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A clinical trial and fMRI study of the cognitive neuroscience of cocaine addiction and of the efficacy of the cognitive enhancer D-Cycloserine for its treatment

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A clinical trial and fMRI study of the cognitive neuroscience of cocaine addiction and of the efficacy of the cognitive enhancer D-Cycloserine for its treatment

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An abstract of
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Abstract

A clinical trial and fMRI study of the cognitive neuroscience of cocaine addiction and of the efficacy of the cognitive enhancer D-Cycloserine for its treatment
By Ashley Paige Kennedy

Cocaine dependence is a chronically relapsing disorder for which its predominant behavioral therapies are associated with only partial efficacy. The learning objectives of my dissertation research plan were primarily addressed by determining if the N-methyl-D-aspartate glutamate receptor partial agonist and cognitive enhancer, D-Cycloserine (DCS), could boost the cocaine abstinence and treatment retention goals of cognitive behavioral therapy (CBT). The research tested an overall hypothesis that enhanced brain glutamate neurotransmission facilitates the therapeutic learning and memory goals of addiction behavioral therapies and thus promotes recovery and relapse prevention in cocaine-dependent persons. The first placebo-controlled, randomized, double-blind study (Study 1) tested the safety and efficacy of once-weekly oral DCS (50 mg) combined with a condensed version of a manual-based CBT in cocaine-dependent men enrolled in the 4-week outpatient Substance Abuse Treatment Program at the Atlanta Veteran's Administration Medical Center. Relative to a 12-step based treatment-as-usual, an under-dosed CBT was associated with significant improvements in drug abstinence and treatment retention at 4-weeks and enhanced maintenance of drug abstinence after four more weeks of post-treatment follow-up. However, the CBT response posed a ceiling effect and DCS was no more effective than placebo in enhancing the response to CBT at the treatment endpoints of 4- and 8-weeks. The second study was an extended replication of Study 1 that differed in the dose of DCS (250 mg), the use of computer- rather than therapist-delivered behavioral therapy (cCBT) and repeated measures of functional magnetic resonance imaging (fMRI) to determine the impact of cCBT and adjunct DCS on the neural processing correlates of attentional bias for conditioned drug-associated cues. DCS was ineffective in facilitating the cCBT response and was associated with an increase in the frequency of cocaine-positive urine samples relative to placebo and did not enhance treatment retention in a cocaine-dependent sample. Neural responses to an addiction Stroop task partially supported this measure as a neurocognitive marker of relapse. These studies provide a more definitive picture of the efficacy of cognitive enhancement as a means of facilitating behavioral therapy outcomes for drug addiction and of the brain changes that code therapeutic response to treatment in cocaine-dependent populations.

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List of Abbreviations

Addiction Severity Index	ASI
African American	AA
anterior cingulate cortex	ACC
attention deficit hyperactivity disorder	ADHD
blood oxygen level dependent	BOLD
Barratt Impulsiveness Scale	BIS-11
Brain Imaging Research Center	BIRC
Brodmann area	BA
Caucasian	CA
central nervous system	CNS
childhood trauma questionnaire	CTQ
cocaine craving questionnaire	CCQ
cocaine Stroop task	cocStroop
Conners adult ADHD rating scale	CAARS
cognitive behavioral therapy	CBT
computerized cognitive behavioral therapy	cCBT
conditioned place preference	CPP
conditioned stimulus	CS
conditioned response	CR
contingency management	CM
cue exposure therapy	CET
d-Cycloserine	DCS
diagnostic and statistical manual of mental disorders--fourth edition	DSM-IV
dopamine transporter	DAT
dorsal anterior cingulate cortex	dACC
dorsal lateral prefrontal cortex	dlPFC
drug use-neutral	CS-
drug use-related	CS+
echo planar imaging	EPI
electronic medical records	EMR
false discovery rate	FDR
full-width- half-maximum	FWHM
functional magnetic resonance imaging	fMRI
general linear model	GLM
Hardy Weinberg equilibrium	HWE
hemodynamic response function	HRF
Hispanic	HS
inferior frontal cortex	IFC
life experience survey	LES
linkage disequilibrium	LD
long term depression	LTD
long term potentiation	LTP
minor allele frequency	MAF
Montreal Neurological Institute	MNI
multi-source interference task	MSIT
nucleus accumbens	NAc
N-methyl-D-aspartate	NMDA
obsessive compulsive disorder	OCD
orbitofrontal cortex	OFC
polymerase chain reaction	PCR

positive and negative affect schedule	PANAS
positron emission tomography	PET
posttraumatic stress disorder	PTSD
prefrontal cortex	PFC
Psychiatric Research Institute	PRI
psychophysiological interaction	PPI
reaction time	RT
region of interest	ROI
right inferior frontal cortex	rIFC
serotonin	5-HT
serotonin reuptake inhibitor	SSRI
single nucleotide polymorphism	SNP
small volume correction	SVC
social anxiety disorder	SAD
substance abuse treatment program	SATP
substantia nigra	SN
subthalamic nucleus	STN
superior parietal lobule	SPL
superior temporal sulcus	STS
supplementary motor area	SMA
state-trait anxiety inventory	STAI
Statistical analysis software	SAS
Stroop task	cStroop
structured clinical interview for DSM-IV disorders-non patients	SCID-NP
supplemental motor area	SMA
treatment as usual	TAU
tryptophan hydroxylase	TPH
tryptophan hydroxylase 2 gene	*TPH2*
unconditioned stimulus	US
University of Arkansas for Medical Sciences	UAMS
urinalysis drug screen	UDS
Veteran's Administration Medical Center	VAMC
volume of interest	VOI
5-hydroxyindoleacetic acid	5-HIAA

INTRODUCTION

For my multidisciplinary dissertation research project, I focused on controlled clinical trial methodology to evaluate a novel combination therapy for the treatment of cocaine dependence. The overall objective of the dissertation research project was to facilitate the following learning goals through the use of multiple vehicles:

i. Knowledge about clinical trial methodology, specifically in the conceptualization, design, conduct, analysis, and interpretation of clinical studies, was aided through the Masters of Science in Clinical Research (MSCR) track and hands-on experience conducting controlled clinical trials assessing the interaction of d-Cycloserine (DCS) and behavioral therapies for cocaine addiction.

ii. Training in the field of human neuroimaging was facilitated by exploring the neural mechanisms involved in the implicit processing of conditioned drug-associated cues and emotionally valenced words, and how altering the neural processing of these cues regulates susceptibility to relapse in cocaine-dependent subjects.

iii. Development of an understanding of the principles, efficacy, and limitations of addiction behavioral therapies was faciliated through the use of cognitive behavioral therapy (CBT) in clinical trials testing the efficacy of a novel combinational therapy for recovery and relapse prevention in a cocaine-dependent population.

iv. Knowledge and research skills related to clinical psychopharmacology and medication development, specifically in the translation of glutamate receptor pharmacology-related learning and memory to the development of more effective treatments for drug dependence, was enabled by investigating the response of cocaine-dependent individuals to the novel adjunct use of DCS as a means of boosting the clinical response to CBT for cocaine dependence.

v. Knowledge and skills related to the roles of genetic variation as a source of individual variation in neural processing correlates of psychiatric disorders was facilitated through an

fMRI study of the regulation of cognitive control processes by allelic variation for a haplotype of the tryptophan hydroxylase 2 (*TPH2*) gene.

vi. Development of a sound working knowledge of the practice of responsible conduct of research was accomplished throughout the dissertation research projects via courses in the MSCR track, ethics seminars in the Molecular and Systems Pharmacology (MSP) program, and mentored research supervision.

The following background represents the general theoretical framework for the dissertation research project. The subsequent chapters (1-5) represent the specific research training efforts related to my learning objectives.

Cocaine dependence and the neural basis of relapse

Cocaine dependence is characterized by the inability to control compulsive drug seeking and use despite the presence of clear adverse consequences, and is associated with a high rate of relapse following periods of abstinence. According to the 2008 National Survey on Drug Use and Health (Office of Applied Sciences, NSDUH Series H-36, HHS Publication No. 09-4434), approximately 36.8 million Americans aged 12 and older had tried cocaine at least once in their lifetimes with an estimated 722,000 new users of cocaine. Cocaine is a psychostimulant that binds to the dopamine transporter (DAT) and blocks the neuronal reuptake of dopamine (Kuhar et al., 1991). This action results in an increase in dopamine concentration in the synapse, which contributes to the pleasurable effects of cocaine. Intense drug craving precipitated by conditioned drug-associated reminders or cues is recognized as a risk factor for relapse (Kilts et al., 2004). Cue-induced craving is evoked by environmental cues such as people, places, and objects or interoceptive cues such as mood states (*e.g.*, negative affect state) that have been paired with the reinforcing effects of frequent, intermittent cocaine use through associative learning. Previous studies have revealed that drug craving increases with exposure to drug-related cues (Sinha et al., 2000). Functional neuroimaging approaches such as positron emission tomography (PET) and functional Magnetic Resonance Imaging (fMRI) have been applied to identify changes in

distributed neural activity that link conditioned drug cues with intense drug craving behaviors in cocaine addicted individuals (Bonson et al., 2002; Childress et al., 1999; Garavan et al., 2000; Grant et al., 1996; Kilts et al., 2004; Kilts et al., 2001; Kosten et al., 2006; Maas et al., 1998; Wang et al., 1999; Wexler et al., 2001). These studies indicated a functional anatomy for cue-induced craving that included the amygdala, anterior cingulate cortex (ACC), insula and nucleus accumbens (NAc) area (Bonson et al., 2002; Childress et al., 1999; Garavan et al., 2000; Grant et al., 1996; Kilts et al., 2004; Kilts et al., 2001; Maas et al., 1998; Wang et al., 1999; Wexler et al., 2001; Wilson et al., 2004). Dysregulated function of the ACC and the orbitofrontal cortex (OFC) is suggested to mediate core characteristics of drug addiction (Goldstein et al., 2007). Drug cue-related increases in drug craving could represent a marker for relapse vulnerability. However, although there are many studies associating drug use cues such as drug use mental imagery and drug paraphernalia with heightened drug motivation (Childress et al., 1999; Grant et al., 1996; Kilts et al., 2004; Kilts et al., 2001), no consistent association between drug cue-induced cocaine craving and time to cocaine relapse or frequency and amount of cocaine abuse in cocaine-dependent men and women was observed (Sinha et al., 2006). These findings suggest a complex relationship between the experiencing of drug use reminders, motivation for drug use, and patterns of drug abuse. Targeting the attenuation of responses to conditioned drug cues however remains a plausible strategy for the development of new treatments that target relapse prevention and recovery in drug-dependent populations. Indeed, this typically represents the target of current behavioral therapies for drug addiction (Carroll and Onken, 2005), though such treatments are associated with only partial efficacy in preventing relapse (Dutra et al., 2008).

Current behavioral therapies for drug dependence

Extant therapies promoting recovery and relapse prevention in drug-dependent individuals are largely represented by behavioral approaches such as CBT, twelve-step, and contingency management (Carroll and Onken, 2005). These behavioral therapies represent the oldest, most prevalent forms of therapy for the treatment of drug dependence and have been

established to be moderately effective in various drug-dependent populations. As a relapse prevention therapy, CBT is based on the principle that learning processes have an integral role in the acquisition and maintenance of drug dependence and that these same learning processes can be used to reduce drug use (Carroll, 1998). CBT has been demonstrated to be efficacious in reducing cocaine use (Covi et al., 2002), in the maintenance of cocaine abstinence (Maude-Griffin et al., 1998b) and may have long term durability in promoting drug abstinence (Carroll et al., 1994). Unlike CBT, which is rooted in associative learning processes, contingency management is based on operant conditioning in which behavior that is followed by positive consequences is more likely to be repeated in the future. In contingency management, subjects receive incentives or rewards (*e.g.,* vouchers) for meeting specific addiction-related treatment goals (*e.g.*, negative urine drug screen) (Carroll and Onken, 2005). Several studies utilizing contingency management have demonstrated the effectiveness of voucher-based incentives contingent on drug-free urine specimens in improving outcomes such as enhancing treatment retention, reducing drug use, and maintaining cocaine abstinence (Higgins et al., 2000a; Higgins et al., 2003; Higgins et al., 2000b; Petry et al., 2004). Twelve-step recovery programs such as Alcoholics Anonymous are widely used in the drug-dependent community and are spiritually based in which drug-dependent individuals adhere to a set of guiding principles for recovery. The efficacy of twelve-step therapies is limited in the literature; however, twelve-step participation in a given month predicted less cocaine use in the next month and subjects who increased their twelve-step participation during the first 3 months of treatments had significantly less cocaine use and lower addiction-related functional impairments (Weiss et al., 2005b). Although the aforementioned behavioral therapies are efficacious treatments for drug dependence, these behavioral therapy approaches are not effective in all drug-dependent individuals as treatment response is highly variable among treated individuals. The limited efficacy of these treatment approaches is evidenced by relapse rates of 40% to 60% within a year following treatment discharge (McLellan, 1998). Therefore, opportunities exist to pair traditional addiction behavioral

therapies (*i.e.*, CBT) with medications (*i.e.*, D-Cycloserine) to facilitate treatment-related recovery and relapse prevention.

The role of DCS, a partial agonist at the NMDA glutamate receptor, in learning and memory

N-methyl-D-aspartate (NMDA) glutamate receptors are ligand-gated ion channels that play a major role in learning and memory (Kaye et al., 2006). These receptors play a prominent role in excitatory transmission in the central nervous system (CNS) (Monahan et al., 1989) and are a subtype of glutamate receptors that requires both glutamate and glycine for activation (Priestley and Kemp, 1994). The receptor is a heterotetrameric cation channel comprised of two NR1 subunits that contain the glycine-binding site along with two NR2 subunits that contain the glutamate-binding site (Kaye et al., 2006). The activation of NMDA receptors opens the cation channels that are permeable to sodium, potassium, and calcium ions (Narahashi et al., 2004). An increase in intracellular sodium leads to neuronal depolarization and enhancement of synaptic activity. Repeated receptor activation results in long term potentiation (LTP) which is considered a cellular/molecular model for learning and memory (Collingridge and Bliss, 1987; Narahashi et al., 2004). NMDA receptor activation has been shown to be a necessary component for LTP induction, and blockade of this receptor by antagonists blocks induction of LTP (Collingridge and Bliss, 1987). NMDA receptors are important for the acquisition, reconsolidation and extinction of memories (Lee et al., 2006).

DCS, an analogue of d-alanine, was originally used clinically as an antibiotic for the treatment of tuberculosis but it has since been characterized as a partial agonist at the NMDA glutamate receptor (Hood et al., 1989; Priestley and Kemp, 1994; Watson et al., 1990). DCS does not bind to the NMDA receptor glutamate-binding site subunit but to the NR1 binding site on the NMDA glutamate receptor, the glycine recognition site. The effects of DCS on the NMDA receptor are dose-dependent and dependent on the occupancy of the glycine binding site by glycine. DCS at low doses could enhance the stimulating effects of endogenous glycine on the

NMDA receptor, whereas at high doses, DCS is expected to compete with glycine and act as a relative antagonist at the receptor (van Berckel et al., 1998).

Previous preclinical studies show that the partial agonist DCS facilitates learning and memory through actions at the NMDA glutamate receptor in a foot shock avoidance task (Flood et al., 1992), linear maze learning task (Quartermain et al., 1994), passive avoidance task and spatial learning task (Monahan et al., 1989), inhibitory avoidance task (Land and Riccio, 1999) and elevated plus-maze task (Rodgers et al., 2011). DCS has also been shown to enhance conditioned taste aversion learning (Davenport and Houpt, 2009; Nunnink et al., 2007), acquisition of conditioned flavor-taste preference (Golden and Houpt, 2007) and relearning of an olfactory discrimination task (Villarejo-Rodríguez et al., 2010). In healthy human populations, DCS facilitated recall of a conditioned fear memory (Kalisch et al., 2009) and declarative learning in both item-category and object location association tasks (Onur et al., 2010). The aforementioned preclinical and human studies emphasize the ability of DCS to augment learning acquisition and memory consolidation, thus serving as the theoretical impetus in the current study for DCS to boost the therapeutic learning and memory goals of behavioral therapy in cocaine-dependent subjects.

Facilitation of extinction with an NMDA receptor partial agonist

DCS has been shown to facilitate the extinction of conditioned fear and drug-related associations in animal models. Extinction is defined as the decline in the frequency or intensity of a conditioned response to a stimulus following the withdrawal of stimulus reinforcement and is thought to reflect learning rather than unlearning processes (Richardson et al., 2004). Extinction is an active learning process that forms a new conditioned stimulus (CS)-no unconditioned stimulus (US) association that inhibits the previously conditioned response (CR). Extinction also involves the consolidation of new memory traces that compete with or inhibit memories of prior conditioned associations.

DCS administration facilitated the extinction of conditioned fear in an animal model (Bouton et al., 2008; Ledgerwood et al., 2003; Ledgerwood et al., 2005; Lee et al., 2006; Walker et al., 2002; Weber et al., 2007; Woods and Bouton, 2006). These studies further indicate that the actions of DCS on extinction manifest when given either before or after the extinction session and perhaps involve facilitation effects on the consolidation of the extinction memory. These studies served as the impetus for the recent clinical studies of DCS facilitation of extinction processes related to exposure psychotherapy in fear-related anxiety disorders (see below). Most animal studies documenting the facilitating effect of DCS on extinction learning utilize paradigms involving fear conditioning to an aversive US such as foot shock. DCS has also been demonstrated to augment extinction following appetitive operant conditioning (*e.g.,* level press for food) (Shaw et al., 2009) and in a conditioned place preference (CPP) paradigm pairing an environmental space with cocaine administration, DCS facilitated extinction of the CPP (Botreau et al., 2006; Kelley et al., 2007; Paolone et al., 2009; Thanos et al., 2009; Yang et al., 2010).

Consistent with the ability of DCS to facilitate the extinction of appetitively conditioned behaviors, DCS also facilitated the extinction of cocaine self-administration in rats and attenuated reacquisition of cocaine self-administration to a CS in rats and monkeys (Nic Dhonnchadha et al., 2009). In two separate studies, DCS enhanced extinction of conditioned ethanol-seeking behavior (Vengeliene et al., 2008) and impaired subsequent reconditioning of the extinguished ethanol-conditioned place preference (Groblewski et al., 2009). These findings demonstrate that the facilitating effect of DCS on extinction is not restricted to aversive conditioning but can be extended to appetitive conditioning, and specifically to the extinction of conditioned drug reinforcement. The current thesis research assessed the extent to which the pro-extinction effects of DCS in animal models of cocaine reinforcement and relapse generalize to human addiction. In animals, reinstatement can occur after extinction of conditioned fear upon re-exposure to the US in the absence of the CS, and this phenomenon may underlie the return to long-term drug abuse in addicts that relapse. DCS has been demonstrated to prevent reinstatement-induced relapse

(Ledgerwood et al., 2004) and reduce cue-induced reinstatement (Torregrossa et al., 2010) in animal models. This preclinical behavioral pharmacology suggests that DCS could potentially facilitate new therapy-related learning and memory in human drug abuse disorders and prevent cue-induced relapse long after DCS administration, a contention supported by recent DCS augmentation of the clinical response to exposure-based therapies in human anxiety- and fear-related disorders.

Augmentation of exposure-based therapy for anxiety disorders with a cognitive enhancer, DCS

Similar to the preclinical literature demonstrating the facilitating properties of DCS in fear conditioning paradigms, DCS has also been shown to facilitate exposure-based therapy in fear- and anxiety-related disorders. Adjunct DCS was associated with reduced symptomatology in panic disorder (Otto et al., 2010), social anxiety disorder (SAD) (Guastella et al., 2008; Hofmann et al., 2006), obsessive compulsive disorder (OCD) (Kushner et al., 2007; Wilhelm et al., 2008) and acrophobia (*i.e.*, fear of heights) (Ressler et al., 2004). However, DCS administration did not facilitate the reduction of spider fear (Guastella et al., 2007) or the extinction of conditioned fear (Guastella et al., 2007) in non-clinical populations. As a combination therapy, DCS did not further reduce associated symptoms in two separate OCD populations - patients with concomitant serotonin reuptake inhibitor (SSRI) treatment (Storch et al., 2007) and in a pediatric sample (Storch et al., 2010). In all of the aforementioned clinical studies, DCS was well tolerated and was not associated with adverse side effects even at the 500 mg dose used in the acrophobia study (Ressler et al., 2004). Another commonality across the clinical studies was the use of short-term dosing of DCS. The use of short-term, once-a-week dosing of DCS as opposed to longer-term, more frequent daily dosing of DCS was guided by preclinical evidence of dependence of the pro-cognitive effects of DCS on DCS dosing frequency. In a linear maze learning task, mice chronically treated daily with 3 mg/kg of DCS for two weeks prior to training on the maze did not have enhanced performance compared to acute treatment with 3 mg/kg of DCS (Quartermain et al., 1994). It has also been demonstrated that the

effectiveness of DCS in enhancing the extinction of conditioned fear is eliminated by chronic exposure to the drug (Parnas et al., 2005; Werner-Seidler and Richardson, 2007). Desensitization of the NMDA glutamate receptor complex at its strychnine-insensitive glycine binding site has also been demonstrated in cell culture with prolonged exposure to DCS (Boje et al., 1993).

D-Cycloserine as an adjunctive therapy for drug dependence

The goal of many addiction therapies is relapse prevention due to the drug conditioned stimulus (CS) no longer predicting the conditioned response (CR) represented by drug seeking and use behaviors. This can be accomplished by either extinction of the CR to a CS (*e.g*., cue exposure therapy (CET)) or by changing contingencies such that the drug CS now predicts a different CR (*e.g.,* CBT). However, the use of comparable exposure-based therapies such as CET to extinguish the learned associative values of drug use cues in drug-addicted individuals have met with largely negative results (Conklin and Tiffany, 2002) and, in at least one instance, an increases in relapse rates (Marissen et al., 2007). In contrast, CBT focuses on treating drug dependence as a learned behavior with the underlying assumption that learning and memory processes play an important role in the development and maintenance of drug dependence. The goal of CBT is to enable drug-dependent individuals to learn and remember a distinct, non-predictive association between conditioned drug cues and drug seeking and use behaviors operationalized as the learning and deploying of coping skills to counter drug use urges (Carroll, 1998). Unlike CET (*e.g.*, effect size = 0.0868) (Conklin and Tiffany, 2002), controlled clinical trials have demonstrated the efficacy of CBT in producing significant, enduring drug abstinence in cocaine-dependent populations (Carroll et al., 2004; Carroll et al., 2005). Although addiction behavioral treatments such as CBT that reduce the susceptibility to relapse by targeting the attenuation of drug cue-related cocaine cravings, they are not effective in all addicted individuals and response is highly variable among treated individuals (Epstein et al., 2003). Current treatments for drug addiction exhibit weaknesses in studies where long term abstinence is the primary outcome of interest and no treatment method has yet been shown to completely and

effectively treat cocaine dependence (Penberthy et al., 2010). The consistent observation of limited efficacy of current addiction therapies (Dutra et al., 2008) represents a major driving force to test a combined pharmacotherapy and psychotherapy treatment plan to decrease recidivism in treatment seeking cocaine-dependent individuals**.**

Estimating the incentive motivational properties of drug-conditioned stimuli in drug-dependent persons

Cocaine addiction is a chronic relapsing disorder, consisting of periods of abstinence followed by relapse to drug use, often precipitated by exposure to drug-related cues (Shaham et al., 2003). Relapse to drug seeking in drug-dependent individuals is strongly dependent on the learned associations formed between drug-related cues and the rewarding effects of the drug (Robinson and Berridge, 1993). These long-term associative memories can be associated with attentional bias for drug-associated stimuli that reflects their acquired incentive motivational properties (Field and Cox, 2008). The level of attentional bias for drug-related stimuli and its neural processing correlates could serve as a biomarker of risk for relapse, and a measure of response to treatment.

The Stroop task is a test of control of cognitive interference in which a non-target stimulus attribute (*e.g.*, word meaning) interferes with the processing of a target stimulus attribute (*e.g.*, word color) (Stroop, 1935). The addiction Stroop task is a measure of an individual's ability to suppress cognitive interference from drug use-related words (Field and Cox, 2008). In this thesis research, we used an fMRI-compatible Stroop task version, the counting Stroop task (Bush et al., 1998), as a foundation task for assessing attentional bias to conditioned cocaine use cues. This addiction Stroop task involves attending to a relevant feature (*e.g.*, counting of words) while ignoring a salient but task-irrelevant feature (*e.g.*, semantic content of drug-related word). The level of cognitive interference (attentional bias) for drug-related stimuli has been demonstrated in various versions of addiction Stroop tasks in diverse drug-dependent populations (*e.g.*, cocaine, alcohol, heroin) (Carpenter et al., 2006; Copersino et al., 2004; Cox et al., 2002; Field et al.,

2007; Hester et al., 2006; Marissen et al., 2006; Vadhan et al., 2007). Several studies have utilized variations of the addiction Stroop task to predict treatment outcomes in drug-dependent populations (Cox et al., 2002; Janes et al., 2010; Marissen et al., 2006; Waters et al., 2003). In a cocaine-dependent sample, a greater Stroop interference effect was associated with decreased treatment retention and a greater proportion of cocaine-positive urines (Carpenter et al., 2006). Similarly, the level of attentional bias (interference effect) for drug-use related stimuli has been associated with the strength of cocaine cravings and heroin cravings (Copersino et al., 2004; Franken et al., 2000a; Franken et al., 2000b). Performance on modified versions of the Stroop task has also predicted binary outcomes measures such as treatment retention and relapse in cocaine- and heroin-dependent subjects (Marissen et al., 2006; Streeter et al., 2008).

In the current study, a counting addiction Stroop task (cocStroop) assessed the impact of cocaine dependence and of a novel combination therapy on the inhibition of cognitive interference by personal cocaine use-related stimuli (Chapters 2, 3 and 5). Moreover, dysphoria is often associated with withdrawal in drug dependence and with heightened risk for relapse (Koob and Volkow, 2009). To disambiguate the impact of conditioned drug cues and dysphoric states on relapse an emotional Stroop task (eStroop) served as a measure of an addict's ability to suppress cognitive interference from negative emotionally valenced words (Chapter 5). The classical counting Stroop task (cStroop) (*e.g.*, congruent and incongruent stimuli) served as a measure of general cognitive control for the specific cocStroop and eStroop measures. Understanding the comparative cognitive and neural processing of drug-associated cues afforded an opportunity for this dissertation research project to inform the mechanisms of relapse in cocaine dependence and of the brain responses to treatment that regulate susceptibility to relapse.

<u>CHAPTER 1:</u> A common TPH2 haplotype regulates the neural processing of cognitive control for the Multi-Source Interference Task (MSIT) (Submitted to the American Journal of Medical Genetics Part B: Neuropsychiatric Genetics)

INTRODUCTION

According to the National Institute of Mental Health (NIMH), about 26% of adults age 18 and older suffer from a psychiatric disorder in a given year with mental health disorders the leading cause of disability in the US (http://www.nimh.nih.gov/). Putative risk factors that increase susceptibility to psychiatric disorders include psychosocial risk factors such as lack of social support or childhood trauma, and biological risk factors such as genetic predisposition, sex, and family history, as well as the interaction between these two types of risk factors (Kendler et al., 2003). Biological risk factors prove to be the more complex as sex and family history are fixed with genetic predisposition varying across the population. The investigation of the role of genetic variation in conferring vulnerability for psychiatric disorders has focused on serotonin (5-HT) neurotransmission, specifically for the involved receptors and transporters, and enzymes responsible for 5-HT synthesis and degradation (D'Souza and Craig, 2008).

The synthesis of 5-HT from tryptophan is catalyzed by the rate-limiting enzyme tryptophan hydroxylase (TPH) and thus TPH critically regulates the availability of serotonin for neurotransmission (Brown et al., 2005; Harvey et al., 2007; Van Den Bogaert et al., 2006; Zhang et al., 2004; Zhou et al., 2005). Two isoforms of TPH exist as distinct gene products with TPH1 preferentially located in the periphery while TPH2 is solely localized in the brain (Walitza et al., 2005) where it is present predominantly in the serotonin cell body-rich pontine raphe nuclei (Bach-Mizrachi et al., 2006). The gene (*TPH2*) encoding the human TPH2 is located on chromosome 12q21, consists of 11 exons, and so far more than 300 single nucleotide polymorphisms (SNPs) have been reported for this locus (de Lara et al., 2007; Harvey et al., 2007; Zhang et al., 2006; Zhou et al., 2005). Genetic association studies indicate that common putatively functional polymorphisms in *TPH2* influence susceptibility to diverse psychiatric disorders linked to a dysregulation in serotonin neurotransmission and alterations in self-regulation (Bach-Mizrachi et al., 2006; Brown et al., 2005; Van Den Bogaert et al., 2006; Zhou et al., 2005). Single marker and haplotype associations of *TPH2* variation with suicidality (De Luca et al., 2005; Haghighi et al., 2008; Ke et al., 2006; Lopez et al., 2007; Luca et al., 2006; Van Den

Bogaert et al., 2006; Yoon and Kim, 2009; Zhang et al., 2004; Zhou et al., 2005; Zill et al., 2007), bipolar disorder (Lin et al., 2007; Lopez et al., 2007; Roche and McKeon, 2009), attention deficit hyperactivity disorder (ADHD) (Baehne et al., 2008; Brookes et al., 2006; Manor et al., 2008; Sheehan et al., 2005; Walitza et al., 2005), borderline personality disorder, (Perez-Rodriguez et al., 2010)and anxiety disorders (Kim et al., 2009; Zhou et al., 2005) have been reported.

Deficits in decision-making and other cognitive functions are proposed to represent vulnerability factors for psychiatric disorders (Aron and Poldrack, 2005; Becker et al., 1999; Dombrovski et al., 2008; Jollant et al., 2005; Jollant et al., 2007; Marzuk et al., 2005; Rubinsztein et al., 2006; Taylor Tavares et al., 2007; Westheide et al., 2008). Manipulations of brain serotonin content by tryptophan depletion and augmentation (Williams et al., 1999) indicate that serotonergic neurotransmission modulates prefrontal cortical activity related to cognitive and inhibitory control (Evers et al., 2006; Morgan et al., 2007; Rubia et al., 2005; Talbot and Cooper, 2006). The association of *TPH2* variants with risk for mental disorders may thus be mediated by their regulation of intermediate cognitive phenotypes of dsyregulated behavior. Indeed, putative functional *TPH2* variants such as a promoter SNP (rs4570625) or of an intron 8 SNP (rs1386483) were associated with impaired cognitive control and response inhibition task performance (Baehne et al., 2008; Osinsky et al., 2009; Reuter et al., 2008; Reuter et al., 2007; Stoltenberg et al., 2006).

This study sought to inform the genetics of risk for psychiatric phenotypes by the use of intermediate cognitive and neural processing phenotypes (*i.e.*, interference suppression) in an imaging genomics approach (Meyer-Lindenberg and Weinberger, 2006) in a general population sample. [Intermediate cognitive and neural processing phenotypes are defined as such that “gene effects at the level of the brain are a more direct effect of genetic variation than complex behavior and will show associations in carrier of risk alleles even if carriers show no clinical diagnostic characteristics” (Meyer-Lindenberg and Weinberger, 2006).] We focused on the two most common *TPH2* 6-locus haplotypes that are in a yin and yang configuration with the yin haplotype

representing the risk haplotype for suicidality, mood and anxiety disorders, and elevated CSF 5-HIAA (Zhou et al., 2005) and the yang haplotype as a risk variant for ADHD (Manor et al., 2008). We hypothesized that allelic variation for the *TPH2* yin haplotype confers risk for psychiatric phenotypes by regulating the neural processing of demands for cognitive control. As a cognitive control demand, the Multi-Source Interference Task (MSIT) combines multiple sources of cognitive interference (Stroop, Eriksen Flanker, Simon effect) with decision making and activates the dorsal anterior cingulate cortex (dACC) and associated brain areas involved in a cognitive control network (Bush and Shin, 2006; Bush et al., 2003). We observed that increasing numbers of copies for the *TPH2* yin haplotype was associated with increasing magnitude of MSIT-related response by the dACC and other brain areas, and with decreased functional connectivity for dACC pathways subserving cognitive control.

MATERIALS AND METHODS

Subjects

Thirty one study volunteers were enrolled, of which 28 healthy adult (ages 18-50 years, 32.9 ± 8.2 years) men and women (12 M/16F) of varying racial backgrounds (15 Caucasian, 8 African-American, 5 Pacific Asian) contributed intact behavioral and fMRI datasets. Subject demographics are summarized in Table 1.1. Written informed consent was obtained for participation in a research protocol approved by the Emory University Institutional Review Board. All subjects were evaluated using the Structured Clinical Interview for DSM-IV Disorders (SCID-NP) (First, 2002) to rule out any Axis-1 psychiatric diagnoses. Exclusion criteria included any history of or current mental illness, history of or current medical illness, history of or current drug or alcohol abuse except nicotine, current psychotropic medications, loss of consciousness greater than 1 min, and pregnancy or breastfeeding for female subjects. All subjects were screened for any metallic objects, (*e.g.*, cardiac pacemakers), medications (*e.g.*, beta-blockers), or claustrophobic contraindications for the fMRI component of the study. Genomic DNA was prepared from blood samples collected from an antecubital vein. All subjects were compensated

Table 1.1 Subject demographic and task performance (Mean +/- SD) (C= Caucasian, AA= African America, PA= Pacific Asian) (*p < 0.001).

Sex (M/F)	12/16
Age, y	32.9 ± 8.2
Race/Ethnicity	15C/8AA/5PA
Education, y	17.6 ± 3.3
Handedness (R/L)	22/6
MSIT Performance	
*Reaction Time, msec	
Control Trial	716 ± 121
Interference Trial	954 ± 160
*Task Accuracy, %	
Control Trial	99.1 ± 2.7
Interference Trial	91.1 ± 8.3

for their participation.

Genotyping

16 SNP markers within the *TPH2* locus were selected for genotyping based on a previous study (Zhou et al., 2005) and to tag 93 kb of *TPH2* and 5 kb of the flanking sequence (Table 1.2). The 6 SNPs used for haplotype analysis are highlighted in Table 1.2. Genotyping was conducted using pre-designed TaqMan allelic discrimination assays developed for use on the 7900HT instrument (Applied Biosystems Inc, Foster City, CA). Reactions were performed in 384-well plates in a 5.0 ul volume containing 1.0 ng/ul of genomic DNA, 2.5 ul of Master Mix, and 0.08 ul of On Demand Assay Mix (Applied Biosystems Inc). Polymerase chain reactions were performed at 50°C for 2 minutes, and 95°C for 10 minutes, followed by 40 cycles each of 95°C for 15 seconds and 60°C for 1 minute. Sequence Detection System (SDS 2.2) software was used for allelic discrimination. Genotyping results were tested for Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) and haplotype block structure were evaluated using Haploview version 3.3.2. *D*' values were computed for all marker pairs and haplotype boundaries were defined using the method of Gabriel et al., (2002) (Figure 1.1). We estimated individual haplotypes of the 6 SNP haplotype using SNPHAP (David Clayton: http://wwwgene.cimr.cam.ac.uk/clayton/software/). These six SNPs span a 52 kb region at the 5' end of *TPH2* and encompass intron 5, exon 7 and intron 8 (Figure 1.1). A particular cis-acting variant of these six SNPs constituting the previously reported *TPH2* yin (212121) or risk haplotype (Zhou et al., 2005) (Table 1.2) was the focus of the proposed study of the influence of allelic variation for *TPH2* on the neural representation of cognitive control. Subjects were classified as carrying 0, 1, or 2 copies (*e.g*., -/-, +/- and +/+) of the *TPH2* yin haplotype.

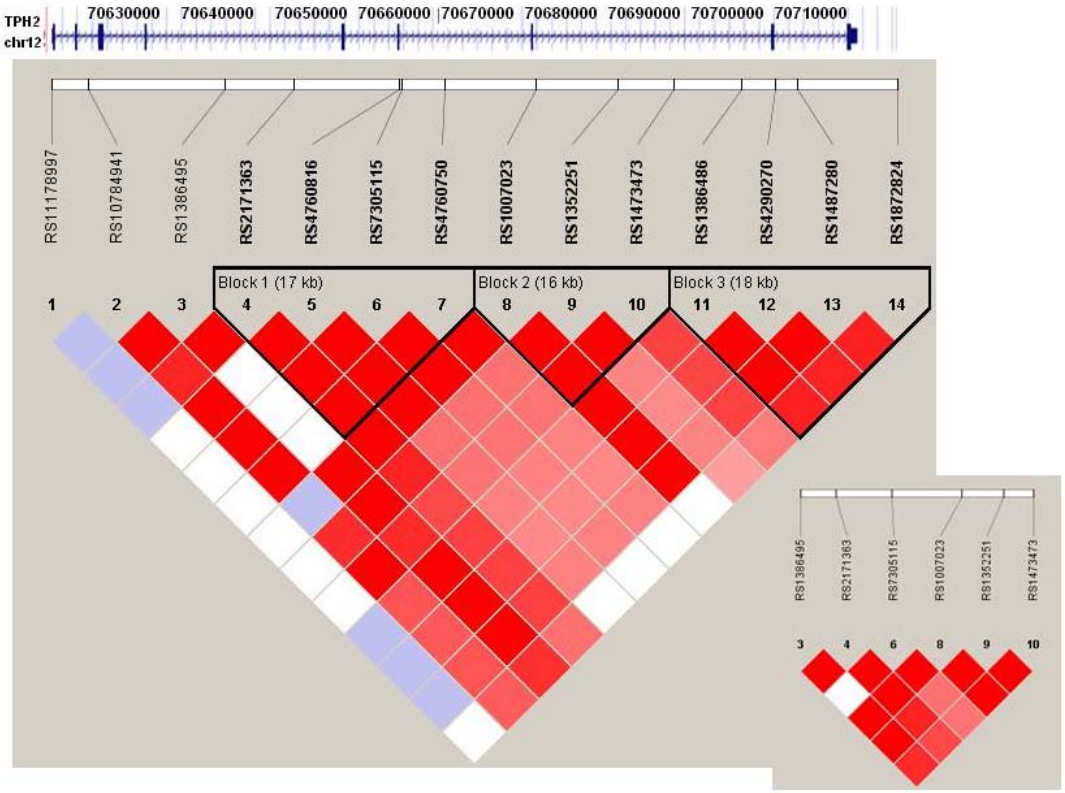
Multi-Source Interference Task (MSIT)

Cognitive control-related brain activity was measured via blood oxygen level dependent (BOLD) contrast fMRI during the MSIT (Bush and Shin, 2006). The effect of the *TPH2* yin

Table 1.2 Single Nucleotide Polymorphisms Identified in *TPH2*. 6 SNPs (red) define the *TPH2* yin haplotype. (Position according to the University of California Santa Cruz (UCSC) Genome Browser; http://genome.ucsc.edu/cgi-bin/hgGateway.) Resulting changes in amino acids for exons: rs34115267 Exon2 Leu36Val; rs7305115 Exon 7 Pro312Pro; rs4290270 Exon 9 Ala375Ala. MAF = minor allele frequency, HWE = p value for test of Hardy Weinberg equilibrium.

#	I.D. No.-SNP ID	Location	Position on chr. 12	MAF	HWE
1	rs11178997	Promoter	70618420	0.083	0.153
2	rs34115267	Exon 2	70621631	0.000	n/a
3	rs10784941	Intron 2	70622779	0.400	0.480
4	rs1386495	Intron 5	70638589	0.300	0.420
5	rs2171363	Intron 5	70646531	0.457	0.499
6	rs4760816	Intron 6	70658868	0.387	0.475
7	rs7305115	Exon 7	70659129	0.387	0.475
8	rs4760750	Intron 7	70664156	0.387	0.475
9	rs1007023	Intron 8	70674641	0.194	0.312
10	rs1352251	Intron 8	70684161	0.452	0.495
11	rs1473473	Intron 8	70690645	0.242	0.367
12	rs1386486	Intron 8	70698487	0.484	0.499
13	rs4290270	Exon 9	70702502	0.500	0.500
14	rs1487280	Intron 9	70705094	0.484	0.499
15	rs41317120	Intron 10	70711517	0.065	0.701
16	rs1872824	Intergenic	70716581	0.467	0.498

Figure 1.1 Linkage disequilibrium of 14 SNPs across 93 kb of the *TPH2* gene. The relative positions of the 14 markers are shown at the top of the figure. Haplotype boundaries were defined using the method of Gabriel et al., (2002). LD is represented with the D' measure with red squares connecting SNPs in complete LD (D' = 1) and white squares connecting SNPs with no LD (D' = 0). Shades of pink represent D' values between 0 and 1. Inset: *TPH2* 6 SNP haplotype. SNP-based haplotypes were constituted by six SNPs spanning a 52 kb region including haplotype block 1 and 2.



haplotype on the engagement of cognitive control processes was assessed by the influence of allelic variation for the *TPH2* yin haplotype on neural activity related to suppressing cognitive interference. For the MSIT (Bush et al., 2003), subjects were instructed to use their index, middle and ring fingers to report the identity of the number in a 3-number array that was different from the other two numbers, regardless of its position in the array. Individuals were instructed that the buttons on a response keypad represented the numbers “one”, “two”, and “three” from left to right. For *interference* trials, the non-target distracters were other numbers, and target numbers were either larger or smaller than distracter numbers and their location in the array was always incongruent with their designated position on the response pad. For *control* trials, the non-target distracters were the letter “x”, and target numbers were always larger than distracters and their location always congruent with their designated position on the response pad (Bush et al., 2003). Stimuli were presented in the center of the visual field for 2 sec with an interstimulus interval of 2 sec. The contrast of successful responses to interference and control trials isolated the neural processing correlate of attempts to suppress cognitive interference while controlling for sensorimotor task demands. Following instruction of the task conditions, a practice session consisting of 10 interference and 10 control trials was used to familiarize subjects with the task demands and to ensure task comprehension. A session contained 56 control and 33 interference trials presented in a random order. An analysis of variance (ANOVA) compared MSIT error rates and mean stimulus reaction times between groups differing in *TPH2* yin haplotype carrying status (0, 1, or 2 copies) with statistical significance defined by $p < 0.05$.

A possible non-specific effect of *TPH2* variation on task-related BOLD response was assessed by the effect of *TPH2* yin haplotype on the response of primary and association visual cortex regions-of-interest (ROI) to a visual checkerboard task. The visual checkerboard task consisted of two alternating visual stimulus conditions. The *fixate* condition consisted of a small, centrally located crosshair onto which subjects were instructed to visually focus. The *checkerboard* condition consisted of a checkerboard pattern alternating at a frequency of 1 Hz.

The BOLD contrast for the checkerboard > fixate condition was examined for main effects of task.

fMRI Acquisition and Image Analysis

fMRI data were acquired on a Siemens 3T Trio scanner using standard echo planar (EPI) BOLD contrast imaging. A total of 125 scans (TR = 2.02 sec, slices = 30, 3 x 3 x 3 mm voxel size) were collected for the MSIT. All image pre-processing and analyses were carried out in MATLAB and SPM5. Images for each subject were slice time-corrected, realigned to the first volume in the time series, and motion corrected. Images were spatially normalized to a standard stereological space (Montreal Neurological Institute (MNI)) using a 12-parameter affine model. Images were resampled to a 2 x 2 x 2 mm voxel size and spatially smoothed using a Gaussian filter (8 mm full-width – half-maximum (FWHM)). Cognitive control-related activation was defined by planned contrasts of the interference and control task conditions (Int-Cont) in a first-level analysis [$p < 0.05$, false discovery rate (FDR) corrected]. These contrasts were entered into a second-level group analysis treating intersubject variability as a random effect. Contrast values from select ROIs (5 mm sphere) from this analysis (main effect of task) were plotted to illustrate a relationship between neural activation and number of copies of the *TPH2* yin haplotype. To assess an effect of allelic variation for the *TPH2* yin haplotype on MSIT-related neural activity the first-level contrast images were entered into a second-level, brain-wide ANOVA general linear model (GLM) with *TPH2* yin haplotype as the independent variable coded as 0,1, or 2 according to the number of copies of the *TPH2* yin haplotype. Post hoc analyses compared the effects of the different *TPH2* haplotypes on the magnitude of the MSIT-related neural activation. To assess a dose-dependent effect of the *TPH2* yin haplotype, we entered the first-level contrast images into a second-level regression analysis with number of the *TPH2* yin haplotypes as a covariate. A similar regression analysis of the effect of *TPH2* yin haplotype was performed for the striate and extrastriate cortex response to the visual checkerboard task. In both cases, regression contrasts were explicitly masked by the main effect of task defined by entering each

subject's first-level contrast image into a one-sample t-test ($p < 0.05$, FDR corrected for multiple comparisons).

Functional Connectivity Analysis

To test the hypothesis that observed increased MSIT-related activation of the dACC and right inferior frontal cortex (IFC) with increasing allele load for the *TPH2* yin haplotype is associated with decreased functional integration within the cognitive control network we determined the impact of *TPH2* genotype on functional connectivity for the dACC and right IFC seeds. For the dACC and right IFC, functional clusters identified from the analyses of the main effect of task (Table 1.3) were used as seed regions in functional connectivity analyses using the psychophysiological interaction (PPI) routine in SPM5. This form of functional connectivity estimation (Friston et al., 1997) defines by correlation if the reference waveform of temporal activity in the seed region related to the MSIT task covaries with that of voxels in other brain areas. Each seed region was defined by a 5 mm radius sphere centered on the voxel maxima for the Int-Cont condition contrast ($p < 0.05$, FDR corrected). Seed region time-series were extracted for each individual, adjusting for motion artifacts and time and dispersion derivatives. Linear regressors for the Int-Cont contrast and the seed x contrast interaction were created for each individual. A first-level model for each individual was constructed that included the adjusted seed time series, Int-Cont contrast, seed x contrast interaction, and motion parameters as regressors. Contrast maps for each individual were then created for the comparison of the seed x contrast interaction relative to zero. To assess the influence of the number of copies of the *TPH2* yin haplotype on the mode of dACC and right IFC functional connectivity, these contrast values for the interaction term were entered into a second-level multiple regression analysis to identify those functionally coupled regions in which intercorrelated task-related activity was modulated by the number of copies of the *TPH2* yin haplotype across subjects. Since the neuractivation magnitude analysis of the main effect of *TPH2* yin haplotype revealed a significant difference between individuals with two versus no copy of the *TPH2* yin haplotype, functional connectivity analysis

Table 1.3 Effect of *TPH2* yin haplotype on MSIT-related neural activation and functional connectivity. ([a]F-statistic) (Abbreviations: SMA = supplementary motor area, dACC = dorsal anterior cingulate cortex, dlPFC = dorsal lateral prefrontal cortex, PFC = prefrontal cortex, rIFC = right inferior frontal cortex, BA = Brodmann Area)

Regions	BA	Voxel T	Cluster size (# of voxels)	Talairach Coordinates		
				X	Y	Z
Main Effect of Task (p< 0.05, FDR corrected)						
Left Superior Parietal Lobule	7	5.47	69	-24	-66	45
Left Precentral Gyrus/SMA	4/6	5.22	14	-33	-6	63
Right Superior Parietal Lobule	7	5.2	16	24	-69	60
Right Middle Occipital Gyrus	19	4.65	2	36	-84	15
dACC/pre-SMA	6/32	4.44	2	-6	9	54
Right Caudate Nucleus		4.39	6	21	-3	21
Right Inferior Frontal Gyrus	44	3.97	13	45	3	33
Main Effect of Genotype (Threshold: p< 0.005, uncorrected) (k ≥ 5)						
Left Inferior Frontal Gyrus	46	16.2[a]	28	-27	30	12
Right Caudate Nucleus		14.2[a]	34	6	21	12
Right SMA	6	11.8[a]	11	6	-12	66
Left Medial Frontal Gyrus	9	11.3[a]	32	-18	42	24
Left Middle Frontal Gyrus	46	11.0[a]	10	-27	42	3
Functional Connectivity (Threshold: p< 0.005, uncorrected) (k ≥ 5)						
Reference voxel: dACC						
Increased Connectivity						
Left SMA	6	3.48	5	-15	-9	60
Superior Temporal Sulcus (STS)	22	3.33	7	-36	-45	21
Decreased Connectivity						
Left Inferior Frontal Sulcus (pars triangularis)	45	3.22	6	-42	24	21
Right Middle Frontal Gyrus	46	3.41	5	51	39	15
Right Dorsomedial PFC	10	3.26	7	24	57	9
Reference voxel: rIFC						
Increased Connectivity						
Left Inferior Frontal Gyrus	47	3.08	5	-27	12	-12
Decreased Connectivity						
Right Precuneus	7	3.35	6	9	-36	48
Left Middle Frontal Gyrus	10	3.27	12	-36	42	21
Left Paracentral Lobule	7	2.91	6	-6	-45	60

was restricted to comparisons between individuals with 0 versus 2 copies of the *TPH2* yin haplotype. Regions exhibiting significant temporal correlation were identified by thresholding at $p < 0.005$, uncorrected, and a spatial cluster extent of 5 contiguous voxels.

RESULTS

Linkage Disequilibrium of TPH2 SNPs and Haplotype Frequencies

All SNPs were in Hardy-Weinberg equilibrium. Three haplotype blocks for the *TPH2* gene could be defined in the ethnically heterogeneous sample population: block 1 (17 kb, SNP 4 to SNP 7 encompassing intron 2, intron 5 and intron 8), block 2 (16 kb, SNP 8 to SNP 10, encompassing exon 7 and intron 8), and block 3 (18 kb, SNP 11 to SNP 14, encompassing intron 8 and exon 9) (Figure 1.1). For the analysis, the previously reported 6 SNP haplotype (see inset Figure 1.1) was considered that spanned haplotype block 1 and 2 which are still in high LD. Here nine haplotypes were observed in this population and the two most common haplotypes were in a yin and yang configuration (Table 1.4). Of the study volunteers, eight subjects had 0 copies of the *TPH2* yin haplotype, twelve had 1 copy of the *TPH2* yin haplotype, and eight had 2 copies of the *TPH2* yin haplotype.

MSIT Performance

As a group, the study participants demonstrated a significant ($p < 0.001$) cognitive interference effect for the interference versus control trials of the MSIT (Table 1.1). The subjects also exhibited a significantly lower ($p < 0.001$) response accuracy for the interference versus control trials (Table 1.1). Individuals differing in the number of copies of the *TPH2* yin haplotype did not exhibit significant differences in mean reaction times or error rates for interference trials, control trials, or for the interference effect (p's > 0.159).

Main Effect of Task and Effect of TPH2 Haplotype

Consistent with previous findings (Bush and Shin, 2006; Bush et al., 2003; Yucel et al., 2007), the contrast of interference and control trials for the MSIT was associated with activation ($p < 0.05$, FDR corrected) of the bilateral posterior parietal cortex, visual cortex, dACC/pre-

Table 1.4 Common *TPH2* 6 Locus Haplotypes.

Haplotype	1	2	3	4	5	6	Frequency
Yin	A	G	G	T	T	T	0.468
Yang	G	A	A	G	C	C	0.194
Hap3	A	A	A	T	C	T	0.129
Hap4	G	A	G	T	C	T	0.081
Hap5	A	A	A	T	T	T	0.048
Hap6	A	A	G	T	T	T	0.032
Hap7	A	A	G	T	C	C	0.016
Hap8	A	G	G	T	C	C	0.016
Hap9	G	A	A	T	C	C	0.016

supplementary motor area (SMA), dorsal sensorimotor striatum, and pars opercularis of the right inferior frontal cortex (Table 1.3, Figure 1.2A). This localization analysis was followed by a volume of interest (VOI) analysis of the MSIT-related dACC response. The BOLD response for a spherical dACC VOI (5 mm radius) survived ($p < 0.05$) a small volume correction (SVC) for multiple comparisons. For the ROI analyses of the influence of *TPH2* variation, BOLD contrast values for the dACC increased as the number of copies of the *TPH2* yin haplotype increased ($p < 0.05$) (Figure 1.3). Similarly, homozygous carriers of the *TPH2* yin haplotype exhibited significantly greater activation in the dorsolateral prefrontal cortex (dlPFC) and right inferior frontal gyrus ROI ($p < 0.05$) relative to individuals lacking the *TPH2* yin haplotype; heterozygotes exhibited intermediate responses. These results suggest an increased cognitive control-related neural activation with increasing numbers of copies for the *TPH2* yin haplotype. The brain-wide ANOVA indicated that allelic variation for the *TPH2* yin haplotype regulated MSIT-related neural activity in the left IFC, dorsal associative striatum, supplementary motor area (SMA), and dlPFC (Table 1.3, Figure 1.2B). Subsequent post hoc analyses indicated that there was no significant difference between 0 and 1 copies of the *TPH2* yin haplotype but a significant difference between 0 and 2 copies and 1 and 2 copies of the *TPH2* yin haplotype in the left IFC and caudate nucleus. These results are consistent with a non-linear regulation of MSIT-related neural activity by *TPH2* haplotypes. A linear regression restricted to an anatomical mask representing those brain areas identified by the main effect of task analysis demonstrated no voxel clusters exceeding the a priori statistical threshold and thus suggested the lack of a linear dose-dependent effect of *TPH2* yin haplotypes. The checkerboard task was associated with robust activation in the primary visual cortex (22 -94 19 mm). The regression model for the visual checkerboard task demonstrated the absence of a relationship between increasing *TPH2* yin haplotype load and the visual cortex response to the checkerboard task.

TPH2 Haplotype Effect on Functional Connectivity for MSIT-related Neural Pathways

To further investigate *TPH2* yin haplotype effects on the neural representation of MSIT-

Figure 1.2 A. Main effects of task. Statistical parametric map of brain activation during the processing of the MSIT. Maps were thresholded at ($p < 0.05$, FDR corrected) B. Main effect of genotype. Maps were thresholded at ($p < 0.005$); see Table 1.3 for coordinates and statistical information. (Abbreviations: SMA= supplementary motor area, dACC=dorsal anterior cingulate cortex, SPL= superior parietal lobule, IFC= inferior frontal cortex)

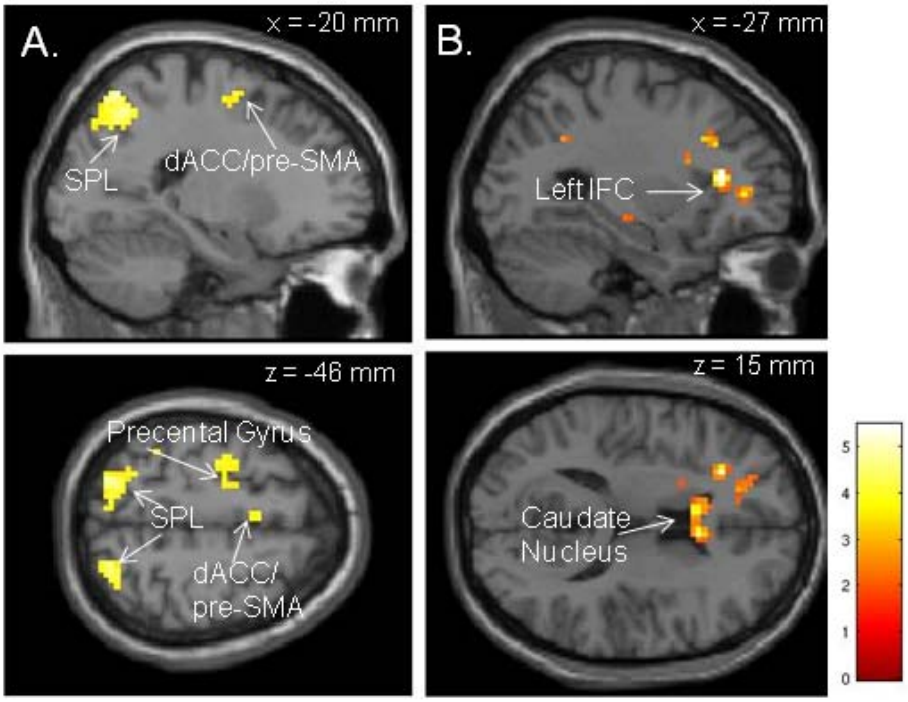
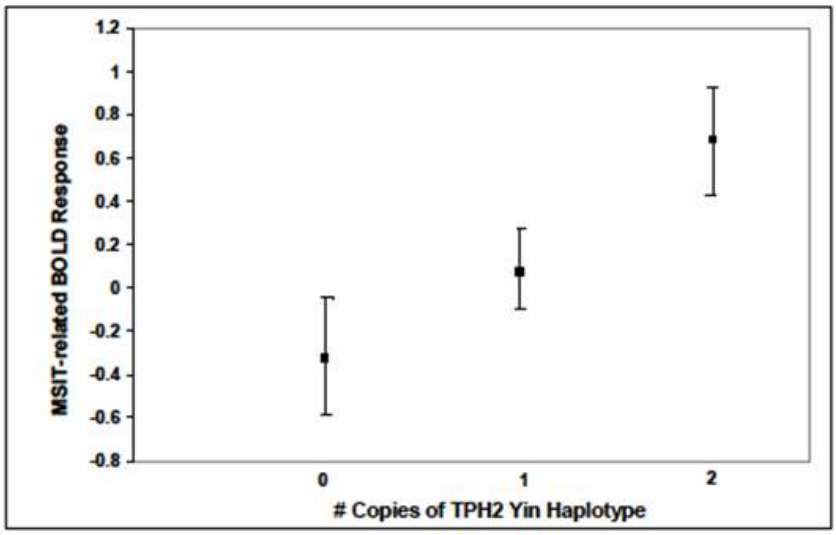


Figure 1.3 Blood oxygen level dependent (BOLD) contrast values for the dorsal anterior cingulate cortex (dACC) region of interest (ROI).



related cognitive interference, functional clusters identified from the analyses of the main effect of the task were used as seed regions for a voxel-wise, brain-wide analysis of allelic regulation of MSIT-related functional connectivity. This analysis focused on the cluster-level responses of the dACC and the right IFC to the MSIT. For the dACC seed, carrying two versus no copies of the *TPH2* yin haplotype was associated with decreased functional connectivity with the right dorsolateral and dorsomedial prefrontal cortex, and the left inferior frontal cortex (pars triangularis), and increased functional connectivity with the left SMA and posterior superior temporal sulcus (STS). For the right IFC seed, carriers with two copies of the *TPH2* yin haplotype exhibited increased functional connectivity with the left inferior frontal gyrus and decreased functional connectivity with the right precuneus, left middle frontal gyrus and left paracentral lobule (Table 1.3).

DISCUSSION

The findings of this study indicate that a *TPH2* yin haplotype linked to psychiatric phenotypes regulates the level of engagement of brain areas subserving cognitive control, as well as in their mode of functional connectivity. These *TPH2* effects on neural representations of cognitive control occur in the absence of significant gene effects on manifest behavior for the MSIT. The checkerboard task served as a negative control for non-specific gene effects on hemodynamic coupling or task-related neural processing. The results suggest that increasing numbers of the *TPH2* yin haplotype is associated with a deficit in neural mechanisms that regulate attentional control and interference suppression.

The dACC/pre-SMA, IFC, and dorsal striatum were engaged by the cognitive control demand posed by the MSIT. The dACC represents the cognitive subdivision of the anterior cingulate cortex (Devinsky et al., 1995) and plays a critical role in monitoring and detecting conflict and deploying dlPFC functions related to executive attention (Botvinick et al., 2004). The dACC also expresses relatively high levels of serotonin (Varnäs et al., 2004). The pre-SMA is responsible for conflict-related modulation that enables the dual process mechanism of switching

from automatic to controlled actions (Isoda and Hikosaka, 2007; Nachev et al., 2005). The right IFC is implicated in the inhibitory control of behavior (Aron and Poldrack, 2006), and the precommissural or associative subdivision of the striatum is involved in motor cognitions (Haber and Knutson, 2009). The presence of two copies of the *TPH2* yin haplotype could be associated with an inefficient dACC and right inferior frontal gyrus response to cognitive control demands as well as their decreased functional connectivity with executive function/response regulatory brain areas. The important role of the dACC/pre-SMA in conflict monitoring and resolution, and suppressing cognitive interference apparently might function in some degree of isolation in individuals with two copies of the *TPH2* yin haplotype.

The results also suggest a co-dominant effect for the *TPH2* yin haplotype, with the presence of two copies having a greater impact on the neural representation of cognitive control and, perhaps, conferred risk for psychiatric disorders compared to individuals who have 0 or 1 copies of the *TPH2* yin haplotype. The functional inference from this study is that individuals with genetic variants that alter serotonin synthesis and confer risk for psychiatric disorders would be compromised in the engagement of integrated activity within neural circuits necessary for inhibitory behavior control, response selection and executive attention such that the tendency for dysregulated behavior would be less checked by opponent cognitive processes.

Only a few studies have investigated the functional relevance of the *TPH2* yin haplotype, but overall these studies suggest a decreased TPH2 function and thus decreased serotonin neurotransmission in individuals carrying this haplotype. Homozygosity for the *TPH2* yin haplotype has been preliminarily associated with decreased cerebrospinal fluid 5-hydroxyindoleacetic acid (5-HIAA), a measure of brain serotonergic neurotransmission (Zhou et al., 2005). A reduced brain serotonergic activity associated with the *TPH2* yin haplotype is also supported by the observation that the G allele of an exon 7 *TPH2* polymorphism (rs7305115), a marker SNP for the yin haplotype, was associated with low TPH2 mRNA expression in the human pons (Lim et al., 2006). Decreased serotonin neurotransmission has, in turn, been

associated with an increased response of the dACC to cognitive control functions related to cognitive interference suppression (Evers et al., 2006) and a decreased response of the right ventrolateral prefrontal cortex (PFC) to the cognitive control process of response inhibition (Rubia et al., 2005). Collectively, these findings are consistent with the view that individuals homozygous for the *TPH2* yin haplotype with presumed lower serotonergic neurotransmission have diminished neural response representatives of cognitive regulation.

Modest *TPH2* association signals were noted for both a risk yin haplotype and for the opposite configuration or yang haplotype, with the latter exhibiting protective effects against suicide and major depression (Zhou et al., 2005) and risk for ADHD (Manor et al., 2008). Due to insufficient representation of the *TPH2* yang haplotype in our subject sample we were not able to assess differential effects of the *TPH2* yin and yang haplotypes on cognitive control-related neural processing in this study. Limitations of the present study include the study of a sample of varying racial and ethnic backgrounds and thus the contribution of population stratification effects to the observed results. Also, subjects had varying handedness and a relatively small sample size was studied that limits the control of Type I error.

CONCLUSIONS

The results of this imaging genomics study suggest that a common *TPH2* yin haplotype regulates cortical functions related to decision-making such that it confers susceptibility for psychiatric disorders due to an inefficient neural processing of demands for cognitive control. A functional connectivity analysis further demonstrated that increasing number of copies of the *TPH2* yin haplotype was associated with decreased engagement of a network of cerebral areas involved in executive functioning. Given the association of the *TPH2* yin haplotype with suicidality and other psychiatric disorders characterized by deficits in self-regulation, this study may inform the causes and risk factors related to individual variation in the expression of suicidality, bipolar disorder and ADHD.

<u>CHAPTER 2:</u> Clinical correlates of attentional bias for a personalized addiction Stroop task in a cocaine-dependent population (Unpublished)

INTRODUCTION

Drug addiction is characterized by pathological learned associations and strongly consolidated memories of drug use. Learned associations of drug use with the exteroceptive (*e.g.*, people, places) and interoceptive (*e.g.*, mood states) contexts of often ritualized drug abuse render these contexts as having conditioned incentive salience (Moeller et al., 2009; Robinson and Berridge, 1993). Due to their acquired properties as incentive motivational stimuli, attentional responses to such conditioned drug use cues represent powerful precipitants of drug seeking and use behaviors in drug-addicted individuals (Kalivas and Volkow, 2005). Typically assessed by their cognitive interfence effect, an attentional bias for drug use cues has been observed for alcohol (Cox et al., 2003; Lusher et al., 2004), nicotine (Waters et al., 2003; Wertz and Sayette, 2001), marijuana (Field et al., 2006), heroin (Franken et al., 2000b), and cocaine (Carpenter et al., 2006; Copersino et al., 2004; Hester et al., 2006) stimuli. These collective findings indicate that an attentional bias for conditioned drug cues represents a general feature of drug addiction. The level of attentional bias for drug cues, and their associated neural responses (Ersche et al., 2010; Janes et al., 2010), represent candidate neurocognitive markers of drug dependence.

The significance of attentional bias to the addiction process is further heightened by the observations that the level of attentional bias for drug stimuli predicts motivation for drug use, relapse, and treatment outcome in drug-abusing populations for heroin (Marissen et al., 2006), 2006), alcohol (Cox et al., 2002), and nicotine (Janes et al., 2010; Waters et al., 2003) stimuli. Additionally, adolescents at-risk for alcohol use disorders exhibited a significant attentional bias for alcohol-related stimuli (Zetteler et al., 2006). For cocaine abusers, the level of attentional bias was significantly correlated with the magnitude of self-rated drug cravings (Copersino et al., 2004) and drug seeking behavior (Cox et al., 2003), and predicted the frequency of cocaine use lapses and treatment duration (Carpenter et al., 2006). These findings suggest that measures of

attentional bias for drug cues have further significance as a cognitive marker of risk of relapse. We sought in this study to further assess the relationship between level of attentional bias for cocaine use cues and treatment outcome in a sample of treatment-seeking, cocaine-dependent men.

Attentional bias for drug-related stimuli has been estimated using visual probe tasks, which directly assess the effects of drug cues on attentional orientation (Field et al., 2006), and, more frequently, Stroop tasks (Cox et al., 2006), which assess cognitive interference when the processing of a task-irrelevant stimulus feature interferes with the simultaneous processing of a task-relevant stimulus attribute (Stroop, 1935). The majority of the Stroop tasks that incorporate drug use cues (addiction Stroop tasks) share a common feature – the use of generalized drug use stimuli to facilitate stimulus control. The cocaine Stroop task (cocStroop) used in the present study deviated from this pattern by the use of personalized, rather than generalized, drug stimuli to respect the fact that the associative learning underlying drug cue conditioning is a highly individually variable, and serves to potentially enhance the incentive salience of the drug cues. The level of cognitive interference for non-drug stimuli in a classical color Stroop task also discriminated cocaine-dependent individuals who completed versus those who did not complete a treatment trial, suggesting that level of global cognitive control predicted treatment retention (Streeter et al., 2008). We therefore compared the ability of the level of cognitive interference for drug stimuli (cocStroop) versus non-drug stimuli for a counting word Stroop task (cStroop) to predict treatment outcome in the same sample of cocaine-dependent subjects. This study tested the hypotheses that personalized drug stimuli are associated with significant attentional bias in cocaine-dependent individuals, and that the relationship between the level of attentional bias and relapse and treatment retention is stronger for drug stimuli versus non-drug stimuli in Stroop tasks.

MATERIALS AND METHODS

Study Participants

Thirty five male adult cocaine-dependent individuals participated in this study. All study subjects provided written informed consent to participate in a research protocol approved by the Emory University Institutional Review Board and the Atlanta Veteran's Administration Medical Center (VAMC) Research and Development Committee. Eligible subjects were cocaine-dependent men between 18 and 65 years of age and met the Diagnostic and Statistical Manual of Mental Disorders--- Fourth Edition (DSM-IV) criteria for diagnosis of cocaine dependence. Potential subjects were excluded for any current Axis I diagnosis other than cocaine or alcohol dependence or nicotine use, current or prior neurological disease, history of a major medical illness, or current use of psychotropic medications.

Treatment Program

The study was conducted in the Substance Abuse Treatment Program (SATP) at the Atlanta, GA VAMC. The outpatient treatment program consisted primarily of 3- or 5- day weekly sessions of twelve-step recovery in a group setting. Following the initial four weeks of treatment, subjects could opt to continue voluntary treatment in the Aftercare portion in which subjects could receive weekly group therapy sessions. Drug abstinence was assessed by urinalysis for cocaine and its metabolites, and other drugs of abuse, at random time points twice weekly over the first 4 weeks of active treatment, and weekly in the Aftercare component. Relapse was defined as a documented use of cocaine as determined by urinalysis and/or self-report or other report as entered in the electronic medical records (EMR) entries for each subject. Subjects were actively followed for a period of 90 days from study enrollment to include active treatment and long-term follow-up.

Assessment Instruments

All study-eligible subjects were evaluated using the Structured Clinical Interview for DSM IV Axis-I Disorders (SCID) (First, 2002) to assess the presence of current psychiatric disorders. The SCID was administered by the clinical coordinator (*e.g.*, > 8 years of SCID experience; SCID training was facilitated by video tape training sessions and mentored

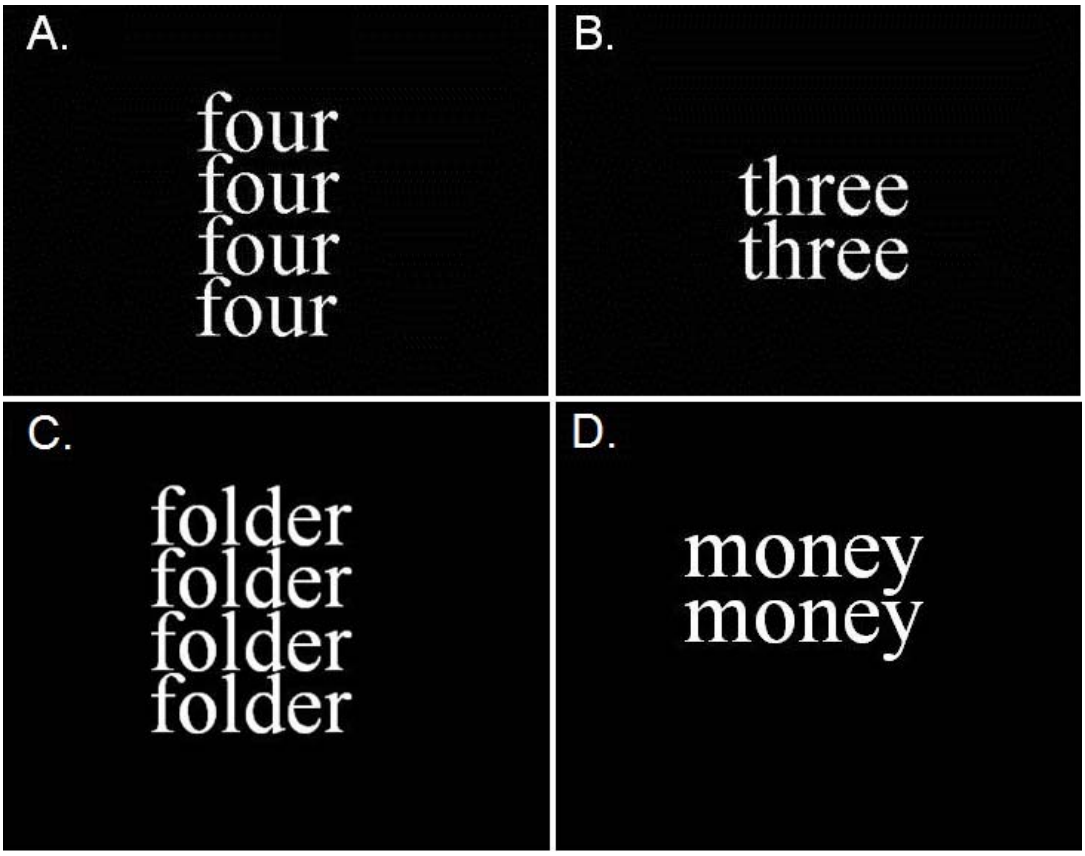
supervision of SCID interviews) which allowed for quality of uniformity amongst all interviews; however, no inter-rated reliability was assessed. Subjects also completed the Addiction Severity Index (ASI) (McLellan et al., 1992) to assess the severity of addiction-related functional impairment, and the Connors Adult ADHD Rating Scale (CAARS) (Conners et al., 1999) to assess the severity of ADHD traits. Demographic information (*e.g.*, age, education) was collected from study participants using a study-specific collection instrument. All assessments were completed at the initial screening visit.

Stroop Tasks

In this study, we utilized a word counting Stroop task that incorporated words representing personal drug use reminders (cocStroop task). All subjects at study entry identified eight cocaine cue words that were associated with their personal drug craving and use. The cocStroop task comprised eight personal cocaine-related (CS+) words (*e.g.*, money, stem) and drug use-neutral (CS-) words (*e.g.*, shelf, table). The counting Stroop task (cStroop) was comprised of congruent stimuli in which the number and name of the words are the same and incongruent stimuli in which they differ (Bush et al., 2006). The cStroop served as a measure of global cognitive control relative to that specifically engaged by cocaine stimuli in the cocStroop task. For each of the tasks, a trial involved the presentation of 1-4 identical words in a vertical array (Figure 2.1) with instructions to indicate by button press, on a 4-button response pad, the numbers of words represented. Subjects were instructed to respond as quickly as possible. Each Stroop task involved 89 word counting trials in which 33 of the words were semantic primes (cocStroop) or incongruent (cStroop) word stimuli presented for 750 msec with an interstimulus interval of 3 sec. Task training prior to data acquisition involved repeated trials of the cStroop task. Word stimulus trials for both Stroop tasks were presented in a random order with a run consisting of 178 words (Total run time = 11 min). Behavioral measures of reaction time (RT) and response accuracy were collected for each task trial at the initial screening or study baseline.

Personalized versus Generalized Drug Use Words

Figure 2.1 Stimulus trials for congruent (A.) and incongruent (B.) stimuli for the cStroop task and neutral (C.) and cocaine use (D.) words for the cocStroop task.



In this study, the addiction Stroop task (cocStroop) utilized personal drug use-related words instead of a generalized word list. Previous studies that have utilized addiction Stroop tasks have relied on general drug use-related words (Carpenter et al., 2006; Goldstein et al., 2007; Hester et al., 2006; Montgomery et al., 2010). However, this study used personalized drug use reminders as they are more representative of the individualized context-specific nature of learned drug associations and therefore offer greater addiction-relatedness. Eight drug use-related words were generated from each participant by using a cocaine cue word generator in which subjects disclosed their personal triggers associated with patterns of drug seeking and use behaviors.

Statistical Analyses

Continuous data were analyzed using analysis of variances (ANOVAs). Kruskall-Wallis tests were used when the respective data set violated assumptions of distribution normality. Paired t-tests were also performed to assess differences in reaction times between neutral and drug-use related words in the cocStroop task and between congruent and incongruent stimuli in the cStroop task among all subjects. Percent accuracy was also determined for responses for both Stroop tasks. Logistic regression was used to determine if the magnitude of the Stroop interference effect predicted relapse in cocaine-dependent subjects. Analyses employed the statistical analysis system SAS Version 9.2.

RESULTS

Subject demographic and clinical variables

Subjects reported using crack cocaine an average of 1.09 (± 1.46) and 14.2 (± 8.08) in the past 7 and 30 days, respectively. All subjects were male with 98% of the subjects self-reporting African-American as their racial background. Subjects were classified as non-relapsers if they did not exhibit a documented relapse to cocaine use in the 0-90 day treatment and follow-up periods. Within this subset of 35 treatment-seeking, cocaine-dependent subjects, 20 were classified as non-relapsers and 15 as relapsers. Relapsers and nonrelapsers did not differ in any of the measured variables at baseline including age, education and cocaine use patterns. However, the

two groups did exhibit significant differences on the CAARS subscale for DSM-IV inattentive symptoms, and for the ASI psychiatric and alcohol subscales (Table 2.1) ($p < 0.05$). Non-relapsers also had significantly greater treatment retention (days in treatment) as compared to relapsers ($p < 0.05$).

Stroop task performance

Attentional Bias for Conditioned Drug Cues: Of the drug use-related words provided by the 35 study participants, only 33 (18%) were shared by two or more participants while 155 were unique to individuals. The most frequently provided words (*i.e.*, money, sex, and smoke) accounted for 14%, 6% and 6% of the words, respectively. Analyses of the personalized drug use-related words determined that the order of presentation of drug use-related words and the reaction times for individual drug use-reminder words (*e.g.*, money versus sex) were not significant within subjects. Cocaine addicts exhibited a significant increase ($p < 0.05$) in reaction time for personal drug use-related words versus neutral words for the cocStroop task (Figure 2.2). The mean attentional bias effect for the group was 30.9 (± 72.4) msec, with evidence of wide interindividual variability reflected in a range of 191 to -211 msec. Relapsers and non-relapsers exhibited a significant difference ($p < 0.05$) in reaction time to cocaine use-related words versus neutral words with those individuals who later relapsed exhibiting a greater attentional bias effect compared to those who maintained cocaine abstinence (Table 2.1). Response accuracy across all subjects was 94.8% (± 6.31) with no significant difference between groups.

Counting Stroop: For the cStroop task, subjects had significantly longer reaction times ($p < 0.001$) for incongruent stimuli versus congruent stimuli (Figure 2.3). There was no significant difference in response times for incongruent versus congruent stimuli between the two groups (Table 2.1). Response accuracy across all subjects was 95.4% (± 5.42) with no significant difference between groups.

Logistic Regression

A logistic regression analysis was used to predict relapse in this sample of cocaine-

Table 2.1 Demographic and Clinical Characteristics of Subjects in SATP at Atlanta VAMC. All Subjects were male. *Kruskal-Wallis test, χ^2, (Non-parametric statistic tests were used when the data violated assumptions of distribution normality.) Values represent X +/- SD. (** p-value <0.05)

Variable	Nonrelapsers	Relapsers	F	df	P
N	20	15	-	-	-
Age (yrs)	43.7 +/- 4.9	43.0 +/- 6.8	0.11	1,33	0.743
Education (yrs)	13.3 +/- 1.4	13.4 +/- 1.3	0.015*	1	0.904
Cocaine Use					
Use past 7 days	1.0 +/- 1.5	1.2 +/- 1.6	0.166*	1	0.684
Use past 30 days	14.5 +/- 8.3	13.9 +/- 8.7	0.00*	1	1.00
Addiction Severity Index (ASI)					
Medical	0.35 +/- 0.39	0.28 +/-0.38	0.405*	1	0.525
Employment	0.68 +/- 0.24	0.68 +/- 0.20	0.00	1,32	0.967
Alcohol	0.13 +/- 0.20	0.32 +/- 0.29	3.89*	1	0.049**
Drug	0.22 +/- 0.07	0.26 +/- 0.08	1.84	1,32	0.184
Legal	0.06 +/- 0.11	0.04 +/- 0.15	0.914*	1	0.339
Family	0.24 +/- 0.26	0.31 +/- 0.27	0.923*	1	0.337
Psychiatric	0.13 +/- 0.20	0.28 +/- 0.20	5.13*	1	0.024**
Medical	0.35 +/- 0.39	0.28 +/-0.38	0.405*	1	0.525
ADHD Traits (CAARS)					
DSM-IV Inattentive	43.0 +/- 12.9	51.6 +/- 12.5	4.64*	1	0.031**
Hyperactive-Impulsive	45.7 +/- 9.9	49.1 +/- 9.1	1.02	1,31	0.320
DSM-IV ADHD Scale	44.1 +/- 12.2	50.9 +/- 11.2	2.69	1,31	0.111
Addiction Stroop					
Cocaine Words (msec)	901.5 +/- 136.7	979.3 +/-193.6	1.90	1,32	0.089
Neutral Words (msec)	887.8 +/- 134.3	923.7 +/- 151.6	0.53	1,32	0.236
Stroop Effect (msec)	13.7 +/- 71.7	55.6 +/- 69.5	2.89	1,32	0.050**
Counting Stroop					
Congruent (msec)	848.2 +/- 136.9	877.5 +/- 140.3	0.38	1,33	0.270
Incongruent (msec)	946.7 +/- 142.6	996.4 +/- 153.4	0.97	1,33	0.156
Stroop Effect (msec)	98.5 +/- 64.5	118.9 +/- 51.7	1.01	1,33	0.163
Days in Treatment	122.7 +/- 95.7	52.7 +/- 70.0	6.25*	1	0.012**

Figure 2.2 Reaction times for drug use-related and neutral words for a sample of cocaine-dependent men who performed a modified addiction Stroop (cocStroop) task. The group mean reaction time (± SD) for neutral words and cocaine use-related was 902.6 (±140.6) and 933.5 (± 164.4) msec, respectively ($p < 0.05$*, paired T-test). The group mean for the cocStroop interference effect (cocaine - neutral) was 30.9 (± 72.8) msec.

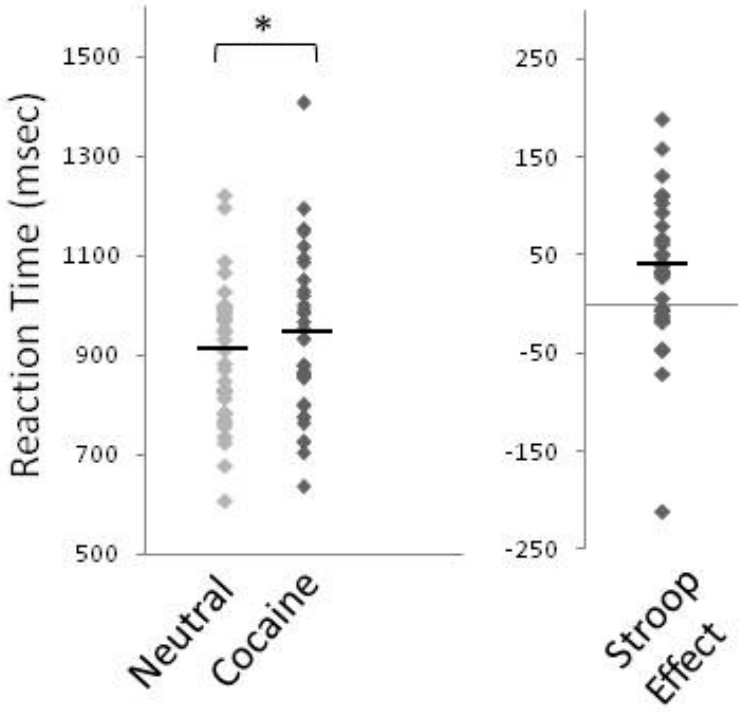
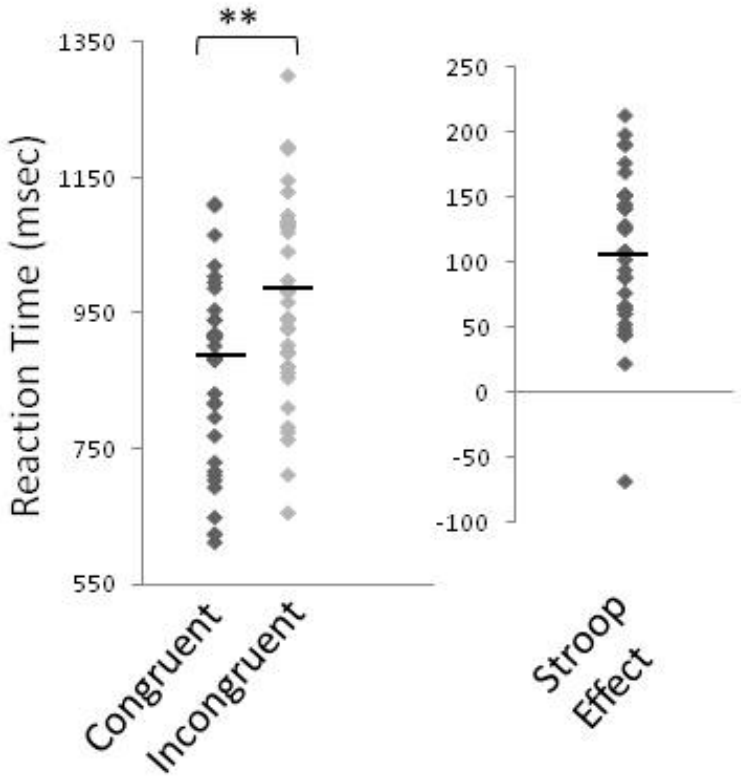


Figure 2.3 Reaction times for congruent and incongruent stimuli for a sample of cocaine-dependent men who performed a word counting Stroop (cStroop) task. The group mean reaction time (± SD) for congruent and incongruent stimuli was 860.8 (± 137.1) and 968.0 (± 147.2) msec, respectively ($p < 0.001$**, paired T-test). The group mean for the cStroop interference effect (incongruent - congruent) was 107.2 (± 59.4) msec.



dependent subjects. Two Stroop task models (interference effect: cStroop and cocStroop) were compared to assess their relative ability to predict relapse in this sample. The results indicate that interference effects for the cocStroop (cocaine-neutral) and cStroop (incongruent-congruent) were not statistically significant predictors of relapse (cocStroop model: $\chi^2 = 2.54$, $p = 0.1108$ and cStroop model: $\chi^2 = 1.003$, $p = 0.3164$). Because there was a significant difference between relapsers and non-relapsers on the CAARS subscale for DSM-IV inattentive symptoms and for the ASI psychiatric and alcohol subscales, these variables were subsequently analyzed to determine their association with relapse prediction. The CAARS DSM-IV Inattentive ($\chi^2 = 3.11$, $p = 0.0777$) and ASI-psychiatric subscales ($\chi^2 = 3.84$, $p = 0.064$) did not discriminate the groups; however, the ASI alcohol subscale ($\chi^2 = 4.31$, $p = 0.0378$, odds ratio (OR) = 1.37, confidence intervals (CI) = 1.02, 1.84) was predictive of relapse in this sample of cocaine-dependent subjects. The results also indicate that total number of days in treatment is a statistically significant predictor of relapse ($\chi^2 = 4.44$, $p = 0.035$, OR = 0.998, CI = 0.976, 0.999).

DISCUSSION

This study sought to assess whether the level of pre-treatment attentional bias predicts relapse in a treatment-seeking, cocaine-dependent population. A prior study reported that level of pre-treatment attentional bias predicted a binary measure of relapse in opiate-dependent subjects (Marissen et al., 2006); however, abstinence was based solely on self-report and was not confirmed by urinalyses. Other studies have noted that the level of attentional bias for drug-use related stimuli is associated with the magnitude of cocaine and heroin craving (Copersino et al., 2004; Franken et al., 2000a; Franken et al., 2000b), and proportion of cocaine positive urines (Carpenter et al., 2006). These treatment outcome studies suggest that attentional bias for drug-related cues could precipitate drug taking or relapse following treatment. This contention is supported by the observation that attentional training reduced attentional bias and post-training drug use by alcohol abusers (Fadardi and Cox, 2009). As previously demonstrated for addiction

Stroop tasks incorporating generalized drug-related stimuli (Carpenter et al., 2006; Copersino et al., 2004; Franken et al., 2000a; Hester et al., 2006; Vadhan et al., 2007), the results indicate that cocaine-dependent subjects demonstrated a significant attentional bias effect for personalized drug use-related stimuli. As a group, eventual relapsers had a significantly greater attentional bias effect for cocaine stimuli compared to non-relapsers; however, both relapsers and non-relapsers exhibited a similar level of difficulty controlling cognitive interference related to incongruent stimuli in the cStroop task. This result suggests that risk for relapse is associated with an impairment in attentional control related to conditioned drug cues, and is not attributable to global deficits in cognitive control associated with cocaine dependence (Di Sclafani et al., 2002; Hester and Garavan, 2004). However, although subjects exhibited a significant attentional bias for personal drug use reminders and incongruent stimuli, neither factor at baseline significantly predicted relapse group membership in this sample.

In a drug-unrelated color Stroop task, the level of performance interference by incongruent stimuli predicted treatment completion in a sample of cocaine-dependent individuals (Streeter et al., 2008). A similar observation was also seen in which neural responses to a cognitive control demand posed by the same Stroop task predicted clinically relevant outcomes such as treatment retention (Brewer et al., 2008). Both studies suggest that variation in general cognitive control ability, rather than acquired incentive motivational or other properties of drug-related stimuli that underlie their attentional bias effect, is predictive of clinical treatment outcomes for drug-dependent individuals. This contention is not supported by the findings of the present study as the cocStroop, but not cStroop, task performance discriminated relapsers and non-relapsers. Furthermore, logistic regression analysis did not support the value of attentional bias as a cognitive marker of risk for relapse in a sample of cocaine-dependent men. There are plausible reasons as to why attentional bias may be a valid marker of drug dependence, but have more limited value as a marker of treatment outcome. Multiple and varying factors contribute to temporally extended incidences of relapse in treatment-seeking drug-dependent populations, and

thus to the ability of a baseline measure to predict risk for future relapse. The varying experiences of psychological or social stressors (*e.g.*, negative affect state, interpersonal problems, and craving) as well as environmental factors (*e.g.,* presence and/or availability of drug) undoubtedly contribute to individually varying rates of relapse over time. Variation in individual traits (*e.g.*, compulsivity, genotype) or states (*e.g.,* drug craving, perceived need for help or treatment) also contributes to variation in relapse rates. Moreover, the multi-determined nature of attentional bias in drug use disorders (Field and Cox, 2008) supports individual variation in the ability to predict treatment outcomes. Finally, the state versus trait characteristics of attentional bias has not been defined in drug dependence; perhaps the level of attentional bias has more time-limited value as a cognitive marker of risk for relapse.

In this treatment study, personalized drug use-related words were used in a modified addiction Stroop task. However, the majority of drug addiction studies utilize one of several lists of drug use-related words for addiction Stroop tasks. Although generalized word lists have task advantages such as the ability to lexically standardize drug and neutral words to presumably isolate the attentional response to the addiction relatedness of drug use-related word stimuli (Cox et al., 2006), this study opted for personalized drug-use words to similarly enhance their addiction relatedness for each subject based on their individually differing learned associations with drug use. Indeed, in this study, 82% of the drug-use related words were unique to subjects. An analysis of the effect on task performance of lexical and semantic differences between the personalized cocaine use and neutral words indicated that part of speech, number of syllables, or word frequency did not significantly determine relative reaction time. The only significant variable was the number of words (1-4) for each trial of the counting word Stroop task, which was controlled for in the drug use and neutral word sets.

The study design does have clear limitations that temper the number of meaningful conclusions to be drawn. As personalized and generalized drug use words were not directly compared it remains unknown as to whether drug cues reflecting personal learned association

with drug use represent comparable or superior measures of attentional bias effect. Such a methods development study would be worthwhile to future efforts to develop attentional bias as a possible cognitive marker of drug dependence or treatment outcome. The drug use and neutral word stimuli were clearly not matched for the possible confounds of familiarity or frequency of use in the English language, though this is rarely attained or relevant in case-control studies of attentional bias related to addiction, and word familiarity is independent from attentional bias in addiction Stroop tasks (Field, 2005). Finally, hypothesis testing used logistic regression analysis based on models of a single variable (level of the cognitive interference effect); however, models incorporating additional explanatory variables would perhaps have greater predictive value (Streeter et al., 2008).

CONCLUSIONS

Attentional bias for drug-related stimuli constitutes a clinically relevant candidate neurocognitive marker of drug dependence (Ersche et al., 2010). The use of personalized versus generalized drug use-related words in a counting word Stroop task was associated with a significant interference effect relative to drug-neutral words (*i.e.*, attentional bias). The results of the present study provide partial support (*e.g.*, the attentional bias effect for cocaine stimuli discriminated between relapsers and non-relapsers; however, this variable was unable to predict subsequent relapse in the sample) for the predictive value of individual variation in attentional bias to forecast probability of relapse in cocaine-dependent men.

***CHAPTER 3:* The neural basis of attentional bias for drug-related stimuli associated with cocaine addiction (Unpublished)**

INTRODUCTION

Attentional and motivational responses to conditioned drug cues represent powerful precipitants of relapse to drug seeking and use behaviors in drug addicted individuals (Kalivas and Volkow, 2005). Attentional bias for drug-related stimuli has been estimated using visual probe tasks, which directly assess the facilitating effect of drug cues on attentional orienting

(Field and Cox, 2008), and, more frequently, Stroop tasks (Cox et al., 2006) which assess cognitive interference when the processing of a task-irrelevant stimulus feature interferes with the simultaneous processing of a task-relevant stimulus attribute (Stroop, 1935). An excellent theoretical and procedural discussion of addiction Stroop tests is provided by Cox et al., (2006). An attentional bias for drug-related stimuli has been demonstrated by drug-abusing individuals for alcohol (Cox et al., 2003; Lusher et al., 2004), nicotine (Drobes et al., 2006; Waters et al., 2003; Wertz and Sayette, 2001), marijuana (Field et al., 2006), heroin (Franken et al., 2000b), and cocaine (Carpenter et al., 2006; Copersino et al., 2004; Hester et al., 2006) stimuli. The attentional bias for conditioned drug cues in drug addicted individuals is attributed to their acquired incentive salience, an overvaluation of drug reinforcers that redirects cognitive resources towards drug-related behaviors (Cox et al., 2006). Recent studies also support a relationship between the attentional command for conditioned drug stimuli and relapse to drug seeking and use behaviors in drug-addicted individuals. For drug Stroop tasks, the magnitude of the estimated attentional bias for drug-related stimuli was significantly correlated with the magnitude of drug craving (Copersino et al., 2004) and drug seeking (Cox et al., 2003), and predicted relapse (Carpenter et al., 2006; Cox et al., 2002; Marissen et al., 2006). Moreover, attentional bias for drug cues may also represent a risk factor for drug abuse (Zetteler et al., 2006).

In spite of the established clinical relevance of attentional bias to drug addiction, the neural representation of attentional bias for drug-related stimuli is currently unknown. Such knowledge would inform the mechanisms of relapse and treatment outcome for drug-addicted individuals, and perhaps the neural basis of risk for drug abuse. The present study used event-related fMRI and a word counting Stroop task (Bush and Shin, 2006; Bush et al., 1998) to define the neural processing correlates of attentional bias in treatment-seeking, cocaine addicted men in an acute state of drug abstinence.

MATERIALS AND METHODS

Subjects

Twenty four treatment-seeking men (42.2 ± 5.0 years of age, 23 African American and one Caucasian) with a DSM-IV diagnosis of cocaine dependence were recruited for study participation. The subject sample was free of DSM-IV Axis 1 diagnoses other than cocaine dependence. Subjects reported using cocaine an average of 17 days in the past month (range, 4-27 days). Subjects also reported using cocaine an average of 4.6 days per week (range, 1-7) and spending an average of $148 (range, $25-$1,000) per day of cocaine use on and off over the last 13.5 years (range, 1-32 years). On the day of fMRI acquisition the subjects had been cocaine abstinent for an average of 6 days (range 1-16 days) as assessed by a combination of self-report and urinalysis results. Eighteen of the subjects had negative urinalysis results for cocaine and its metabolites at that time. Participants were also the subject of long-term follow up of relapse events. All study participants provided informed consent to participate in a study protocol approved by the Emory University Investigational Review Board and the Research and Development Committee of the Atlanta Veterans Administration Medical Center.

Tasks

Associative learning and memory processes underlie the conditioning of drug cues in the addiction process (Robinson and Berridge, 1993) and are thus highly individually contextualized by the differing internal and external contexts associated with ritualistic drug abuse. This makes it unlikely that a set of generalized drug stimuli will have similar addiction relatedness (the goal of addiction Stroop tasks) across a sample of addicted individuals and argues for the use of personalized drug stimuli in assessing attentional bias related to addiction (Cox et al., 2006). In the present study individualized drug-related words were used to assess attentional bias in an fMRI-compatible word counting Stroop task (Bush et al., 1998). The cocaine Stroop (cocStroop) task was a word counting task involving the random presentation of eight personal cocaine use-related words presented in a fixed categorical order with eight neutral words across subjects. Cocaine use-related words were provided in an interview with each participant while the eight cocaine use-unrelated neutral words were standardized across subjects unless they corresponded

to a cocaine-related word. Each trial consisted of the visual presentation (500 msec) of 1-4 identical words in a vertical array. Subjects were instructed to indicate the number of words viewed by pressing the corresponding button of a four-button response pad. A total of 89 trials (33 cocaine, 56 neutral) were presented with a variable interstimulus interval (2.3-9.3 sec).

MRI Acquisition

All scans were acquired using a Siemens Magnetom Trio 3T whole body MRI scanner with TIM gradients (Siemens Medical Solutions, Inc., Malvern, PA, USA). T2*-weighted images were acquired using a gradient echo-planar (EPI) pulse sequence with the following parameters: 30 axial slices, matrix = 64 x 64, FOV = 192 mm, slice thickness = 3 mm, gap = 1 mm, TR = 2.02 sec; TE = 30 msec; flip angle = 90°, voxel size = 3 x 3 x 3 mm^3. A total of 166 images were collected during task performance.

Image Preprocessing and fMRI Analyses

Image pre-processing involving slice timing, realignment, normalization, and smoothing (8 mm FWHM Gaussian kernel) was performed with Statistical Parametric Mapping version 5 (SPM5, Welcome Department of Imaging Neuroscience, University College London, U.K.). After preprocessing, statistical analysis was performed using the general linear model implemented in SPM5.

Individual Subject Level Model Fitting: Events corresponding to correct responses were modeled with a stick function at the stimulus onset time and convolved with a canonical hemodynamic response function (HRF). Cocaine and neutral word events were modeled separately. Prior to fitting the model the data were high-pass filtered with a cutoff of 128 sec and serial correlations were accounted for with an autoregressive model. The subject's motion parameters as well as time and dispersion derivatives of the HRF were included as covariates in the model. A linear contrast of cocaine versus neutral word conditions was then used to generate a statistical parametric map for each individual that was then used in the second-level analyses.

Random Effects Analysis: The resulting summary statistics images from the individual-level (fixed effects) models were then entered into a one-sample t-test to account for intersubject variability. For this contrast, a voxel-level significance threshold of $p_{\text{uncorrected}} < 0.001$ was used with a contiguity threshold of 5 voxels to minimize type I error (Forman et al., 1995).

Correlation analysis: The summary statistics images from the fixed effects models were entered into a linear regression model with the Stroop effect (average cocaine word reaction time - average neutral word reaction time) for each individual entered as a covariate. The results from this analysis are reported at $p_{\text{uncorrected}} < 0.001$ for positive correlations and $p_{\text{uncorrected}} < 0.001$ for negative correlations unless otherwise noted.

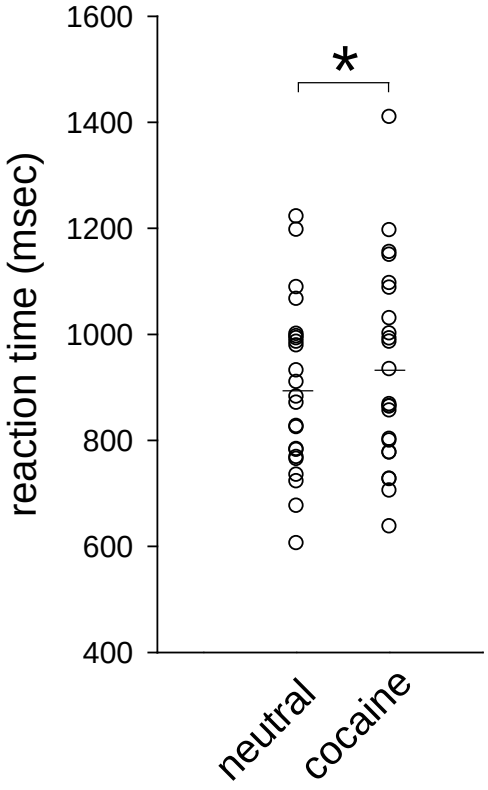
Subgroup analysis: The cocaine versus neutral word images from the fixed effects models for those subjects representing the extremes in Stroop effect ("negative" Stroop effect, N = 6, "positive" Stroop effect, N = 7) were entered into a two sample t-test. The results from this analysis are reported at $p_{\text{uncorrected}} < 0.005$.

RESULTS

Task Performance

Of the cocaine-related words provided by 23 addicts only 28 were shared by two or more participants while 112 were unique. The frequency distribution for the 140 endorsed cocaine use-related words demonstrated that one-half of the words were unique and that the most frequently provided words (*i.e.*, money, lighter, smoke) accounted for 10, 7, and 6% of the words, respectively. Consistent with prior results (Hester et al., 2006), cocaine addicts in a state of early drug abstinence exhibited a significant increase ($p < 0.01$) in reaction time for cocaine use-related versus neutral words for the cocStroop task (Figure 3.1). However, the subject-averaged Stroop effect for cocaine words exhibited marked interindividual variability, ranging from -211 to +187 msec. It is noteworthy that 7 of 23 subjects (30%) exhibited averaged reaction times for cocaine words that were less that those observed for neutral words (-44 ± 89 msec; a "negative" Stroop effect) that differed significantly ($p < 0.001$) from those 7 individuals representing the other

Figure 3.1 Reaction times for cocaine use-related and neutral words for a sample of cocaine-dependent men who performed a word counting cocaine Stroop (cocStroop) task. The group mean reaction time (± SD) for cocaine use-related and neutral words was 932 ± 190 and 896 ± 160 msec, respectively ($p < 0.05$, one-tailed paired T-test).



phenotypic extreme of the attentional bias effect (118 ± 45 msec).

fMRI Responses

Word Counting Stroop Task: Cocaine versus Neutral Words. Cocaine use-related words were associated with activation of the posterior cingulate cortex spanning the retrosplenial cortex, rostral posterior cingulate cortex, and precuneus ($p < 0.001$) (Table 3.1, Figure 3.2). The left inferior parietal cortex and dorsolateral prefrontal cortex also exhibit differential activation for cocaine versus neutral words in the word counting task. Lesser responses involved the middle temporal gyrus, right inferior parietal cortex, right inferior frontal cortex, and right ventrolateral and dorsolateral prefrontal cortex ($p < 0.005$).

Relationship between Behavioral and Neural Cocaine Stroop Effects. The variable Stroop effect associated with cocaine use-related words exhibited significant negative and positive correlations with the BOLD response for cocaine versus neutral words (Table 3.1). Negative correlations ($p < 0.001$) representing less activation with increasing attentional bias were observed for the right ventral striatum (Figure 3.3), left inferior parietal cortex, right superior temporal sulcus, left amygdala, left dorsolateral prefrontal cortex, and thalamus; lesser correlations ($p < 0.005$) were observed for the right posterior ventromedial prefrontal cortex, right substantia nigra/subthalamic nucleus, right inferior frontal cortex, and dorsal striatum. Positive correlations ($p < 0.001$) representing greater activation with increasing attentional bias involved the right occipital-temporal junction (Figure 3.3), right premotor cortex, left middle temporal gyrus, and right lingual/parahippocampal gyrus; lesser correlations ($p < 0.005$) were observed for the anterior ventromedial prefrontal cortex and left inferior frontal cortex.

Comparison of Neural Correlates for Phenotypic Extremes of Attentional Bias. fMRI data for one of the participants in the subgroup representing “negative” Stroop effects for cocaine-related words was not used in data analysis due to motion corruption. Compared to those individuals (N = 7) with the most “positive” Stroop effects for cocaine-related words (118 ± 45 msec), those (N = 6) with “negative” Stroop effects (-44 ± 89 msec) exhibited greater activation

Table 3.1 Stereotaxic and anatomical location of neural responses related to attentional bias for drug-related stimuli in cocaine addicts

Region[a]	Talairach coordinates			Cluster	Voxel T
Cocaine > neutral stimuli ($p < 0.001$)					
Posterior cingulate cortex (23)	0	-28	26	34	4.73[b]
Precuneus (R, 19)	33	-77	34	12	4.36
Posterior cingulate (23)	3	-58	14	5	4.28
Inferior parietal lobule (L, 40)	-59	-42	38	5	4.19
Middle frontal gyrus (L, 9)	-48	22	35	5	4.11
Correlation between level of attentional bias and BOLD response					
Negative correlations ($p > 0.001$)					
Ventral striatum	6	20	-1	10	4.65
Inferior parietal lobule (L, 40)	-36	-53	44	8	4.62
Superior temporal gyrus (R, 22)	42	-40	19	8	4.47
Amygdala (L)	-18	-6	-10	6	4.27
Superior frontal gyrus (L, 9)	-21	45	28	13	4.08
Thalamus	3	-23	7	8	4.06
Ventromedial prefrontal (R, 11/32)	18	29	-9	20	4.66[c]
SN/STN (R)	12	-12	-9	20	4.29[c]
Inferior frontal gyrus (R, 45)	45	29	4	10	4.07[c]
Dorsal striatum (L)	-12	4	14	24	3.45[c]
Positive correlations ($p < 0.001$)					
Middle occipital gyrus (R, 19)	36	-80	23	52	5.71[b]
Precentral gyrus (R, 6)	53	-13	28	5	4.93
Middle temporal gyrus (L, 21)	-53	-29	-6	5	4.49
Parahippocampal gyrus (R)	18	-44	0	12	4.39
Medial OFC (11)	6	49	-15	19	4.08[c]
Inferior frontal cortex (L, 45)	-53	35	4	18	4.03[c]
Rostral anterior cingulate cortex (24)	-3	30	10	8	3.88[c]
Comparison of phenotypic extremes for attentional bias[d]					
Negative > positive ($p < 0.005$)					
Dorsal striatum	-9	4	19	28	4.44
Inferior parietal lobule (R, 40)	50	-28	26	9	4.49
dlPFC/IFC (L, 9/44)	-50	10	33	16	4.10
Putamen (R)	21	-8	6	9	4.06
Positive > negative ($p < 0.005$)					
Middle temporal gyrus/hippocampus	-42	-21	-9	11	4.99
Posterior cingulate cortex (R, 30)	12	-49	8	10	4.12
Medial OFC (11)	3	43	-15	5	3.62
Anterior cingulate cortex (L, 32)	-12	10	33	5	3.50

[a]information in parentheses refer to hemisphere and Brodmann's areas
[b]$p < 0.05$ corrected at cluster level
[c]$p < 0.005$, uncorrected
[d]contrast of cocaine versus neutral words for 6 individuals with "negative" Stroop effects compared to 7 individuals with the most positive Stroop effects

Figure 3.2 Parasagittal representation (x = -3 mm) of greater BOLD responses for cocaine versus neutral word stimuli for the cocStroop task. Responses for the dorsal posterior cingulate cortex and precuneus are illustrated.

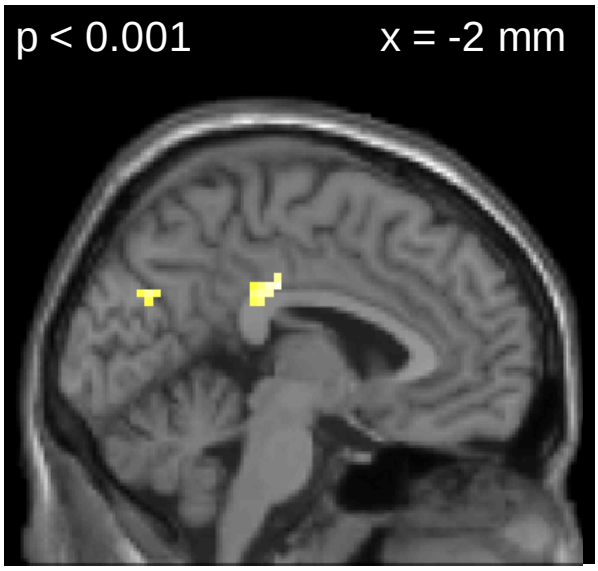
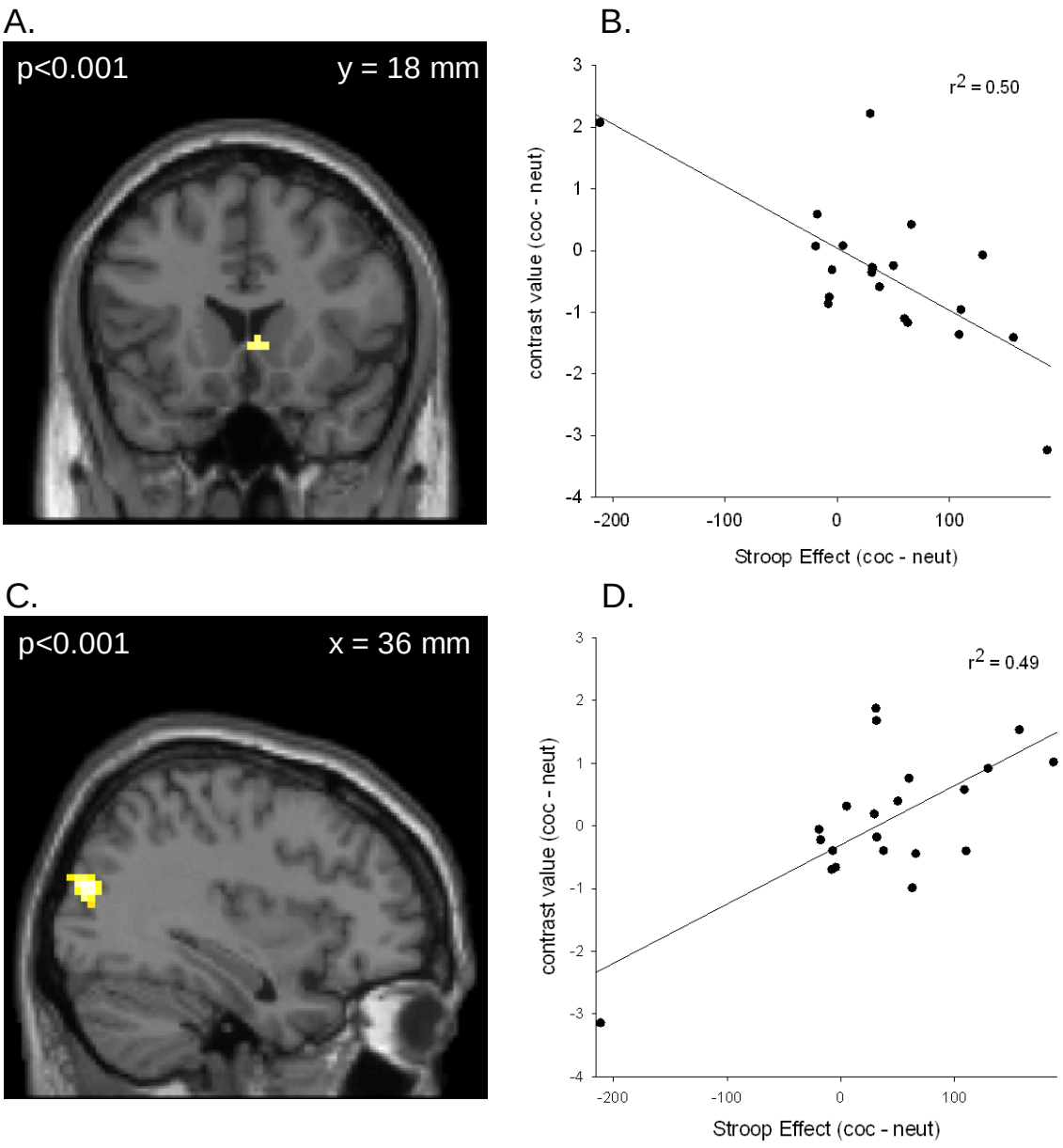


Figure 3.3 A. Region of ventral striatum in which activity correlated ($r^2 = 0.50$, $p < 0.001$) with the magnitude of their attentional bias effect for cocaine stimuli across participants and illustrate in B for a spherical volume of interest centered on 6 20 -1 mm. C. Region of occipital-temporal cortex in which activity correlated ($r^2 = 0.49$, $p < 0.001$) with the magnitude of their attentional bias effect for cocaine stimuli across participants and illustrated in D for a spherical volume of interest centered on 36 -80 23 mm.



of the dorsal striatum (left caudate nucleus and right posterior putamen), right inferior parietal cortex, and left dorsolateral/inferior frontal cortex. The subgroup of individuals exhibiting the most "positive" Stroop effects demonstrated greater activation of the left anterior temporal cortex, posterior cingulate cortex, anterior ventromedial prefrontal cortex, and dorsal anterior cingulate cortex (Table 3.1).

DISCUSSION

The goal of the present study was to define the neural representation of the cognitive bias for drug-related stimuli that is frequently noted for drug-dependent individuals and attributed to their conditioned motivational salience. A specific sample of treatment-seeking, cocaine-dependent men in a state of early drug abstinence was studied. The observed neural responses for the cocaine Stroop task are more consistent with the engagement of behavioral control mechanisms than with the processing of incentive motivation and salience. A prominent activation for cocaine versus neutral word stimuli spanned Brodmann's areas 19, 23, and 31 of the posterior cingulate cortex and precuneus. Prior functional neuroimaging studies of cocaine-dependent individuals have noted posterior cingulate cortex responses to conditioned drug cues (Kilts et al., 2004; Kosten et al., 2006). This specific response is consistent with the engagement of self-monitoring (Buckner et al., 2008; Fransson and Marrelec, 2008) and autobiographical memory recall (Maddock et al., 2001; Spreng et al., 2009) processes. With the left parietal cortex and dorsolateral prefrontal cortex, and right inferior frontal cortex (pars opercularis) responses, the posterior cingulate response may reflect the engagement of a network of brain areas that effect self-regulation (Aron and Poldrack, 2006; Stevens et al., 2007) and attentional control (Rossi et al., 2009) processes that regulate attentional bias for drug-related stimuli. This inference represents an extension of a biased competition model of attentional allocation for the simpler color Stroop effect in which the neural processing by posterior cortex of task-relevant stimulus features (font color) is enhanced and the processing of task-irrelevant features (the word) is suppressed (Polk et al., 2008). In the case of the addiction Stroop effect, the suppression of drug

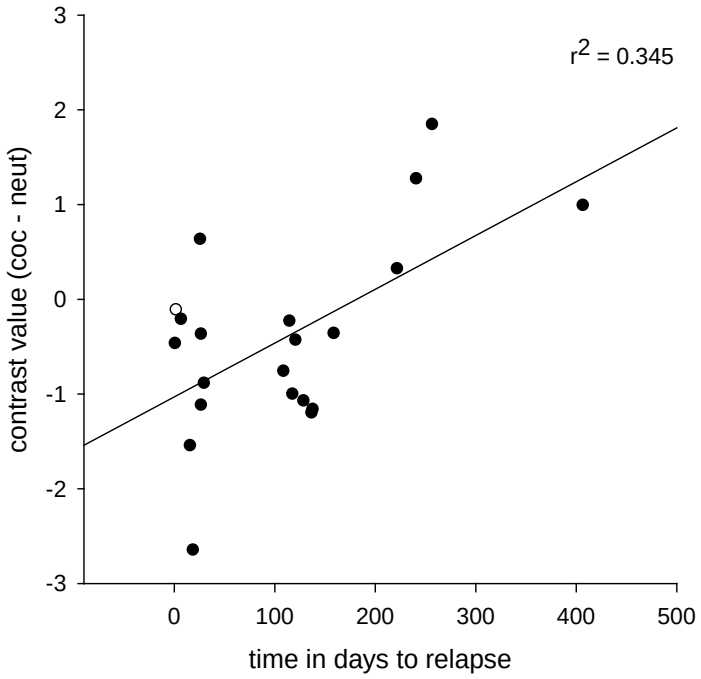
motivational and other corollaries of processing conditioned drug cues necessitates the involvement of higher order behavioral and cognitive control mechanisms to attend to the task-relevant stimulus feature (*i.e*., number of words).

It is highly probable that the neural correlate of attentional bias for drug use-related stimuli reflects multiple underlying processes (Field and Cox, 2008) involving motivational, affective, mnemonic, social, and cognitive processes, and individually varying factors related to stress (Duncan et al., 2007), drug abstinence state (Mogg and Bradley, 2002), mood state (Bradley et al., 2007), treatment outcome (Brewer et al., 2008), and genetics (Lusher, 2009). As a result, group-level estimates of attentional bias may obscure the estimation of its neural representation – a possibility supported by the observed wide inter-individual variation in differential RTs that define the addiction Stroop effect. We therefore sought to further define the neural basis of attentional bias and explore the functional roles of involved brain areas by examining correlations between the individual RT and BOLD responses for the cocaine and neutral word stimuli. Negatively correlated brain responses involved striatal, paralimbic, limbic, brainstem, parietal, thalamic and prefrontal brain areas. The observation that striatal responses to drug stimuli were negatively correlated suggests that their role in attentional bias does not reflect the processing of the incentive salience (Zink et al., 2006) or reward valuation (Knutson et al., 2001) of drug-related stimuli. Alternatively, the ventral striatal correlation may reflect the generation of a prediction error signal (Hare et al., 2008; McClure et al., 2003; Pagnoni et al., 2002; Schultz and Dickinson, 2000) related to the mismatch between the automatic detection of drug predictive cues and drug non-availability or treatment motivation, an adaptive response to the uncertainty of drug use resulting in lesser attentional bias. The observed negative correlation of ventral striatal, dorsal striatal, and ventral mesencephalic responses with the level of attentional bias is consistent with deployment of a striato-nigral-striatal circuit mediating control of a cocaine-seeking habit (Belin and Everitt, 2008). This interpretation is consistent with the observation that the magnitude of the ventral (9, 20, -4 mm) and dorsal (24, -14, 12 mm) striatal

response predicted later time to relapse in this sample ($p_{uncorrected} < 0.001$; Figure 3.4). In a tentative neural processing model of attentional bias, a striatal prediction error signal marshals the dorsolateral prefrontal cortex for top-down control (Montague et al., 2004) and the thalamus to gate sensory information projecting to the prefrontal cortex (Steriade M, 1997) to regulate the response to conditioned drug cues. The magnitude of attentional bias was also negatively correlated with the response of the right subthalamic nucleus/substantia nigra and inferior frontal cortex, brain areas central to behavioral inhibitory control (Aron and Poldrack, 2006), further suggesting the engagement of specific control efforts to suppress cognitive interference by the task-irrelevant drug-related words. Finally, the observed negative correlation for the ventromedial prefrontal cortex suggests the recruitment of this stimulus valuation system (Hare et al., 2008) in controlling attentional bias, perhaps by representing the aversive value of drug use.

Attentional bias was also associated with positively correlated brain responses involving occipital/temporal, motor, limbic, paralimbic, prefrontal and anterior cingulate areas. These associations may represent the sources of cognitive interference that underlie the attentional bias effect. The observed greater activation of the occipital/temporal junction with increasing attentional bias may reflect visual attention consistent with the incentive motivation property of conditioned drug cues (Moeller et al., 2009; Mogg et al., 2003). Correlated parahippocampal gyrus and anteroventral medial prefrontal cortex activity may represent the recall of drug use-related associative memories (Rekkas and Constable, 2005; Tsukiura et al., 2002) and the appetitive valuation of drug-related stimuli (Hare et al., 2008), respectively. Similarly, correlated rostral anterior cingulate cortex activity is consistent with the involvement of its roles in cognitive control processes related to error detection (Klein et al., 2007) and emotional conflict (Etkin et al., 2006) in modulating attentional bias for drug-related stimuli. Finally, correlated motor cortex activation is consistent with the presence of competing motor response tendencies related to drug use. The presence of attentional bias for drug-related stimuli seems to be multidimensionally determined by their affective, memory, motor, and incentive salience associations that define

Figure 3.4 Correlation between the ventral striatal response to cocaine-related stimuli for the cocStroop task during early drug abstinence and time to relapse for a sample of cocaine-dependent men. Treatment-seeking participants were enrolled in a 12-step-based outpatient addiction treatment program consisting of a 4 weeks of 3 or 5 day/week behavioral therapy followed by voluntary participation in aftercare treatment (1 day/week). Relapse date was established by a positive urinalysis for cocaine metabolites or by entries in the subjects' electronic medical record.



their conditioned motivation and drive selective attention to interfere with non-drug related behaviors.

Though apparently little considered, the absence of Stroop effects or presence of "negative" Stroop effects (*i.e.*, faster RT for drug than neutral stimuli) for drug-related stimuli has been observed previously for drug-abusing individuals (Drobes et al., 2006). The possibility therefore exists that there are categorically distinct attentional responses to drug-related stimuli across individuals with drug dependence. We approached a test of this possibility by comparing the neural responses to cocaine word stimuli between subgroups of addicts representing the phenotypic extremes of the observed attentional bias effects. The subgroup with "negative" Stroop effects exhibited greater dorsal striatal, right inferior parietal, and left dorsolateral/inferior prefrontal responses to cocaine use-related stimuli consistent with the greater engagement of prediction error monitoring and control processes. In contrast, "positive" Stroop effects were associated with the greater engagement of neural representations of appetitive stimulus valuation and the recall of addiction-related memories. These results suggest similarities between the mechanisms underlying the addiction Stroop effect and the Stroop effect model of competition bias (Polk et al., 2008) in which the incentive motivational features of drug-related stimuli compete with the deployment of control processes to modulate the level of attentional bias.

Limitations

The present study differed from typical addiction Stroop tasks in the use of personalized drug use stimuli to enhance the ecological validity of the cocaine Stroop task. A recent ecological study of the relationship between putative relapse triggers and drug craving and use by cocaine addicts demonstrated negligible association between 12 generalized relapse factors and cocaine craving (Epstein et al., 2009), suggesting that the generalized factors were only abstractly related to the specific and individually varying relapse precipitants for the sample studied. All of the participants studied were treatment-seeking and enrolled in a behavioral addiction therapy program. This quality of being, versus not-being, treatment seeking alters the

prefrontal cortical response to drug cues (Wilson et al., 2004) and thus could alter the behavioral and neural representations of attentional bias for drug-related stimuli. Indeed, attentional bias for cocaine-related stimuli was observed for treatment-seeking but not nontreatment-seeking cocaine-dependent men (Vadhan et al., 2007). All of the study participants were also male. Observed sex differences in the explicit neural processing of cocaine cues (Kilts et al., 2004) suggest that men and women may differ in the neural correlates of attentional bias for drug stimuli.

CONCLUSIONS

The results of this fMRI study suggest that the level of attentional bias reflects the product of competition between the incentive motivation property of conditioned drug cues and the engagement of sensory, cognitive and motor control mechanisms that inhibit conditioned drug seeking and use behaviors by cocaine addicts. For addicts exhibiting greater attentional bias the computational processing of drug use-related stimuli is dominated by computing their value as behavioral incentives, while for individuals with lesser attentional bias their processing is dominated by control efforts to reduce their value as incentives. Observations that the magnitude of attentional bias predicts level of drug craving, relapse and treatment retention (Carpenter et al., 2006; Copersino et al., 2004; Cox et al., 2003; Cox et al., 2002; Marissen et al., 2006) are consistent with this competition model of attentional bias. The recent observations that behavioral and neural responses to the drug-unrelated color Stroop task also predicted treatment outcomes and retention for cocaine-dependent individuals (Brewer et al., 2008; Streeter et al., 2008) suggest that the cognitive control rather than the incentive motivation responses to drug-related stimuli determine the clinical significance of attentional bias for addiction Stroop tasks.

<u>CHAPTER 4:</u> A randomized trial of the adjunct use of D-Cycloserine to facilitate cognitive behavioral therapy outcomes in a cocaine-dependent population (Submitted to Addictive Behaviors)

INTRODUCTION

Cocaine addiction is a chronically relapsing disorder associated with high rates of

recidivism in treatment-seeking individuals. To date, treatment for cocaine addiction remains solely reliant on behavioral therapies; there are currently no FDA approved pharmacotherapies for cocaine addiction. The major behavioral therapies including cognitive behavioral therapy (CBT), 12-Step, and contingency management approaches are effacious in promoting recovery and relapse prevention for drug-dependent individuals (Garcia-Rodriguez et al., 2009; Maude-Griffin et al., 1998a; Weiss et al., 2005a). However, the available addiction behavioral treatments have limited or partial efficacy in reducing the susceptibility to relapse (*e.g.*, 40% – 60% relapse rates, (McLellan, 1998)). While drug antibody (Haney et al., 2010; Martell et al., 2009) and targeted enzyme (Brimijoin et al., 2008; Collins et al., 2009) approaches may eventually provide therapeutic advantages, a more proximal approach might be represented by the adjunct use of a cognitive enhancer to boost the therapeutic cognitions and thus efficacy associated with a behavioral therapy based strongly on learning and memory processes.

Recent theories related to the drug addiction process emphasize the roles of learning and memory processes (Kelley, 2004; Robbins et al., 2008). The formation of addiction memories are dynamic processes reflecting associative learning mechanisms by which conditioned drug cues can elicit intense drug seeking and wanting (Robinson and Berridge, 2001). Neural processing networks exhibiting drug use experience-dependent plasticity represent addiction learning and its associated memories (Everitt et al., 2008). Similarly, in modifying cognitions and behaviors associated with drug addiction, certain types of CBT rely for their effectiveness on engaging competing learning and memory processes represented by differing networks modified by treatment experience-dependent neuroplasticity. One of the goals of addiction therapy is relapse prevention in which the drug conditioned stimulus (CS) no longer predicts the conditioned response (CR) of drug seeking and use behaviors. This can be accomplished by either extinction of the CR to a CS or by changing contingencies such that the drug CS now predicts a different CR. Both processes involve new learning and memory. Extinction-based psychotherapies such as prolonged imaginal exposure are effective in extinguishing pathological learned associations in

anxiety disorders such as posttraumatic stress disorder (PTSD) (Foa et al., 2005). However, the use of analogous cue exposure therapy (CET) approaches to extinguish the learned associative values of drug use cues in drug-addicted individuals have met with largely negative results (Conklin and Tiffany, 2002; Price et al., 2009) and, in at least one instance, an increase in relapse rates (Marissen et al., 2007). Cognitive behavioral therapy for cocaine addiction involves the identification of the thoughts, feelings, and events that precede and follow episodes of cocaine use and the learning and deployment of coping skills to counter drug use urges (Carroll, 1998). Unlike CET, controlled clinical trials have demonstrated the efficacy of CBT in producing significant, enduring drug abstinence in cocaine-addicted populations (Carroll et al., 2004). The maximal beneficial effects of CBT appear to be delayed, suggesting that the coping skills take time to be learned and consolidated (Carroll et al., 2000; Epstein et al., 2003).

The N-methyl-D-aspartate (NMDA) glutamate receptor is critical for the activity-dependent control of synaptic efficacy, exemplified by long term potentiation (LTP) or depression (LTD) of synaptic signals, a molecular mechanism of experience-dependent neuroplasticity that underlies learning and memory formation (Malenka and Bear, 2004; Rebola et al., 2010). D-Cycloserine (DCS), a partial agonist at the NMDA glutamate receptor, binds to the glycine binding regulatory site to facilitate glutamatergic neurotransmission (Kaye et al., 2006). DCS also enhances NMDA receptor-dependent synaptic potentials and facilitates the long-term synaptic plasticity of glutamate neurotransmission (Billard and Rouaud, 2007; Nitsche et al., 2004; Rouaud and Billard, 2003). Several preclinical studies have established that DCS can enhance learning and memory processes related to extinction (Walker et al., 2002) and operant behavior (Hood et al., 1989; Lelong et al., 2001; Monahan et al., 1989). Recent controlled clinical trials have explored the use of adjunct DCS to enhance learning and memory processes related to behavioral therapies for patients with anxiety disorders. The major effect of the adjunct use of DCS with extinction-based psychotherapies for anxiety disorders seems to be an acceleration of extinction learning and memory (Hofmann et al., 2006; Kushner et al., 2007; Ressler et al., 2004;

Wilhelm et al., 2008). This effect is mirrored in a facilitation of the rate of extinction of learned associations to cocaine cues in animal models (Botreau et al., 2006; Nic Dhonnchadha et al., 2009; Paolone et al., 2009), suggesting that DCS facilitates the extinction of both aversively and appetitively-conditioned associations. A recent report (Otto et al., 2010) indicated that DCS also facilitates the learning and memory processes associated with CBT in individuals with panic disorder. A seemingly logical extension of these findings was that DCS might facilitate the rate and extent of therapeutic learning and memory processes associated with CBT for cocaine addiction, and thus lead to enhanced relapse prevention and treatment retention outcomes. This hypothesis was tested using a placebo-controlled, double blind clinical trial design that assessed the interaction between DCS and a condensed version of a manualized CBT for cocaine addiction (Carroll, 1998).

MATERIALS AND METHODS

Study Design

A randomized, double blind, placebo-controlled study design was used to determine the efficacy and safety of the adjunct use of DCS with CBT to facilitate drug abstinence in a treatment-seeking sample of cocaine-dependent men. The clinical trial was conducted over a period of 23 months (April 2007 to March 2009) in the Substance Abuse Treatment Program (SATP) at the Atlanta Veteran's Administration Medical Center (VAMC) and consisted of 8 weeks of possible outpatient treatment of which the first 4 weeks consisted primarily of twelve-step recovery techniques in a group setting. Based on the number of past treatment enrollments, subjects were either assigned to 3- or 5-day per week treatment plans for four weeks. The last 4 weeks (Aftercare) following SATP and/or CBT and DCS/placebo administration consisted of voluntary treatment in which subjects could receive once-weekly group therapy sessions and served as a means of estimating whether any effects of CBT or DCS endured after therapy. Drug abstinence was assessed by an urinalysis drug screen (UDS) for cocaine and its metabolites, and other drugs of abuse, at random time points twice weekly over the first 4 weeks (SATP phase)

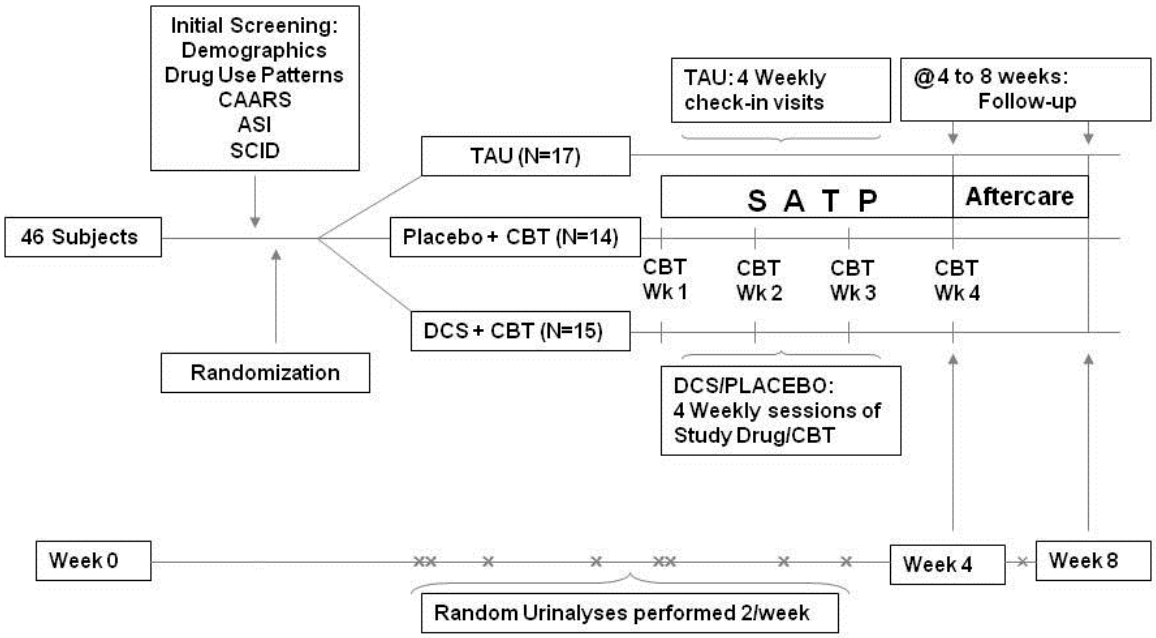
and weekly in the Aftercare component. All urinalysis results (+/- UDS) were reported in the electronic medical records (EMR) entries for each subject.

Following screening, eligible study volunteers were randomized to one of three parallel treatment arms (*i.e.*, DCS + CBT, Placebo +CBT, or Treatment as Usual (TAU)). The TAU group represented the delivery of standard SATP/Aftercare quality of care and was a control group for the effect of CBT. In addition to assignment to the DCS + CBT, and Placebo +CBT groups, these subjects also received the standard care for treatment for substance dependence (TAU) in the SATP (*i.e.*, DCS + CBT +TAU, Placebo +CBT + TAU). This study used a fixed dose of 50 mg of DCS administered 60 minutes prior to the onset of a CBT session. DCS (Seromycin, 250 mg; Eli Lilly and Co, Indianapolis, IN) was reformulated by the research pharmacist into 50 mg with identical placebo capsules. Previous studies of the efficacy of DCS to facilitate exposure-based psychotherapies in fear- and anxiety-related disorders demonstrated that 50 mg of DCS was efficacious in reducing associated symptoms and was well tolerated (Hofmann et al., 2006; Otto et al., 2010; Ressler et al., 2004). Subjects received 50 mg of DCS one hour prior to once-a-week CBT with a licensed clinical psychologist for four weeks during the SATP phase. All subjects, irrespective of treatment arm, met with the study research coordinator once a week for assessments (Figure 4.1). Relapse was defined as one positive UDS as confirmed by the urinalysis results in the EMR entries. Abstinence outcomes and self-reports of drug use were also verified by UDS. The EMR entries served primarily as a tool to gather additional information concerning relapse and abstinence in study subjects. Unlike most controlled trials in drug-dependent samples, loss to follow-up was not coded as relapse because the clinical team could not definitively determine whether loss to follow-up was due to reasons other than relapse (*e.g.*, obtainment of employment).

Randomization

The simple random allocation sequence was achieved by generating a randomized list of numbers (1, 2, 3) in which a number corresponded to a certain treatment group (*e.g.*, 1 = study

Figure 4.1 Study design for a randomized, double blind, placebo-controlled study of the adjunct use of DCS to boost the therapeutic response to CBT for male cocaine addicts.



medication (DCS or placebo), 2 = study medication (DCS or placebo), 3 = TAU). The clinical coordinator (RG) generated this random allocation sequence. To further randomize subjects to the DCS or placebo groups, the research pharmacist flipped a coin in which heads corresponded to DCS and tails corresponded to placebo. This level of randomization allowed for a double blind to be achieved in which research staff (except for the research pharmacist who never interacted with the subjects) and study participants were blinded to the medication (DCS/placebo) treatment groups.

Sample Size Calculation

A sample size of 15 was proposed for each group and was based on the results of power calculations for effect size and variance estimates from prior controlled clinical trials of the adjunct use of DCS with exposure-based behavioral therapies for persons with anxiety disorders. These studies randomized 13-16 subjects per arm and demonstrated that DCS facilitated the clinical response to behavioral therapies with moderate to large effect sizes ($d = 0.73 – 1.06$) (Hofmann et al., 2006; Kushner et al., 2007; Ressler et al., 2004).

Inclusion/Exclusion Criteria

All study subjects provided written informed consent to participate in a research protocol approved by the Emory University Institutional Review Board and the Atlanta VAMC Research and Development Committee. Eligible subjects were cocaine-dependent persons between 18 and 65 years of age enrolled in the SATP at the Atlanta VAMC. All subjects met the Diagnostic and Statistical Manual of Mental Disorders--- Fourth Edition (DSM-IV) criteria for diagnosis of cocaine dependence. The SCID was administered by the study coordinator (*e.g.*, > 8 years of SCID experience; SCID training was facilitated by video tape training sessions and mentored supervision of SCID interviews) which allowed for quality of uniformity amongst all interviews; however, no inter-rated reliability was assessed. Potential subjects were excluded for any current Axis I diagnosis other than cocaine or alcohol dependence or nicotine use, current or prior neurological disease, history of a major medical illness (Axis III diagnosis), or current use of

psychotropic medications. Prior to study participation, medication reconciliation was completed by the study addiction psychiatrist (KPGD) to assess possible adverse drug interactions between DCS and currently prescribed medications.

Assessment Instruments

All study-eligible subjects were evaluated using the Structured Clinical Interview for DSM IV Axis-I Disorders (SCID) (First, 2002) to diagnosis psychiatric disorders, the Addiction Severity Index (ASI) (McLellan et al., 1992) to assess the severity of addiction-related functional impairment and the Connors Adult ADHD Rating Scale (CAARS) (Conners et al., 1999) was used to assess ADHD traits. Demographic information (*e.g.*, age, education) was collected from study participants using a study-specific collection instrument. All assessments were completed at screening (baseline).

Cognitive Behavioral Therapy

Study participants engaged in four condensed sessions of a 12-session manual-based Cognitive Behavioral Therapy for Cocaine Addiction (Carroll, 1998) at one-week intervals (http://archives.drugabuse.gov/txmanuals/CBT/CBT1.html). This abbreviated CBT protocol was used to allow the assessment of possible response-enhancing effects of DCS and is consistent with the fear- and anxiety-related disorders literature (Hofmann et al., 2006; Kushner et al., 2007; Ressler et al., 2004). Five of the eight topics (*e.g.*, Coping with Craving, Shoring Up Motivation and Commitment to Stop, Refusal Skills/Assertiveness, Seemingly Irrelevant Decisions, and All-Purpose Coping Plan) in the manual were selected based on relevancy to the sample population and covered in the four group or individual CBT sessions. The groups were open groups so that new participants could join the group at any time. Each session started with a review of the prior session to reinforce topics and skills that had been addressed in the previous session. The therapist for the CBT sessions was a clinically trained PhD level licensed psychologist (NW) who is certified in CBT. The first CBT session focused on understanding their drug craving experiences. Subjects learned the properties of their drug cravings (*e.g.*, the duration of craving),

and learned to identify the triggers and cues that precipitated drug cravings. This session also focused on learning strategies to combat or cope with cravings (*e.g.*, recall of negative consequences of cocaine abuse or avoidance of cues). The second CBT session involved increasing motivation and a commitment to stop using illicit drugs. The psychologist (NW) clarified realistic treatment goals for each subject and challenged a subject's ambivalence towards drug abstinence. Subjects also learned a systematic approach to identify and cope with thoughts about cocaine that involved recognizing, avoiding and coping with cue-associated drug cravings. The third CBT session addressed refusal skills in which subjects first learned ways to break contact with individuals who supply or use cocaine. Subjects learned and actively engaged in practicing drug refusal skills in role-playing exercises. The final CBT session encompassed an all-purpose coping plan. Subjects learned to anticipate high-risk situations and to understand seemingly irrelevant stimuli that lead to drug relapse. Subjects also developed a comprehensive coping plan that could be recalled when encountering triggers or cravings. All sessions involved a series of practice exercises in which the skills taught in that particular session were further reinforced by each subject via repetition in an attempt to consolidate the therapeutic memories. All four sessions involved the recall of typical drug use situations and the prevention of responses to the urge to use drugs through a variety of learned and recalled coping strategies.

Statistical Analyses

Data were analyzed using Statistical Analysis Software (SAS) (SAS Institute Inc., Cary, NC, USA). Kaplan-Meier survival analyses were performed on data for 4- and 8-week study time points to determine group effects on cocaine abstinence and treatment retention between the three treatment arms. Loss to follow-up was not coded as relapse in the study and these subjects were censored in the Kaplan-Meier survival analysis. Post hoc analyses were subsequently performed on the survival curves and an adjusted p-value was calculated to determine significance of all pair-wise comparisons of the survival curves. Effect sizes (Cohen's d) were calculated to estimate the effects of CBT and DCS relative to the control (TAU) group for both the 4-week and 8-week

study endpoints. These estimations were conducted for the drug abstinence outcome variable only.

RESULTS

Subjects

The flow diagram for study enrollment is illustrated in Figure 4.2. Utilizing electronic medical records (EMR), 877 cocaine-dependent males were identified as presenting to the SATP for treatment from April 2007 to May 2009. Of that pool of potential subjects, 584 were excluded by EMR review due to Axis I or III diagnoses, other drug dependence (*e.g.,* opiate, methamphetamine), refusal of treatment, assignment to another treatment facility, drug free time > 60 days and age > 65. Of the 293 remaining persons who qualified for further study evaluation, 238 declined study participation. Of the remaining 55 subjects who consented for the clinical trial, nine were not randomized to treatment due to loss to follow-up, SCID failure, or diagnosis of a new Axis-I or III diagnosis as determined by the psychiatrist on call. Two subjects withdrew after randomization but before their first treatment session. Subjects were considered enrolled in the study upon completion of the first day of treatment. Of the forty-four men actively enrolled in the clinical trial study, 15 were randomized to the DCS + CBT arm, 13 to the placebo + CBT arm and 16 to the TAU arm. Three of the study subjects were court-mandated to complete the SATP (1 in the TAU group and 2 in the Placebo + CBT arm). Ten subjects were actively being treated for nicotine dependence via nicotine replacement therapy (nicotine patch or gum). Four subjects were prescribed naltrexone for alcohol dependence (1 each in the DCS + CBT and Placebo + CBT arms, and 2 in the TAU group).

Demographics

The clinical and demographic variables for the randomized subject sample are described in Table 4.1. Subjects in the randomized sample reported using crack cocaine an average of 9 days (+/- 8) in the past 30 days and had an average of 3.7 (+/- 3.5) prior treatment enrollments. The majority of subjects seeking treatment was homeless and had been assigned halfway house

Figure 4.2 Flow diagram of study participants. A total of 46 subjects were randomized to the three treatment arms of the study.

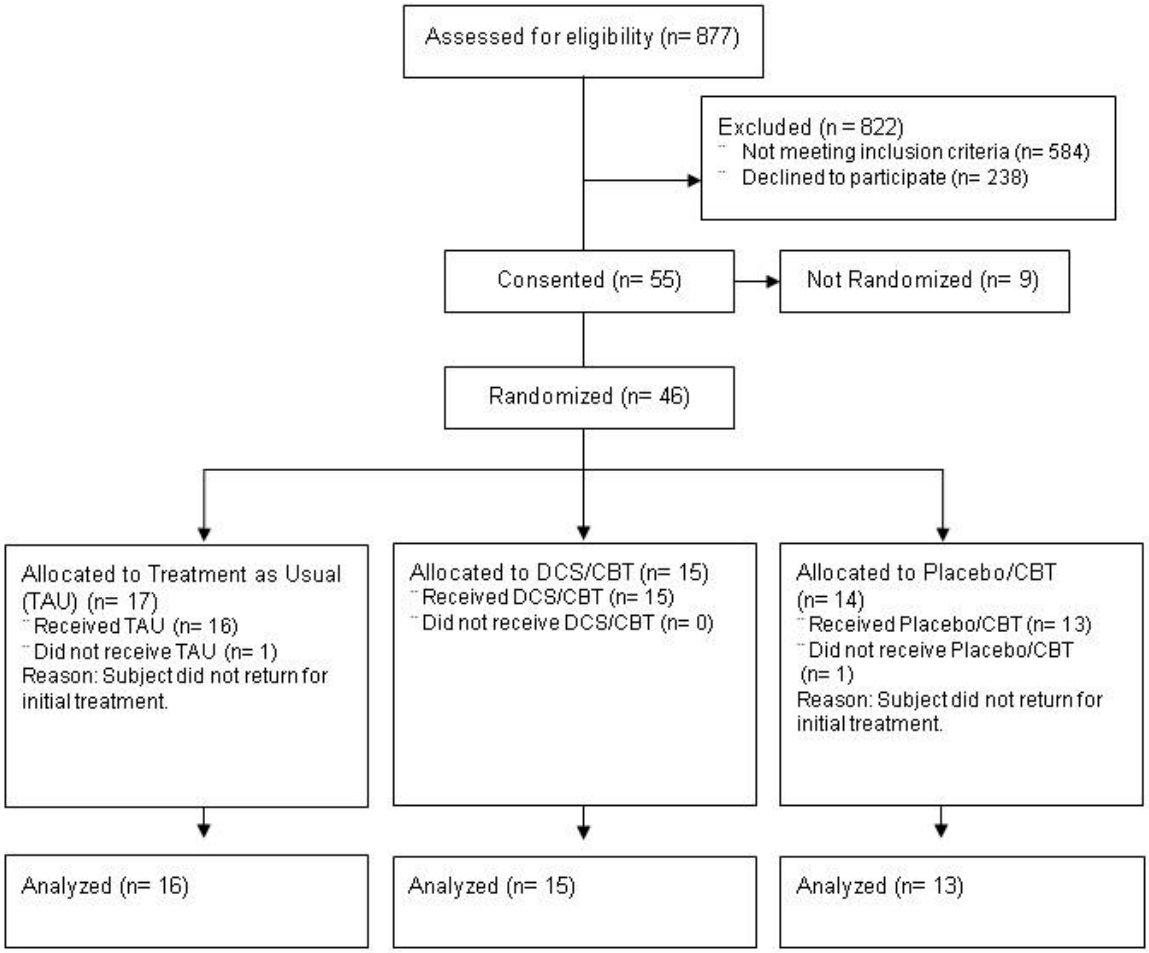


Table 4.1 Demographic and Clinical Characteristics of the Randomized Subject Sample (Race: AA = African America; CA = Caucasian); **(**Half way house: Focus =1; Stepping Stone =2; Gateway =3; Relative/ Friend =4; Hope House =5; Grace =6); Deviations in the n are listed as (n) in the columns for the respected variables. Values represent X +/- SD. (* Fisher Exact Test) (** p-value <0.05)

	DCS + CBT	**Placebo + CBT**	**TAU**
Variable			
N	15	13	16
Age	48.3 +/- 5.4	48.3 +/- 5.2	47.6 +/- 6.1
Race (AA, CA)	15 AA	13 AA	15 AA, 1 CA
Education-yrs	13.7 +/- 1.8	12.5 +/- 1.4	13.4 +/- 1.4
Handedness (R/L)*,**	14/1	13/0	11/5
Half-Way House Assignment (1/2/3/4/5/6)*	8/2/2/1/0/2	7/1/1/3/1/0	8/3/1/3/0/1
Treatment Team (3-day/5-day/Evening Prg.)*	10/5/0	7/5/1	5/10/1
Drug Related Functional Impairment (ASI)			
Medical	0.42 +/- 0.42 (14)	0.51 +/- 0.38 (11)	0.38 +/- 0.42 (12)
Employment	0.70 +/- 0.17 (14)	0.74 +/- 0.26 (11)	0.82 +/- 0.23 (12)
Alcohol	0.45 +/- 0.33 (14)	0.31 +/- 0.27 (11)	0.22 +/- 0.28 (12)
Drug	0.27 +/- 0.06 (14)	0.18 +/- 0.10 (11)	0.21 +/- 0.08 (12)
Legal	0.08 +/- 0.19 (14)	0.11 +/- 0.14 (11)	0.04 +/- 0.09 (12)
Family	0.21 +/- 0.25 (14)	0.22 +/- 0.21 (11)	0.27 +/- 0.27 (12)
Psychiatric	0.12 +/- 0.18 (14)	0.05 +/- 0.12 (11)	0.23 +/- 0.21 (12)
ADHD Traits (CAARS)			
ADHD Symptoms Total	50.6 +/- 11.6 (14)	49.5 +/-12.4	50.8 +/-13.5
Inattentive Symptoms Total	48.9 +/- 11.5 (14)	49.9 +/-12.5	46.7 +/- 13.4
Hyperactivity Symptoms Total	49.9 +/- 11.5 (14)	48.7 +/- 8.2	50.1 +/- 9.2
Cocaine Use			
Age at first cocaine use	27.4 +/- 7.1 (14)	26.2 +/- 7.3	26.7 +/- 6.2
Age at monthly cocaine use	31.7 +/- 7.3	28.8 +/- 5.3	30.2 +/- 7.8
Days of past use in last 7 days	0.53 +/- 1.1	0.38 +/- 0.8	0.72 +/- 1.4
Days of past use in last 30 days	11.0 +/- 8.6	6.3 +/- 6.5	9.2 +/- 8.5
Previous Treatments	2.93 +/- 3.3	3.7 +/- 3.8	4.4 +/- 3.6
Nicotine Use			
Age at first smoking	15.6 +/- 2.2 (11)	17.2 +/- 5.7 (12)	17.2 +/- 3.8 (9)
Age at first smoking daily	20.4 +/- 8.6 (11)	19.9 +/- 4.6 (12)	19.1 +/- 2.8 (8)

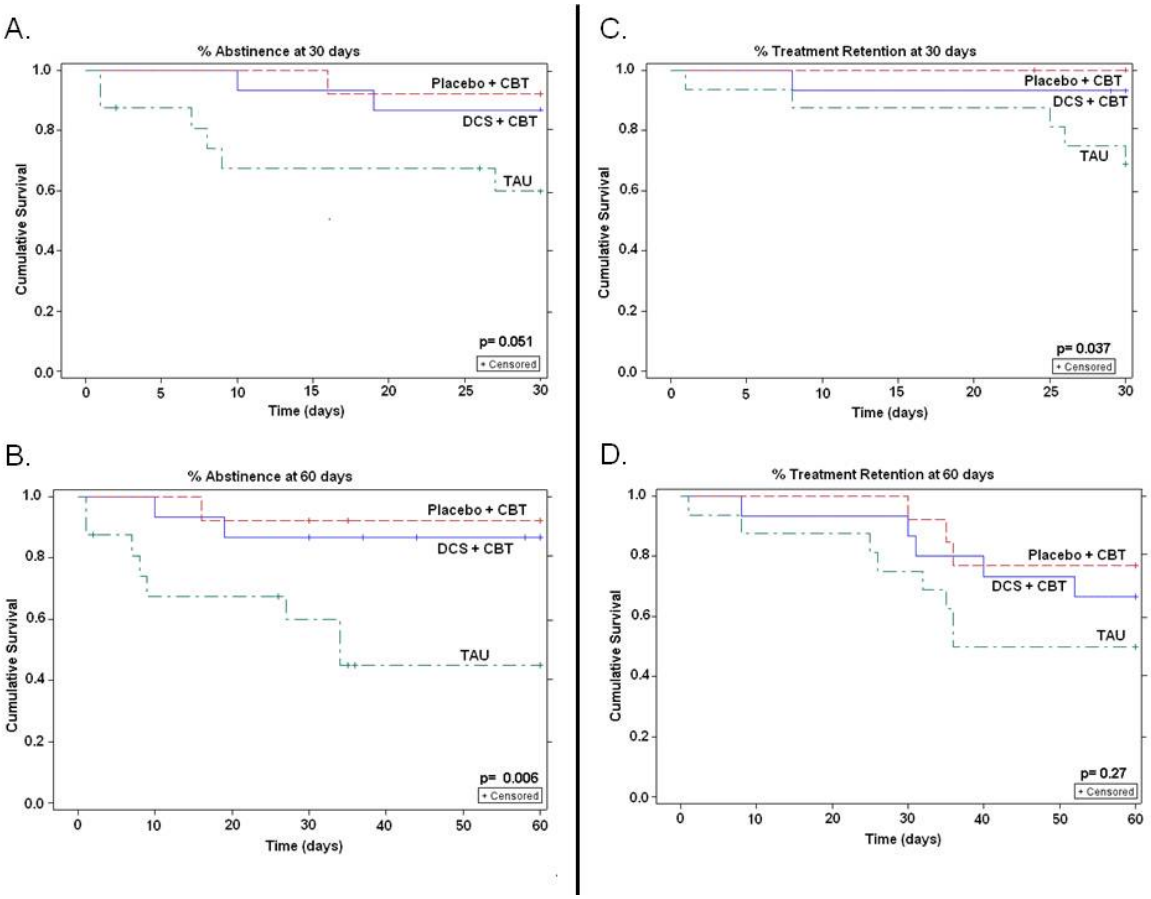
Alcohol Use			
# Alcohol Dependent/Abuse***,****	4/8	9/3	10/2
Age at first drinking	14.6 +/- 5.2 (14)	14.7 +/- 3.2	16.6 +/- 4.2 (14)
Total Days in Treatment	131.0 +/- 128.8	108.8 +/- 78.2	80.1 +/- 74.2

placement (77%) with four primary halfway house settings. 98% of the subjects were African-American. The individuals in each treatment arm did not differ in any of the measured demographic variables including age, education, half-way house assignment, or treatment team assignment (3 or 5 days/week). Groups also did not differ in the levels of addiction-related functional impairment (ASI), ADHD traits (CAARS subscales), and cocaine, nicotine and alcohol use patterns. However, the three groups did exhibit significant differences in handedness, and in the number of subjects who were alcohol dependent or alcohol abusers.

Drug Abstinence and Treatment Retention

The survival analysis indicated that subjects who received DCS + CBT or placebo + CBT exhibited numerically greater rates of cocaine abstinence, 86.7% and 92.3% respectively, compared to TAU (59.8%) at 4 weeks, though the effect was marginally statistically significant ($p = 0.0516$, Log rank test) (Figure 4.3A). Small effect sizes were noted for the placebo + CBT group ($d = 0.34$) and DCS + CBT group ($d = 0.13$) relative to the TAU group at 4 weeks. However, the survival analysis for the 8-week data indicated a significant group difference in rates of cocaine abstinence for groups randomized to the CBT arms versus the TAU group. The DCS + CBT or placebo + CBT groups maintained 86.7% and 92.3% cocaine abstinence, respectively, compared to the TAU group (44.9%) ($p = 0.0058$, Log rank test) (Figure 4.3B). The DCS + CBT and placebo + CBT groups did not differ in rates of cocaine abstinence ($p = 0.637$); however, there was a significant difference in the rates of cocaine abstinence at 8-weeks for the TAU and placebo + CBT arms ($p = 0.011$). Moderate effect sizes were noted for the placebo + CBT group ($d = 0.55$) and DCS + CBT group ($d = 0.39$) relative to the TAU group at 8 weeks. The survival curves for treatment retention at 4 weeks indicated a significantly greater treatment retention for those groups receiving CBT versus the TAU subjects; 93.3% and 100% retention for the DCS + CBT or placebo + CBT groups, respectively, compared to 68.8% for the TAU group ($p = 0.0366$, Log rank test) (Figure 4.3C). The DCS + CBT and placebo + CBT groups did not differ in rates of treatment retention ($p = 0.352$). At 8 weeks, there were no significant group

Figure 4.3 A**.** Kaplan-Meyer survival curves for drug abstinence for the three treatment groups (DCS + CBT, Placebo + CBT, TAU) at the 4-week (p=0.0516, Log rank test) and B. 8-week endpoints (p=0.0058, Log rank test). C**.** Kaplan-Meyer survival curves for treatment retention for the three treatment groups at the 4-week (p=0.0366, Log rank test) and D. 8-week endpoints (p=0.27, Log rank test). (Censored = no "event" or relapse)



differences in the survival curves for % treatment retention ($p = 0.27$, Log rank test) (Figure 4.3D). Subjects in all three treatment arms exhibited a decrease in % treatment retention (DCS + CBT, 66.7%; placebo + CBT, 76.9%; TAU, 50%) relative to values at 4 weeks. There were no significant group differences in the percent of subjects opting for the Aftercare extension of treatment - 86.7% of subjects in the DCS + CBT opted for Aftercare as compared to 76.9% and 56.3% for the placebo + CBT and TAU groups, respectively.

DISCUSSION

The primary goals of this controlled clinical trial were to ascertain the acute treatment efficacy (0-4 weeks) and post-treatment durability of DCS administration as a means of boosting the relapse prevention and treatment retention goals of CBT, an empirically supported therapy with partial efficacy for the treatment of cocaine addiction. A major barrier to drug abstinence in this population is the uncountered drug use motivation triggered by conditioned external and interoceptive drug-related cues that predict drug seeking and use behaviors. These learned associations are associated with strongly consolidated drug use memories that are highly resistant to extinction (Weiss et al., 2001), a property that perhaps underlies the general ineffectiveness of extinction-based CET approaches to prevent relapse (Conklin and Tiffany, 2002). Alternative treatment approaches such as CBT seek to counter addiction by modifying cognitive and behavioral representations of drug use that oppose the prior learned associations such that conditioned drug cues no longer predict drug abuse. A premise of this study was that CBT, like extinction, involves therapeutic learning and memory processes that are mediated by glutamatergic neurotransmission and are thus amenable to enhancement with DCS administration. This premise was tested by pairing weekly DCS pretreatments with four weekly sessions of CBT representing a condensed version of a 12-session CBT (Carroll, 1998). A similar strategy was recently used to demonstrate DCS augmentation of exposure-based CBT outcomes for individuals with panic disorder (Otto et al., 2010).

The results of this clinical trial indicate that a condensed and abbreviated version of a manual-based 12-session CBT for cocaine addiction (Carroll, 1998) resulted in significant improvements in relapse prevention and treatment retention, the primary study outcome variables. The gains relative to the TAU group also endured over a one-month follow-up period. The effect sizes for CBT versus TAU, irrespective of receiving placebo or DCS, reflected small to moderate enhancements (d = 0.19-0.55) of the relapse prevention outcomes for TAU. This clinical trial also revealed that DCS as an adjunct treatment to CBT offered no advantage relative to placebo in promoting drug abstinence and treatment retention in a cocaine-dependent population; both CBT arms were comparably more effective than a relatively intensive 12-Step-based TAU. Therefore, the intent of the study design to assess the ability of DCS to boost the partial clinical response to an "under-dosed" version of CBT was not realized. In a dose-response study of the efficacy of CBT in cocaine addicts, Covi and colleagues (Covi et al., 2002) determined that the efficacy of a highly individualized CBT was independent of "dose" over a three-fold range of frequency of therapy sessions (*e.g.,* twice weekly, once weekly, biweekly). This clinical trial seemingly replicated that outcome. The result is that a ceiling response to brief CBT precluded the opportunity to assess the ability of co-administered DCS to facilitate the clinical response to CBT. Attrition in addiction treatment programs and loss to follow-up for clinical trials in drug-dependent populations are well-recognized problems. Clearly, the possibility of DCS to facilitate a more efficient treatment process (*i.e.*, fewer CBT sessions) would be of significant clinical value. The lack of dose-dependence observed for highly individualized CBT in cocaine addicted samples (Covi et al., 2002; Fiorentine, 2001) suggests that the pairing of DCS with a more standardized form of CBT such as computerized CBT (Carroll et al., 2008) would be an improved experimental design of heightened translational significance.

Strengths and Limitations

Strengths of the study include that all of the study participants were involved in active treatment. Adherence to the regimen of DCS study medication was 100% as noted by observation

and no adverse events occurred throughout the clinical trial. An added strength of the study was that it was an outpatient study, which allowed the assessment of treatment response by subjects exposed to cocaine cues in the environment. An inpatient study could have possibly caused inflated treatment effects while in the controlled setting of the hospital and dramatic decreases in treatment efficacy once the patient was re-exposed to his drug use environment. Spot urinalysis was conducted in halfway house settings when subject's behavior required further surveillance and was indicated in the EMR, and thus completed the random UDS measures of relapse in the SATP. Another strength of this study was that a manualized CBT was implemented which could facilitate the generalization of a combination therapy to other treatment facilities.

Limitations of the study include that the majority of the participants were homeless and with varying shelter environments making this a difficult clinical population for longitudinal study. Another limitation of the study is that SATP is an abstinence-based treatment program, meaning that a common measure of treatment response – the percentage of drug positive urines as a function of time - could not be ascertained. The robust response to CBT also precluded the ability to estimate the variance of the effect size.

CONCLUSIONS

The results of this clinical trial replicated the efficacy of CBT for cocaine dependence and demonstrated that adjunct DCS with a condensed version of CBT was comparable to the placebo +CBT arm in treatment retention and drug abstinence at 4- and 8-week treatment endpoints. The results also suggest that the addition of an under-dosed, manualized CBT is more effective than a standard 12-step treatment approach alone in promoting relapse prevention and functional recovery in a cocaine-dependent sample. A major premise of this study is that DCS would facilitate the retention of therapeutic learning and memory consolidation. Recent fMRI studies have demonstrated that DCS facilitates hippocampal mechanisms of learning and memory consolidation in humans (Kalisch et al., 2009; Onur et al., 2010). Future studies of the value of DCS as a treatment adjunct in cocaine-addicted individuals could be enhanced by a similar use of

in vivo functional neuroimaging technology to define its effect on putative neural mechanisms of therapeutic learning and memory processes.

***CHAPTER 5:* A controlled clinical trial and fMRI study of the adjunct use of D-Cycloserine with a computerized cognitive behavioral therapy for cocaine dependence (Unpublished)**

INTRODUCTION

In a double-blind, randomized controlled clinical trial in a sample of cocaine-dependent men enrolled in a Substance Abuse Treatment Program (SATP) at the Atlanta Veteran's Administration Medical Center (VAMC), 50 mg of d-Cycloserine (DCS) prior to four weekly sessions of a condensed version of a manual-based cognitive behavioral therapy (CBT) was no more effective than placebo in facilitating drug abstinence or treatment retention goals of CBT (Chapter 4). However, the combination of an under-dosed CBT with a 12-step based treatment approach was associated with significant improvements in treatment retention and drug abstinence at the 4- and 8-week treatment endpoints, relative to 12-step therapy alone. A major limitation of the design of this clinical trial was that SATP is an abstinence-based treatment program in which lapses to drug use triggered treatment exclusion and thus precluded the ability to assess the effect of treatment on the primary outcome variable (relapse prevention) as a continuous variable. This initial clinical trial had additional design limitations that posed barriers to a definitive assessment of the possible benefit of cognitive enhancement on behavioral therapy outcomes and are enumerated below:

I. A high rate of homelessness and varying shelter environments (*e.g.*, half-way houses) in the sample population enrolled in the SATP at the VAMC represented a major barrier for longitudinal assessment and subsequent follow-up.

II. The participants enrolled in the clinical trial were African American men and military service veterans and was thus not generally representative of the larger populations of cocaine-dependent individuals in the community (*e.g.*, females and other ethnic backgrounds, non-military veterans).

III. The frequency of urinalysis (*e.g.*, twice weekly over the first 4 weeks (SATP phase) and weekly in Aftercare) was sub-optimal to the surveillance of drug use throughout the duration of the study.

IV. The ceiling response to a condensed CBT protocol precluded the ability to determine if the cognitive enhancer, DCS, could boost the treatment retention and drug abstinence goals of CBT.

We therefore sought to address these limitations in the re-design of an independent controlled clinical trial assessing the efficacy of adjunct DCS administration as a means to boost the therapeutic learning and memory processes of CBT. Piloted by the initial trial, the current trial incorporated the following design changes:

I. The initial trial utilized a dose of 50 mg of DCS that was based on prior controlled clinical trials of the adjunct use of DCS with exposure-based behavioral therapies for persons with fear- and anxiety-related disorders (Guastella et al., 2008; Hofmann et al., 2006; Kushner et al., 2007; Ressler et al., 2004; Wilhelm et al., 2008). The current trial used a dose of 250 mg of DCS that was effective in facilitating hippocampus-dependent declarative learning in a healthy sample of subjects (Onur et al., 2010). The facilitating effect of adjunct DCS on behavior therapy outcomes in individuals with anxiety disorders have been documented for a dose range of 50 to 500 mg with no significant adverse side effects even at a 500 mg dose (Ressler et al., 2004).

II. In the initial trial, study participants engaged in an under-dosed, manual-based CBT for cocaine addiction (Carroll, 1998) at once-weekly therapist-delivered sessions. Since the ceiling response to an abbreviated CBT protocol precluded the opportunity to assess the ability of adjunct DCS to facilitate a clinical response to CBT, a more standardized form (*e.g.*, control of dosing and duration at each treatment session) of behavioral therapy was implemented into the trial design. The current clinical trial

utilized thrice weekly computerized CBT (cCBT) sessions that included fluency-based skills training. Fluency building training requires subjects to be actively engaged in the acquisition and consolidation of the information presented in the cCBT treatment modules and thus represented a defined target for the cognitive enhancing effect of DCS.

III. The patient population for the initial DCS clinical trial sampled a specific subsample of cocaine-dependent persons who were male military veterans enrolled in the SATP at the Atlanta VAMC. However, the current clinical trial at the University of Arkansas for Medical Sciences (UAMS) used a community-based sample of cocaine-dependent men and women of varying racial backgrounds.

IV. Unlike the abstinence-based VAMC approach to enhancing treatment adherence, the current DCS trial used a contingency management (CM) voucher system to improve treatment retention among subjects**.**

V. In the initial DCS clinical trial, the binary treatment outcome of relapse/abstinence was largely defined by any single positive urine drug screen (UDS) which typically resulted in termination from the treatment program and loss-to-follow-up. In the current DCS trial, percentage of all UDSs positive for cocaine metabolites (lapses) and percentage of all treatment sessions completed were assessed thrice weekly as continuous measures of treatment outcome over the 4 weeks of active treatment.

VI. The use of functional neuroimaging approaches to monitor the brain responses to therapy was deemed to be premature for the initial DCS trial. However, the current trial employed task-related fMRI to explore the impact of cCBT and DCS on the neural representation of the CR to a drug CS using an addiction Stroop task (cocStroop) (Chapter 3). As a complement to the primary outcome variables, the repeated measures fMRI studies were conceptualized as a sensitive means of assessing the

mechanisms underlying a treatment-related inhibition of a major determinant of relapse

The initial DCS clinical trial demonstrated the efficacy of CBT for cocaine dependence and guided the design of the current DCS trial to provide a more definitive study of the efficacy of adjunct DCS, relative to placebo, to facilitate the therapeutic learning and memory goals associated with cCBT and thus promote relapse prevention and functional recovery.

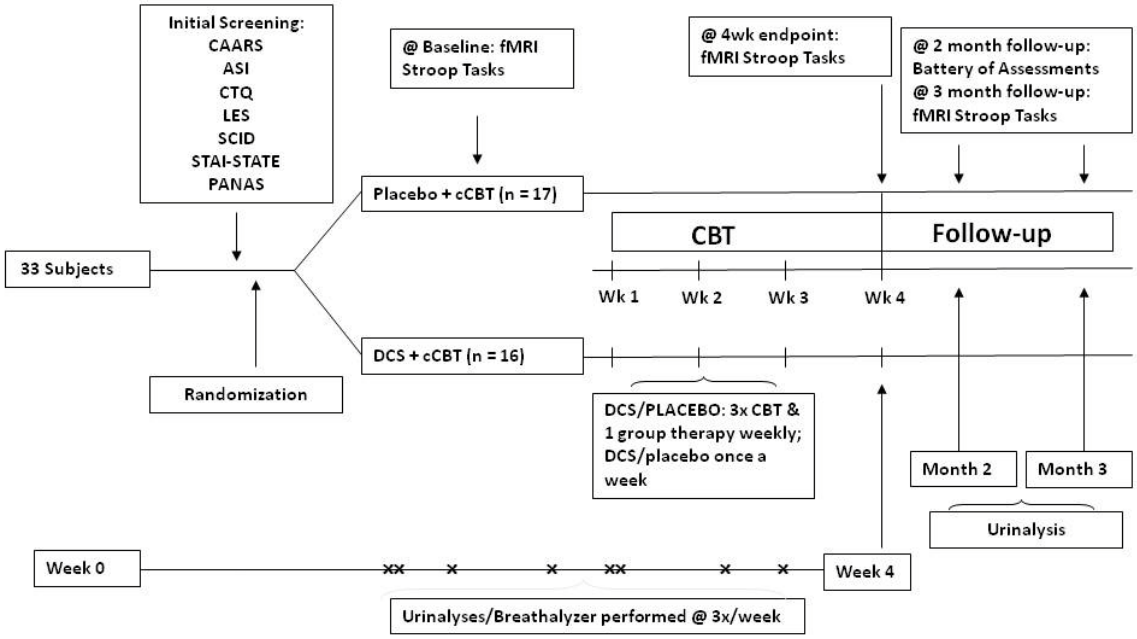
MATERIALS AND METHODS

A randomized double blind, placebo-controlled study design (Figure 5.1) was used to determine the efficacy of short-term oral administration of the adjunct use of DCS relative to placebo prior to computerized cognitive behavioral therapy (cCBT) sessions in a treatment-seeking sample of cocaine-dependent subjects. The two-arm clinical trial was conducted over a period of 9 months (September 2010 to May 2011) in the Psychiatric Research Institute (PRI) and Brain Imaging Research Center (BIRC) at the University of Arkansas for Medical Sciences (UAMS) in Little Rock, Arkansas. Study participants were recruited from the surrounding Little Rock area through self-referrals, response to advertisements in newspapers and the internet, and to study flyer distribution at outpatient and inpatient alcohol and drug abuse clinics, and public spaces (*e.g.,* bus stops, grocery stores, etc). The treatment plan for this clinical trial consisted of 4 weeks of outpatient treatment in which subjects had 30-minute sessions of cCBT three times per week, and one session per week was preceded by dosing of study medication (*i.e*., DCS or placebo). All subjects received once-weekly group therapy. Following prescreening and intake assessments, eligible study subjects were randomized to one of two parallel treatment arms (*i.e.,* DCS or placebo). This study used a fixed dose of 250 mg of DCS administered 60 minutes prior to the onset of cCBT. DCS and placebo groups received the study medication once a week for four weeks.

Inclusion/Exclusion Criteria

All study subjects provided written informed consent to participate in a research protocol

Figure 5.1 Study design for a randomized, double blind, placebo-controlled study of the adjunct use of DCS to boost the therapeutic response to cCBT for cocaine-dependent subjects.



approved by the University of Arkansas for Medical Sciences (UAMS) Institutional Review Board. Eligible subjects were cocaine-dependent, treatment-seeking women and men between 18 and 65 years of age. A structured pre-screening interview was conducted to determine appropriateness of this study for each participant. The pre-screening procedures for study eligibility included a review of medical diagnoses, conditions, or medications by self-report. Potential subjects were excluded based on a medical history of loss of consciousness of greater than 10 min, neurologic disorder (*e.g.*, epilepsy), severe hepatic insufficiency, significant current or prior cardiovascular disease (*e.g.*, hypertension, arrhythmias), current psychiatric disorder, history of hospitalization within the previous six months for a medical illness, and current use of psychotropic medications. Deafness, blindness or other significant sensory impairment represented exclusion criteria as all subjects had to be able to perform basic functions on a computer. Prior to study enrollment, each candidate was evaluated for study safety related to medication-DCS interactions or DCS contraindications due to medical disorders. Urinalyses were performed to assess the presence of drugs of abuse other than cocaine, marijuana, and/or nicotine, or pregnancy in female participants, with both conditions representing exclusion criteria. The menstrual stage status of all female subjects was also estimated by the self-reported time of last menses.

Randomization and Study Blind

Randomization to treatment arms (*e.g.*, placebo or DCS) was performed by a computer-generated program that randomly assigned each subject identifier to each experimental treatment group. In order of enrollment, each participant was assigned a subject number. The DCS medication and the placebo pills were identically encapsulated by the research pharmacy. The study double-blind on the treatment arms was maintained by the research pharmacist with the

research subjects, therapists, investigators, and clinical staff blinded as to treatment randomization. In the instance of a severe adverse event during the study, the blind was to be immediately broken for investigation of said severe adverse events. If at that time the study was continued, the investigator to whom the blind was broken was not involved in any direct subject or therapist interactions that could jeopardize the remainder of the blinded trial.

Assessment Instruments

Demographic and clinical information for study subjects was collected prior to randomization using a study-specific data collection form (*e.g*., age, race, education) (Refer to Table 5.1 for list of assessment instruments). The severity, frequency and amount of recent cocaine use and duration of lifetime cocaine use were also determined using the data collection form. The Addiction Severity Index (ASI) was administered to assess the severity of addiction-related functional impairment (McLellan et al., 1992). All study subjects were evaluated for the absence of drug dependencies other than cocaine and nicotine by the Structured Clinical Interview for DSM IV Axis-I Disorders (SCID) interview (First, 2002). The SCID was administered by the clinical research coordinator (*e.g.,* < 1 year of SCID experience; SCID training was facilitated by video tape training sessions and mentored supervision of SCID interviews) which allowed for quality of uniformity amongst all interviews; however, no inter-rated reliability was assessed. Current and past Axis I disorders (*e.g*., depression, anxiety disorder) were also assessed by the SCID interview and represented exclusion criteria for all subjects. The presence and/or absence of childhood trauma (*e.g.,* physical or sexual abuse, emotional or physical neglect) was evaluated using the abbreviated version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). Recent (past year) stress in study subjects was determined by the Life Experiences Survey (LES) (Sarason et al., 1978). Current anxiety features and mood states were assessed using the State-Trait Anxiety Inventory (STAI-S) (Spielberger, 1983) and Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988b). The absence of attention deficit hyperactivity disorder (ADHD) was confirmed by the Conners

Table 5.1 Quantitative instruments used to characterize cocaine-dependent subjects in the clinical trial study.

Assessment/Instruments	***Measures***
Brief demographic inventory and prior treatment history	Sex, race/ethnicity, marital status, handedness, treatment history
Recent and past drug use	Current and lifetime drug use patterns
Addiction Severity Index (ASI)	Multi-dimensional measure of addiction severity
Structured Clinical Interview for DSM-IV (SCID)	Clinical interview for psychiatric disorders (First et al., 1995)
Childhood Trauma Questionnaire (CTQ)	Measure of nature and severity of childhood maltreatment (Bernstein et al., 2003)
Positive and Negative Affect Scale (PANAS)	20-item measure of mood state (Watson et al., 1988a)
State anxiety subscale of the State-Trait Anxiety Inventory (STAI-S)	20-item measure of current level of anxiety (Speilberger, 1983)
Conners Attention Deficit Hyperactivity Disorder (ADHD) Rating Scale (CAARS)	ADHD symptoms and their severity (Conners, 1997)
Significant recent life events using the Life Experiences Survey (LES)	Assesses positive and negative life events in the previous year (Sarason et al., 1978)
Cocaine Craving Questionnaire (CCQ)	10-item measure of current cocaine cravings (Tiffany et al., 1993)
Barratt Impulsiveness Scale (BIS-11)	30-item measure of impulsive personality traits (Patton et al., 1995)
Substance Use Calendar	Assesses lapse and relapse to drug use (Hersh et al., 1999)

Adult ADHD Rating Scale (CAARS) (Conners et al., 1999). Current cravings for cocaine were evaluated by the abbreviated version of the Cocaine Craving Questionnaire (CCQ) (Tiffany et al., 1993). The Barratt Impulsiveness Scale (BIS-11) was used to assess factors of impulsivity related to attention, motor and behavioral control (Patton et al., 1995). All assessments were completed at screening (baseline) except for the STAI-S that was collected on a weekly basis and the PANAS and CCQ that were also collected at subsequent follow-up visits (*i.e.*, 1- and 2-month) (Table 5.2). The ASI was also collected at the 4-week treatment endpoint, and at the 1- and 2-month follow-up assessments to assess possible treatment-related recovery of functioning across multiple domains. Along with scheduled urine analysis screens (see below), lapse and relapse to cocaine use was monitored using the Substance Use Calendar (Hersh et al., 1999) at the 1- and 2-month follow-ups. All subjects were screened for any internal or external ferromagnetic objects (*e.g.*, cardiac pacemakers, orthodontics), medication (*e.g.*, beta-blocker) or claustrophobic contraindication for fMRI studies.

Drug Monitoring

Study subjects had urine specimens collected at all study-related visits (*i.e.*, intake, fMRI scans, thrice weekly during active treatment and at monthly follow-up visits) under staff observation and were screened on-site using qualitative urinalysis procedures (Multi-Drug Screen Test Panel; Innovacon, Inc.). All urine specimens were screened for cocaine and its metabolites, and other drugs of abuse (*i.e.*, methamphetamine, opioids, benzodiazepines, marijuana, and amphetamines). Breath samples were analyzed using an Alco-Sensor III Breathalyzer (Intoximeter Inc.) at study-related visits and, if a participant had a blood alcohol level > 0.08, the subject was dismissed from therapy for that day and assessments were not performed.

Computerized Cognitive Behavioral Therapy (cCBT)

The cCBT consisted of 12 possible sessions over 4 weeks with weekly treatment visits of three cCBT sessions and one group therapy session. Prior to each cCBT session, subjects were asked if they were experiencing any significant life events or thoughts about suicide. Subjects

Table 5.2 Timeline of study procedures, instruments/assessments, and treatment randomization for a randomized, double blind, placebo-controlled study of the adjunct use of DCS to facilitate cCBT in cocaine-dependent subjects (For a comprehensive list of assessments and associated abbreviations, please refer to Table 5.1.)

Screening/Intake	
Pre-Screening Eligibility for Study	If subject eligible, continue to intake. If subject not eligible, subject cannot further participate in research study.
Intake	Informed consent and HIPAA document process Assessments: ASI, LES, CAARS, PANAS, STAI-S, CTQ, SCID, BIS-11, CCQ, Cocaine Cue Word Generator Self-report of medical history: drug use, mental and physical health Specimens: Urinalysis, Breathalyzer, Pregnancy Test
Month 1: Post Randomization	
cCBT	Thrice weekly Group therapy session once weekly
Medication	250 mg DCS once weekly Placebo once weekly
Drug Screening	Specimens thrice weekly: Urinalysis, Breathalyzer
fMRI	1st fMRI - Prior to 1st CBT session 2nd fMRI – Following the end of the 4-week treatment period
Assessments	STAI-S once weekly ASI at the end of the 4-week treatment period
Month 2	
1-Month Follow up	Assessments: ASI, STAI-S, PANAS, CCQ, Substance Use Calendar Specimens: Urinalysis, Breathalyzer
Month 3	
2-Month Follow up	Assessments: ASI, STAI-S, PANAS, CCQ, Substance Use Calendar 3rd fMRI Specimens: Urinalysis, Breathalyzer, Pregnancy Test

who disclosed suicidal ideation and/or perceived a need to further disclose sensitive information about significant adverse life events were referred to the clinical staff and/or on-call therapist. All study subjects received a cCBT based on the behavioral treatment approach outlined in the National Institute for Drug Abuse (NIDA) Therapy Manual for Drug Addiction: Manual 2 (Budney, 1998). This cCBT has previously been demonstrated to be effective for the treatment of opiate dependence (Bickel et al., 2008). A total of 24 modules were selected for topic content related to the subject sample and are listed in Table 5.3 in the order that they were experienced. The content of all of the modules was written at a 7th grade level of reading ability. Prior to the initial cCBT session, all subjects completed a training module on the computer to acclimate them to the presentation format of the computer-delivered treatment and to allow them to practice responding to the fluency training-related queries (see below). Subjects had the option to have the therapy-related information read aloud via headphones by the computer, concurrent with its visual presentation. The format of the cCBT included fluency-building training which actively engages subjects in effectively learning and retrieving the cCBT information presented by requiring them to respond to a series of questions using multiple choice and fill-in-the-blank answers. Each question was presented in various permutations so that the wording was different. During each topic, participants were given immediate feedback on their responses by the computer. Fluency building requires the participant to develop a predetermined level of content fluency related to each of the cCBT modules based on their accuracy and speed in responding. If fluency criteria were not met the module was repeated so that the content of the interactive cCBT is adjusted based on a given participant's level of acquired comprehension of the module contents. Study subjects were allowed to repeat any CBT modules after the completion of all 24 modules.

Group Therapy

Group therapy was conducted to engage all study participants in an evidence-based therapy and involved four weekly 45 minute sessions that followed the Group Drug Counseling

Table 5.3 Order of Modules selected for project-specific computerized cognitive behavioral therapy.

Training Module
What is a Functional Analysis?
Conducting a Functional Analysis
Self Management Planning
Managing Thoughts about Using
Managing Negative Moods and Depression
Drug Refusal Skills Training
Introduction into Problem Solving
Effective Problem Solving
Seemingly Irrelevant Decisions
Taking Responsibility for Choices
Coping with Thoughts about Using
Decision Making Skills
Increasing Self Confidence in Decision Making
Identifying and Managing Triggers for Risky Drug Use
Introduction into Assertiveness
How to Express Oneself in an Assertive Manner
Communication Skills
Nonverbal Communication
Introduction to Anger Management
How to Become More Aware of the Feeling of Anger
Coping with Anger
Introduction into Relaxation Training
Progressive Muscle Relaxation Generalization

manual (Daley, 1998). Each session was comprised of three parts: 1) group members stated the substance(s) to which they were addicted, how long they have been sober, and a summary of their previous week's activities to stay sober, 2) there was a group discussion on a topic pertaining to addiction and recovery, and 3) members told the group their plans for their recovery for the upcoming week. Group topics covered in session included psychoeducation about addiction, triggers, managing negative emotions, relationships and addiction, behavioral and cognitive strategies to manage triggers and craving, etc. Group therapy sessions were led by a Ph.D.-level clinical psychologist (*e.g.*, experience ~ 2.5 years).

Contingency Management Voucher System

Sustained attendance at treatment visits is typically poor among cocaine-dependent subjects with failure to participate in treatment leading to worse clinical outcomes (McKay et al., 2001). In this clinical trial, we employed a contingency management procedure to reinforce attendance as has been demonstrated to be effective in other trials (Garcia-Rodriguez et al., 2009; Rash et al., 2008). Subjects were not penalized for cocaine-positive urine specimens or reinforced for cocaine-negative specimens. Rather, a voucher system (Bickel et al., 2010) involved systematically reinforcing the completion of three successive treatment visits. Completion of three treatment visits meant attendance at a weekly group therapy session, attendance at the three cCBT sessions, providing three urine specimens, and completion of all required weekly assessments. The first completion of a scheduled visit was worth 10 points (each point is equivalent to $0.25) or $2.50. Each subsequent consecutive attendance at a treatment visit increased the value of the voucher by five points (2^{nd} = 15 points, 3^{rd} = 20 points, etc.). As an additional incentive for continuous completion of treatment visits, a $10.00 bonus was provided to participants for each set of 3 consecutive completed treatment visits. Continuous attendance throughout the 4-week study treatment period resulted in a participant receiving vouchers equivalent to $152.50. Points earned were redeemed by the participant by check. Failure to complete a treatment visit (*e.g.*, missing group therapy) resulted in no vouchers being earned for

that visit. If a participant was a no-show for a treatment visit, the value of the voucher was reset to the initial $2.50 level. Completion of the next three consecutive clinic visits would return the value of the vouchers to the level obtained before the reset. Points, once earned, could not be lost.

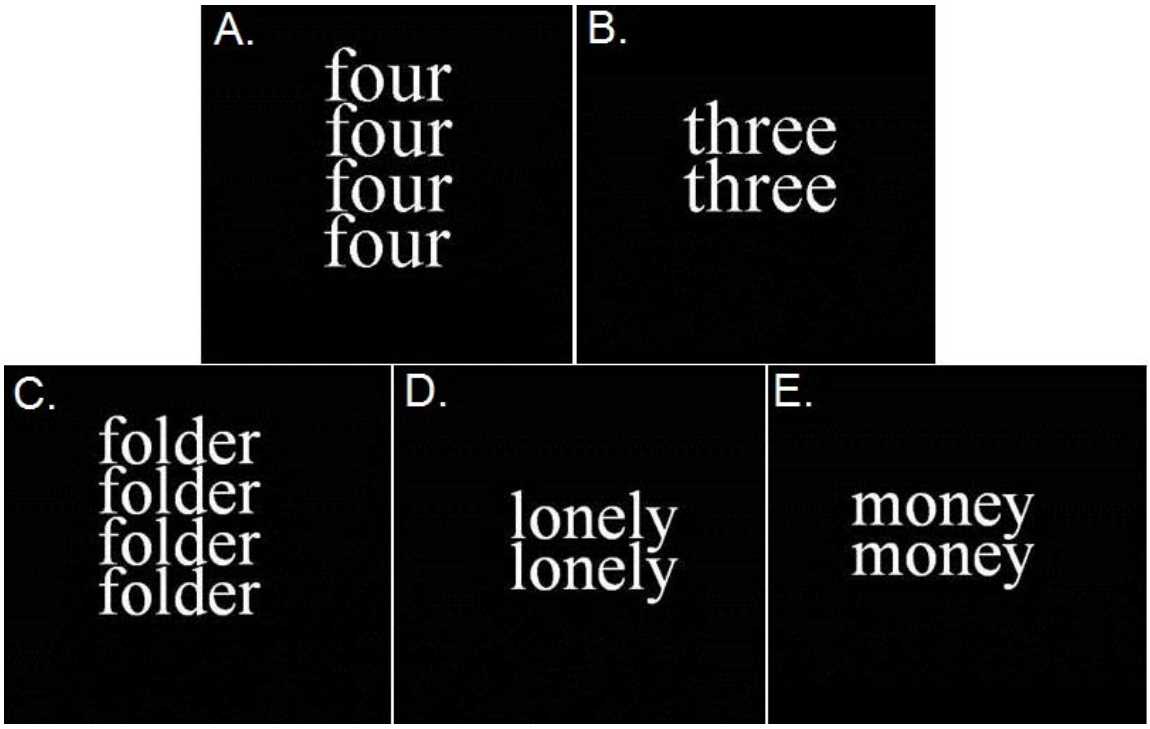
Follow-up Visits

After completion of the 4-week active treatment program, participants were contacted by research staff for follow-up visits at 1- and 2-months post-treatment to assess the durability of a treatment effect. The participants provided urine and breath samples for drug analysis and pregnancy testing, and completed a battery of assessments (*i.e.*, ASI, STAI, PANAS, CCQ, Substance Use Calendar) at each follow-up visit. Participants completed an fMRI session at the 2-month follow-up visit.

Stroop Tasks

In this clinical trial, we assessed the effect of treatment on the conditioned responses (CR) to drug use cues (CS) using a modified word counting Stroop task that incorporated words representing personal drug use reminders (cocStroop task) and drug use-neutral words (see Chapters 2 and 3). All subjects at study entry provided eight cocaine cue words that were associated with their drug craving and use behaviors. The subjects ranked, on a scale of 1-6, each provided drug-related cue word as to its relative strength as a drug use reminder. The cocStroop task comprised eight personal drug use-related (CS+) words (*e.g.*, money, stem) and drug use-neutral (CS-) words (*e.g.*, shelf, table). As a control for general cognitive control ability, a counting Stroop (cStroop) task was comprised of congruent stimuli in which the number and name of the words are the same and incongruent stimuli in which they differ (Bush et al., 2006). The repeated measures cStroop task assessed non-specific effects of treatment on cognitive control and executive functioning that can be altered by cocaine withdrawal as well as prolonged treatment-related cocaine abstinence. For each of the three tasks, a trial involved the presentation of 1-4 identical words in a vertical array with instructions to indicate the number of words presented by depressing one of four pre-assigned buttons on a response device (Figure 5.2).

Figure 5.2 Stimulus trials for congruent (A.) and incongruent (B.) stimuli for the cStroop task and neutral (C.) emotional (D.) and cocaine use (E.) words for the cocStroop and eStroop tasks.



Subjects were instructed to respond as quickly as possible. Each Stroop task run involved the presentation of 285 word counting trials in which 96 of the words were emotionally valenced (eStroop), drug use-related (cocStroop) or incongruent (cStroop) word stimuli. Stimuli were presented for 2000 msec followed by the presentation of a fixation cross with an interstimulus interval of 300 msec. Task training prior to fMRI acquisition involved repeated trials of the cStroop task until an accuracy of ≥ 95% was attained. Word stimulus trials representing the cStroop, eStroop and cocStroop tasks were presented in a mixed blocked, event-related design in a pseudorandom order with a total run time of approximately 12 minutes. The first and third blocks of stimulus trials consisted of emotionally valenced, drug use-related and neutral stimuli with the second block compromised of congruent and incongruent stimuli. Each block was separated by the presentation of a fixation cross for 20 sec. Each fMRI session [*e.g.*, baseline ("session 1"), 4-week treatment endpoint ("session 2"), 2-month follow-up ("session 3")] involved three runs with a 1 minute rest period between each run for a total on-task scanner time of approximately 38 minutes. Stroop task-related fMRI responses and behavioral measures of reaction time (RT) and response accuracy were collected for each task trial at each fMRI session.

Statistical Analyses

All analyses are for subjects that were "actively" enrolled in treatment as defined as completion of a minimum of three cCBT sessions and one dose of study medication (*i.e.*, DCS or placebo). Continuous data (*e.g.*, demographic and clinical characteristics) were analyzed using a series of t-tests. Wilcoxon tests were used when the respective data set violated assumptions of distribution normality. Categorical data were analyzed using chi-square or fisher's exact when sample sizes were relatively small (*e.g.*, $n < 5$). Paired t-tests were performed to assess differences in reaction times between neutral, emotional, and drug-use related words in the cocStroop and eStroop tasks (*e.g.*, neutral versus emotional; neutral versus cocaine) and between congruent and incongruent stimuli in the cStroop task among all subjects at baseline. To assess possible differences between the placebo and DCS treatment arms on the baseline interference

effects for the cStroop (*e.g*., incongruent – congruent), eStroop (*e.g*., emotional – neutral) and cocStroop (*e.g*. cocaine – neutral), a series of t-tests was performed. A series of repeated-measures ANOVAs were performed to determine differences between runs 1-3 within session 1 for all subjects in the cStroop, eStroop and cocStroop tasks. Baseline carry-over effects (*i.e*., the effect of one trial "carries over" to the next trial) were calculated and analyzed by paired t-test for the following trial pairs: emotional - neutral > neutral - neutral, cocaine - neutral > neutral - neutral, incongruent - incongruent > congruent - incongruent, and incongruent - congruent > congruent - congruent. Paired t-tests were also used to determine differences between session 1 and 2 data for the neutral, emotional and drug use-related words and the incongruent and congruent stimuli for all subjects. A series of two-way ANOVAs was performed for each Stroop task to determine differences for arm, session and arm*session interaction. Data were analyzed using SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA).

fMRI Acquisition and Analyses

fMRI data was acquired and analyzed at the Brain Imaging Research Center (BIRC). Study subjects completed the Stroop tasks while positioned in a Phillips 3T Achieva X-series MRI scanner. fMRI data were collected using an echo planar (EPI) sequence with thin axial slices (3 mm thickness) to minimize signal loss in susceptible regions of interest (*e.g*., ventral prefrontal cortex). The EPI sequence had TR = 2500 ms, TE = 30 ms, FOV = 192 x 192 mm, matrix = 64 x 64 with 30 slices of 3 mm thickness for 3 x 3 x 3 mm voxel volumes. Structural scout images were acquired using a spin echo, T1-weighted pulse sequence (TR = 500 ms, TE = 20 ms, flip angle = 90 degrees). In addition, a 3D MPRAGE sequence were used to acquire 3D anatomical data at an isotropic resolution of 1 x 1 x 1 mm to enable activation localization and co-registration with functional neuroimaging data. In addition to task-related scans, we acquired a "resting-state" fMRI scan (7 min, 20 sec) in which the subject simply rested awake in the scanner.

Image processing and analysis was conducted with SPM8, AFNI and MATLAB. Images were resliced and corrected for motion by registration to the first functional image acquired for

each subject using a 6 parameter transformation (Friston et al., 1995). Images were spatially normalized to the Montreal Neurological Institute (MNI) template by applying a 12 parameter affine transformation followed by nonlinear warping using basis functions (Ashburner and Friston, 1999). Images were smoothed using a Gaussian kernel of 5 mm full width at half maximum to enhance signal to noise ratios and enable group comparisons. Global differences were controlled by proportional scaling. Low frequency noise was removed using a high-pass filter and serial correlations accounted for by an autoregressive model of the first order.

After standard image preprocessing (slice timing, realignment/motion correction, linear detrending, global normalization, smoothing) images were entered into a two-level statistical analyses. *General linear model (GLM):* The first-level fit subject-specific models and the resulting individualized parameters were combined in the second level model to obtain group level estimates. At the first-level, SPM8 and/or AFNI were used to fit the canonical hemodynamic response function (HRF) plus the time and dispersion derivatives across the conditions of interest (*e.g*., cocaine versus neutral stimuli). These summary statistical images were then subjected to second-level tests to assess differences within and across subjects. *Correlation analyses:* Effects of task and treatment conditions on responses in functional regions-of-interest (ROI) were defined by a functional mask created by the results of the group activation maps for contrast images of interest (*e.g*., cocaine > neutral words). The boundaries of the functional ROI were defined by either a 5 mm radius sphere centered on activation maxima, or defined by the activation cluster extents. The summary statistics images from the first-level subject-specific models were entered into a linear regression model with residual Stroop interference effect (the average neutral reaction times were regressed on average cocaine reaction times and residuals were extracted from the model.) for each subject as a covariate. The results from all these analyses are reported at $p < 0.005$, uncorrected unless otherwise specified. A cluster-level corrections for multiple comparison corrections used permutation analyses.

RESULTS

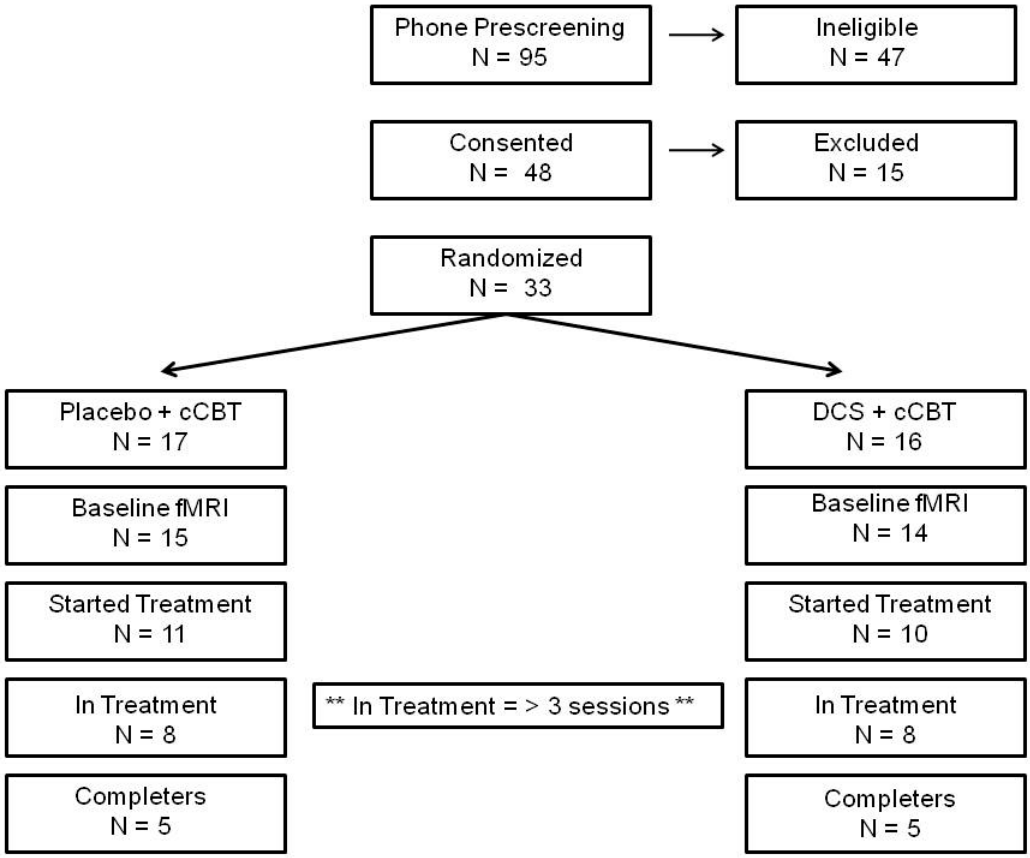
Subjects

The participant screening, assessment, randomization, and treatment enrollment outcomes for the study are depicted as a flow diagram in Figure 5.3. Ninety-five possible study volunteers were contacted between September 2, 2010 and April 15, 2011. Utilizing a phone pre-screening tool to determine preliminary study eligibility, 47 out of 95 subjects were deemed ineligible based on study exclusion criteria defined by self-reported medical diagnoses, conditions, and/or medications. Of the remaining 48 subjects who provided written informed consent to participate in the clinical trial and were further assessed for study eligibility, 15 were excluded due to Axis I disorders (*e.g.*, depression, other drug dependencies) as determined by the SCID, and/or reports of unstable medical conditions or diagnoses. The remaining 33 study-eligible subjects completed a battery of qualitative and quantitative assessments and were randomized to either the placebo or DCS treatment arms. Of the 17 and 16 subjects in the placebo and DCS arms, a total of 15 and 14 subjects completed the baseline fMRI scan, respectively. Reasons for failing to complete the fMRI scan included discomfort in the scanner environment or loss to follow-up. Upon completion of the fMRI scan, 10 subjects in the DCS arm and 11 subjects in the placebo arm were randomized. Subjects were considered "actively" enrolled in the treatment study following completion of a minimum of three cCBT sessions and receiving at least one dose of study medication (*i.e.*, DCS or placebo); five subjects failed to meet this post-randomization requirement for study inclusion. Reasons for failing to satisfy these active enrollment criteria included loss to follow-up after the baseline scan or having completed only one cCBT session. Of the 16 subjects actively enrolled in the clinical trial study, 8 were randomized to the DCS arm and 8 to the placebo arm. One additional subject was subsequently withdrawn from the study due to failure to report his/her enrollment in a concurrent clinical trial for medication development.

Baseline Demographic and Clinical Variables

There was no significant difference between the treatment arms on any of the baseline

Figure 5.3 Flow diagram of study participants. A total of 33 subjects were randomized to the placebo and DCS treatment arms of the clinical trial.



demographic or clinical characteristics, indicating that randomization had been successful (Table 5.4). However, there were significant differences between the treatment groups for certain subscales of the CTQ (*e.g.*, sexual abuse and emotional neglect, $p < 0.05$) and the BIS-11 (*e.g.*, attentional impulsiveness, $p < 0.05$).The main route of administration for crack-cocaine use was smoking for all of the subjects. 56.3% of the subjects self-reported anti-social behaviors with no significant difference between the treatment arms in their prevalence.

Outcome Variables: Drug Abstinence and Treatment Retention

Prior to treatment, 50% of subjects for each study arm had positive urinalysis outcomes for cocaine metabolites and/or other drugs. There was a significant difference between the placebo and DCS treatment arms for the percentage of subjects with cocaine-positive urinalysis outcomes across the 4-weeks of study treatment ($p < 0.05$) (Figure 5.4). Relative to the placebo condition, adjunct DCS was associated with a *greater* percentage of cocaine-positive urines. There was no significant effect of week on this outcome variable across the four weeks of treatment, or for the arm x week interaction. For weekly percentage of completed treatment sessions, there was no significant difference between the placebo and DCS treatment arms across the 4-week treatment trial (Figure 5.5). There was also no significant effect of week on this outcome variable across the four weeks of treatment, or for the arm x week interaction.

Stroop Task Performance

Baseline. Consistent with prior results (Carpenter et al., 2006; Copersino et al., 2004), cocaine-dependent subjects exhibited significant cognitive interference effects for drug use-related ($p < 0.005$) and negative emotional ($p < 0.005$) words for the cocStroop and eStroop tasks, respectively (Figure 5.6). Subjects also exhibited significant cognitive interference effects ($p < 0.0001$) for the processing of incongruent versus congruent stimuli for the cStroop task (Figure 5.7). There was no significant difference at baseline between subject groups comprising the two treatment arms for the interference effects associated with either the cocStroop, eStroop or cStroop tasks (Table 5.5). An analysis of the effect of task replication within session runs on

Table 5.4 Demographic and Clinical Characteristics of the Randomized Subject Sample (Race: AA = African American; CA = Caucasian; HS= Hispanic); Values represent X +/- SD. (*Wilcoxon test; ** Fisher Exact Test).

	Placebo	DCS
Variable		
N	8	8
Age (yrs)	44.3 +/- 5.2	45.3 +/- 5.3
Education (yrs)*	11.8 +/- 0.89	12.4 +/- 1.1
Race (AA/CA/HS)**	7/1/0	4/3/1
Sex (M/F)**	7/1	6/2
Handedness (R/L)**	7/1	8/0
Drug Use		
Age: daily cocaine use	28.0 +/- 7.4	30.3 +/- 5.6
Use past 7 days*	2.9 +/- 2.0	1.6 +/- 2.3
Use past 30 days	17.3 +/- 8.8	10.1 +/- 9.4
# of treatment enrollments*	2.3 +/- 3.5	3.5 +/- 1.9
Age: 1st Alcohol Use*	15.9 +/- 3.0	15.4 +/- 2.7
Age: 1st Nicotine Use	22.1 +/- 10.0	18.8 +/- 7.6
Addiction Severity Index (ASI)		
Medical*	0.20 +/- 0.28	0.30 +/- 0.35
Employment*	0.77 +/- 0.18	0.76 +/- 0.35
Alcohol	0.20 +/- 0.25	0.18 +/- 0.19
Drug*	0.21 +/- 0.10	0.20 +/- 0.13
Legal*	0.07 +/- 0.10	0.01 +/- 0.03
Family*	0.09 +/- 0.17	0.20 +/- 0.22
Psychiatric*	0.06 +/- 0.11	0.004 +/- 0.01
Quantitative Assessments (Total Scores)		
CTQ*	43.5 +/- 17.5	57.6 +/- 21.6
BIS-11	62.9 +/- 9.9	69.5 +/- 9.7
CAARS - ADHD Index	47.0 +/- 9.5	51.6 +/- 11.8

Figure 5.4 Percentage of participants with cocaine-positive urinalysis results for the placebo/cCBT and DCS/cCBT treatment arms at intake and at weekly intervals during treatment.(two-way ANOVA; arm: $p < 0.05$*, week: $p = 0.912$, arm x week interaction: $p = 0.500$)

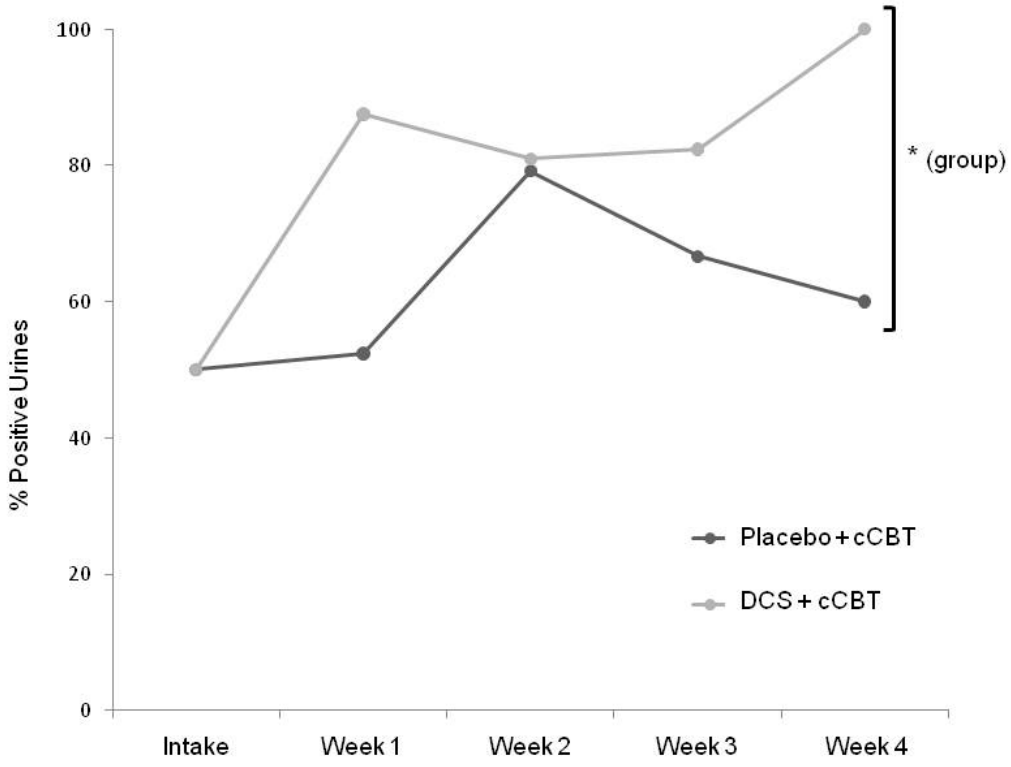


Figure 5.5 Percentage of completed weekly cCBT sessions for the placebo/cCBT and DCS/cCBT treatment arms for the 4-weeks of treatment. (two-way ANOVA; arm: $p = 0.648$, week: $p = 0.281$, arm x week interaction: $p = 0.710$)

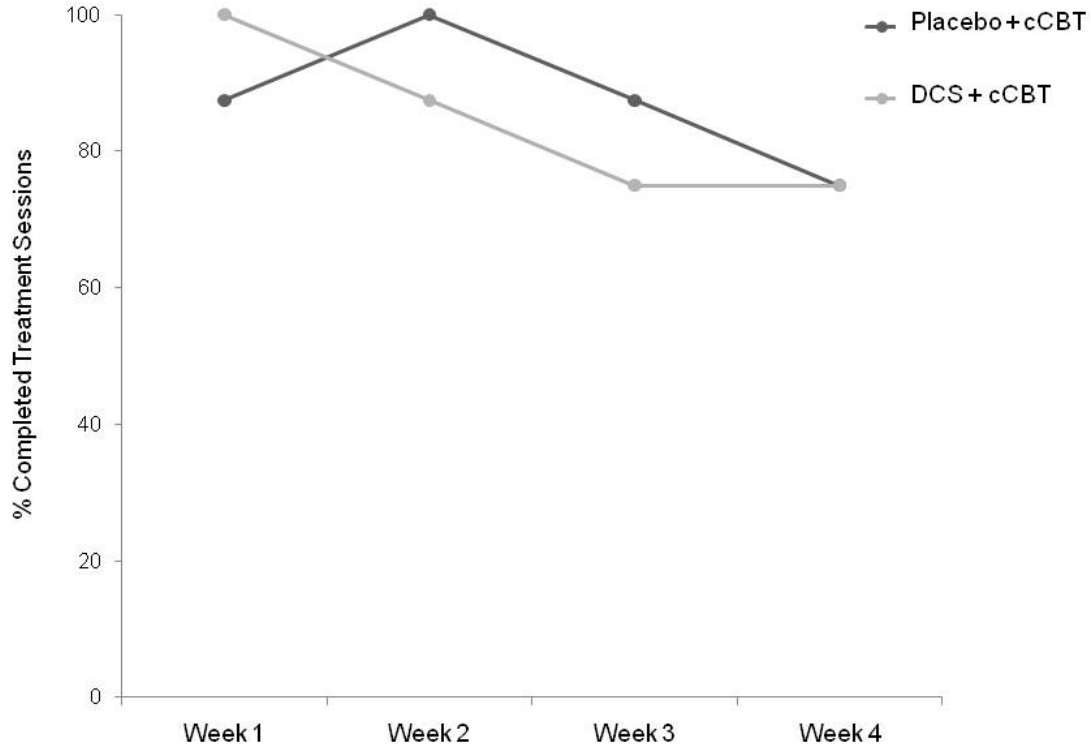


Figure 5.6 Reaction times for drug use-related, negative emotionally-valenced and neutral words for the cocStroop and eStroop tasks. The group mean reaction time (± SD) for neutral words, emotional words, and cocaine use-related was 896.7 (± 96.5), 918.2 (± 96.0) and 966.0 (± 130.7) msec, respectively (paired t-test, $p < 0.005$**). The group mean for the cocStroop and eStroop interference effects (cocaine – neutral; emotional - neutral) was 69.3 (± 83.1) and 21.5 (± 19.9), respectively.

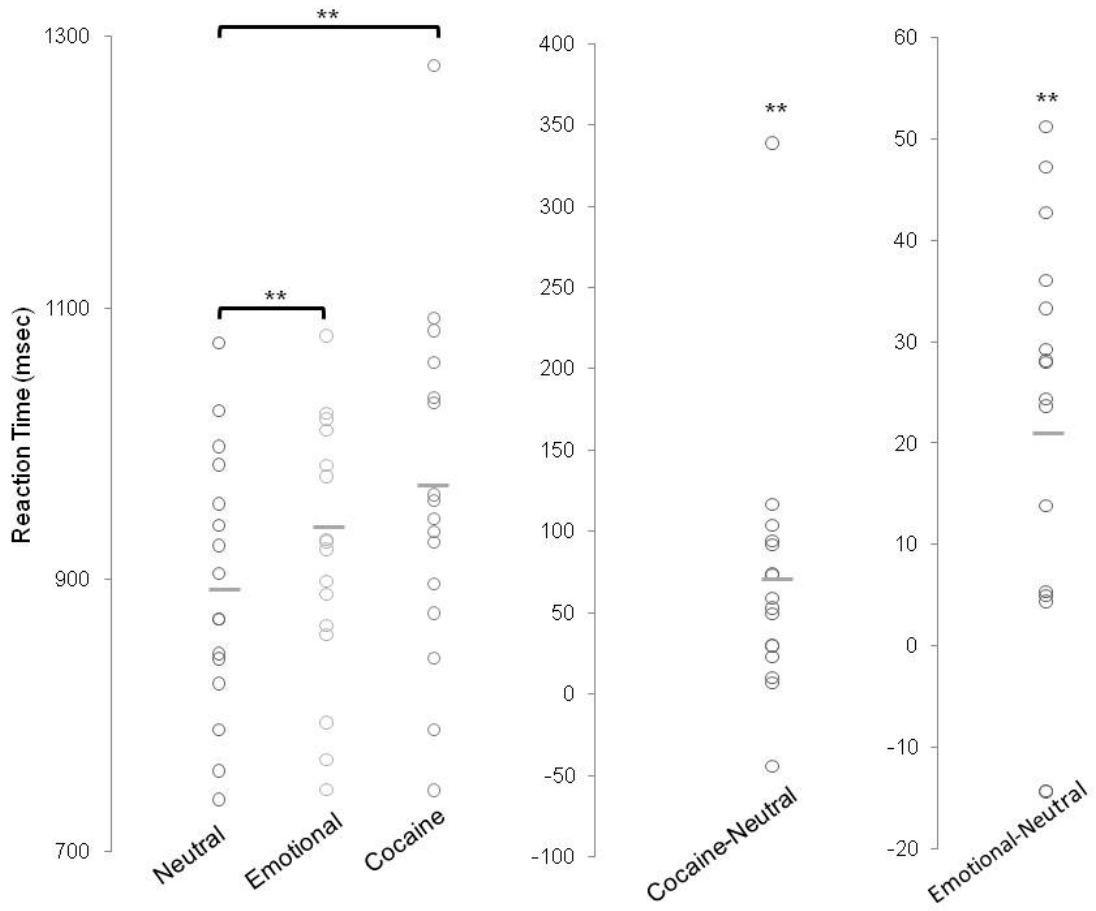


Figure 5.7 Reaction times for congruent and incongruent stimuli for the cStroop task. The group mean reaction time (± SD) for congruent and incongruent stimuli was 811.7 (± 94.6) and 943.6 (± 118.7) msec, respectively (paired t-test, $p < 0.0001$***). The group mean for the cStroop interference effect (incongruent - congruent) was 131.8 (± 55.3).

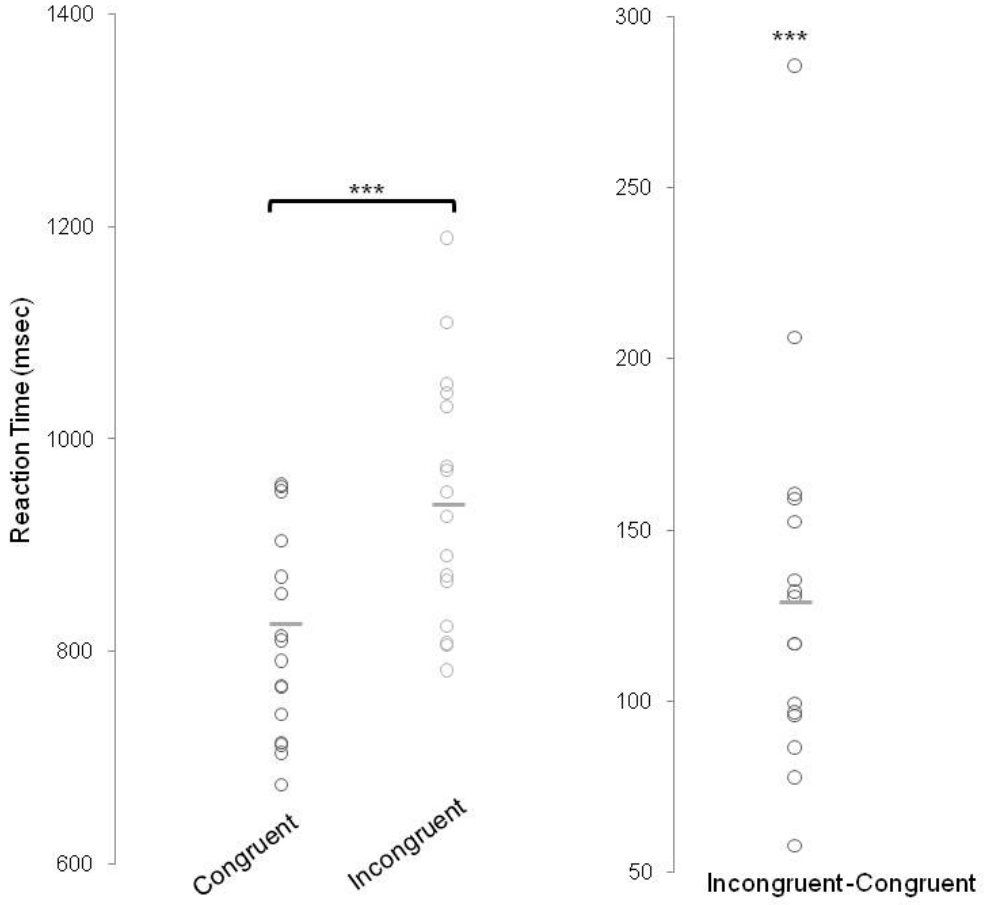


Table 5.5 Baseline interference effects (mean ± SD) for the cocaine Stroop (cocStroop), emotional Stroop (eStroop), and counting word Stroop (cStroop) tasks for the placebo/cCBT and DCS/cCBT treatment arms of the controlled clinical trial (N = 8).

Stroop task	Placebo + cCBT	DCS + cCBT	p-value
cocStroop	107.9 ± 95.5	30.7 ± 47.7	0.060
eStroop	22.9 ± 18.6	20.0 ± 22.3	0.781
cStroop	117.5 ± 35.8	146.2 ± 69.3	0.316

measured interference effects indicated a significant effect of run for all three of the Stroop tasks (Figure 5.8, 5.9). An analysis of carryover effects related to the processing of drug use-related or negative emotional word stimuli indicated that reaction times for the processing of neutral words preceded by cocaine use-related words ($p < 0.005$) or emotional words ($p < 0.0001$) were significantly longer than for neutral words preceded by neutral words (Figure 5.10). Similarly, for the cStroop task, reaction times were significantly greater for congruent stimuli preceded by incongruent stimuli versus congruent stimuli preceded by congruent stimuli ($p < 0.005$) (Figure 5.11). Additionally, reaction times were greater for incongruent stimuli preceded by congruent stimuli as opposed to incongruent stimuli preceded by incongruent stimuli ($p < 0.05$).

Effect of Treatment. For all subjects, there was no significant difference between the pre- and post-treatment sessions in the magnitude of the interference effects for the cocStroop, eStroop, or cStroop tasks (Table 5.6). There was a significant difference between sessions for the attentional bias effect for cocaine use-related words ($p < 0.05$); there was no significant main effect of arm or session x arm interaction effect for the cocStroop task. In contrast, there was no significant effect of session for the cognitive interference effect associated with the eStroop or cStroop tasks (Table 5.6), or for the main effect of treatment arm or the session x arm interaction term.

fMRI Responses

Baseline Stroop Tasks. For the cocStroop task, drug use-related words were associated with robust activation of the inferior/middle frontal gyrus ($p < 0.05$, corrected), as well as the inferior frontal gyrus, posterior cingulate cortex, superior temporal gyrus (relative deactivation), and occipital cortex ($p < 0.005$, uncorrected) (Table 5.7, Figure 5.12). The contrast of drug use-related versus negative emotional word stimuli was used to assess the contention that attentional bias is related to negative emotional responses to drug use reminders (Cox et al., 2006). This contrast was also associated with robust activation of the left middle frontal gyrus ($p < 0.05$, corrected), as well as the middle temporal gyrus, inferior frontal gyrus, fusiform gyrus, and

Figure 5.8 Reaction times for baseline congruent and incongruent stimuli across Runs 1, 2, and 3 for the cStroop task. The mean reaction time (± SD) for the congruent and incongruent stimuli for Runs 1, 2, and 3 are 956.3 (± 134.0), 876.6 (± 93.1), 857.2 (± 88.2) and 978.9(± 133.0), 908.7 (± 102.7), 867.0 (± 86.4) msec, respectively (repeated-measures ANOVA, $p < 0.005$**; $p < 0.0001$***).

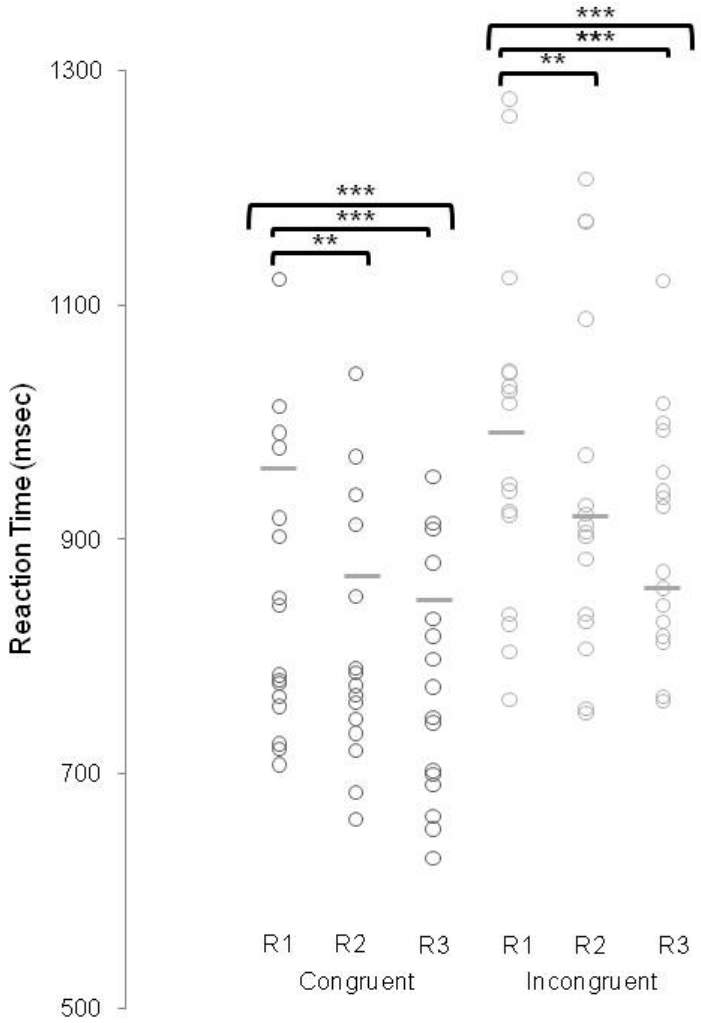


Figure 5.9 Reaction times for baseline neutral, emotional, and cocaine stimuli across Runs 1, 2, and 3 for the cocStroop and eStroop tasks. The mean reaction time (± SD) for the neutral, emotional, and cocaine stimuli for Runs 1, 2, and 3 are 1023.4 (± 155.7), 954.2 (± 128.6), 920.5 (± 125.0) and 852.5 (± 123.0), 807.2 (± 107.0), 775.5 (± 101.6) and 986.7 (± 148.0), 940.5 (± 145.8), 903.5 (± 100.0) msec, respectively (repeated-measures ANOVA, $p < 0.05$*; $p < 0.005$**; $p < 0.0001$***).

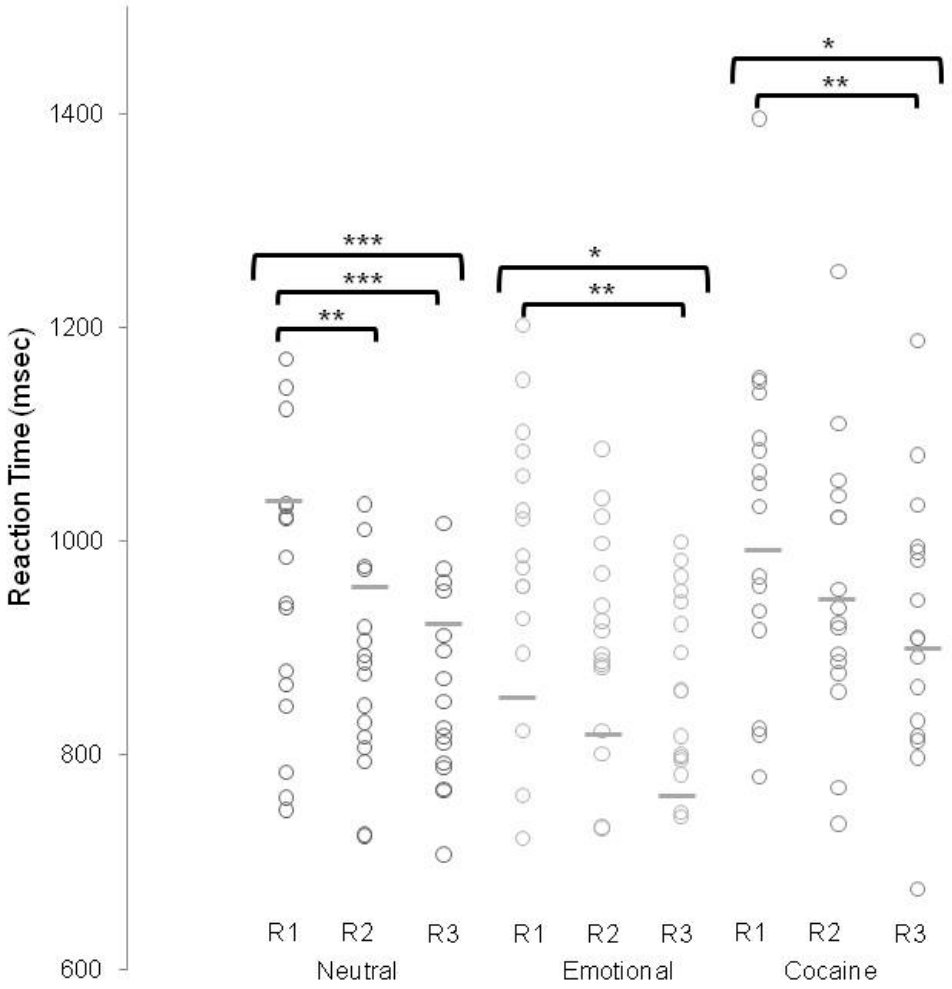


Figure 5.10 Reaction times for carry-over effects (e.g. emotional_neutral, cocaine_neutral, and neutral_neutral) for the cocStroop task. The group mean reaction time (± SD) for the emotional_neutral, cocaine_neutral, and neutral_neutral carry-over effects was 929.3 (± 107.6), 914.8 (± 101.9) and 878.7 (± 97.1) msec, respectively (paired t-test, $p < 0.005$**; $p < 0.0001$***).

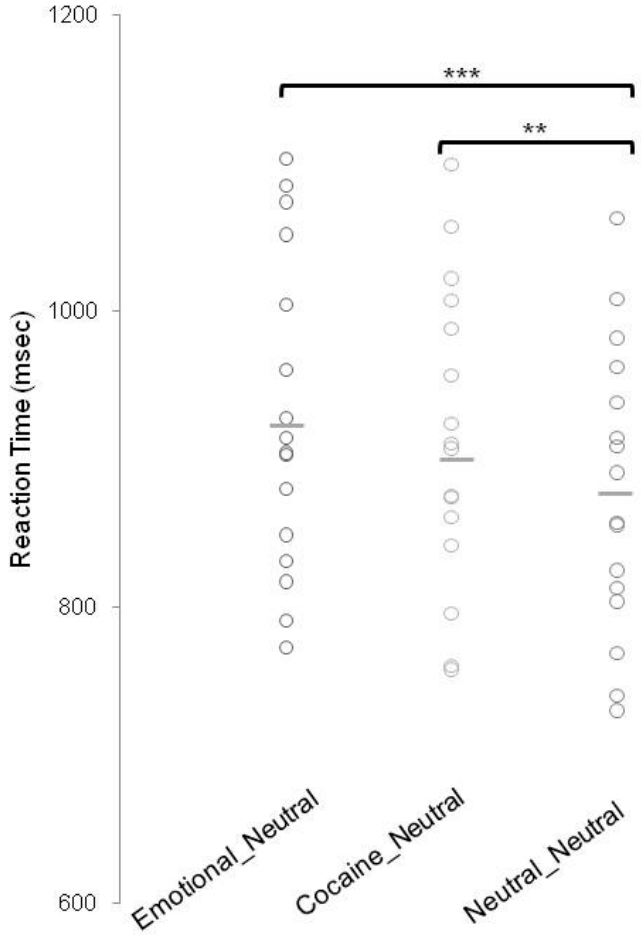


Figure 5.11 Reaction times for carry-over effects (e.g. incongruent_incongruent, congruent_incongruent, incongruent_congruent, and congruent_congruent) for the cStroop task. The group mean reaction time (± SD) for the incongruent_incongruent, congruent_incongruent, incongruent_congruent, and congruent_congruencarry-over effects was 931.7 (± 104.9), 952.6 (± 127.6), 828.8 (± 103.9) and 799.8 (± 98.8) msec, respectively (paired t-test, $p < 0.05$*; $p < 0.005$**).

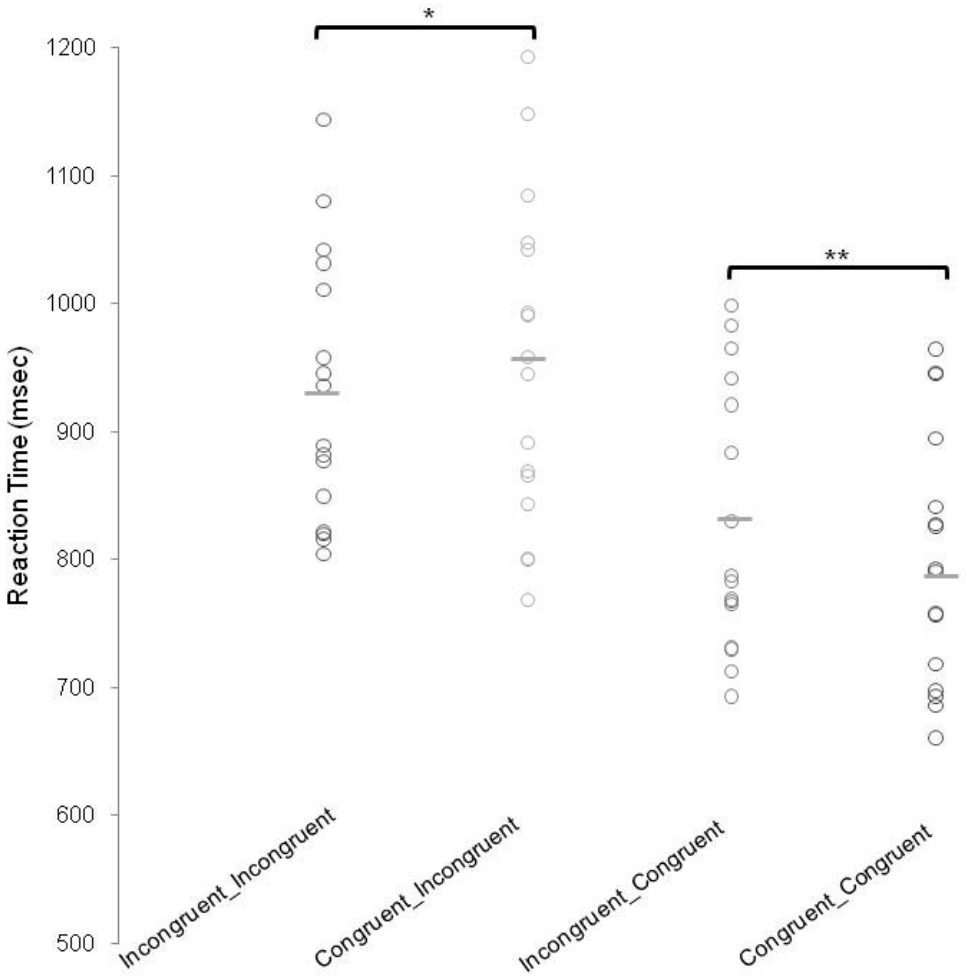


Table 5.6 Effect of treatment on level of interference effects (msec) for the cocaine Stroop (cocStroop), emotional Stroop (eStroop), and counting word Stroop (cStroop) tasks. Mean interference effects (± SD) represent the mean latency of correct responses for the cocaine, emotional, or incongruent words minus the mean latency of correct responses to the neutral or congruent words. Data are summarized for the treatment completers only (N = 5) with data representing the pre-treatment (S1) and post-treatment (S2) sessions for the placebo/cCBT and DCS/cCBT treatment arms of the controlled clinical trial.

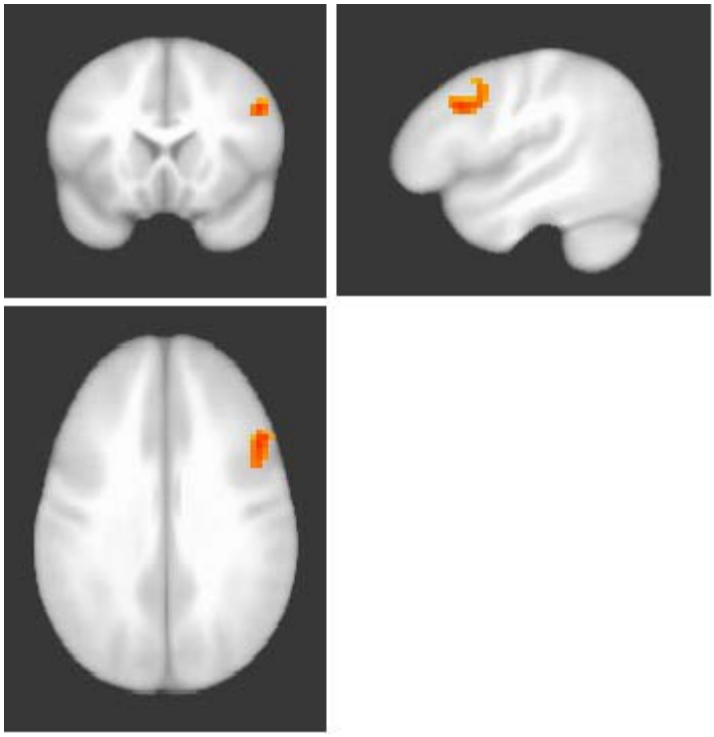
	Arm		By session	By arm
Stroop task	Placebo + cCBT	DCS + cCBT		
S1: cocaine	123.7 ± 121.3	51.4 ± 43.8		
S2: cocaine	50.9 ± 29.7	5.2 ± 47.5	$p < 0.05$*	$p = 0.158$
S1: emotional	18.8 ± 23.4	25.4 ± 26.7		
S2: emotional	29.2 ± 36.1	17.5 ± 29.1	$p = 0.912$	$p = 0.953$
S1: counting word	100.7 ± 31.4	112.1 ± 30.8		
S2: counting word	100.0 ± 39.0	99.8 ± 22.7	$p = 0.651$	$p = 0.685$

Table 5.7 Anatomical location of neural responses related to attentional bias for the cocStroop and eStroop tasks and cognitive interference for the cStroop task in cocaine-dependent subjects. (MNI = Montreal Neurological Institute)

Region[a]	MNI coordinates			Cluster	Voxel T
Cocaine > neutral stimuli (cocStroop)					
L. Inferior/middle frontal gyrus[b] (BA 44/9)	-46.5	16.5	32.5	58	5.15
L. Inferior frontal gyrus[c] (BA 45)	-49.5	22.5	17.5	21	5.66
L. Posterior cingulate cortex[c] (BA 23)	-4.5	-52.5	14.5	14	4.72
L. Superior temporal gyrus [c] (BA 22)	-49.5	-1.5	-0.5	14	-4.82
R. Cerebellum[c]	25.5	-73.5	-21.5	9	4.42
R. Middle occipital gyrus[c] (BA 19)	34.5	-91.5	17.5	8	4.48
Cocaine > emotional stimuli (cocStroop and eStroop)					
L. Middle frontal gyrus[b] (BA 9)	-49.5	22.5	35.5	44	5.31
L. Middle temporal gyrus[c] (BA 21)	-58.5	-34.5	-12.5	20	5.26
L. Inferior frontal gyrus[c] (BA 45)	-52.5	25.5	17.5	18	4.24
R. Inferior frontal gyrus[c] (BA 45)	37.5	31.5	14.5	13	4.42
L. Fusiform gyrus[c] (BA 36)	-40.5	-34.5	-21.5	11	4.58
L. Precuneus[c] (BA 19)	-34.5	-76.5	41.5	10	4.27
Emotional > neutral stimuli (eStroop)					
L. Middle temporal gyrus [c] (BA 37)	-55.5	-61.5	2.5	11	-4.77
R. Inferior frontal cortex [c] (BA 46)	37.5	34.5	11.5	7	-4.08
Incongruent > congruent stimuli (cStroop)					
L. Inferior frontal gyrus[b] (BA 44)	-49.5	13.5	23.5	38	5.26
L. Inferior parietal cortex [c] (BA 40)	-52.5	-31.5	47.5	19	4.40
L. Precentral gyrus[c] (BA 4)	-31.5	-7.5	50.5	7	4.31
R. Middle frontal cortex [c] (BA 6)	19.5	-1.5	56.5	7	4.80
Correlation between level of attentional bias (cocaine-neutral) and BOLD response					
Positive correlation					
L. Fusiform gyrus[b] (BA 37)	-28.5	-61.5	-12.5	38	6.46
Dorsomedial prefrontal cortex[b] (BA 6)	-1.5	16.5	62.5	37	7.42

[a]information in parentheses refer to hemisphere and Brodmann's areas
[b]$p < 0.05$, cluster-level correction for multiple comparisons using permutation analyses
[c]$p < 0.005$, uncorrected

Figure 5.12 BOLD responses corresponding to the attentional bias effect for personalized drug use-related words versus neutral words for the cocStroop task. Response for the left inferior/middle frontal gyrus is illustrated.



precuneus ($p < 0.005$, uncorrected) (Figure 5.13). For the eStroop task, negative emotionally valenced words were associated with (de)activation of the middle temporal gyrus and inferior frontal cortex ($p < 0.005$, uncorrected). For the cStroop task, the contrast of incongruent versus congruent stimuli was also associated with robust activation of the left inferior frontal gyrus ($p < 0.05$, corrected), as well as the inferior parietal cortex, precentral gyrus and middle frontal cortex ($p < 0.005$, uncorrected) (Figure 5.14).

Relationship between the Behavioral and Neural cocStroop Effects. The attentional bias effect for personalized drug use-related words exhibited significant ($p < 0.005$) positive correlations with the BOLD response for drug-associated versus neutral words. Positive correlations representing greater neural activation with increasing attentional bias for cocaine-related stimuli involved the left fusiform gyrus and dorsomedial prefrontal cortex (0.05, corrected) (Table 5.7, Figures 5.15 and 5.16).

DISCUSSION

Clinical Use of DCS for Cocaine Dependence

The primary goal of this controlled clinical trial was to determine the efficacy of once-weekly DCS administration relative to placebo in facilitating the drug abstinence and treatment retention outcomes associated with cCBT in a community-based sample of cocaine-dependent women and men. This clinical trial follows a previous study which determined that DCS was no more effective than placebo in boosting relapse prevention and treatment retention in a VAMC-based cocaine-dependent sample (Chapter 4). This DCS trial demonstrated that adjunct DCS was ineffective in facilitating the treatment outcomes for cCBT. Rather, DCS administration was associated with an <u>increase</u> in the frequency of drug-positive urine samples relative to placebo. This outcome is distinct from the facilitating effects of DCS when combined with exposure-based psychotherapies for anxiety disorders that reflect the processes of aversive conditioning and raises the possibility that DCS has distinct effects on appetitively conditioned behaviors. This possibility is not supported by results from animal models of drug relapse indicating that DCS

Figure 5.13 BOLD responses corresponding to the attentional bias effect for personalized drug use-related words versus negative emotionally valenced words for the cocStroop and eStroop tasks. Response for the left middle frontal gyrus is illustrated.

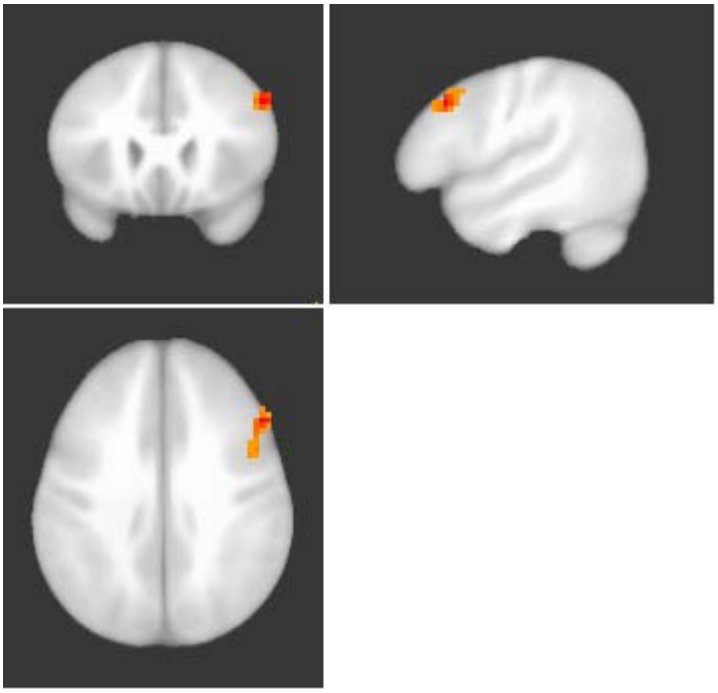


Figure 5.14 BOLD responses for incongruent versus congruent stimuli for the cStroop task. Response for the left inferior frontal gyrus is illustrated.

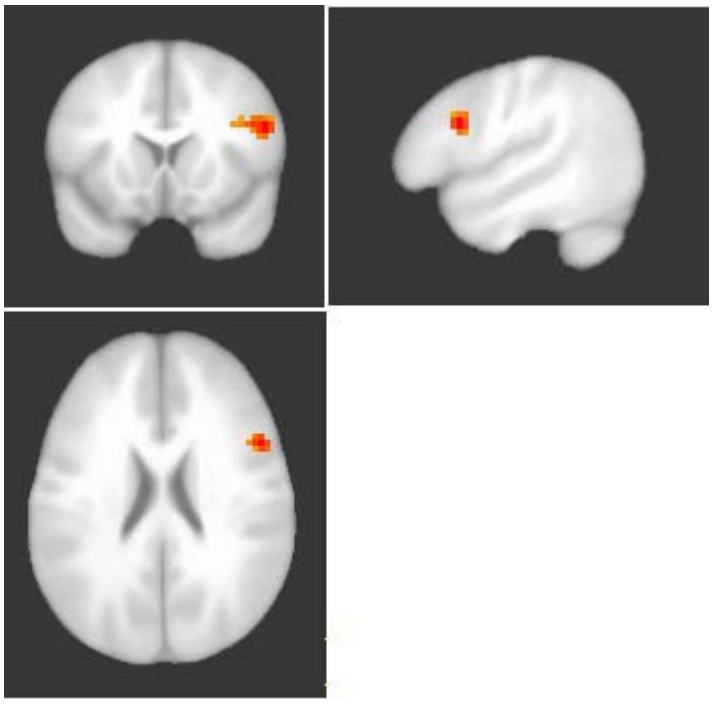


Figure 5.15 BOLD response in the left fusiform gyrus (left) correlated ($r = 0.931$, $p < 0.001$) with the magnitude of the attentional bias effect for drug use-related stimuli across cocaine-dependent subjects and is illustrated (right) for a spherical (5 mm radius) volume of interest centered on -28.5,-61.5,-12.5 mm.

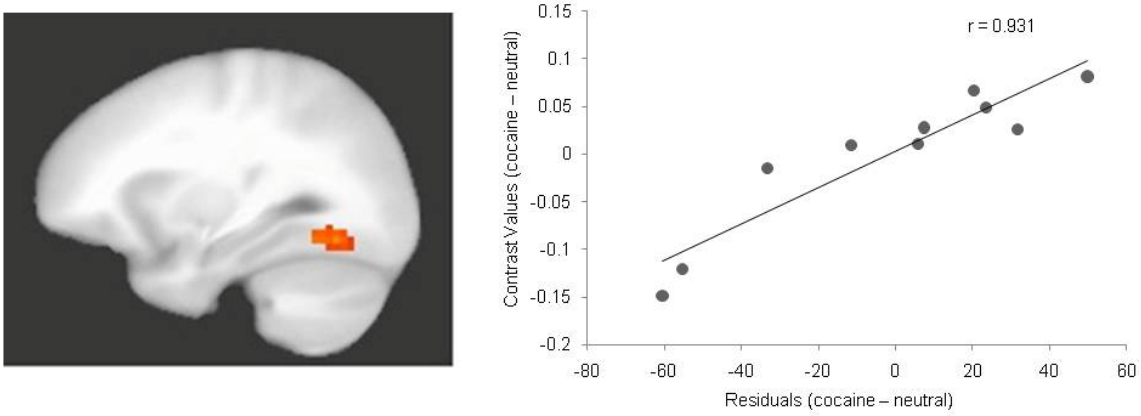
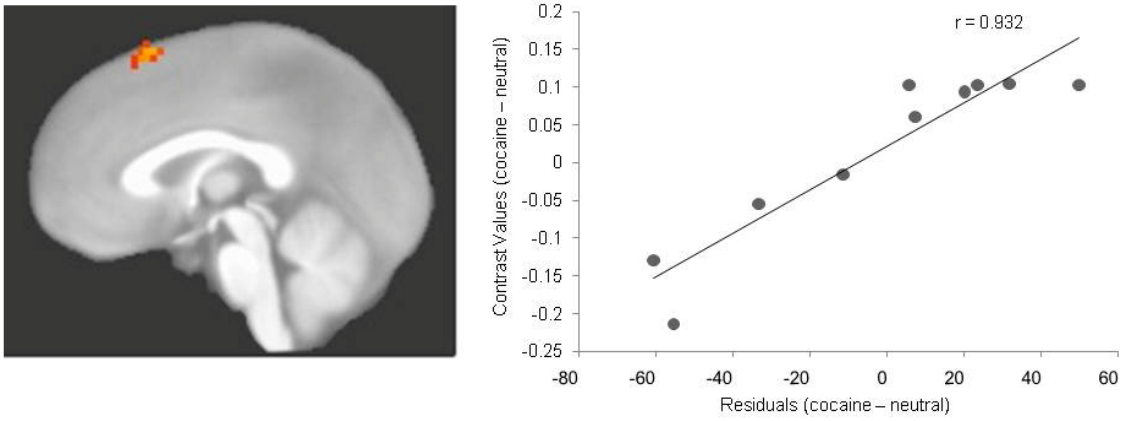


Figure 5.16 BOLD response in the dorsomedial prefrontal cortex (left) correlated ($r = 0.932$, $p < 0.005$) with the magnitude of the attentional bias effect for drug use-related stimuli across cocaine-dependent subjects and is illustrated (right) for a spherical (5 mm radius) volume of interest centered on -1.5, 16.5, 62.5 mm.



facilitates the extinction of cocaine self-administration and its reinstatement by conditioned drug cues (Nic Dhonnchadha et al., 2009; Torregrossa et al., 2010). Perhaps the difference in the interaction effects of DCS with anxiety and drug use disorders relate to fundamental differences between the cognitive neuroscience of these disorders. The paradoxical response to adjunct DCS observed in the present study suggests that DCS is facilitating aspects of the addiction process rather than the therapeutic learning and memory processes associated with cCBT. In this regard, it is possible that DCS is facilitating reconsolidation of the drug conditioned stimulus (CS)-unconditioned stimulus (US) association. Reconsolidation of the associative CS-US relationship could increase the propensity to seek and use drugs in response to an experienced CS. Unlike the majority of DCS trials in subjects with anxiety disorders, the target of DCS in the present study was the learning and memory processes related to CBT and not those related to extinction (of the anxiogenic CS). The possibility therefore exists that the facilitating effects of DCS on therapy outcomes are dependent on an interaction with the specific processes related to extinction. However, in a recent study, 50 mg DCS prior to extinction-based cue exposure therapy (CET) was associated with increased cravings and reactivity to cocaine cues in a cocaine-dependent sample (Price et al., 2009). Similarly, DCS was also shown to be no more effective than placebo in facilitating CET in abstinent alcohol-dependent subjects (Watson et al., 2011). These outcomes suggest that the observed inhibitory effect of DCS on cCBT outcomes are not due to the absence of extinction processes. While both anxiety and addictive disorders are arguably rooted in pathological learning and memory processes, the paradoxical response to DCS in cocaine-dependent persons suggests that cognitive enhancement interacts with distinct neurocognitive processes in the distinct disorders.

A major challenge to this DCS trial was the poor retention of subjects beyond the baseline fMRI session to initiate and/or complete the treatment sessions for the duration of 4-weeks in active treatment. More than half of the randomized subjects were lost-to-follow-up prior to initiating cCBT or following a single cCBT session. The lack of motivation to start and/or

complete treatment represents an endemic problem of drug use disorders. In a 2005 survey by the Substance Abuse and Mental Health Services Administration (SAMSHA), of 20.9 million illicit drug users who were classified as needing substance abuse treatment, 94.4% did not perceive the need for treatment. Out of the remaining 5.6% who perceived a need for treatment, 4.1% made no effect to receive treatment and only 1.4 % making an effort to initiate treatment. Several studies have demonstrated that drug-dependent persons who perceive a need for treatment are more likely to enter treatment as opposed to those who don't perceive a need for treatment (Siegal et al., 2002). Similarly, there is also evidence that suggest that perceiving a need for treatment is predictive in treatment retention (Longshore and Teruya, 2006). The inclusion of qualitative and quantitative measures of treatment motivation (*e.g*., perceived need for treatment) in the assessment battery prior to randomization may explain individual variation in treatment enrollment, engagement and outcome. Unfortunately, such measures were not collected in the present DCS study. Among the subjects who were actively enrolled in the treatment study, the percentage of completed treatment sessions was maintained at > 75% across the duration of the study. Contingency management (CM) was used to improve treatment retention across all subjects; however, DCS relative to placebo did not boost treatment retention as both arms had gradual declines in the percentage of completed treatment sessions over the 4-weeks of treatment.

Attentional Bias for Conditioned Drug Use Cues

Consistent with the results described in Chapters 2 and 3, cocaine-dependent subjects at study baseline demonstrated a significant attentional bias effect for personalized drug use-related words consistent with their conditioned incentive salience. Subjects also exhibited the predicted attentional bias effect for negative valence emotional stimuli for the eStroop task and a cognitive interference effect for incongruent word stimuli in the cStroop task. Reaction times for all stimulus types decreased across the session trials indicative of task habituation or scanner acclimation processes, or a possible task learning effect. Cocaine use cues also exhibited a significant carryover effect on subsequent neutral stimulus trials for the cocStroop task, a finding

consistent with the contention that the attentional bias effect is attributable to difficulty in disengaging attention from drug use reminders (Waters et al., 2005). The cStroop task further evidenced processes of conflict adaptation (Gratton et al., 1992) as incongruent stimuli were processed more rapidly following an incongruent versus a congruent stimulus.

A tested hypothesis of this DCS trial was that cCBT would be associated with a diminished conditioned response (attentional bias) to the drug CS (personalized drug use-related words). Indeed, the level of attentional bias related to cocaine cues was decreased from baseline to treatment endpoint for both study arms. It is of note that no effect of treatment was observed for the attentional bias and cognitive interference effects for the eStroop and cStroop tasks, respectively. This selective cognitive influence of treatment is consistent with a treatment-related diminution of the conditioned incentive properties of the drug CS. However, there was an observed disconnect between this effect of treatment on attentional bias and the lack of effect on the primary treatment outcome variable. This dissociation suggests that compulsive cocaine abuse is driven more by automatic, habit-based processes than by conditioned responses to drug stimuli. A long-standing theoretical model of relapse associated with drug addiction posits that conditioned drug cues maintain their motivational power reflecting over-learned behaviors and automatic processes of drug craving that leads to compulsive drug consumption (Tiffany, 1990).

Neural Correlates of Attentional Bias for a Stroop Task

The fMRI component of this study represented an extension of the assessment of the attentional bias effect for drug cues as a neurocognitive marker of cocaine addiction (Chapter 3). In the present study, the goal was to determine if the attentional bias effect further represented a neurocognitive marker of treatment response in cocaine-dependent subjects. A planned contrast of the BOLD response to cocaine use and neutral word stimuli indicated a robust activation of the left inferior/middle frontal gyri (BA 44/9). This brain area represents a critical node in cognitive regulatory brain networks (Aron and Poldrack, 2006) and suggests that the experience of cocaine use reminders engages efforts to control cocaine use urges. The observation that this area was

activated by all three of the Stroop tasks further supports the view that executive or supervisory control is required in all three tasks related to conflict monitoring and error detection, attentional control, and inhibitory control. The attentional bias effect for cocaine cues was also associated with significant, though less robust, activation of the posterior cingulate cortex as was previously identified in Chapter 3 and in prior imaging studies of neural responses to conditioned drug use-related cues (Duncan et al., 2007; Garavan et al., 2000; Kilts et al., 2004; Kosten et al., 2006). This neuroactivation may reflect the roles of the posterior cingulate cortex in autobiographical memory retrieval (Maddock et al., 2001), in the modulation of memory by emotionally salient stimuli (Maddock, 1999), and in motivation and decision making (Maddock, 1999; Mohanty et al., 2008).

The cocaine > emotional stimulus processing contrast was associated with further activation in the fusiform gyrus and the precuneus. The fusiform gyrus is involved in visual word recognition and could serve to categorize drug use-related as salient stimuli. The precuneus is involved in target detection (Corbetta et al., 2000) which suggests that cocaine-dependent subjects may be processing drug use-related words as visual targets. This processing mechanism is consistent with the property of attentional command by conditioned drug cues in drug-dependent populations. To further define the neural basis of attentional bias and explore the functional roles of involved brain areas, correlation analyses were performed that examined the relationship between individual variation in the level of attentional bias effect and BOLD responses for cocaine cues. The observation that dorsomedial prefrontal cortex responses to cocaine use-related stimuli were positively correlated with level of attentional bias suggests that the engagement of response selection processes related to decision making may contribute to the attentional bias effect. The neural representation of the effect of treatment on the attentional bias effect will be evaluated upon completion of the enrollment goals.

CONCLUSIONS

The results of the present study do not support the therapeutic advantage of using the cognitive enhancer DCS to boost the response to a cognitive behavioral therapy for cocaine addiction. The DCS trial results are decidedly at odds with the cumulative evidence supporting the use of adjunct DCS as a means of boosting the therapeutic response to exposure-based psychotherapies for anxiety disorders. The results further the understanding of the neural basis of the attentional bias effect in cocaine-dependent individuals that, when partnered with the treatment outcome results for the fully enrolled sample, will be novelly informative of the functional brain changes that code the individually varying response to treatment. Independent replication studies are needed to further explore the use of cognitive enhancement approaches, both psychopharmacological and cognitive skills training, as a means of enhancing the only partial efficacy of behavioral therapies for drug use disorders.

DISCUSSION

A critical guide to my thesis research was the visualization early on of my career goals and the development of a career development plan to prepare me for that career. This thesis research plan focused on the use of targeted research projects to address the learning objectives reflecting that career development plan. The following discussion represents my interpretation of the role of each project in acquiring the requisite knowledge and skills related to each learning objective. The Discussion sections of each of the preceding 5 chapters fulfill the need for critical scientific interpretation of each research project and will not be reiterated here.

The targeted learning objectives of my thesis research plan were to (1) acquire expertise related to clinical trial methodology, (2) acquire training in the field of human functional neuroimaging, (3) develop an understanding of the principles, efficacy, and limitations of addiction behavioral therapies, (4) acquire knowledge and research skills related to clinical psychopharmacology and medication development, (5) acquire knowledge and skills related to the roles of genetic variation as a source of individual variation in the neural processing correlates

of human behavior and of psychiatric disorders, and (6) develop a sound working knowledge of the practice of responsible conduct of research.

In Chapter 1, the primary learning objective was to use an imaging genomics approach to explore the relationship between genetic variation and variation in the neural representation of behavior. This learning objective focused specifically on how intermediate cognitive and neural processing phenotypes can be used to inform the mechanisms of genetic vulnerability for psychiatric disorders. Learning was fulfilled through the use of Functional Magnetic Resonance Imaging (fMRI) to assess the influence of *TPH2* genetic variation on cerebral cortical function related to cognitive control using the Multi-Source Interference Task (MSIT). Knowledge and skills related to imaging genomics focused on polymorphic variation in the tryptophan hydroxylase 2 gene (*TPH2*) and was enabled by hands-on training in the following areas: DNA extraction and amplification of DNA through polymerase chains reactions (PCR), single nucleotide polymorphism (SNP) genotyping, and construction of haplotype block structures based on mapping linkage disequilibrium. Skills training also included MR image processing and analysis, specifically in image pre-processing and functional connectivity. The finding from this study suggests that a common *TPH2* yin haplotype regulates the neural representation of cognitive control and that the association of risk for psychiatric disorders with allelic variation for the *TPH2* yin haplotype is related to the inefficient functional engagement of cortical areas involved in cognitive control demands (*e.g.*, conflict monitoring and resolution). This project enhanced my general understanding of how human functional neuroimaging approaches can be used to define the neural mechanisms related to suppressing cognitive interference and how genetic variation (*e.g.*, *TPH2* haplotype) can modulate the processing of a cognitively demanding task (*e.g.*, MSIT). The skills training related to fMRI in this research project were implemented in Chapters 3 and 5 to determine if the attentional bias effect for drug-use related stimuli represented a neurocognitive marker of treatment response in cocaine-dependent subjects.

In Chapter 2, a modified addiction Stroop task was developed and applied to a relatively large sample of cocaine-dependent individuals to assess attentional bias for conditioned drug cues, a measure of their properties as conditioned incentives. The study sought to further characterize the ability of the attentional bias effect for drug cues to forecast treatment response in cocaine-dependent persons and thus to further evaluate its value as a cognitive marker of risk for relapse. This project facilitated skills training in the use of quantitative patient assessments, and the acquisition of knowledge related to the cognitive basis of drug addiction and of skills related to cognitive task development. This study demonstrated that personalized drug use-related words, as opposed to generalized drug-associated words, are associated with a significant attentional bias effect in cocaine-dependent men that could possibly be more informative in predicting risk for relapse during and following treatment. The results of this study provided partial support for the predictive value of individual variation in attentional bias to forecast risk of relapse in cocaine-dependent subjects. This project facilitated a greater depth of understanding of how individual variation in attentional bias for over-learned conditioned drug-associated cues codes individual variation in risk for relapse.

As an extension of Chapter 2, efforts described in Chapter 3 involved the application of fMRI to elucidate the neural mechanisms involved in the implicit processing of personalized drug use-related stimuli and to better understand the mechanisms of relapse for drug-dependent individuals. Specifically, fMRI was used to explore the neural response to drug-related stimuli in a word counting Stroop task in a sample of cocaine-dependent men. This project was my introduction to the cognitive neuroscience of drug addiction. I deliberated on the imaging study design that would bring the attentional bias task (cocStroop) described in Chapter 2 into the fMRI environment. Issues of stimulus type, number of events, and timing were addressed with experimental and control tasks that resulted in a blocked, event-related design tailored to a data acquisition and analysis plan. Additional training in the field of neuroimaging was acquired through processing and analyzing the imaging data in this study. Here I gained proficiency in the

use of Presentation® software to design and implement the task in the scanner, and gained proficiency with Statistical Parametric Mapping (SPM8), Analysis of Functional NeuroImaging (AFNI), and Matlab software tools. The results from this study suggest that attentional bias for drug use-related stimuli reflects coordinated activity within a specific network of brain areas. This network could reflect drug use decision-making in which the incentive motivational features of drug-related stimuli compete with the deployment of control processes to modulate the level of attentional bias. Defining the neural representation of attentional bias for personalized drug-related stimuli further informs the addiction research field as to neural mechanisms of relapse and the clinical significance of addiction-related Stroop tasks.

Chapter 4 represents a training vehicle for acquiring and integrating multiple learning objectives including implementation of clinical trial methodology, specifically in the design, conduct, analysis, and interpretation of controlled clinical trials, and in understanding the principles, efficacy, and limitations associated with 12-Step and CBT approaches to addiction treatment. Another targeted objective was to acquire hands-on knowledge and research skills related to clinical psychopharmacology, specifically in the use of glutamate receptor pharmacology (*e.g*. DCS) to boost the clinical response to CBT. Knowledge about designing and conducting a randomized, double-blind, placebo-controlled clinical trial was enhanced by skill training in the following areas: the use of qualitative and quantitative assessment instruments for the clinical diagnosis and characterization of cocaine-dependent subjects, and the use of psychotherapy in faciliating relapse prevention in drug-dependent populations. I also applied the skills and knowledge related to clinical trial methodology that I acquired during the Masters in Science in Clinical Research (MSCR) Program at Emory. These skills included trial conceptualization and design, research ethics of working with a vulnerable human population, and the statistical analysis of trial data. The goal of this study was to determine if the N-methyl-D-aspartate (NMDA) glutamate receptor partial agonist and cognitive enhancer, D-Cycloserine (DCS), could boost the cocaine abstinence and treatment retention goals of cognitive behavioral

therapy (CBT). The results demonstrated that although an under-dosed CBT was associated with significant improvements in drug abstinence and treatment retention, there was no demonstration of the ability of DCS to boost the relapse prevention or treatment retention goals of CBT. This study served to enhance my understanding of the clinical presentation and human cost of drug addiction, specifically in the barriers associated with treatment and the obstacles involved in enrolling and retaining drug-dependent subjects.

Chapter 5 was a culmination of Chapter 2, 3, and 4 in which the goal was to utilize knowledge acquired from the previous research projects to conduct a clinical trial that would be a conclusive study of the ability of adjunct DCS to facilitate the drug abstinence and treatment retention goals associated with computerized CBT. This study would also serve as a vehicle to define the neural mechanisms involved in the processing of conditioned drug-associated cues and negative emotionally valenced words, and how altering the neural processing of these cues regulates susceptibility to relapse in cocaine-dependent subjects. Skills acquired in the previous research studies allowed the study to be reconceptualized to fit the new training site (*i.e*., UAMS), and to take advantage of the significant addiction research resources afforded by the UAMS Center for Addiction Research (CAR). Guided by the DCS trial outcomes from Chapter 4, significant changes were implemented into the UAMS clinical trial design such as dose of DCS, format of behavioral therapy, higher frequency assessment of drug abstinence/lapse, representation of cocaine-dependent women and men of diverse racial backgrounds, and longer-term follow-up of subjects. The Stroop tasks (*i.e*., cocStroop, eStroop and cStroop tasks) were modified to increase the overall power of the design to inform the cognitive neuroscience of relapse and to maximize the time spent in the scanner environment. The results from this in-progress clinical trial suggest a diminishing rather than a facilitating effect of adjunct DCS on the cCBT response, as DCS was associated with a greater percentage of positive drug urines during treatment. This research project gave me further opportunity to utilize the application of a

neuroimaging technique to clinical problem solving, specifically in the treatment of cocaine addiction.

Finally, in developing my career goal of patient-oriented research I have had many and continuous opportunities throughout my thesis research plan to acquire sound competence in the practice of the responsible conduct of research. Ethical conduct amongst research scientists is of utmost importance in fostering collaborative and cooperative research, protecting the rights and safety of research volunteers, and advancing the scientific goals of the research community. Researchers frequently face ethical dilemmas and issues that can influence the integrity and interpretation of research data**.** Ethical standards should be maintained throughout the scientific community to ensure continual public trust and support for research. Many research-based institutions (*e.g.*, universities) and funding agencies have demonstrated a heightened awareness of the importance of ethics in research and have taken an expanded initiative to educate current and future researchers about the ethical and societal dimensions of research. Throughout my dissertation research, responsible conduct of research has been an integral part of my training plan and has been implemented throughout my mentored supervision on every thesis research project. My education in research ethics has been further supported through formal courses and seminars that examine concepts inherent to the ethical and responsible conduct of research.

The goal of the ethics courses and seminars was to identify and solve ethical problems and conflicts arising in clinical research, using methods of ethical decision making. Topics of importance included: the history of the protection of human subjects; informed consent and vulnerable patient populations (*e.g.* children, minorities, and mentally ill); the priorities and processes of the institutional review board (IRB); industry/university/patient relationships and conflict of interest; the impact of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) on clinical research; and ethical issues in research in the developing world. General policies of discussion also included proper reporting of scientific misconduct, ownership or copyright of scientific property, proper data collection, data management, and data protection,

and proper record keeping. My continual education in research ethics has served as a vital preparation for my career as a future independent clinical researcher focusing on vulnerable human populations to apply established professional norms and ethical principles in the performance of all scientific research-related activities.

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