuals also had higher self-reported impulsivity scores. Studies show that mRNA for the 2A receptors is most abundant in the frontal cortex (Pompeiano, Palacios et al. 1994). The 5-HT_{2A} receptor has been associated with impulsivity: lower receptor density has been observed in impulsive and violent aggressive individuals (Meyer, Wilson et al. 2008). In preclinical studies, 5-HT_{2A} receptor antagonists have been associated with decreasing cue-reinstated behavior in rats. Blocking the 2A receptors attenuates the locomotor response that's induced by cocaine (Fletcher, Grottick et al. 2002). The authors suggested that 2A receptor blockade modulates locomotor-stimulant properties of cocaine but not the reinforcing effects of the drug (Fletcher, Grottick et al. 2002). In our results we did not observe a difference in

motor impulsivity between the genotypes, but perhaps if the sample was larger we would be able to observe an effect.

An inverse relationship between 5-HT_{2A} receptor density and serotonin levels have been implicated by the literature. Lower CSF 5-HIAA has been observed in people with aggressive and impulsive behavior (Brown, Ebert et al. 1982). Increased 5-HT_{2A} receptor density however has been associated with reduced reactivity to threat related stimuli. Fisher et al. measured receptor binding using PET and BOLD activation using fMRI in the amygdalas of healthy volunteers (Fisher, Meltzer et al. 2009). The authors suggested that serotonin plays a role in integrating affective information between amygdala and prefrontal cortex, with the 2A receptor being predominant in this modulation (Fisher, Meltzer et al. 2009). While 5-HT_{2A} receptor mRNA is concentrated in several brain areas, mRNA for the 2C receptor is more widespread in the CNS, and almost exclusively in the central nervous system (Pompeiano, Palacios et al. 1994). Li and colleagues studied alterations in 5-HT_{2A} and 5-HT_{2C} receptors in serotonin transporter knockout mice (Li, Wichems et al. 2003). They found changes in the density of both receptors, with region- specific alterations. The 2A receptors were increased in hypothalamus and septum and decreased in the striatum while the 2C receptors were increased in the amygdala (Li, Wichems et al. 2003). The T allele is associated with higher expression of the 2A receptor and perhaps, in our sample, this correlated with reduced serotonin function (Polesskaya and Sokolov 2002). The functional effects of the T allele could be due to differential splicing, increased binding of enhancers or similar mechanisms that would lead to higher gene expression .

Tryptophan Hydroxylase 1

TPHI has been a vulnerability gene for suicidality, and has been thought to be involved in suicidal behavior, especially violent suicide attempts (Mann, Malone et al. 1997; New, Gelernter et al. 1998; Nielsen, Virkkunen et al. 1998; Abbar, Courtet et al. 2001; Courtet, Jollant et al. 2005). Many variants have been identified in the gene, with several associations found relating *TPHI* and alcoholism, nicotine dependence, antisocial behavior and heroin addiction, (Nielsen, Goldman et al. 1994; Nielsen, Barral et al. 2008;

Nielsen, Ji et al. 2008; Yuferov, Levran et al. 2010). We did not find any significant association between genotypes of *TPH1* when we analyzed attentional bias and self-report impulsivity scores. Perhaps *TPH1* is not related to behavioral aspects of drug dependence that involve prefrontal cortex or top-down processing. TPH1 plays an important role in central nervous system development but it may not be important to brain areas involved in executive functioning or sensory processing simply due to anatomical localization (TPH2 on the other hand would be hypothesized to play a larger role here).

Summary of genetic results

We observed significant findings for *HTR2C*, *5-HTTLPR* and *TPH2* variants. The *HTR2C* Cys23Ser SNP was observed to make a difference in average attentional bias scores of cocaine-dependent individuals. This implies that *HTR2C* is associated with a general attentional bias effect in the Cocaine Stroop task.

The *5-HTTLPR*, on the other hand, showed an effect on performance in the first block of the task, which was also when all subjects displayed the highest attentional bias scores. We hypothesize that this first part of the task relates to attentional impulsivity, although, this was not observed in the data. The *5-HTTLPR* results suggest that synaptic serotonin levels (modified by the serotonin transporter) might be related to the processes responsible for the interference that cocaine-related words exert on color naming. S-carriers had the highest attentional bias scores for the first block of the Cocaine Stroop task, but it was the other genetic group, the L/L' individuals, that had significantly higher attentional impulsivity scores. These results suggest that the serotonin transporter or synaptic serotonin levels might play different roles in attentional bias and impulsivity. There was no correlation between attention bias scores for the first block of the task and BIS attentional impulsivity, suggesting that the two tasks measure different constructs, and serotonin transporter function exerts differential effects on attentional bias and impulsivity.

We also observed an interaction effect between 5-HTTLPR genotype and BIS attentional impulsivity in our study sample. This interaction effect might be indicative of a gene environment interaction. Individuals with the L'L' genotype who were cocaine users displayed the highest BIS attentional impulsivity scores. On the other hand, it was controls with the S'S' genotype that displayed the highest attentional impulsivity scores. Cocaine directly interacts with the serotonin transporter, and could have caused this difference in impulsivity between controls and cocaine-dependent individuals, increasing attentional impulsivity in individuals who perhaps had lower scores before cocaine use.

Lastly, for the *TPH2* variant we studied, individuals with the TT genotype displayed the highest attentional bias scores for the second block of the task. During the second block, the attentional bias scores were generally reduced for all subjects. We hypothesize that this block of the task represents learning or an extinction effect. Subjects who continue to display high attentional bias in this last block of the task might be experiencing reduced capacity to learn. On the other hand, the second block of the task could be representing a completely different and separate process that subjects experience while performing the task.

Therapeutic implications

In agreement with recent preclinical findings, the current study suggests that serotonergic medications may be utilized as pharmacotherapeutic treatment for cocaine addiction. Our results indicate that the serotonin transporter, 2C receptor and tryptophan hydroxylase 2 should be targeted. There are several FDA approved pharmacological treatments that target these systems already, for example, selective serotonin reuptake inhibitors (SSRIs) target the serotonin transporter. Our study results suggest that increasing serotonin transporter function will reduce attentional bias. Some other possible therapeutic agents might be partial 5-HT_{2C} receptor agonists, such as *meta*-Chlorophenylpiperazine (mCPP). Serotonin receptor agonists however, might lead to different treatment responses for different genetic backgrounds.

Limitations

One limitation of this study is the sample size. The control group was too small. In the total African American group, if the sample sizes were larger, there would likely be more significant findings. The present study was only on average, 0.50 powered to detect an association between serotonin variant genotypes and attentional bias or self-reported impulsivity in cocaine dependent individuals. This low power is due to the small number of subjects in the study. We expect that if we were to increase the sample size (essentially double it), the study would be .80 powered to have detect a $p < 0.05$ significant difference.

In addition, the case and control populations have different age and sex compositions. It's possible that the results in the study are confounded by these age and sex differences. For *HTR2C*, especially, which is found on the X chromosome, we would expect sex differences. However, when we covaried for age or sex, we did not find a difference in the results. Subsequent studies could examine genetic variation of these genes in larger, better selected populations of cocaine-dependent individuals and control subjects. Restricting the age range to make sure that age in the study sample is not responsible (a confounder) for cognitive performance would be beneficial. In future studies, it would also be beneficial to recruit an equal number of subjects with specific genotypes for *HTR2A* and *HTR2C* as well as *TPH1* and *TPH2* to participate. This was not done in the current study.

For behavioral testing, the picture version of the cocaine Stroop Task could be administered to reduce inter-trial variability for the subjects and reduce the variance in response. Pictures might provide stimuli that are closer to the environmental triggers that subjects are exposed to in nature. We observed the biggest attentional bias difference between the cocaine and control groups in the first block of the Cocaine Stroop task. Before the subjects performed this block of the task, they completed 60 practice trials. In the future, we can also record subject responses during the practice trials to see if the attentional bias is even higher at this earliest stage. Dividing the task in more blocks might also be beneficial in allowing us to analyze learning processes that might take place in the progression of the task.

Early studies using variations of the Stroop task observed an effect for therapy. For example, patients with depression showed a greater response latency in naming the color of words associated with depressive symptoms compared to neutral and positive words. When these patients were tested again after therapy and clinical improvement, they did not display this difference in performance (Gotlib and Cane 1987). Similar results were observed in patients with specific phobias and anxiety (reviewed in (Williams, Mathews et al. 1996). Perhaps in future studies, we can test subjects before and after they undergo treatment. This way we can investigate the role of serotonin genetics and treatment on attentional bias scores in cocaine dependent subjects.

In animal models, the serotonin transporter is reported to interact with the products of other genes, including ones encoding serotonin receptors, other neurotransmitter systems and neurodevelopmental proteins (Homberg, Nijman et al. 2010) Serotonin transporter knockout mice provide a low transporter activity model that is similar to cocaine use. We may choose to focus on other variants that have been reported to be in linkage disequilibrium with the *5-HTTLPR*. For example, it's been reported that individuals with a low *5-HTTLPR* variant and a gain of function 5-HT₃ receptor variant were 2.5 more likely to be diagnosed with alcohol and drug dependence (Enoch, Gorodetsky et al. 2010) In future studies, we may study this variant.

Another aspect of genetic variation that needs to be addressed is the possibility that observed changes are due to epigenetic mechanisms, such as DNA methylation, histone modifications and miRNA. A complex fine-tuning of neurotransmitters occurs in the central nervous system and individuals with different genetic backgrounds all undergo adaptations that establish a homeostatic level of functioning. Epigenetic changes, however, might reflect more recent changes in gene regulation which might be better suited to extrapolation due to environmental exposures such as cocaine.

7 – CONCLUSIONS

Our results suggest that the serotonin system is related in the modification of attentional bias, and to a lesser extent, impulsivity in this population of cocaine dependent subjects. The data collected for this thesis did not support our hypothesis regarding variants that lead to lower serotonin function also lead to higher attentional bias and impulsivity. For the genetic variants in which we observed significant differences, our data supported the opposite relationship. Variants that led to higher serotonin function increased attentional bias.

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9 - CURRICULUM VITAE

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