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Serotonin 5-HT2C Receptor-Mediated Modulation of the Behavioral and Neurochemical Effects of Cocaine in the Squirrel Monkey

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By

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B.A., Tufts University, 2003

Advisor: Leonard L. Howell, Ph.D.

An abstract of a dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Neuroscience

2011

Abstract

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Despite substantial efforts, there are currently no pharmacotherapeutics available for the treatment of cocaine abuse. Accumulating evidence indicates that indirect serotonin receptor agonists attenuate the behavioral and neurochemical effects of cocaine in animals and, accordingly, attenuate the subjective effects of cocaine in humans, but the specific receptor subtypes mediating these effects remain unknown. Recent studies have demonstrated that compounds exhibiting selectivity for both the serotonin 5-HT2A receptor (5-HT_{2A}R) and 5-HT2C receptor (5-HT_{2C}R) differentially modulate the behavioral and neurochemical effects of cocaine in rodents, but such compounds have yet to be systematically evaluated in nonhuman primates. The goals of the present experiments were therefore to determine the impact of pretreatment with the selective 5-HT_{2A}R antagonist M100907, the preferential and selective 5-HT_{2C}R agonists mCPP and Ro 60-0175, and the selective 5-HT_{2C}R antagonist SB 242084, upon the behavioral-stimulant, reinforcing, reinstatement, and neurochemical effects of cocaine in squirrel monkeys. The results indicated that 5-HT_{2C}R agonism attenuated, whereas 5-HT_{2C}R antagonism enhanced, the behavioral-stimulant and reinstatement effects of cocaine in a dose-dependent manner. Pretreatment with Ro 60-0175 also dose-dependently attenuated the direct reinforcing effects of cocaine. In contrast, administration of SB 242084 alone produced modest behavioral-stimulant effects and this compound also exhibited reinforcing effectiveness when substituted for cocaine availability in a self-administration procedure. The impact of pretreatment with Ro 60-0175 and SB 242084 upon the behavioral effects of cocaine were correlated with a selective reduction or enhancement, respectively, of cocaine-induced dopamine increases within the mesolimbic dopamine system. Finally, pretreatment with M100907 did not alter any behavioral effects of cocaine. These results suggest that 5-HT_{2C}R agonists may represent a novel class of pharmacotherapeutics for the treatment of cocaine abuse as they functionally antagonized the behavioral and neurochemical effects of cocaine in nonhuman primates. Furthermore, the selective 5-HT_{2C}R antagonist SB 242084 exhibited a behavioral profile similar to other psychostimulants and may warrant consideration as a candidate substitute agonist therapy for the treatment of cocaine abuse, although the demonstration of its reinforcing effectiveness may be an indication of abuse liability.

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List of Abbreviations

5-HT	5-hydroxytryptamine; serotonin
$5\text{-HT}_2\text{R}$	serotonin 2 receptor family
$5\text{-HT}_{2A}\text{R}$	serotonin 2A receptor subtype
$5\text{-HT}_{2B}\text{R}$	serotonin 2B receptor subtype
$5\text{-HT}_{2C}\text{R}$	serotonin 2C receptor subtype
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
DA	dopamine
DAT	dopamine reuptake transporter
DOI	1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane
ED_{Max}	maximally-effective unit dose of cocaine (self-administration)
ED_{Peak}	maximally-effective dose of pre-session cocaine prime (reinstatement)
FR20	fixed-ratio 20
GABA	γ-aminobutyric acid
HPLC	high-performance liquid chromatography
i.m.	intramuscular
i.p.	intraperitoneal

i.v.	intravenous
mCPP	*meta*-chlorophenylpiperazine
mg/kg	milligrams per kilogram
mg/kg/inf	milligrams per kilogram, per infusion
MDMA	3,4-methylenedioxymethamphetamine
NAcc	nucleus accumbens
NE	norepinephrine
NET	norepinephrine reuptake transporter
PFC	prefrontal cortex
SERT	serotonin reuptake transporter
SEM	standard error of the mean
SN	substantia nigra
SNpc	substantia nigra pars compacta
SSRI	serotonin-selective reuptake inhibitor
Veh	vehicle
VTA	ventral tegmental area

Chapter I. Introduction

A. Cocaine: History and Pharmacology

1. Prevalence of Misuse

According to the 2009 National Survey on Drug Use and Health (NSDUH), 1.6 million Americans aged 12 and older used some form of cocaine in the past month, comprising an estimated 0.7 percent of the population (Substance Abuse and Mental Health Services Administration 2010a). Additionally, it was reported that 1.1 million Americans met diagnostic criteria within the past year for cocaine abuse or dependence based upon the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), with only marijuana, alcohol, and pain relievers exhibiting higher rates of substance abuse diagnosis. The misuse of cocaine extends to youth populations as well, as the 2009 Monitoring the Future survey revealed that approximately 3.5% of 8th-12th graders reported use of any form of cocaine within the past 12 months, and nearly 40% of students reported that cocaine was easily obtainable (Johnston et al 2010). In addition to its addictive properties, cocaine use has also been highly correlated with serious physiological health risks. For example, the Drug Abuse Warning Network reported in 2007 that cocaine was the most commonly-involved of all illicit substances in emergency-room visits resulting from drug use or misuse, comprising a remarkable 29 percent of all such visits (Substance Abuse and Mental Health Services Administration 2007), a figure which rose to 43 percent in 2009 (Substance Abuse and Mental Health Services Administration 2010b). Furthermore, cocaine use has recently been associated with increased risk of unintentional injury (Ryb et al 2009), as well as HIV transmission and mortality (Muhuri and Gfroerer 2011).

Despite the prominence of cocaine abuse, and substantial research efforts spanning several decades, there are currently no FDA-approved medications available for the treatment of

cocaine addiction or dependence. Therefore, one important aim of the research described presently was to identify novel pharmacotherapeutic targets for the treatment of cocaine abuse, assessed using nonhuman primate models of cocaine use and relapse.

2. Pharmacological Mechanism of Action

The primary pharmacological mechanism of action of cocaine is binding to, and subsequent functional inhibition of, the three monoamine reuptake transporter proteins: the norepinephrine transporter (NET), the serotonin transporter (SERT), and the dopamine transporter (DAT) (Reith et al 1986; Madras et al 1989; Ritz et al 1990). Such reuptake inhibition prevents the normal clearing of excess neurotransmitter from the synaptic cleft, thus prolonging neurotransmitter-induced activation of receptor targets. Although cocaine binds with relatively equal potencies to each of the monoamine reuptake transporters, its abuse-related effects have been largely attributed to actions upon the DAT (Ritz et al 1987). For example, animals will readily self-administer DAT-selective inhibitors (Howell and Byrd 1991; Roberts 1993; Howell et al 2000; Lindsey et al 2004), but not NET-selective inhibitors (Woolverton 1987) or SERT-selective compounds (Tessel and Woods 1975; Howell and Byrd 1995). Therefore, it appears that the direct reinforcing effects of cocaine and related psychostimulants are mediated primarily through increases in extracellular concentrations of dopamine.

B. Neuroanatomical Substrates of Cocaine Behavioral Pharmacology

1. Overview of Brain Dopamine Systems

It is generally accepted that three major dopamine (DA) pathways exist in the brain. These are commonly referred to as the mesocorticolimbic, nigrostriatal, and tuberohypophyseal systems (Moore and Bloom 1978). The mesocorticolimbic and nigrostriatal DA pathways have

been well-established to mediate the abuse-related behavioral effects of cocaine and other psychostimulants. In contrast, the tuberohypophyseal system, comprised of DA cell bodies originating in the arcuate and periventricular nuclei of the hypothalamus that project to various subregions of the pituitary gland, serve to regulate neuroendocrine functions such as prolactin release. It is also important to note that the tuberohypophyseal pathway exhibits only modest expression of DAT at its terminal sites (Meister and Elde 1993; Lorang et al 1994), and is thus unlikely to mediate the prominent behavioral effects of cocaine and related psychostimulants. Therefore, the sections below will further describe only the mesocorticolimbic and nigrostriatal systems in greater detail.

2. The Mesocorticolimbic and Nigrostriatal DA Systems: Roles in Cocaine-Reinforced Behavior

a. Mesocorticolimbic System

The mesocorticolimbic DA system originates from somata within the ventral tegmental area (VTA) and the dorsal portion of the substantia nigra pars compacta (SNpc) (Moore and Bloom 1978; Haber and McFarland 1999). These cells project axons to limbic and cortical areas including the nucleus accumbens (NAcc), amygdaloid nuclei, anterior cingulate cortex, and frontal cortical regions (Moore and Bloom 1978; Bjorklund and Dunnett 2007). The association between mesocorticolimbic DA signaling and the reinforcing effects of both natural and drug stimuli has been well-established (Di Chiara and Imperato 1988; Schultz et al 1997; Wise 2004). For example, studies in both rodents and nonhuman primates have demonstrated that mesolimbic DA activity is increased in response to food presentation (Richardson and Gratton 1996; Schultz 2007) and drugs of abuse, such as the psychostimulants cocaine and amphetamine, are self-administered when infused directly into the NAcc or prefrontal cortex of rats (Phillips et al 1994; McBride et al 1999). Additionally, lesions of the VTA (Roberts and Koob

1982) or NAcc (Roberts et al 1977; Zito et al 1985), or local infusion of the DA D_1 receptor antagonist SCH23390 within the NAcc (Maldonado et al 1993), reduced the reinforcing effects of self-administered cocaine. Similarly, local infusion of DA receptor antagonists into the basolateral amygdala, which receives dense DA innervation from the VTA, attenuated the conditioned-reinforcing effects of drug-associated stimuli (See et al 2001; Di Ciano and Everitt 2004). Such findings have strongly implicated the mesocorticolimbic DA pathway as mediating the reinforcing effects of cocaine and other drugs of abuse. Indeed, virtually all drugs of abuse (e.g. psychostimulants, opiates, nicotine, alcohol, etc.) share the common effect of increasing DA levels within the terminal regions of the mesocorticolimbic system, regardless of their initial pharmacological mechanisms of action (Di Chiara et al 1988). However, more recent studies have elucidated an important role for extra-limbic DA signaling in the abuse-related effects of cocaine, specifically within the sensorimotor regions of the dorsal striatum (Wise 2009).

b. Nigrostriatal System

The caudate nucleus and putamen within the neostriatum receive dense, topographically-organized DA projections from cell bodies localized primarily within the ventral portion of the SNpc, comprising the nigrostriatal DA pathway (Moore and Bloom 1978). The selective degeneration of these DA-releasing neurons has long been associated with the onset and motoric symptomatology of Parkinson's Disease (Hornykiewicz 2001; Hornykiewicz 2004) and are not classically associated with the abuse-related effects of cocaine. However, recent evidence suggests that the dopaminergic nigral afferents to the dorsolateral striatal region may mediate important aspects of chronic and compulsive reinforcement-based learning (Wise 2009). In particular, the nigrostriatal system has been implicated in the maintenance of reinforced habitual behaviors (Yin et al 2004; Faure et al 2005). Such a finding is not surprising

as some parkinsonian patients present with impairments of habit-learning (Witt et al 2002; Frank et al 2004), whereas treatment with L-Dopa to partially restore normal DA signaling may result in the emergence of undesirable compulsive behaviors (for review, Voon et al 2009). Given the role of the nigrostriatal DA pathway in habitual learning and compulsive behavior, it is not surprising that the nigrostriatal system may mediate some of the abuse-related effects of cocaine. For example, DA levels increased within the dorsal, but not ventral, striatal territories during presentation of response-contingent cocaine-associated cues in rats trained to self-administer cocaine (Ito et al 2000; Ito et al 2002), and infusion of the nonselective DA receptor antagonist flupenthixol into the dorsal striatum decreased cocaine-seeking behavior under a second-order schedule of cocaine reinforcement (Vanderschuren et al 2005). Moderate increases in DA levels within the dorsal striatum have also been demonstrated in nonhuman primates trained on a second-order schedule of cocaine self-administration during the first drug-free component, when behavior is maintained by response-contingent cocaine-associated cues but not cocaine itself (Kimmel et al 2005). Furthermore, cocaine-induced functional activity in nonhuman primates, as measured using 2-deoxyglucose autoradiography, was found to expand from ventral to progressively more dorsal and lateral regions of the striatum following chronic (100 days) cocaine self-administration (Porrino et al 2004). Finally, in accordance with these preclinical findings, positron emission tomography studies using cocaine-dependent human subjects revealed that exposure to cocaine-associated cues caused decreased binding of [^{11}C]raclopride within the dorsal, but not ventral, striatum, an effect that is putatively indicative of increased release of endogenous DA. Importantly, this cue-elicited DA release within the dorsal striatum positively correlated with self-reported cocaine craving (Volkow et al 2006; Wong et al 2006). Taken together, these findings suggest a role not only for the

mesocorticolimbic DA system, but also for the nigrostriatal DA system, in mediating the reinforcing and abuse-related effects of cocaine and cocaine-associated stimuli.

C. Serotonergic Modulation of Cocaine Effects

Both the mesocorticolimbic and nigrostriatal DA projections are susceptible to modulation by neurotransmission of serotonin (5-hydroxytryptamine, 5-HT) (for review, Alex and Pehek 2007; Fink and Gothert 2007; Navailles and De Deurwaerdere 2011). 5-HT projections arising from neurons localized within the medial and dorsal raphe nuclei terminate not only in the VTA and SNpc, but also within the terminal regions of their respective DA projections, including ventral and dorsal regions of the striatum and the prefrontal cortex (Azmitia and Gannon 1986; Halliday and Tork 1989; Wallman et al 2011). Pharmacological enhancement of 5-HT levels can be achieved *in vivo* via systemic administration of selective serotonin reuptake inhibitors (SSRI, e.g. fluoxetine) or serotonin releasers (e.g. fenfluramine). Studies utilizing such compounds have revealed an important modulatory role for 5-HT upon the neurochemical effects of cocaine. For example, cocaine-induced elevations in DA levels within the striatum were reduced following pretreatment with the SSRI alaproclate in squirrel monkeys (Czoty et al 2002). Importantly, indirect 5-HT agonists also modulate the behavioral effects of cocaine, as acute pretreatment with an SSRI attenuated the behavioral-stimulant effects of cocaine in nonhuman primates (Howell and Byrd 1995). More relevant to human cocaine abuse, acute systemic administration of SSRIs reduced cocaine self-administration in rats (Carroll et al 1990a; Richardson and Roberts 1991; Glatz et al 2002) and nonhuman primates (Kleven and Woolverton 1993; Czoty et al 2002), suggesting that increased 5-HT levels alters the direct reinforcing effects of cocaine. In an elegant series of experiments, the stimulant-like and reinforcing effects of several compounds with varying selectivity for releasing DA vs. 5-HT were

assessed in nonhuman primates. In accordance with the aforementioned findings, compounds with high selectivity for releasing DA vs. 5-HT induced behavioral-stimulant effects in squirrel monkeys (Kimmel et al 2009) and were self-administered by rhesus macaques (Wee et al 2005). However, compounds with a lower ratio of DA/5-HT release exhibited weaker behavioral-stimulant effects (Kimmel et al 2009), maintained lower rates of self-administration (Wee et al 2005), and suppressed cocaine self-administration in nonhuman primates (Rothman et al 2005; Negus et al 2007). Additionally, decreased selectivity of DA vs. 5-HT release by these compounds was associated with a reduced capacity to increase DA within the striatum (Kimmel et al 2009), again correlating increased 5-HT signaling with reductions in their inherent DA-increasing, stimulant-like, and reinforcing effects. These findings lend further support to the hypothesis that a pharmacologically-induced increase in 5-HT neurotransmission attenuates the behavioral effects of psychostimulants, including their reinforcing effectivess, and that these effects are highly correlated with a diminished capacity to increase DA levels in striatal regions.

Despite these promising results, clinical studies investigating indirect serotonin agonists (such as SSRIs) as potential pharmacotherapeutics for the treatment of cocaine abuse have largely failed to alter cocaine-taking behavior in humans (Grabowski et al 1995; Batki et al 1996; Ciraulo et al 2005; Winhusen et al 2005; but see Moeller et al 2007). The reasons for the discrepancy between preclinical and clinical effectiveness for serotonergic compounds remains unclear, but may be related to the nonspecific activation of multiple serotonin receptor subtypes, which might in turn elicit undesirable side effects and thus limit compliance in human cocaine users. For example, acute administration of SSRIs in humans has been linked with self-reported gastrointestinal, neurological, and psychological (e.g. anxiogenesis) adverse effects (for review, Vaswani et al 2003). Interestingly, pretreatment with the SSRI fluoxetine decreased food-maintained responding at doses that also reduced cocaine self-administration in rhesus

monkeys (Kleven and Woolverton 1993), suggesting that indirect serotonin agonists produce measurable reductions of nondrug-maintained behaviors in preclinical studies. It is critical to note that administration of SSRIs results in increased activity at all 5-HT receptor targets throughout the brain. The presynaptic and postsynaptic effects of 5-HT are mediated by at least 14 distinct receptors, grouped into 7 different families (5-HT_1 – 5-HT_7) based upon homology of structure and function (Hoyer et al 2002). Identification of the specific 5-HT receptor subtypes that mediate the reduction of abuse-related cocaine effects following indirect agonist administration could allow for the development of more selective pharmacotherapeutic compounds that may lack some, if not all, of the undesirable side effects elicited by SSRIs, thus increasing compliance and possibly demonstrating better efficacy in human cocaine abusers. The present experiments were therefore designed to examine the modulatory role of a specific subset of 5-HT receptors upon cocaine-induced neurochemical and behavioral effects in nonhuman primates.

D. Serotonin 5-HT_2 Receptor-Mediated Modulation of Cocaine Effects

1. Overview of 5-HT_2 Subtype Receptors

The 5-HT_2 receptor family is comprised of three subtypes: 5-HT_{2A}R, 5-HT_{2B}R, and 5-HT_{2C}R (Hoyer et al 2002). These receptors belong to the superfamily of seven transmembrane-domain G-protein coupled receptors. While 5-HT_{2A}R and 5-HT_{2C}R are expressed throughout the brain, the 5-HT_{2B}R is predominantly expressed within the gastrointestinal and peripheral nervous systems with only low levels localized within the brain (Kursar et al 1994; Bonhaus et al 1995). Accordingly, drugs selectively targeting the 5-HT_{2B}R have failed to alter the behavioral effects of cocaine in rodents (Fletcher et al 2002). Therefore, the 5-TH_{2B}R was not considered as a candidate target for the modulation of cocaine effects in the present experiments.

The 5-HT_{2A}R and 5-HT_{2C}R are highly homologous with respect to amino acid sequence (Boess and Martin 1994). Both receptor subtypes couple to $G_{\alpha q/11}$, whose activations in turn promote the activity of two downstream effectors, phospholipase C and phospholipase A_2. The activation of phospholipase C ultimately results in increased intracellular concentrations of Ca^{2+}, whereas activation of phospholipase A_2 increases levels of intracellular arachidonic acid. Both of these intracellular signaling cascades are believed to confer an overall net stimulatory effect upon the activated cell (Boess and Martin 1994). Studies now indicate that exogenously-administered agonists, antagonists, or inverse agonists at the 5-HT_{2A}R and 5-HT_{2C}R may differentially modulate each downstream effector pathway (Cussac et al 2008). Such diverse actions following ligand-mediated receptor binding, recently termed "functional selectivity", are becoming an increasingly important consideration for pharmacologists as it allows for an extraordinarily diverse behavioral profile for compounds which bind to a given receptor with equal selectivity and affinity, but might elicit different effector responses. A final point of interest regarding 5-HT_{2A}R and 5-HT_{2C}R is their high levels of constitutive activity *in vivo*, as they have been found to induce G-protein coupling in the absence of ligand binding (for review, Teitler et al 2002; Berg et al 2005). This constitutive activity, when considered in combination with functional selectivity, serves to further highlight the complexity of these receptors and the potential for a highly diverse range of pharmacological manipulations (e.g. agonists, antagonists, inverse agonists).

Taken together, these findings suggest that drugs which bind to the 5-HT_2 family of receptors have the potential to exert a wide variety of behavioral effects and, as such, require careful and systematic evaluation in preclinical research. In rodent studies, mounting evidence has suggested that signaling through the 5-HT_{2A}R and 5-HT_{2C}R differentially modulate the activity of the mesocorticolimbic and nigrostriatal DA systems, as well as cocaine-induced

neurochemical and behavioral effects. However, these effects have yet to be replicated in nonhuman primates. The following sections will detail the current status of 5-HT_2R research and provide the rationale for the present experiments.

2. 5-HT_{2C} Receptor

a. Distribution

In situ hybridization and radioligand binding techniques have been used to localize the 5-HT_{2C}R mRNA and protein, respectively, within mesocorticolimbic and nigrostriatal systems in several species. In rats, 5-HT_{2C}R mRNA is detected at high levels in caudate-putamen, nucleus accumbens, amygdala, frontal and cingulate cortices, SNpc, and VTA (Pompeiano et al 1994). A more recent study confirmed the presence of 5-HT_{2C}R protein in similar brain regions, suggesting a predominantly somatodendritic localization for these receptors (Clemett et al 2000). In macaques, 5-HT_{2C}R mRNA was detected in the cerebral cortex, amygdala, VTA, and ventral aspects of the striatum including the nucleus accumbens (Lopez-Gimenez et al 2001a). Interestingly, 5-HT_{2C}R mRNA was not detected in dorsolateral striatal regions, although there was notable binding of the labeled 5-$HT_{2A/2C}$R antagonist [^{3}H]mesulergine within the caudate-putamen, though it could not be clearly differentiated from nonspecific binding. Finally, in humans, 5-HT_{2C}R mRNA mapped more closely to patterns observed in rats, with detection in both ventral and dorsal portions of the striatum, SNpc, amygdala, and cortical regions (Pasquelatti et al 1999). Unfortunately, the VTA was not investigated as a region of interest in this study. Interestingly, while mRNA expression was high within the caudate-putamen, [^{3}H]mesulergine binding was low within this region, suggesting that although striatal neurons express the 5-HT_{2C}R, its localization may be predominantly limited to the terminals of its axonal projections.

The cellular distribution of 5-HT_{2C}R within DA systems has been investigated extensively in the rat brain (Fig. 1). Within both the VTA and SNpc, 5-HT_{2C}R mRNA was detected in both dopaminergic and GABAergic neurons (Eberle-Wang et al 1997; Bubar and Cunningham 2007), although the majority of co-expression occurred in GABAergic cells. Although still a matter of debate, it was proposed by the study's authors that the latter function as locally-projecting interneurons whose activation would inhibit DA neurotransmission. Similarly, 5-HT_{2C}R mRNA in the human SNpc did not colocalize with immunoreactivity for tyrosine hydroxylase, suggesting that 5-HT_{2C}R-positive cells were not dopaminergic, but likely GABAergic in nature (Pasquelatti et al 1999). It remains unclear whether these GABAergic cells projected locally or extra-nigrally, although recent evidence has suggested the existence of locally-projecting GABAergic interneurons within the human SNpc (Hebb and Robertson 2000). 5-HT_{2C}R protein has also been predominantly detected on GABAergic neurons (likely interneurons) within the rodent PFC (Liu et al 2007).

Taken together, it is evident that the 5-HT_{2C}R is situated to modulate DA signaling through diverse mechanisms, within both DA-producing mesencephalic regions as well as their terminal fields. Because patterns of mRNA expression and immunoreactivity are generally detected within the same regions, it has been suggested that these receptors are predominantly postsynaptic and somatodendritic, although there is some evidence for localization on axon terminals in a few areas. Particularly within the VTA and PFC (and possibly SNpc), the localization of 5-HT_{2C}R to GABAergic interneurons suggests that activation of these receptors would serve to inhibit mesolimbic DA neurotransmission. Indeed, functional and behavioral

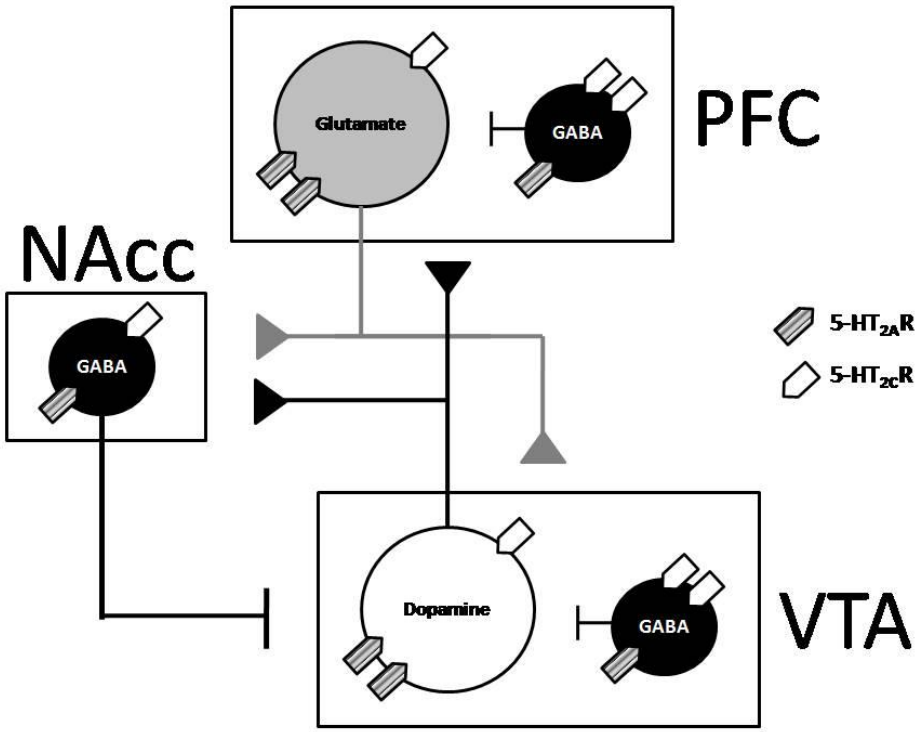

Figure 1. Cellular distribution of the 5-HT_{2A}R and 5-HT_{2C}R within a simplified schematic representation of the rodent mesolimbic DA system. The VTA consists of dopaminergic neurons (*white circle*) which project to both NAcc and PFC, and locally-projecting GABAergic interneurons (*black circle*). The PFC consists of pyramidal glutamatergic neurons (*gray circle*) which project to NAcc and VTA, and locally-projecting GABAergic interneurons (*black circle*). The NAcc is comprised of medium-spiny GABAergic neurons (*black circle*) which project to VTA and other limbic structures not shown. Detailed descriptions of subcellular receptor localization are provided in the text. As all cell-types typically express both 5-HT_{2A}Rs and 5-HT_{2C}Rs, intracellular predominance of expression is visually depicted by the presence of two receptor symbols. Briefly, VTA DA cells and PFC glutamatergic neurons predominantly express 5-HT_{2A}R, whereas VTA and PFC GABAergic interneurons predominantly express 5-HT_{2C}R. Both receptors are expressed at similar levels within NAcc medium spiny neurons. *Black lines with triangular*

terminals, DA projections. *Black lines with perpendicular terminals*, GABA projections. *Gray lines with triangular terminals*, glutamate projections. *Striped polygons*, 5-HT_{2A}R. *White polygons*, 5-HT_{2C}R.

studies have helped to elucidate the modulatory role of 5-HT_{2C}R activity upon DA neurotransmission.

b. 5-HT_{2C}R-Mediated Modulation of Cocaine Effects

As described above, the 5-HT_{2C}R is localized within mesocorticolimbic and nigrostriatal structures, suggesting that it may modulate DA neurotransmission. In agreement with this hypothesis, electrophysiological and microdialysis studies have revealed that systemic administration of selective 5-HT_{2C}R agonists decreased, while antagonists and inverse agonists increased, basal firing rates of VTA DA neurons and subsequent DA release within the NAcc (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008). The impact of the 5-HT_{2C}R upon nigrostriatal activity is less clear. For example, some studies have demonstrated little-to-no modulatory effect of 5-HT_{2C}R agonism or antagonism upon SNpc neuronal firing and DA release within the dorsal striatum (Di Matteo et al 1999; Di Giovanni et al 2000; Marquis et al 2007) whereas others have demonstrated more pronounced effects (Di Giovanni et al 1999; Gobert et al 2000; De Deurwaerdere et al 2004; Navailles et al 2004; Alex et al 2005). Additionally, an early investigation of 5-HT_{2C}R knockout mice found an enhancement of DA release following cocaine administration in NAcc, but not dorsal striatum (Rocha et al 2002). However, a later study found that although 5-HT_{2C}R knockout mice had increased basal DA levels in the dorsal striatum and NAcc and correlated increased tonic activity in SNpc neurons, changes in DA levels following administration of amphetamine or the selective DAT inhibitor GBR 12909 did not differ between knockout and wild-type mice, although a nigrostriatally-mediated behavioral response to amphetamine (stereotypy) was enhanced in the knockout animals (Abdallah et al 2009). Therefore, it appears that the 5-HT_{2C}R may indeed modulate both

mesolimbic and nigrostriatal DA activity, although actions upon the nigrostriatal system remain unclear.

The impact of 5-HT_{2C}R pharmacological manipulations upon the neurochemical and behavioral effects of cocaine has been investigated extensively in rodents. Studies have consistently and reliably found that 5-HT_{2C}R agonists attenuate, while antagonists enhance, the behavioral and neurochemical effects of cocaine. Specifically, administration of 5-HT_{2C}R agonists in rodents attenuated cocaine-induced elevations of DA within the NAcc (Navailles et al 2008), cocaine-induced hyperlocomotion (Grottick et al 2000; Filip et al 2004), the discriminative stimulus effects of cocaine (Callahan and Cunningham 1995; Frankel and Cunningham 2004), as well as the direct reinforcing effects of cocaine as measured by self-administration procedures (Grottick et al 2000; Fletcher et al 2008). Additionally, 5-HT_{2C}R agonism reduces reinstatement of previously-extinguished cocaine-maintained responding, a procedure commonly described as a model of relapse to drug use (Neisewander and Acosta 2007; Burbassi and Cervo 2008). Conversely, systemic administration of 5-HT_{2C}R antagonists exert effects opposite to those following agonist administration, thus enhancing cocaine-induced DA release within the NAcc (Navailles et al 2004), cocaine-induced hyperlocomotion (Fletcher et al 2002; Fletcher et al 2006), the discriminative stimulus effects of cocaine (Filip et al 2006), cocaine self-administration (Fletcher et al 2002), and cocaine-induced reinstatement (Fletcher et al 2002). Taken together, these findings suggest that signaling through the 5-HT_{2C}R exerts inhibitory control over cocaine-mediated neurochemical and behavioral effects in rodents. However, such effects have not been systematically tested in nonhuman primates. Therefore, the present experiments were designed to directly assess the impact of 5-HT_{2C}R agonism and antagonism upon the behavioral and neurochemical effects of cocaine in nonhuman primate subjects.

3. 5-HT_{2A} Receptor

a. Distribution

Similar to the 5-HT_{2C}R, the regional distribution of the 5-HT_{2A}R has been described using both in situ hybridization techniques and radioligand binding assays. Across species, the 5-HT_{2A}R is most densely localized to cortical regions, including frontal and cingulate areas which receive DA innervation from the VTA in the mesolimbic system (Pompeiano et al 1994; Cornea-Hebert et al 1999; Hall et al 2000; Lopez-Gimenez et al 2001b; Varnas et al 2004). Most studies indicate that these cortical 5-HT_{2A}Rs are predominantly postsynaptic in nature. In contrast to the 5-HT_{2C}R in cortical regions, which is predominantly expressed by GABAergic interneurons, the 5-HT_{2A}R appears to be localized to either the apical dendrites or somata of glutamatergic pyramidal neurons throughout the cortex and medium-spiny projection neurons within the striatum, although some evidence of presynaptic localization and localization to GABAergic interneurons exists (Santana et al 2004). Given that glutamatergic pyramidal neurons within the PFC send efferent projections to NAcc and VTA, it could be proposed that signaling through PFC-localized postsynaptic 5-HT_{2A}Rs could indirectly enhance mesolimbic DA neurotransmission and, consequently, the effects of cocaine and related psychostimulants, whereas 5-HT_{2A}R antagonists would be expected to attenuate such effects. Furthermore, 5-HT_{2A}R receptors have also been detected within the VTA, SNpc, amygdala, and striatum of rats (Cornea-Hebert et al 1999; Nocjar et al 2002). However, studies have elucidated considerable species differences with respect to localization in these brain regions. For example, 5-HT_{2A}R is detected in dense “patchy” distributions throughout the rodent caudate-putamen, but is found at dramatically lower levels in the striatum of nonhuman primates and humans (Lopez-Gimenez et al 1999; Hall et al 2000; Lopez-Gimenez et al 2001b; Varnas et al 2004). Differences between rodent and primate 5-

$HT_{2A}R$ mRNA have also been described for both the SN and VTA. Specifically, 5-$HT_{2A}R$ mRNA has been described at moderate levels in the SN and VTA of rats (Pompeiano et al 1994), at lower levels in humans (Ikemoto et al 2000), but was not detected in the SN of monkeys in one study (Lopez-Gimenez et al 2001b). Although such discrepancies may be accounted for by varied methodological protocols, these studies do suggest the possibility of a significant species difference as primate 5-$HT_{2A}R$ distribution is dramatically different from rodents, and to a lesser extent, humans. Further research is needed to better define the receptor distribution in the nonhuman primate basal ganglia.

A number of studies in rodents have investigated the subcellular localization of the 5-$HT_{2A}R$ within the VTA (Fig. 1). The majority of 5-$HT_{2A}R$ immunolabeling colocalized with tyrosine hydroxlyase, suggesting that the receptors are expressed by DA-releasing neurons, although there was some evidence for colocalization with enzymatic markers of GABA (Doherty and Pickel 2000; Nocjar et al 2002). Within the NAcc, the 5-$HT_{2A}R$ protein was predominantly detected on GABAergic medium-spiny neurons (Cornea-Hebert et al 1999), although a double-labeling study also found 5-$HT_{2A}Rs$ on axon terminals of corticostriatal and pallidostriatal projections putatively synapsing on striatal interneurons (Bubser et al 2001). Unfortunately, the subcellular localization of 5-$HT_{2A}R$ in SNpc of rats, or in any region of primate brain, has yet to be adequately described. However, given the subcellular localization of 5-$HT_{2A}R$ in PFC and VTA, 5-$HT_{2A}R$ activation would be expected to facilitate DA release and cocaine-induced effects, whereas 5-$HT_{2A}R$ antagonism would be expected to attenuate such effects.

b. 5-$HT_{2A}R$-Mediated Modulation of Cocaine Effects

In contrast to the 5-$HT_{2C}R$, antagonism of the 5-$HT_{2A}R$ does not alter basal firing rate of VTA neurons nor alter DA levels within either the NAcc, caudate-putamen, or PFC of rats,

suggesting that this receptor does not modulate tonic DA tone (Di Giovanni et al 1999; Gobert et al 2000; Bonaccorso et al 2002; Pehek et al 2006). However, pharmacological activation of the 5-HT_{2A}R via systemic administration of the 5-$HT_{2A/2C}$R agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) modestly, but significantly, enhanced the firing rate of VTA DA neurons in rodents, and has also been shown to increase DA levels within mesolimbic terminal regions in rodents (Bortolozzi et al 2005; Pehek et al 2006) and, more recently in our laboratory, in rhesus monkeys (unpublished observations). Furthermore, the dopaminergic effects of DOI were reversed via pretreatment with the selective 5-HT_{2A}R antagonist M100907. These studies are in accordance with expected results predicted by the 5-HT_{2A}R localization in midbrain and cortical regions. One might speculate based upon these dopaminergic effects that DOI and related 5-HT_{2A}R agonists may modulate the neurochemical and/or behavioral effects of cocaine. However, this has not been systematically tested as many 5-HT_{2A}R agonists elicit behavioral effects in experimental animals that disrupt reliable behavioral measurements. Additionally, the human potency of various hallucinogenic drugs was found to be highly correlated with affinity for the 5-HT_{2A}R (Sadzot et al 1989). As such, agonists for the 5-HT_{2A}R have not been considered as a plausible pharmacological intervention for the treatment of substance abuse and have therefore garnered little attention as modulators of cocaine effects in animals.

As described above, 5-HT_{2A}R antagonism does not modulate basal DA tone. Interestingly though, systemic administration of the selective 5-HT_{2A}R antagonist SR 46349B attenuated amphetamine-induced elevations of DA within the rat NAcc (Auclair et al 2004) as well as amphetamine-induced reductions of [^{11}C]raclopride binding within the dorsal striatum (Egerton et al 2008), suggesting that 5-HT_{2A}R antagonism attenuates stimulated DA release within both the nigrostriatal and mesolimbic DA pathways. In accordance with these neurochemical findings, systemic administration of selective 5-HT_{2A}R antagonists has been shown to attenuate many of

the behavioral effects of cocaine in rodents, including cocaine-induced hyperlocomotion (McMahon and Cunningham 2001; Fletcher et al 2002; Filip et al 2004), the discriminative-stimulus effects of cocaine (McMahon and Cunningham 2001; Filip et al 2006), and reinstatement of cocaine-maintained responding following exposure to either cocaine-associated cues or a cocaine prime (Fletcher et al 2002; Nic Dhonnchadha et al 2009). Importantly, studies employing microinjection techniques have identified the VTA as one brain region in which 5-HT_{2A}R antagonists modulate cocaine-induced hyperlocomotion (McMahon et al 2001), whereas M100907 infused directly into the PFC has recently been found to attenuate cocaine cue-induced reinstatement (Pockros et al 2011). These findings are in accordance with predicted outcomes based upon 5-HT_{2A}R cellular localization in VTA and PFC. Overall, it appears that 5-HT_{2A}R is situated throughout the mesolimbic system in locations where it can functionally modulate the behavioral effects of cocaine in rodents.

It is interesting to note that, in contrast to compounds acting upon the 5-HT_{2C}R, administration of a selective 5-HT_{2A}R antagonist (M100907) failed to alter the ongoing self-administration of cocaine in rats (Fletcher et al 2002; Nic Chonnchadha et al 2009), suggesting that 5-HT_{2A}R antagonism is incapable of modulating the direct reinforcing effects of cocaine. This lack of effect of M100907 pretreatment upon cocaine reinforcement has been replicated in rhesus monkeys at doses which suppressed the self-administration of stereoisomers of 3,4-methylenedioxymethamphetamine (MDMA) (Fantegrossi et al 2002). To our knowledge, this is the only study that has investigated the effect of selective 5-HT_{2A}R antagonism upon any behavioral effect of cocaine in nonhuman primates. An important aim of the current experiments was therefore to extend the nonhuman primate methodologies tested to include not only ongoing self-administration of cocaine, but also the behavioral-stimulant and

reinstatement effects of cocaine, two measures which are affected by 5-HT_{2A}R antagonism in rodents as described above.

E. Advantages of Nonhuman Primate Models of Pharmacological Effects

As has been described thus far, the impact of pharmacological manipulations of the 5-HT_{2A}R and 5-HT_{2C}R upon cocaine-induced effects has been exclusively investigated in rodents, with the aforementioned exception of Fantegrossi et al (2002). However, there are important considerations to acknowledge that may diminish the generalization of such findings to human cocaine abusers.

First, there are important neuroanatomical differences between rodents and humans in brain regions which are known to mediate the abuse-related effects of cocaine and other drugs of abuse. For example, when compared to humans, rats display a markedly different distribution of dopamine D_1-like and D_2-like receptors within the striatum as the D_1/D_2 receptor ratio is higher in rats compared to nonhuman primates and humans (for review, Weerts et al 2007). Differences in serotonin transporter (SERT) and serotonin receptor distributions have also been characterized between rodents and primates; such differences have already been described in detail for the 5-HT_{2A}R and 5-HT_{2C}R. Indeed, it has been argued in a recent review that nonhuman primates are a more valid animal model than rodents when studying the pharmacological effects of compounds acting upon the 5-HT_2 receptor family (Weerts et al 2007). Furthermore, species differences apply not only to neurotransmitter systems, but also to regional interconnectivity, especially with respect to the DA-receiving prefrontal cortical regions and striatum, where afferent and efferent connections in humans is more homologous to that seen in monkeys as compared to rats (Haber et al 2000; Uylings et al 2003; Seamans et al 2008; Haber and Knutson 2010). Perhaps related to these notable differences in neuroanatomy and

neurotransmitter systems is the finding that the effects of cocaine upon measures of neuronal metabolic activity in rodents are in complete opposition to the effects observed in nonhuman primates and humans (London et al 1990; Lyons et al 1996, Porrino et al 2002; Porrino 2003; Mandeville et al 2011), again suggesting that the impact of novel pharmacotherapeutics upon cocaine effects may be more accurately predicted in nonhuman primates.

A second consideration when investigating the pharmacotherapeutic potential of experimental compounds are the differences in pharmacokinetics between rodents and primates. Pharmacokinetics refers to the rate of drug uptake, clearance, and metabolism. Because rodents have higher rates of metabolism compared to nonhuman primates and humans, it is plausible that studies employing nonhuman primates will more accurately predict the timecourse and effectiveness of drug treatments when administered to humans. Additionally, some studies have demonstrated differences in drug metabolism in rodents compared to nonhuman primates and humans for several drug classes, including psychostimulants (Banks et al 2007; Weerts et al 2007). Given that drugs can be metabolized in various ways to produce both active and inactive metabolites across species, it is possible that drug-induced effects observed in rodents may be stronger, weaker, or absent in nonhuman primates and humans, or vice versa, depending on the rate of drug metabolism and the resulting metabolites. Such effects are not limited to therapeutic benefits, but may also include undesirable or even toxic side effects, and evidence suggests that the emergence and magnitude of these adverse effects in humans are more concordantly predicted in nonhuman primates compared to rodents (Olson et al 2000).

Finally, there are important methodological differences when investigating the self-administration of abuse-related compounds in rodents compared to nonhuman primates. The

long lifespan of nonhuman primates allows for within-subjects experimental designs, therefore reducing the number of required subjects. Furthermore, the duration of self-administration studies in rats is typically limited to several weeks or months due to the difficulty in maintaining intravenous catheter patency, and even less so for mice. However, a single catheter preparation can be kept patent for several years in nonhuman primates. Additionally, nonhuman primates can safely endure multiple, sequential catheter preparations, further extending the duration of drug-taking history to many years. This is a critical factor in that the majority of human drug abusers present with several years of drug-taking history, and often polydrug abuse, that can be effectively modeled in nonhuman primates but are nearly impossible to parallel in rodents, given their limited window for intravenous catheterization. Long-term drug-taking history is also an important variable to model in experimental animals because it likely incorporates any neuroadaptations that may have occurred as a result of extended drug exposure. If, for example, 5-HT_{2A}Rs or 5-HT_{2C}Rs are differentially expressed following chronic cocaine exposure, the reactivity to pharmacological treatments acting at these receptor subtypes may also be modified. Depending on the time required for these adaptations to occur, the effects of such compounds in humans may be more accurately measured in nonhuman primates as compared to rodents, as rodents may not achieve levels of drug-taking necessary to induce some of these neuroadaptations.

Taken together, these factors indicate a clear advantage and necessity of using nonhuman primate subjects for the investigation of potential pharmacotherapeutic interventions in the treatment of drug abuse and further highlight the necessity and value of the present experiments.

F. Summary and Experimental Rationales

Evidence derived from animal models of cocaine use and relapse suggests that serotonergic signaling modulates the neurochemical and behavioral effects of cocaine, and therefore may provide potential pharmacotherapeutic targets for the treatment of cocaine abuse in humans. However, indirect serotonin agonists have failed clinically to alter cocaine-taking behavior, possibly due to the pharmacological activation of many serotonin receptor subtypes. More recent studies in rodents have demonstrated that the modulation of cocaine effects following pretreatment with compounds selective for the 5-HT_{2A} and 5-HT_{2C} receptors, but these targets have not been systematically evaluated in nonhuman primates. Such studies would serve to extend and expand upon previous findings derived in rodent models of drug use and lend further support to 5-HT_2 receptors as novel targets for substance abuse medications development. Therefore, the aims of this research project were as follows:

1. To assess the impact of 5-HT_{2C}R activation or antagonism, and 5-HT_{2A}R antagonism, upon the behavioral-stimulant effects of cocaine in nonhuman primates.

2. To identify the modulatory roles of the 5-HT_{2C}R and 5-HT_{2A}R upon cocaine self-administration and cocaine-induced reinstatement, operant procedures designed to model drug use and relapse respectively, in nonhuman primates.

3. To determine the effects of 5-HT_{2C}R activation and antagonism, or 5-HT_{2A}R antagonism, upon cocaine-induced changes in extracellular dopamine levels within dorsal (caudate nucleus) and ventral (nucleus accumbens) striatum in nonhuman primates.

Chapter II. 5-$HT_{2A/2C}$ Receptors: Modulation of the Behavioral-Stimulant Effects of Cocaine

A. Introduction

Among the more prominent behavioral effects of cocaine, amphetamine, and other related psychostimulants is their capacity to increase alertness, attention, wakefulness, and locomotion in humans. Such behavioral-stimulant effects have frequently been measured in rodents by assessing alterations in horizontal and vertical ambulations in response to a pharmacological challenge. These locomotor-activating effects of cocaine and other psychostimulants are believed to be strongly correlated with their capacity to elevate dopamine (DA) levels within the mesolimbic and/or nigrostriatal systems, neurochemical effects that are also known to play a prominent role in the reinforcing and abuse-related effects of drugs of abuse (Wise and Bozarth 1987; Wise 1998; Wise 2004). As such, assays of locomotor activity in rodents have proven useful in identifying potential pharmacotherapeutic targets for the treatment of cocaine abuse, based upon any observed pharmacological modulations of the behavioral-stimulant effects of cocaine.

Studies employing cocaine-induced hyperlocomotion assays in rodents have demonstrated a strong modulatory role for the 5-HT_2 receptor (5-HT_2R) family. Specifically, 5-HT_{2C}R agonism and 5-HT_{2A}R antagonism attenuate, whereas 5-HT_{2A}R agonism and 5-HT_{2C}R antagonism enhance, the locomotor-activating effects of cocaine (Grottick et al 2000; McMahon and Cunningham 2001; Fletcher et al 2002; Filip et al 2004; Fletcher et al 2006). Similar findings have been reported for the hyperlocomotor effects of amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) (Moser et al 1996; Auclair et al 2004; Ball and Rebec 2005; Herin et al 2005). More recent studies have shown that the effects of 5-HT_{2A}R-selective and 5-HT_{2C}R-selective compounds upon cocaine-induced hyperlocomotion are highly

predictive of their impact upon the neurochemical, reinforcing, and reinstatement effects of cocaine in rodents (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008). However, whether 5-$HT_{2A/2C}$R pretreatments can modulate the neurochemical and associated behavioral effects of cocaine in nonhuman primates has yet to be investigated.

Drug-induced behavioral-stimulant effects have long been modeled in squirrel monkeys as a significant increase in rates of lever-pressing maintained by schedules of reinforcement that normally engender low rates of operant responding. Using fixed-interval schedules of stimulus termination, for example, lever-pressing dramatically increases following pretreatment with cocaine, amphetamine, or morphine, all of which also induce hyperlocomotion in rodents (Barrett et al 1977; Spealman et al 1977; Spealman et al 1989; Jones and Holtzman 1994; Howell and Byrd 1995; Bauzo et al 2009; Kimmel et al 2009). In accordance with rodent hyperlocomotion studies, the rate-increasing effects of various drugs in monkeys are highly correlated with their capacity to increase extracellular levels of DA (Spealman et al 1989). Furthermore, drugs which selectively inhibit the function of the dopamine transporter (DAT) increase rates of fixed-interval responding, whereas drugs that selectively inhibit the norepinephrine or serotonin transporters do not (Spealman et al 1989; Howell and Byrd 1995; Ginsburg et al 2005). Taken together, these findings suggest that the fixed-interval stimulus-termination procedure is a simple assay that can be used to predict the impact of pharmacological pretreatments upon the abuse-related and neurochemical effects of cocaine and related psychostimulants in nonhuman primates.

Previous work has demonstrated that the serotonin (5-HT) system can modulate the behavioral-stimulant effects of cocaine in monkeys. For example, in squirrel monkeys, administration of SERT inhibitors or the nonselective 5-HT_2R agonist quipazine attenuated the

behavioral-stimulant effects induced by cocaine or the DAT-selective inhibitor GBR 12909, whereas the nonselective 5-HT_2R antagonists ketanserin, mianserin, or ritanserin enhanced the behavioral-stimulant effects of GBR 12909 or cocaine (Howell and Byrd 1995; Howell et al 1997). Unfortunately, due to the lack of subtype-selective compounds at the time, these studies were unable to identify the specific 5-HT receptor subtypes responsible for the observed effects. However, the recent development of more pharmacologically-selective tools has led to the elucidation of differential modulatory roles for the 5-HT_{2A}R and 5-HT_{2C}R in rodent studies of stimulant-induced hyperlocomotion. The goals of the present experiments were therefore to determine the impact of selective 5-HT_{2C}R agonism and antagonism, and 5-HT_{2A}R antagonism, upon the behavioral-stimulant effects of cocaine in squirrel monkeys trained to lever-press according to a fixed-interval operant schedule of stimulus termination. We hypothesized that 5-HT_{2C}R activation and 5-HT_{2A}R antagonism would attenuate, while 5-HT_{2C}R antagonism would enhance, the behavioral-stimulant effects of cocaine.

B. Methods

1. Subjects

Four adult male squirrel monkeys (*Saimiri sciureus*) weighing 850 – 1200g served as subjects. Between experimental sessions, animals were individually housed in a climate-controlled room and fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal) with ad libitum access to water. Daily enrichment was provided via access to foraging devices, toys, and climbing/swing devices. Animals had served in previous behavioral studies involving administration of compounds acting upon monoaminergic and/or glutamatergic systems (Kimmel et al 2007; Banks et al 2009; Bauzo et al 2009; Fantegrossi et al 2009; Kimmel et al 2009). All studies were conducted in strict

accordance with the National Institutes of Health's "Guide for Care and Use of Laboratory Animals", the American Association for Accreditation of Laboratory Animal Care (AAALAC), and were approved by the Institutional Animal Care and Use Committee of Emory University.

2. Apparatus

During behavioral sessions, animals were comfortably seated in a commercially-available plexiglass chair within a ventilated, sound-attenuating chamber (Med Associates Inc., St. Albans, VT). The chair was equipped with an operant panel consisting of a series of red and white lights, a lever, and a whitenoise amplifier which was activated throughout the duration of all behavioral sessions to further reduce the influence of ambient noise. Med-PC IV software (Med Associates Inc., St. Albans, VT) was interfaced with each chamber to allow for automated output control and lever-press recording.

3. Fixed-Interval Schedule of Stimulus Termination

Daily sessions were conducted five days per week (Monday-Friday) and lasted approximately 90 min. Each session began with the illumination of a pair of red lights. During a 300-sec fixed-interval, lever presses were recorded but had no programmed consequences. Once the 300-sec interval elapsed, the schedule progressed into a 3-sec limited hold. A single response during the limited hold extinguished the red lights and illuminated a white light for 15-sec to signal reinforcement. If the animal failed to press the lever during the limited hold, a mild electrical stimulus (300ms, 3-6mA) was delivered to a shaved portion of the distal end of the tail that was secured within an acrylic tail yoke. Each daily session consisted of 15 consecutive fixed-interval components separated by 60-sec timeout periods during which all lights were extinguished and responses had no scheduled consequences. Lever presses and response rates were recorded for each individual component and then averaged across the session.

Experimental sessions involving drug pretreatments were conducted twice per week (Tuesday, Friday). Cocaine (veh, 0.3-3.0 mg/kg) was administered immediately prior to the onset of the session while the animal was seated in the operant chair. The preferential 5-HT_{2C}R agonist mCPP (veh, 0.1-0.3 mg/kg), the selective 5-HT_{2C}R agonist Ro 60-0175 (veh, 0.1-0.3 mg/kg), and the 5-HT_{2A}R antagonist M100907 (0.1-3.0 mg/kg) were administered 15-min prior to cocaine. The selective 5-HT_{2C}R antagonist SB 242084 (veh, 0.01-0.1 mg/kg) was administered 30-min prior to cocaine pretreatment. Drugs were tested for their impact upon cocaine-induced behavioral-stimulant effects in the following order: M100907, mCPP, Ro 60-0175, SB 242084. For each drug, the order of dose combinations with cocaine was randomized for each individual subject. All drugs were administered intramuscularly into the thigh muscles at a volume of 0.2-0.6 ml.

4. Drugs

Cocaine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), m-chlorophenylpiperazine (mCPP) (Sigma-Aldrich, St. Louis, MO), and Ro 60-0175 (Tocris Bioscience, Ellisville, MO) were dissolved in 0.9% saline. SB 24204 (Tocris Bioscience, Ellisville, MO) was initially dissolved at a concentration of 1.0 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO) , and 0.9% saline, and further diluted to appropriate concentrations using 0.9% saline. M100907, generously provided by Kenner C. Rice, Ph.D. of the Laboratory of Medicinal Chemistry at the NIH/NIDDK, was initially dissolved in sterile water containing a minute amount of 1.0N hydrochloric acid. The solution was then titrated to a pH within the range of 5.0-7.0 and brought to volume with sterile water. All drug solutions were passed through a 0.2 µm-pore polysulfone filter prior to use. Doses were calculated from the salt weights.

5. Data Analysis

Only response rates obtained from the first 10 components (approximately 60-min) of each test session were used for analyses as the behavioral-stimulant effects of lower doses of cocaine returned to baseline values by this time (Fig. 2a). For each subject, rates of responding following each drug-combination test were normalized as a percent of the overall response rate following vehicle treatments of all drugs. Effects of various doses of the pharmacological pretreatments at each cocaine dose were analyzed using repeated-measures ANOVAs with post hoc Tukey's tests (mCPP, Ro 60-0175) post hoc Dunnett's tests (M100907, SB 242084 alone, SB 242084 + 0.1 mg/kg cocaine) or paired t-test (SB 242084 + 1.0 mg/kg cocaine). Data were graphically plotted using GraphPad v. 5.01 (GraphPad Software Inc., La Jolla, CA) and analyzed using SigmaStat v. 3.0 software (Systat Software Inc., San Jose, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

C. Results

1. 5-HT_{2C}R Agonists: mCPP and Ro 60-0175

The effects of various doses of cocaine upon response rates are shown in Figure 2a. The mean response rate (± SEM) following vehicle pretreatments of both mCPP and cocaine was 0.41 ± 0.06 responses/s. A moderate dose of cocaine (0.3 mg/kg) induced rate-increasing effects that peaked at approximately 280% of baseline responding within 10-15 min of drug administration and gradually returned to baseline rates within 60 min. Administration of 1.0 mg/kg cocaine increased response rates to a similar peak magnitude as that engendered by 0.3 mg/kg, but the effect persisted for a longer duration. Increasing the dose further to 3.0 mg/kg suppressed responding to approximately 50% baseline across all subjects for most components.

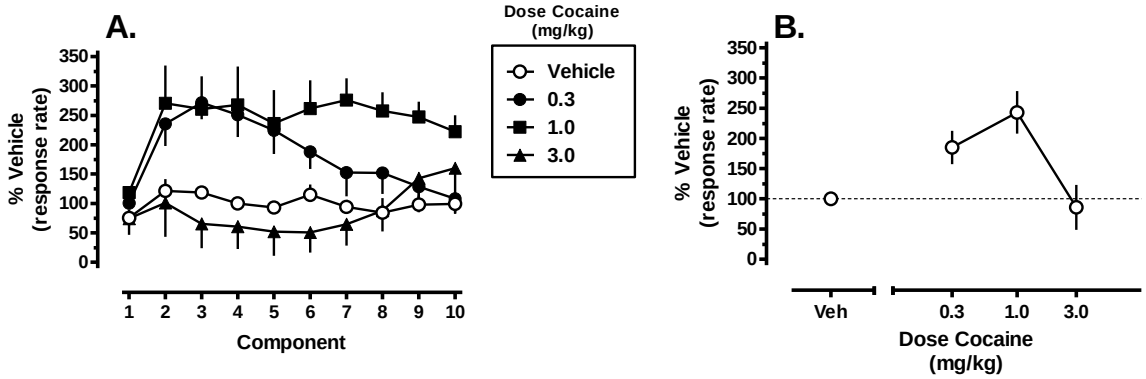

Figure 2. (A) Time-course of the effects of cocaine (0.3-3.0 mg/kg) on response rates maintained by a fixed-interval 300-sec operant schedule of stimulus termination in squirrel monkeys (n=4). (B) Averaging the response rates from individual components for each cocaine dose generates a typical inverted U-shaped dose-response function with respect to cocaine-induced behavioral-stimulant effects. Data (mean ± SEM) are expressed as a percent of the averaged session response rate following administration of the cocaine vehicle (saline). The dotted line represents baseline response rate following vehicle treatment (100%). *Abscissae*: sequential fixed-interval components (A) or dose of cocaine (B). *Ordinates*: normalized response rates.

Averaging the response rates across all components for each cocaine dose produced a prototypical inverted U-shape dose-response function (Fig. 2b). This dose-response curve was replicated in subsequent experiments and served as the basis for determining the modulatory impact of all pharmacological pretreatments tested.

The effects of pretreatment with the preferential 5-HT_{2C}R agonist mCPP alone and in combination with cocaine are shown in Figure 3. Administration of mCPP induced a dose-dependent downward shift of the ascending limb of the cocaine dose-response function. Two-way ANOVA indicated significant main effects for cocaine ($F_{(3,9)} = 9.12$, $p = 0.004$) and mCPP ($F_{(2,6)} = 12.63$, $p = 0.007$) but not a significant interaction ($F_{(6,18)} = 0.85$, $p = 0.56$). One-way ANOVAs with post hoc analyses revealed that the behavioral-stimulant effect of the maximally-effective dose of 1.0 mg/kg cocaine (~243% baseline) was significantly attenuated following pretreatment with 0.3 mg/kg mCPP to ~163% baseline responding ($F_{(2,6)} = 5.41$, $p = 0.045$; Tukey's test, $p < 0.05$). Additionally, the behavioral-stimulant effects of 0.3 mg/kg cocaine were completely abolished by pretreatment with 0.3 mg/kg mCPP compared to vehicle (~100% vs. ~185%, respectively), although the interaction barely failed to reach statistical significance ($F_{(2,6)} = 4.94$, $p = 0.054$). It should be noted that the 0.3 mg/kg dose of mCPP suppressed responding significantly when administered alone to approximately 57% of baseline responding ($F_{(2,6)} = 5.23$, $p = 0.048$; Tukey's test, $p < 0.05$). To confirm that the actions of mCPP were mediated through the 5-HT_{2C}R, the dose combination of 0.3 mg/kg mCPP and 1.0 mg/kg cocaine was retested following pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 in three squirrel monkeys. One-way ANOVA revealed a main effect for treatment condition ($F_{(5,10)} = 3.85$, $p = 0.033$), although post hoc Dunnett's tests failed to identify any significant differences. Visual inspection of the data clearly indicates that SB 242084, at doses which produced only modest

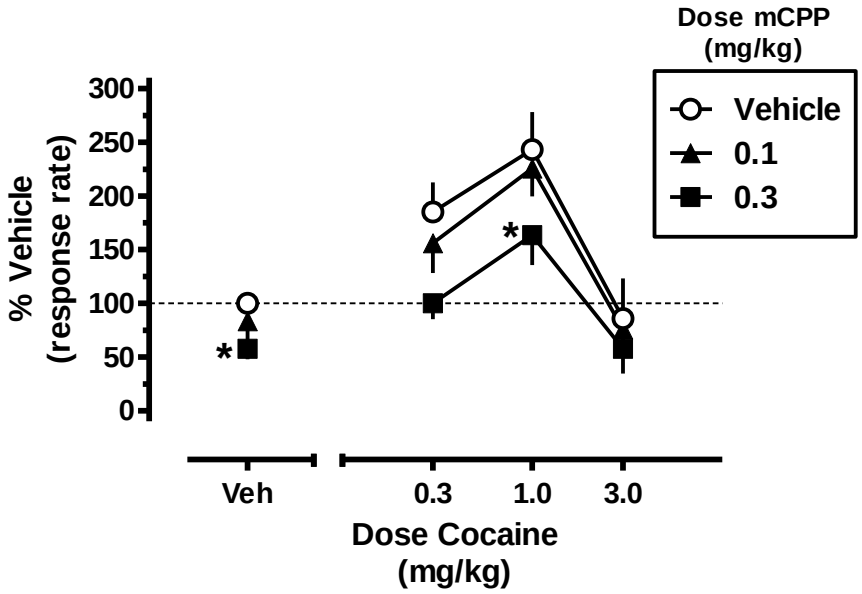

Figure 3. Effects of pretreatment with mCPP (veh, 0.1-0.3 mg/kg) prior to administration of saline or cocaine (0.3-3.0 mg/kg) on rates of responding in squirrel monkeys (n=4) trained to lever-press on a fixed-interval 300-sec schedule of stimulus termination. mCPP dose-dependently caused a downward shift of the ascending limb of the cocaine dose-response function, but only at a dose which also significantly decreased responding when administered alone (0.3 mg/kg). Data (mean ± SEM) are expressed as a percent of responding following administration of the vehicle for both mCPP and cocaine. The dotted line represents baseline response rate following vehicle treatment (100%). Asterisks (*) indicate significant difference ($p < 0.05$) for a given data point compared to "Vehicle" mCPP treatment prior to the same dose of cocaine. *Abscissa*: dose of cocaine. *Ordinate*: normalized response rate.

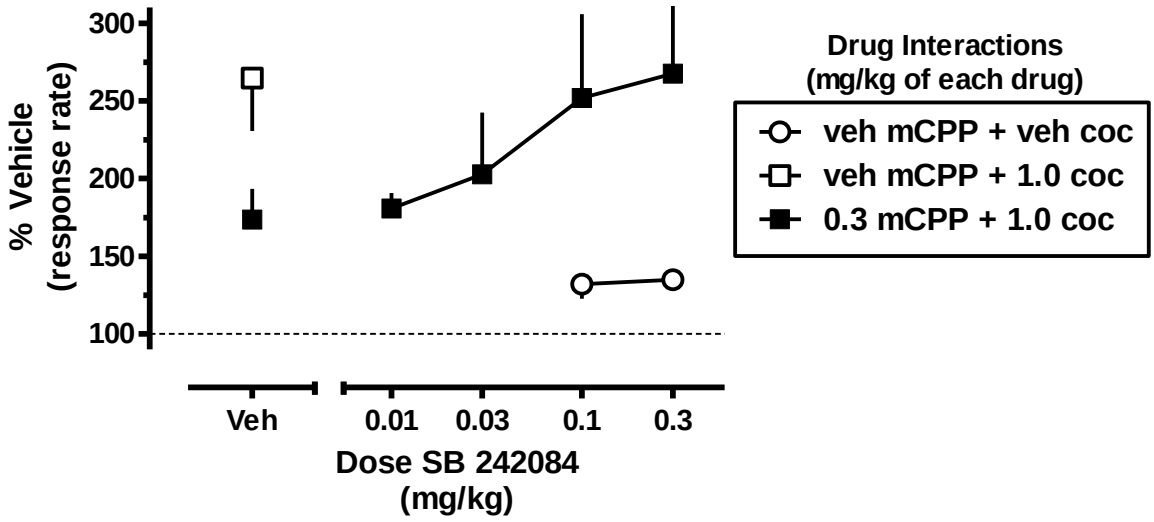

Figure 4. Effects of pretreatment with SB 242084 (veh, 0.01-0.3 mg/kg) on the mCPP-induced attenuation of the behavioral-stimulant effects of cocaine in squirrel monkeys (n=3) responding on a fixed-interval 300-sec schedule of stimulus termination. Administration of 1.0 mg/kg cocaine following vehicle treatments of mCPP and SB 242084 caused an increase in responding ("veh" SB 242084, *open square*). Pretreatment with 0.3 mg/kg mCPP attenuated the rate-increasing effect of cocaine ("veh" SB 242084, *filled square*). Pretreatment with SB 242084 alone induced only modest behavioral-stimulant effects at the highest doses tested (*open circles*), but dose-dependently prevented the attenuating effect of mCPP (*filled squares*). SB 242084 was administered 15 min prior to mCPP, which was administered 15 min prior to cocaine. Data (mean ± SEM) are expressed as a percent of responding from sessions where only the vehicles for SB 242084, mCPP, and cocaine were administered. The dotted line represents baseline response rate following vehicle treatment (100%). *Abscissa*: dose of SB 242084. *Ordinate*: normalized response rate.

behavioral-stimulant effects alone (0.1 and 0.3 mg/kg), dose-dependently blocked the attenuating of effects of mCPP (Fig. 4).

The effects of pretreatment with the highly selective 5-HT_{2C}R agonist Ro 60-0175 alone and in combination with cocaine are shown in Figure 5. The mean response rate (± SEM) following vehicle pretreatments of both Ro 60-0175 and cocaine was 0.44 ± 0.14 responses/s. Similar to mCPP, Ro 60-0175 induced a dose-dependent downward shift of the ascending limb of the cocaine dose-response function with no apparent alteration of the effects of the highest cocaine dose (3.0 mg/kg). Two-way ANOVA indicated significant main effects for cocaine ($F_{(3,9)}$ = 10.99, p = 0.002) and Ro 60-0175 ($F_{(2,6)}$ = 5.16, p = 0.05) but not a significant interaction ($F_{(6,18)}$ = 1.37, p = 0.28). Subsequent one-way ANOVAs with post hoc analyses revealed that the behavioral-stimulant effects of 1.0 mg/kg cocaine (~293%) were significantly attenuated following pretreatment with 0.3 mg/kg Ro 60-0175 to ~226% baseline responding ($F_{(2,6)}$ = 5.491, p = 0.044; Tukey's test, p < 0.05). A similar but nonsignificant trend was observed at the 0.3 mg/kg cocaine dose (~241% vs. ~191%) ($F_{(2,6)}$ = 4.34, p = 0.068). In contrast to mCPP, one-way ANOVA with Tukey's post hoc analysis indicated that neither dose of Ro 60-0175 significantly altered response rates when administered alone (0.1 mg/kg, p = 0.62; 0.3 mg/kg, p = 0.07). The highest dose of Ro 60-0175 (0.3 mg/kg) reduced response rates to approximately 75% of baseline responding, nearly half the magnitude of change in responding induced by the highest (0.3 mg/kg) dose of mCPP tested.

Because the attenuation of cocaine-induced behavioral-stimulant effects elicited by Ro 60-0175 was relatively modest compared to that exerted by mCPP, antagonism interaction studies with SB 242084 would be unlikely to provide any significant alterations in the effects of Ro 60-0175 under these conditions. Therefore, to confirm that the actions of Ro 60-0175 were

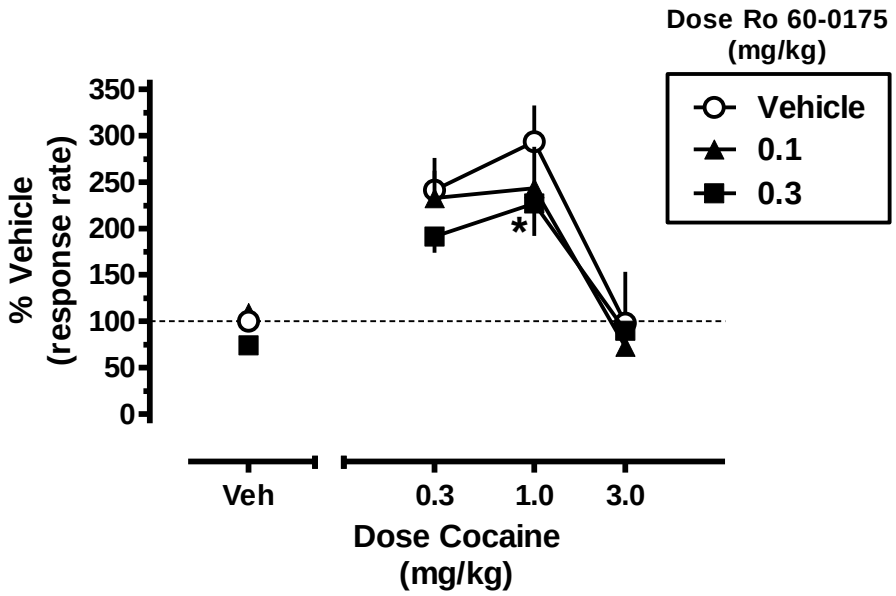

Figure 5. Effects of pretreatment with Ro 60-0175 (veh, 0.1-0.3 mg/kg) prior to administration of saline or cocaine (0.3-3.0 mg/kg) on rates of responding in squirrel monkeys (n=4) trained to lever-press on a fixed-interval 300-sec schedule of stimulus termination. Ro 60-0175 did not significantly alter responding when administered prior to saline, but dose-dependently caused a modest downward shift of the ascending limb of the cocaine dose-response function. Data (mean ± SEM) are expressed as a percent of responding following administration of the vehicle for both Ro 60-0175 and cocaine. The dotted line represents baseline response rate following vehicle treatment (100%). Asterisks (*) indicate significant difference ($p < 0.05$) for a given data point compared to "Vehicle" Ro 60-0175 treatment prior to the same dose of cocaine. *Abscissa*: dose of cocaine. *Ordinate*: normalized response rate.

mediated through the 5-HT_{2C}R, we tested whether the rate-decreasing effects of Ro 60-0175 were 5-HT_{2C}R-dependent by expanding the tested dose range alone and in combination with SB 242084. This dose combination was only investigated in two subjects because these studies were the first to administer Ro 60-0175 or SB 242084 to nonhuman primates and we were therefore hesitant to repeatedly administer high doses of these compounds to large numbers of subjects. Despite the small sample size, pretreatment with 1.0 mg/kg Ro 60-0175 caused a marked reduction of response rates to approximately 45% of baseline, and this effect was dose-dependently blocked by administration of SB 242084 15 min prior to Ro 60-0175 (Fig. 6).

2. 5-HT_{2C}R Antagonist: SB 242084

The effects of pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 alone and in combination with cocaine on response rates maintained by a fixed-interval 300-sec schedule of stimulus termination are shown in Figure 7. The mean response rate (± SEM) following vehicle pretreatments of both SB 242084 and cocaine was 0.39 ± 0.07 responses/s. When administered alone, one-way ANOVA with post hoc analyses indicated that 0.03 and 0.1 mg/kg SB 242084 induced modest but significant behavioral-stimulant effects on response rates compared to vehicle pretreatment (~137% and ~154% baseline, respectively) ($F_{(3,9)}$ = 7.05, p = 0.01; Dunnett's test, $p < 0.05$). We next sought to determine the impact of 5-HT_{2C}R antagonism upon cocaine-induced behavioral-stimulant effects. Because we hypothesized an enhancement of the behavioral effects of cocaine, the cocaine dose range was shifted a half-log dose lower in order to incorporate a dose of cocaine that, when administered alone, did not induce significant stimulant-like effects. Pretreatment with SB 242084 induced an upward shift of the ascending limb of the cocaine dose-response function (Fig. 7). Administration of a low dose of cocaine (0.1 mg/kg) did not significantly increase responding above baseline (~123%, $p > 0.05$). However,

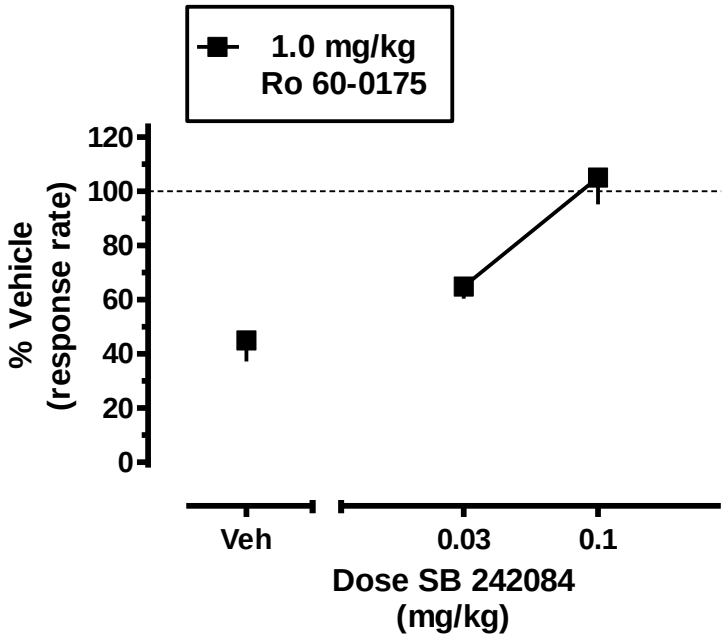

Figure 6. Effects of pretreatment with SB 242084 (veh, 0.03-0.1 mg/kg) on the rate-decreasing effect of 1.0 mg/kg Ro 60-0175 in squirrel monkeys (n=2) trained to lever-press on a fixed-interval 300-sec operant schedule of stimulus termination. SB 242084 dose-dependently blocked the behavioral-suppressant effect of Ro 60-0175. Data (mean ± SEM) are expressed as a percent of responding following administration of the vehicle for Ro 60-0175. The dotted line represents baseline response rate following vehicle treatment (100%). SB 242084 was administered 15 min prior to Ro 60-0175, which was administered 15 min prior to session onset. *Abscissa*: dose of SB 242084. *Ordinate*: normalized response rate.

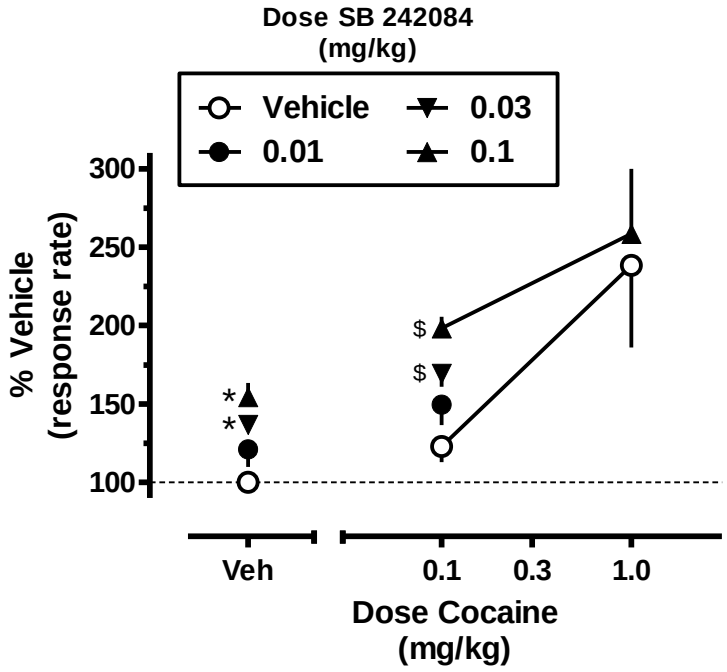

Figure 7. Effects of pretreatment with SB 242084 (veh, 0.01-0.1 mg/kg) prior to administration of saline or cocaine (0.1 and 1.0 mg/kg) on rates of responding in squirrel monkeys (n=4) trained to lever-press on a fixed-interval 300-sec schedule of stimulus termination. SB 242084 demonstrated modest but significant behavioral-stimulant effects when administered prior to saline. SB 242084 induced a dose-dependent upward shift of the ascending limb of the cocaine dose-response function at doses which also increased response rates when administered alone. Data (mean ± SEM) are expressed as a percent of responding following administration of the vehicle for both SB 242084 and cocaine. The dotted line represents baseline response rate following vehicle treatment (100%). Asterisks (*) indicate significant difference ($p < 0.05$) for a given data point compared to vehicle treatments of SB 242084 and cocaine. Dollar symbols ($) indicate significant difference ($p < 0.05$) for a given data point compared to the effects of vehicle SB 242084 prior to 0.1 mg/kg cocaine. *Abscissa*: dose of cocaine. *Ordinate*: normalized response rate.

combined pretreatment with various doses of SB 242084 and 0.1 mg/kg cocaine produced behavioral-stimulant effects that were of greater magnitude than the effects of 0.1 mg/kg cocaine alone ($F_{(4,12)} = 17.53$, $p < 0.001$), and this interaction appeared to be additive. Specifically, post hoc Dunnett's tests revealed a significant effect at dose combinations of 0.03 and 0.1 mg/kg SB 242084 prior to 0.1 mg/kg cocaine (169% and 198%, respectively), compared to vehicle SB242084 prior to 0.1 mg/kg cocaine ($p < 0.05$). The highest dose of SB 242084 tested (0.1 mg/kg) did not significantly alter the effects of the maximally-effective 1.0 mg/kg cocaine dose (paired t-test, $p = 0.30$). The finding that this dose combination did not result in rates of responding that were located on the descending limb of the dose-response function indicates that pretreatment with SB 242084 induced an upward shift, rather than a parallel leftward shift, of the cocaine dose-response function.

3. 5-HT_{2A}R Antagonist: M100907

The effects of pretreatment with the selective 5-HT_{2A}R antagonist M100907 alone and in combination with cocaine on fixed-interval responding are shown in Figure 8. The mean response rate (± SEM) following vehicle pretreatments of both M100907 and cocaine was 0.43 ± 0.09 responses/s. Two-way ANOVA indicated a significant main effect for cocaine ($F_{(3,9)} = 22.41$, $p < 0.001$) no significant main effect for M100907 ($F(3,9) = 3.154$, $p = 0.079$) and a significant interaction ($F_{(9,27)} = 2.82$, $p = 0.018$). Pretreatment with M100907 alone did not alter response rates ($F_{(3,9)} = 2.25$, $p = 0.152$). However, subsequent one-way ANOVAs and post hoc analyses within each cocaine dose failed to identify any significant differences when comparing vehicle treatment to any dose of M100907, suggesting that selective 5-HT_{2A}R antagonism failed to alter the behavioral-stimulant effects of cocaine.

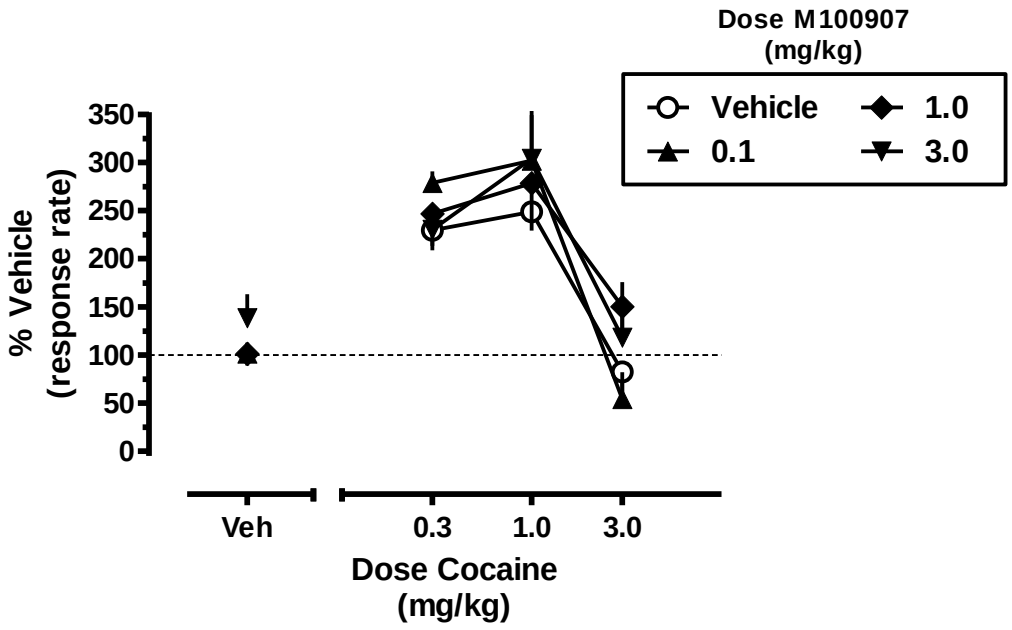

Figure 8. Effects of pretreatment with M100907 (veh, 0.1-3.0 mg/kg) prior to administration of saline or cocaine (0.3-3.0 mg/kg) on rates of responding in squirrel monkeys (n=4) trained to lever-press on a fixed-interval 300-sec schedule of stimulus termination. M100907 did not significantly alter responding when administered prior to saline, nor did it alter the behavioral-stimulant effects of cocaine. Data (mean ± SEM) are expressed as a percent of responding following administration of the vehicle for both M100907 and cocaine. The dotted line represents baseline response rate following vehicle treatment (100%). *Abscissa*: dose of cocaine. *Ordinate*: normalized response rate.

D. Discussion

Although previous work has demonstrated a modulatory role for 5-HT upon the behavioral and neurochemical effects of cocaine in nonhuman primates, the impact of specific 5-HT receptor subtypes has remained unclear. In the present study, using compounds that demonstrate greater inter-subtype selectivity than those used previously, we now report that selective 5-HT_{2C}R activation attenuated, whereas 5-HT_{2C}R antagonism enhanced, the behavioral-stimulant effects of cocaine in squirrel monkeys. Additionally, 5-HT_{2A}R antagonism was ineffective at altering cocaine-induced behavioral-stimulant effects.

Recent studies in rodents have indicated that selective 5-HT_{2C}R agonists attenuate, whereas 5-HT_{2C}R antagonists enhance, the locomotor-increasing effects of cocaine. However, to the best of our knowledge, highly selective 5-HT_{2C}R agonists or antagonists such as Ro 60-0175, SB 242084, and others have not been previously administered to nonhuman primates. We therefore were initially concerned about the safety and tolerability of the administration of such compounds in our subjects. To confirm the 5-HT_{2C}R as a pharmacotherapeutic target and rationalize the administration of Ro 60-0175 and SB 242084, we first assessed the impact of mCPP pretreatment upon the behavioral-stimulant effects of cocaine. mCPP is commonly described as a "preferential" 5-HT_{2C}R agonist because 1) it exhibits modest selectivity for the 5-HT_{2C}R compared to the 5-HT_{2A}R (pEC_{50} = 7.09 vs. 6.65, respectively), 2) it is a more efficacious agonist at the 5-HT_{2C}R compared to the 5-HR_{2A}R, and 3) its behavioral effects in rodents are blocked by 5-HT_{2C}R antagonists, but not antagonists of other receptor subtypes (Porter et al 1999; Bubar and Cunningham 2008). Most importantly, we have previously administered mCPP at doses up to 2.5 mg/kg in rhesus monkeys (nearly 10x higher than the highest dose used in the present study) without deleterious or adverse consequence (Murnane et al 2010). In the present

study, we found that pretreatment with mCPP attenuated the behavioral-stimulant effects of cocaine in squirrel monkeys, extending results derived from rodent studies into monkeys. Furthermore, the effects of mCPP were prevented following pretreatment with the highly selective 5-HT_{2C}R antagonist SB 242084, suggesting that the effects of mCPP were indeed mediated through activation of the 5-HT_{2C}R and not other 5-HT_2R subtypes.

It must be noted that the behavioral-stimulant effects of cocaine were only attenuated by a dose of mCPP which also reduced rates of responding when administered alone. One might therefore speculate that the actions of mCPP may not have been due to an attenuation of direct cocaine effects per se, but rather may have been attributed to nonspecific disruptions in operant performance that were counteracted by the stimulant-like effects of cocaine. In support of this argument, administration of selective 5-HT_{2C}R agonists has been shown to induce hypolocomotive effects in rodents (Kennett et al 2000; Gleason et al 2001; Kimura et al 2004; Stiedl et al 2007). It is critical to note, however, that in addition to direct agonist activity at 5-HT receptors, mCPP also functions as a 5-HT releaser, increasing extracellular concentrations of 5-HT (Baumann et al 1993; Eriksson et al 1999). For example, we have reported that systemic administration of 2.5 mg/kg mCPP in rhesus monkeys increased extracellular concentrations of 5-HT within the caudate nucleus by approximately 400%, and that 5-HT levels remained elevated above baseline for at least 60 min (Murnane et al 2010). This is an important consideration as pharmacological enhancement of 5-HT levels via administration of an indirect agonist, such as a SERT inhibitor or 5-HT releaser, decreases response rates in squirrel monkeys maintained on a fixed-interval schedule of stimulus termination (Spealman et al 1989; Howell and Byrd 1995). Taken together, these findings raise the possibility that the rate-decreasing effects of mCPP observed here may be at least partially mediated by its effects upon 5-HT release, and not solely via 5-HT_{2C}R activation.

To further investigate the effects of 5-HT_{2C}R activation, we replicated the mCPP experiment with Ro 60-0175, a drug that, compared to mCPP, exhibits greater selectivity and agonist efficacy for the 5-HT_{2C}R (Porter et al 1999). Pretreatment with Ro 60-0175 fully recapitulated the effects of mCPP, although the maximal reductions in basal responding and the attenuation of cocaine-induced behavioral-stimulant effects were of lower magnitude as compared to those produced by mCPP pretreatment. Our results obtained with Ro 60-0175 pretreatment are again in agreement with studies in rodents demonstrating that selective 5-HT_{2C}R activation attenuates the locomotor-increasing effects of cocaine (Grottick et al 2000; Filip et al 2004). Importantly, we found that the behavioral-stimulant effects of cocaine were significantly attenuated by a dose of Ro 60-0175 which did not produce signifcant reductions in baseline responding when administered alone (0.3 mg/kg). In contrast, mCPP only attenuated the effects of cocaine at a dose which reduced basal responding to nearly 50% of baseline. One may argue that increasing the pretreatment dose of Ro 60-0175 would have an even stronger attenuating effect upon the behavioral-stimulant effects of cocaine. However, increasing the dose of Ro 60-0175 to 1.0 mg/kg reduced baseline responding to 45%, and was therefore not tested for interactions with cocaine. Because the rate-decreasing effects of Ro 60-0175 were fully reversed by pretreatment with the 5-HT_{2C}R antagonist SB 242084, it seems likely that the nonspecific effects of both mCPP and Ro 60-0175 upon lever-pressing are mediated through the 5-HT_{2C}R. As such, it cannot be argued that greater pharmacological selectivity for the 5-HT_{2C}R would eliminate or minimize nonspecific disruptions in operant performance. Rather, we suggest that greater selectivity/efficacy for the 5-HT_{2C}R engenders superior behavioral specificity of drug effects on responding. The development and availability of even more selective and/or efficacious pharmacological tools would allow us to test this hypothesis in the future.

In contrast to mCPP and Ro 60-0175, the selective 5-HT_{2C}R antagonist SB 242084 dose-dependently but modestly increased rates of responding alone and potentiated the behavioral-stimulant effects of a low dose of cocaine. This interactive effect of combined SB 242084 and cocaine administration is consistent with several previous studies in rodents. For example, genetic mutant mice lacking the 5-HT_{2C}R displayed a greater locomotor response to cocaine relative to wild-type controls (Rocha et al 2002), and systemic administration of selective 5-HT_{2C}R antagonists enhanced cocaine-induced locomotor activity in rats (Fletcher et al 2002; Filip et al 2004; Fletcher et al 2006). There is also evidence to support our finding that administration of SB 242084 alone induces stimulant-like effects, as administration of a high dose of SB 242084 (1.0 mg/kg) significantly increased locomotor activity above baseline values in rats (Zaniewska et al 2009). Interestingly, pretreatment with SB 242084 did not alter the effects of a dose of cocaine that elicited maximum increases in responding (1.0 mg/kg). Given that 3.0 mg/kg cocaine lies on the descending limb of the curve, one might have predicted that the combination of effective doses of SB 242084 with 1.0 mg/kg cocaine would have resulted in a data point located on the descending limb of the dose-response curve. However, rather than this expected parallel leftward shift of the cocaine dose-response function, 5-HT_{2C}R antagonism seemingly affected only the ascending limb of the curve, and in fact slightly enhanced the behavioral-stimulant effect of 1.0 mg/kg cocaine, an effect that may have been limited in magnitude by a ceiling effect. Likewise, rather than a parallel rightward shift, mCPP and Ro 60-0175 induced a downward shift of the ascending limb only and did not alter the descending limb of the dose-response function.

The mechanism underlying such complex interactions with cocaine is difficult to explain, but may be related to a differential capacity of 5-HT_{2C}R agonism or antagonism to modulate mesolimbic vs. nigrostriatal DA pathways. As described earlier (Chapter I, section 2b), 5-HT_{2C}R-

mediated modulation of DA signaling within the NAcc is clear, but less so for the dorsal striatal territories. For example, the selective 5-HT_{2C}R agonist WAY 163909 caused reduced activity within the mesolimbic, but not nigrostriatal, DA system in rats. In the same study, WAY 163909 was found to attenuate stimulant-induced locomotor activity, but had no effect on stimulant-induced stereotypy (Marquis et al 2007). The induction of stereotypy by high doses of psychostimulants is believed to be an important factor underlying the descending limb of dose-response curves with respect to stimulant-induced locomotor activity, as rodents engaging in stereotypical behaviors exhibit less horizontal locomotion. DA signaling within the mesolimbic system has long been argued to mediate the induction of locomotor activity, whereas DA signaling within the nigrostriatal system is believed to mediate the induction of stereotypical behaviors in rodents (Kelly et al 1975; Wise and Bozarth 1987). Extrapolating these theories into our nonhuman primate model, it could be argued that the ascendling limb of the cocaine dose-response function is mediated predominantly by increased DA signaling within the mesolimbic system, whereas the descending limb is mediated predominantly by increased DA signaling within the nigrostriatal DA system. If true, then our observed failure of the 5-HT_{2C}R-selective compounds to modulate the descending limb may be a reflection of a limited capacity to affect DA signaling within the nigrostriatal system. Such a theory is supported by the detection of 5-HT_{2C}R mRNA in the VTA, but not SNpc, in macaque brain (Lopez-Gimenez et al 2001a).

Surprisingly, pretreatment with the selective 5-HT_{2A}R antagonist M100907 had no effect upon the behavioral-stimulant effects of cocaine. This was an unexpected result as systemic administration of 5-HT_{2A}R antagonists attenuates the hyperlocomotive effects of cocaine in rodents (McMahon and Cunningham 2001; Fletcher et al 2002; Filip et al 2004). The reasons for the lack of effect in our model remain speculative. The dose range and pretreatment time were selected based on previous results in our laboratory demonstrating that M100907 dose-

dependently blocked the rate-decreasing effects of MDMA in squirrel monkeys responding under an identical fixed-interval schedule of stimulus termination (Fantegrossi et al 2009). In that study, the behavioral effects of MDMA were argued to be mediated specifically through central 5-HT systems, but not DA systems, as the effects of MDMA were reversed by pretreatment with M100907 or the SERT inhibitor fluoxetine, but not by the selective DAT inhibitor RTI-177. Therefore, although M100907 was found to be behaviorally active in that study, its effects were likely not related to actions on DA signaling. The lack of a modulatory impact upon the behavioral-stimulant effects of cocaine may indicate an inability of 5-HT_{2A}R antagonism to modulate DA signaling in the squirrel monkey. As described previously (Chapter I, section 3a), there are clear species differences between rodents and primates regarding 5-HT_{2A}R distribution. Specifically, studies suggest that the 5-HT_{2A}R is expressed at dramatically lower levels in nonhuman primates and humans, particularly within the striatum and DAergic cell-body regions of the midbrain (Lopez-Gimenez et al 1999; Hall et al 2000; Ikemoto et al 2000; Lopez-Gimenez et al 2001b). The ineffectiveness of 5-HT_{2A}R antagonism to modulate the behavioral-stimulant effects of cocaine in the present study might therefore be due to the lack of adequate receptor protein within the central DAergic systems. Interestingly, similar findings were reported in a study by Fantegrossi et al (2002), where pretreatment with M100907, at doses similar to those used in the current study, abolished the reinforcing effects of R-(-)-MDMA but did not significantly alter the reinforcing effects of cocaine in rhesus monkeys.

Our laboratory previously demonstrated that pretreatment with nonselective 5-HT_2R antagonists (ritanserin, ketanserin) induced an upward shift of the behavioral-stimulant dose-response function for both cocaine and the selective DAT inhibitor GBR 12909 in squirrel monkeys responding on a similar fixed-interval schedule of stimulus termination (Howell and Byrd 1995; Howell and Byrd 1997). Those results could be viewed as being in full agreement

with the present data, if we were to presume that the nonselective 5-HT_2R antagonists in those previous studies were essentially functioning as selective 5-HT_{2C}R antagonists, due to the inability of their antagonistic effects upon the 5-HT_{2A}R to modulate the behavioral effects of cocaine in nonhuman primates. Correspondingly, administration of the nonselective 5-HT agonist quipazine, as well as the SERT inhibitors fluoxetine, clomipramine, and alaproclate, attenuated the behavioral-stimulant effects of cocaine, and our current findings suggest that direct or indirect activation of the 5-HT_{2C}R may have at least partially mediated those effects (Howell and Byrd 1995).

In summary, pretreatment with the 5-HT_{2C}R agonists mCPP and Ro 60-0175 attenuated, whereas the 5-HT_{2C}R antagonist SB 242084 enhanced, the behavioral-stimulant effects of cocaine in squirrel monkeys. Pretreatment with a 5-HT_{2A}R antagonist had no effect. In general, pharmacological compounds which attenuate the behavioral-stimulant effects of cocaine in nonhuman primates have subsequently been found to also reduce the reinforcing and/or neurochemical effects of cocaine (Howell and Byrd 1995; Czoty et al 2002; Bauzo et al 2009). Therefore, the present results suggest that drugs acting selectively at 5-HT_{2C}Rs may modulate other behavioral effects of cocaine, including its reinforcing and reinstatement effects.

Chapter III. 5-$HT_{2A/2C}$ Receptors: Modulation of Cocaine Reinforcement and Reinstatement

A. Introduction

The self-administration procedure serves as a preclinical measure of the abuse-related effects of drugs and is based on the observation that administration of many drugs of abuse will "reinforce", or increase the probability of occurrence of, specific operant-behavioral responses in animals. Such techniques have been utilized not only to identify the neurobiological mechanisms underlying the reinforcing effects of drugs of abuse, but also to assess the pharmacotherapeutic potential of novel medications to reduce drug-taking behavior. Although abundant evidence indicates that the reinforcing effects of cocaine and related psychostimulants are highly correlated with increases in DA signaling (Ritz et al 1987; Bergman et al 1989), numerous studies have demonstrated that manipulations of central 5-HT systems can modulate the reinforcing effects of cocaine. For example, dietary supplementation with the 5-HT precursor L-tryptophan, or pretreatment with SERT inhibitors and 5-HT releasers (each of which results in elevated extracellular levels of 5-HT), attenuated the self-administration of cocaine in rodents and nonhuman primates (Carroll et al 1990a; Carroll et al 1990b; Richardson and Roberts 1991; Kleven and Woolverton 1993; Glowa et al 1997; Czoty et al 2002; Negus et al 2007). In contrast, reductions in 5-HT signaling following central 5-HT neurotoxic lesions enhanced breakpoints in rats self-administering cocaine on a progressive-ratio schedule (Loh and Roberts 1990), and pretreatment with 5-HT_{1a}R agonists, which reduce 5-HT signaling via autoreceptor activation, potentiated the reinforcing effects of cocaine in nonhuman primates (Nader and Barrett 1990; Czoty et al 2005). Importantly, acute administration of SERT inhibitors also attenuated the subjective effects of cocaine in humans with a history of cocaine abuse (Walsh et al 1994; Walsh and Cunningham 1997). Taken together, these results indicate that 5-

HT exerts an inhibitory influence over the reinforcing effects of cocaine, and that this modulatory role can be targeted via pharmacological manipulations.

Despite these promising results, clinical trials evaluating SERT inhibitors as treatments for cocaine dependence have been largely unsuccessful (Grabowski et al 1995; Batki et al 1996; Ciraulo et al 2005; Winhusen et al 2005; but see Moeller et al 2007). Although the cause for the apparent discrepancy between preclinical findings and clinical results of SERT inhibitors is unknown, several possible explanations exist. For example, the indirect activation of all 5-HT receptor subtypes via SERT inhibition may produce a diverse spectrum of undesirable behavioral and physiological effects which could contribute to low compliance rates in humans. There is some preclinical evidence in support of this argument, as some studies have found that administration of SERT inhibitors reduced responding maintained by nondrug reinforcers (e.g. food) at doses equal to or lower than doses required to reduce cocaine self-administration in rats (Carroll et al 1990a) and nonhuman primates (Kleven and Woolverton 1993; Negus et al 2007). Alternatively, if the beneficial effects of indirect 5-HT agonists are mediated through specific receptor subtypes, it is plausible that such effects could be attenuated by the activation of other 5-HT receptors, given the evidence that some 5-HT receptors functionally oppose one another, particularly with regard to modulation of DA neurotransmission (Alex and Pehek 2007; Fink and Gothert 2007; Navailles and De Deurwaerdere 2011). Whatever the cause, the clinical failure of indirect 5-HT agonists should not cause abandonment of the 5-HT system as a target for the treatment of cocaine abuse. Rather, researchers have more recently pursued the intriguing possibility of identifying specific 5-HT receptor subtypes as novel pharmacotherapeutic targets that may selectively and specifically recapitulate the beneficial behavioral profile of SERT inhibitors.

There now exists substantial evidence to suggest that pharmacological manipulations of the 5-HT_{2C}R selectively modulate the reinforcing effects of cocaine in rodents. Pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 attenuated cocaine self-administration maintained by a fixed-ratio or progressive-ratio schedule in rats (Grottick et al 2000; Fletcher et al 2004; Fletcher et al 2008). In contrast, pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced cocaine self-administration under a progressive-ratio schedule (Fletcher et al 2002), a finding that is in agreement with the behavioral profile of genetic mutant mice lacking the 5-HT_{2C}R which responded approximately twice as much for cocaine infusions on a progressive-ratio schedule compared to their wildtype counterparts (Rocha et al 2002). Taken together, these results suggest that activation of the 5-HT_{2C}R functionally attenuates the reinforcing effects of cocaine, an effect that would be predicted based on reports that 5-HT_{2C}R agonists also attenuate the hyperlocomotive, neurochemical, and discriminative-stimulus effects of cocaine (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008).

The effects of 5-HT_{2C}R-selective compounds have also been investigated using the reinstatement procedure, a behavioral assay that has been described as a preclinical model of relapse to drug use. In the reinstatement procedure, previously-extinguished cocaine-maintained behavior is “reinstated” by exposing the subject to one of three stimuli: a drug-associated cue, a stressor, or a “priming” infusion of the drug that is self-administered (de Wit & Stewart 1981; Spealman et al 1999; Shaham et al 2003; Epstein et al 2006). It has been argued that drugs which reduce the reinstatement of responding elicited by any or all of these stimuli may serve to promote abstinence in human drug abusers. In accordance with their effects on the reinforcing effects of cocaine, pretreatment with selective 5-HT_{2C}R agonists has been shown to attenuate cue-induced, stress-induced, and cocaine-induced reinstatement of extinguished cocaine-maintained responding in rats (Grottick et al 2000; Neisewander and Acosta 2007;

Burbassi and Cervo 2008; Fletcher et al 2008; Pentkowski et al 2010), whereas pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced cocaine-induced reinstatement (Fletcher et al 2002). The modulatory role for 5-HT_2 receptors does not lie solely with the 5-HT_{2C}R subtype, as pretreatment with the selective 5-HT_{2A}R antagonist M100907 attenuated both cocaine-induced and cue-induced reinstatement in rats (Fletcher et al 2002; Nic Dhonnchadha et al 2009; Pockros et al 2011). Therefore, the 5-HT_{2A}R and 5-HT_{2C}R exert opposing influences on behavior in rodent models of relapse.

Although previous studies have investigated the impact of 5-HT_2 receptors upon cocaine self-administration in nonhuman primates, these studies were limited by the nonselective pharmacological tools available. For example, pretreatment with the nonselective 5-$HT_{2A/2C}$R antagonist ritanserin enhanced the reinforcing effects of cocaine (Howell and Byrd 1995) and the selective DAT inhibitor GBR 12909 (Howell et al 1997) in squirrel monkeys, but the specific receptor subtypes mediating these effects were not able to be determined. The potential of serotonergic compounds to modulate reinstatement in nonhuman primates has received even less attention. In one recent study, pretreatment with the SERT inhibitors fluoxetine and citalopram attenuated cocaine-induced reinstatement in squirrel monkeys trained to self-administer cocaine according to a second-order operant schedule (Ruedi-Bettschen et al 2010), but the specific receptors mediating this effect were not investigated. Indeed, the effects of 5-HT_2R subtype-selective compounds have not been systematically evaluated in nonhuman primate reinstatement procedures to date.

Therefore, to determine the modulatory role of specific 5-HT_2R subtypes on the reinforcing and reinstatement effects of cocaine, we designed several experimental aims. First, we assessed levels of cocaine-induced reinstatement following pretreatment with a preferential

or selective 5-HT_{2C}R agonist (m-chlorophenylpiperazine and Ro 60-0175, respectively), a 5-HT_{2C}R antagonist (SB 242084), or a 5-HT_{2A}R antagonist (M100907), in squirrel monkeys trained to self-administer cocaine according to a second-order operant schedule of behavior. To discern whether any observed effects could be attributed to nonspecific disruptions in operant performance, effective drug pretreatments were administered to a separate group of squirrel monkeys responding under an identical second-order operant schedule where the maintaining event was a nondrug reinforcer. Second, the impact of pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 upon the direct reinforcing effects of cocaine in monkeys was examined. The capacity of the 5-HT_{2A}R antagonist M100907 to attenuate the reinforcing effects of cocaine was not investigated in the present study as such compounds have failed to alter ongoing cocaine self-administration in rodents (Fletcher et al 2002; Nic Dhonnchadha et al 2009). Finally, given previous results obtained by us and others indicating that the selective 5-HT_{2C}R antagonist SB 242084 exhibits a behavioral profile similar to other psychostimulants, we investigated whether this compound could function as a reinforcer in squirrel monkeys with a recent history of cocaine self-administration. Together, these results are the first to investigate the modulatory role of the 5-HT_{2A}R and 5-HT_{2C}R subtypes upon the reinforcing and reinstatement effects of cocaine in nonhuman primates.

B. Methods

1. Subjects

Eleven adult male squirrel monkeys (*Saimiri sciureus*) weighing 800 – 1350g served as subjects. Between experimental sessions, animals were individually housed in a climate-controlled room and fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal) with ad libitum access to water.

Daily enrichment was provided via access to foraging devices, toys, and climbing/swing devices. Each animal had served in previous behavioral studies involving administration of compounds acting upon monoaminergic, and/or glutamatergic systems (Bauzo et al 2009). All studies were conducted in strict accordance with the National Institutes of Health's "Guide for Care and Use of Laboratory Animals", the American Association for Accreditation of Laboratory Animal Care (AAALAC), and were approved by the Institutional Animal Care and Use Committee of Emory University.

2. Apparatus

During behavioral sessions, animals were comfortably seated in a commercially-available plexiglass chair within a ventilated, sound-attenuating chamber (Med Associates Inc., St. Albans, VT). The chair was equipped with an operant panel consisting of a series of red and white lights, a lever, and a whitenoise amplifier which was activated throughout the duration of all behavioral sessions to further reduce the influence of ambient noise. Med-PC IV software (Med Associates Inc., St. Albans, VT) was interfaced with each chamber to allow for automated output control and lever-press recording. For self-administration and reinstatement studies, a motor-driven syringe pump (Model PHD2000, Harvard Apparatus, Holliston, MA), mounted on the outer wall of the operant chamber, held a 35cc syringe containing appropriate concentrations of drugs made available for intravenous infusion. Each syringe was fitted with a 0.2 µm-pore polysulfone filter and connected via stainless-steel adaptors and polyvinyl chloride tubing to the external portion of the subject's catheter prior to session onset.

3. Surgeries

For self-administration and reinstatement experiments, subjects were prepared with chronic indwelling venous catheters under aseptic conditions. Animals were initially

anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0mg) and ketamine HCl (20mg). Anesthesia was maintained throughout the procedure with inhaled isoflurane (0.5-1.5%). A polyvinyl chloride catheter (diameter: 0.025" inner, 0.035" outer) was inserted into either the left or right femoral vein or external jugular vein and allowed to rest near the right atrium. The distal end of the catheter was routed subcutaneously and exited at the interscapular region of the animal's back. A custom-made nylon mesh jacket (Lomir Biomedical Inc., Malone, NY) protected the external portion of the catheter. Animals were allowed to recover for 5-7 days before resuming operant-behavioral sessions. When not in use, catheters were filled with heparinized saline and locked using 25-guage stainless-steel obturators. To maintain patency, catheters were flushed several days per week with 0.2 ml saline. If a catheter became occluded or damaged during the course of the study, it was promptly removed and a new catheter was implanted into the same vessel when possible, or another vessel. For all surgical procedures, preoperative antibiotics (ceftriaxone) and postoperative analgesics (meloxicam or flunixin) were administered by veterinary staff who closely monitored the animals.

4. Cocaine Self-Administration, Reinstatement, and Substitution

a. Second-Order Schedule of Cocaine Self-Administration

Daily sessions were conducted 5-7 days per week and lasted approximately 60 min. Animals were allowed to intravenously self-administer cocaine according to a second-order operant schedule (Schindler et al 2002). Each session began with the illumination of a pair of red lights. During a 600-sec fixed-interval, a fixed-ratio 20 (FR20) operant schedule was superimposed such that every twentieth lever-press extinguished the red lights and briefly illuminated a white light for 2-sec, followed immediately by reillumination of the red lights. Responding during the 2-sec white light was recorded but did not contribute to the subsequent

ratio requirement. Once the 600-sec fixed-interval elapsed, the schedule progressed into a 200-sec limited hold. The first completed fixed-ratio within the limited hold extinguished the red lights and resulted in an intravenous bolus infusion of cocaine (veh, 0.01-0.3 mg/kg/inf in 0.5 ml; 25 ml/min flow rate). The cocaine infusion was paired with a 15-sec white light, followed by a 60-sec timeout during which all lights were extinguished and responses were recorded but had no programmed consequences. If the animal failed to complete an FR20 during the limited hold, the red lights were extinguished and the schedule moved directly into the timeout. Each daily session consisted of five fixed-interval components. Response rates were calculated for each individual component and then averaged across the session. Responding was deemed stable when response rates for each session varied < 20% across 3 consecutive days. Once responding was stable, the unit dose of cocaine was altered and behavior was allowed to stabilize until the maximally-effective unit dose of cocaine (ED_{max}, i.e. the unit dose of cocaine that maintained highest rates of responding) was identified for each individual subject. The ED_{Max} for most subjects was 0.1 mg/kg/inf, but ranged from 0.03-0.3 mg/kg/infusion across all subjects.

To assess the effects of 5-HT_{2C}R agonism upon the reinforcing effects of cocaine, Ro 60-0175 (veh, 0.1-0.3 mg/kg) was administered 15-min prior to the onset of cocaine self-administration sessions. The effect of all doses of Ro 60-0175 was tested in combination with three unit doses of cocaine (ED_{Max}, and one-half log-step unit doses above and below the ED_{Max} unit dose). Dose-combinations were tested for three consecutive sessions (Tuesday, Wednesday, Thursday). Subjects were allowed to self-administer cocaine without pharmacological pretreatment on other days of the week (Monday, Friday). Each dose of Ro 60-0175 was tested in combination with a unit dose of cocaine for three consecutive sessions for two reasons. First, we considered the possibility that although Ro 60-0175 may alter the reinforcing effects of cocaine, extinction of responding may not emerge within a single session.

In contrast, if Ro 60-0175 reduced response rates immediately within the first session, extending the duration of testing to three consecutive daily sessions could allow for the detection of rapid tolerance to the effects of Ro 60-0175. All doses of Ro 60-0175 were tested against a given unit dose of cocaine before switching the subject to a different cocaine unit dose. For each subject, the dose-order of cocaine self-administration and Ro 60-0175 pretreatment order was randomized. Pretreatment studies did not begin until cocaine self-administration response rates had stabilized, varying < 20% across three consecutive sessions.

b. Cocaine-Induced Reinstatement

For reinstatement experiments, the ED_{max} unit dose for cocaine self-administration was assessed for each individual animal as described in "General Procedure". The reinstatement procedure used consisted of three phases. During "maintenance", animals were allowed to self-administer their respective ED_{max} of cocaine. Response rates were considered stable when responding varied by < 20% across three consecutive self-administration sessions. Once response rate was stable, subjects progressed to the "extinction" phase during which completed fixed-ratios within the 600-sec fixed-interval or the limited hold were recorded but did not produce conditioned reinforcement (i.e. the white light was withheld) and saline infusions were substituted for cocaine. Under extinction conditions, response rates for all subjects rapidly decreased across sessions. Responding was deemed extinguished when the overall response rate within a single session reached ≤ 20% of the mean response rate of the three maintenance sessions. "Reinstatement" tests occurred on the day immediately following successful extinction of responding. Five minutes prior to the onset of the session, animals were administered a noncontingent, intravenous bolus infusion ("prime") of cocaine (veh, 0.03 – 1.0 mg/kg). The white light was reintroduced as per a maintenance self-administration session, but importantly,

saline was still substituted for cocaine infusions throughout the duration of the session. Therefore, all responding during a reinstatement test was dependent upon the dose of the noncontingent prime and the reintroduction of conditioned reinforcement, but not by cocaine reinforcement during the session. For each subject, the dose of cocaine prime that induced maximal rates of responding was deemed the ED_{Peak}. The ED_{Peak} for each individual subject was typically one-half log-step above the ED_{Max} unit dose for maintenance cocaine self-administration sessions. Each pharmacological pretreatment was tested across a range of cocaine prime doses, relative to the ED_{Peak}, within each individual subject. For drug interaction studies, drugs were studied in sequence and pretreatments administered as follows: M100907 (0.03-0.3 mg/kg) 30-min prior to cocaine prime; mCPP (0.1-0.3 mg/kg) 15-min prior to cocaine prime; Ro 60-0175 (0.1-0.3 mg/kg) 15-min prior to cocaine prime; SB 242084 (0.03-0.1 mg/kg) 30-min prior to cocaine prime. Within each drug-interaction experiment, reinstatement tests for each drug dose were separated by the reestablishment of maintenance cocaine self-administration and subsequent extinction. The dose order of drug combinations for reinstatement tests was randomized within subjects.

c. SB 242084 Substitution

In the first substitution experiment, intravenous SB 242084 (veh, 0.01 – 0.1 mg/kg/infusion) was substituted for the availability of intravenous cocaine infusions. The unit dose of SB 242084 was held constant across sessions until response rates stabilized as described above. To prevent long-term disruptions in operant behavioral output, responding was considered extinguished when response rates reached ≤ 20% baseline cocaine self-administration for two consecutive sessions. Animals were allowed to restabilize on ED_{max}

cocaine self-administration between SB 242084 substitution tests. The dose order of SB 242084 substitution was randomized within subjects.

In the second substitution experiment, the reinforcing effects of SB 242084 were assessed when the drug was made available immediately following exposure to saline-extinction sessions. Subjects were first allowed to self-administer their respective ED_{Max} cocaine unit dose until responding had stabilized (< 20% variability) for three consecutive sessions. Responding was then extinguished as described earlier (section 4b). To prevent long-term disruptions in operant behavioral output, responding was considered extinguished when response rates reached ≤ 20% baseline cocaine self-administration for two consecutive sessions. Once extinction criteria were satisfied, parameters for the impending session were restored to that of a normal maintenance session with 0.03 mg/kg/infusion SB 242084 available for self-administration. Daily sessions continued until responding stabilized (varied < 20% across three consecutive sessions). The dose of SB 242084 used was chosen based on results from the prior substitution experiment.

5. Second-Order Schedule of Stimulus Termination

Daily sessions were conducted 5 days per week and lasted approximately 60 min. Each session began with the illumination of a pair of red lights. During a 600-sec fixed-interval, a fixed-ratio 20 operant schedule was superimposed such that every twentieth lever-press extinguished the red lights and briefly illuminated a white light for 2-sec, followed immediately by reillumination of the red lights. Responding during the 2-sec white light was recorded but did not contribute to the subsequent ratio requirement. Once the 600-sec fixed-interval elapsed, the schedule progressed into a 20-sec limited hold. A completed fixed-ratio 20 during the limited hold extinguished the red lights and illuminated a white light for 15-sec to signal

reinforcement. If the animal failed to complete the fixed-ratio during the limited hold, a mild electrical stimulus (300ms, 3-6mA) was delivered to a shaved portion of the distal end of the tail that was secured within an acrylic tail yoke. Each daily session consisted of 5 consecutive components separated by 60-sec timeout periods during which all lights were extinguished. Response rates were recorded for each individual component and then averaged across the session.

The effects of pharmacological pretreatments were assessed in order as follows: mCPP (veh, 0.1-0.3 mg/kg) 15-min prior to session onset; Ro 60-0175 (veh, 0.1-0.3 mg/kg) 15-min prior to session onset; SB 242084 (0.01-0.1 mg/kg) 30-min prior to session onset. The order of dose administration for each drug was randomized within subjects.

6. Drugs

Cocaine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), m-chlorophenylpiperazine (mCPP) (Sigma-Aldrich, St. Louis, MO), and Ro 60-0175 (Tocris Bioscience, Ellisville, MO) were dissolved in 0.9% saline. SB 24204 (Tocris Bioscience, Ellisville, MO) was initially dissolved at a concentration of 1.0 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO) , and 0.9% saline, and further diluted to appropriate concentrations using 0.9% saline. M100907, generously provided by Kenner C. Rice, Ph.D. of the Laboratory of Medicinal Chemistry at the NIH/NIDDK, was initially dissolved in sterile water containing a minute amount of 1.0N hydrochloric acid. The solution was then titrated to a pH within the range of 5.0-7.0 and brought to volume with sterile water. All drug solutions were passed through a 0.2 µm-pore polysulfone filter prior to use. Doses were calculated from the salt weights. Unless otherwise specified, all drugs were administered via the intramuscular route into the thigh muscle.

7. Data Analysis

For self-administration and reinstatement experiments, response rates were normalized to the percent of responding maintained during maintenance cocaine self-administration sessions when the ED_{Max} unit cocaine dose was available. For second-order stimulus termination experiments, response rates following pharmacological pretreatments were normalized to the percent of responding following vehicle drug pretreatment. Data were analyzed using repeated-measures ANOVAs with post hoc Tukey's tests or Dunnett's tests, or paired t-tests, as specified. Data were graphically plotted using GraphPad v. 5.01 (GraphPad Software Inc., La Jolla, CA) and analyzed using SigmaStat v. 3.0 software (Systat Software Inc., San Jose, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

C. Results

1. Cocaine-Induced Reinstatement

a. 5-HT_{2C}R Agonists: mCPP and Ro 60-0175

The effects of pretreatment with the preferential 5-HT_{2C}R agonist mCPP on cocaine-induced reinstatement are shown in Figure 9. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.35 ± 0.28 responses/s. When pretreated with mCPP vehicle, cocaine-induced reinstatement across a range of cocaine doses produced an inverted U-shaped dose-response function, with the maximally-effective priming dose of cocaine (ED_{Peak}) producing responding that was ~109% of the response rate maintained during maintenance cocaine self-administration. Pretreatment with mCPP induced a dose-dependent downward shift in the cocaine dose-response function. Two-way repeated-measures ANOVA indicated significant main effects of cocaine dose ($F_{(3,6)} = 5.30$, $p = 0.04$) and mCPP dose

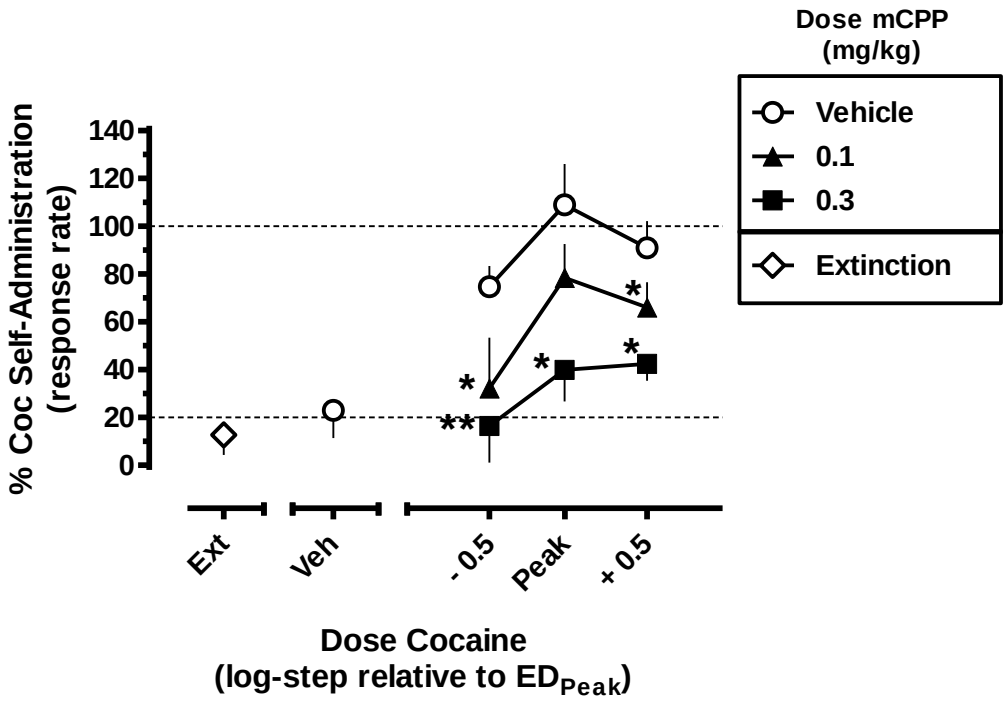

Figure 9. Effects of pretreatment with mCPP (veh, 0.1-0.3 mg/kg) on cocaine-induced reinstatement in squirrel monkeys (n=3). Following stable cocaine self-administration behavior, responding was extinguished (open diamond). Presession priming with cocaine induced a typical inverted U-shaped dose response curve (open circles). mCPP dose-dependently caused a downward shift of the cocaine dose-response function (filled symbols). Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted lines represent baseline self-administration rate (100%) and extinction criterion (20%). Asterisks indicate significant difference (* $p < 0.05$, ** $p < 0.01$) for a given data point compared to "Vehicle" mCPP treatment prior to the same dose of cocaine prime. *Abscissa*: dose of cocaine prime. *Ordinate*: normalized response rate.

($F_{(2,4)}$ = 21.89, p = 0.007). Subsequent one-way repeated-measures ANOVAs with post hoc Tukey's tests revealed that 0.1 and 0.3 mg/kg mCPP attenuated the reinstatement effects induced by both the lower dose of cocaine ($F_{(2,4)}$ = 19.09, p = 0.009) and the higher dose of cocaine ($F_{(2,4)}$ = 78.57, p < 0.001), whereas the reinstatement effect of the ED_{Peak} dose of cocaine was significantly attenuated only following pretreatment with 0.3 mg/kg mCPP ($F_{(2,4)}$ = 9.97, p = 0.028). Indeed, pretreatment with 0.3 mg/kg mCPP reduced cocaine-induced reinstatement across all cocaine doses to a magnitude similar to that observed when subjects were pretreated with mCPP vehicle and administered a pre-session vehicle cocaine (saline) prime, suggesting an almost-complete blockade of cocaine-induced reinstatement.

To confirm that the observed effects of mCPP were mediated via actions at the 5-HT_{2C}R, we redetermined the effect of 0.3 mg/kg mCPP on cocaine-induced reinstatement following pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 (Fig. 10). One-way repeated-measures ANOVA indicated a significant effect of treatment condition ($F_{(4,8)}$ = 8.53, p = 0.006). Post hoc Dunnett's tests revealed that 0.3 mg/kg mCPP significantly attenuated the reinstatement effects of ED_{Peak} cocaine to ~40% of the cocaine self-administration response rate (p < 0.05). Administration of 0.03 mg/kg SB 242084 did not appreciably induce reinstatement alone (30%). However, this same dose of SB 2424084 given prior to mCPP resulted in a magnitude of cocaine-induced reinstatement that was not significantly different from cocaine alone (~84% compared to ~108%). This result indicates that pretreatment with SB 242084 was sufficient to prevent the effects of mCPP and suggests that the observed effects of mCPP were indeed mediated through actions at the 5-HT_{2C}R.

The effects of pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 on cocaine-induced reinstatement are shown in Figure 11. The mean response rate (± SEM) during

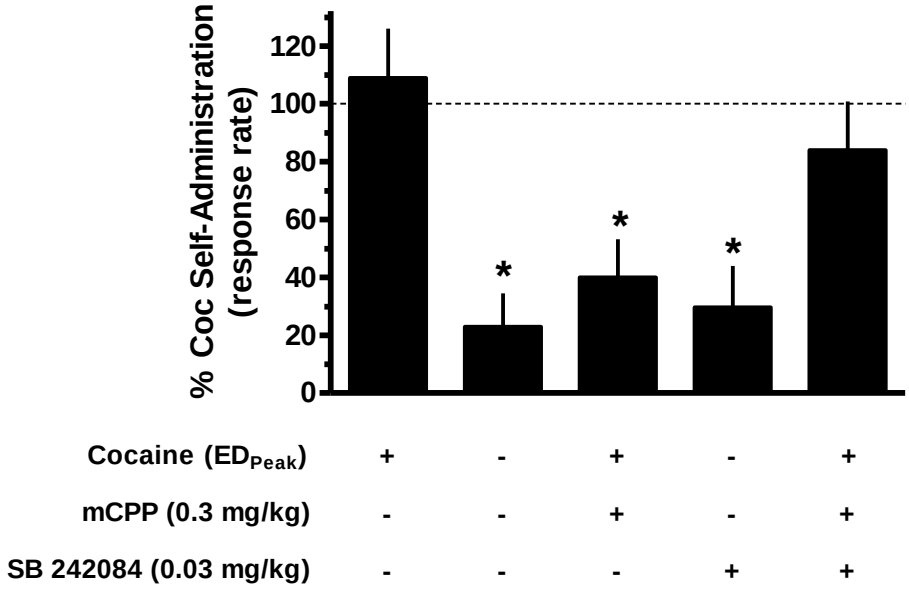

Figure 10. The effects of mCPP alone or following pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 on cocaine-induced reinstatement in squirrel monkeys (n=3). Administration of 0.3 mg/kg mCPP attenuated the reinstatement effect induced by priming with the ED_{Peak} dose of cocaine. Pretreatment with 0.03 mg/kg SB 242084 did not induce reinstatement alone, but prevented the effects of mCPP. Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted line represents baseline self-administration rate (100%). Asterisks indicate significant difference (* $p < 0.05$) for a given data point compared to the reinstatement effect induced by ED_{Peak} cocaine prime alone. *Abscissa*: drug-interaction treatment conditions. *Ordinate*: normalized response rate.

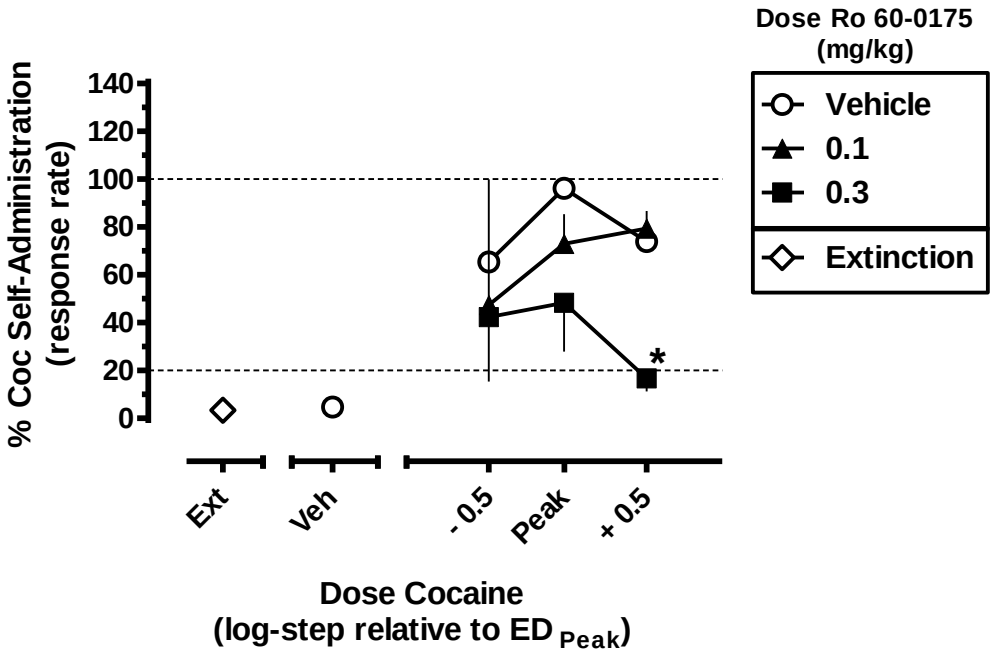

Figure 11. Effects of pretreatment with Ro 60-0175 (veh, 0.1-0.3 mg/kg) on cocaine-induced reinstatement in squirrel monkeys (n=3). Following stable cocaine self-administration behavior, responding was extinguished (open diamond). Presession priming with cocaine induced a typical inverted U-shaped dose response curve (open circles). Ro 60-0175 dose-dependently caused a downward shift of the cocaine dose-response function (filled symbols). Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted lines represent baseline self-administration rate (100%) and extinction criterion (20%). Asterisks indicate significant difference (* $p < 0.05$) for a given data point compared to "Vehicle" Ro 60-0175 treatment prior to the same dose of cocaine prime. *Abscissa*: dose of cocaine prime. *Ordinate*: normalized response rate.

maintenance ED_{Max} cocaine self-administration sessions was 1.14 ± 0.16 responses/s. As was observed in the previous experiment, cocaine-induced reinstatement across a range of cocaine doses resulted in an inverted U-shaped dose-response function following vehicle Ro 60-0175 pretreatment. The maximally-effective priming dose of cocaine (ED_{Peak}) produced responding that was ~96% of the response rate maintained during maintenance cocaine self-administration. Similar to the effects of mCPP, pretreatment with Ro 60-0175 induced a dose-dependent downward shift in the cocaine dose-response function. Two-way repeated-measures ANOVA indicated a significant main effect of Ro 60-0175 dose ($F_{(2,4)} = 59.04$, $p = 0.001$) but not for cocaine dose ($F_{(3,6)} = 2.76$, $p = 0.13$). Subsequent one-way repeated-measures ANOVAs with post hoc Tukey's tests revealed that 0.3 mg/kg Ro 60-0175 attenuated the reinstatement effects induced by the highest dose of cocaine ($F_{(2,4)} = 43.90$, $p = 0.002$), whereas the reinstatement effects engendered by the low dose of cocaine were not affected by either dose of Ro 60-0175 ($F_{(2,4)} = 1.99$, $p = 0.25$). The analysis within the ED_{Peak} dose of cocaine nearly missed significance ($F_{(2,4)} = 3.56$, $p = 0.13$), although it should be noted that ED_{Peak} cocaine-induced reinstatement was approximately halved by pretreatment with 0.3 mg/kg Ro 60-0175 (~96% vs. ~48%, respectively).

We next redetermined the effect of 0.3 mg/kg Ro 60-0175 on cocaine-induced reinstatement following pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 (Fig. 12). One-way repeated-measures ANOVA indicated a significant effect of treatment condition ($F_{(4,8)} = 21.35$, $p < 0.001$). Post hoc Dunnett's tests further revealed that 0.3 mg/kg Ro 60-0175 significantly attenuated the reinstatement effects of ED_{Peak} cocaine ($p < 0.05$). Additionally, and in accordance with the previous mCPP experiment, administration of 0.03 mg/kg SB 242084 did not induce any measurable reinstatement alone (~3%), but prevented the effects of Ro

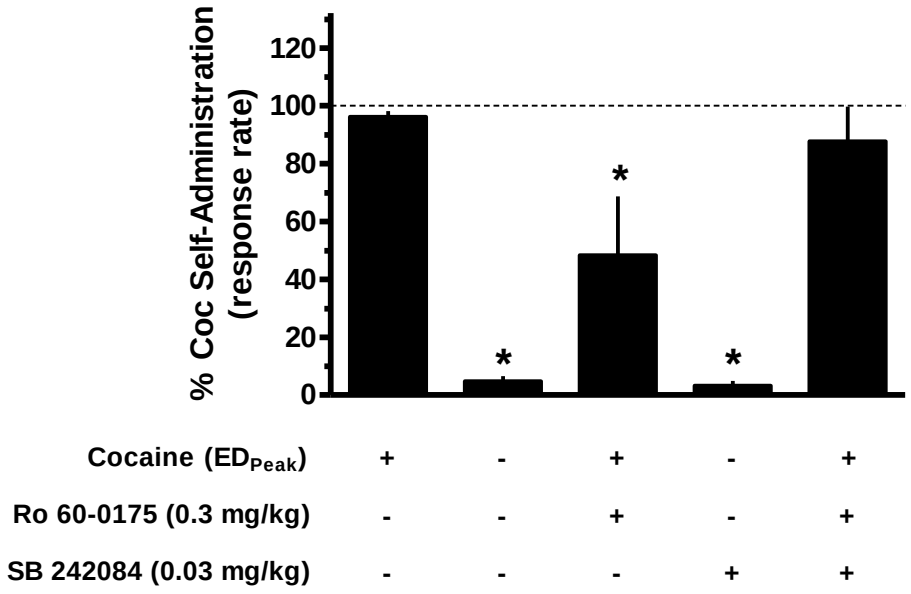

Figure 12. The effects of Ro 60-0175 alone or following pretreatment with the selective 5-HT$_{2C}$R antagonist SB 242084 on cocaine-induced reinstatement in squirrel monkeys (n=3). Administration of 0.3 mg/kg Ro 60-0175 attenuated the reinstatement effect induced by priming with the ED$_{Peak}$ dose of cocaine. Pretreatment with 0.03 mg/kg SB 242084 did not induce reinstatement alone, but prevented the effects of Ro 60-0175. Data (mean ± SEM) are expressed as the percent of responding maintained during ED$_{Max}$ cocaine self-administration sessions. The dotted line represents baseline self-administration rate (100%). Asterisks indicate significant difference (* $p < 0.05$) for a given data point compared to the reinstatement effect induced by ED$_{Peak}$ cocaine prime alone. *Abscissa*: drug-interaction treatment conditions. *Ordinate*: normalized response rate.

60-0175 upon cocaine-induced reinstatement, rescuing response rates to levels (~88%) that were not significantly different from the effect of cocaine alone ($p > 0.05$).

b. 5-HT_{2C}R Antagonist: SB 242084

Based upon the observation that pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced the behavioral-stimulant effects of cocaine (Chapter II), we hypothesized that SB 242084 would similarly enhance the reinstatement effects induced by a presession cocaine prime in the present experiment. To better evaluate this prediction, we shifted the dose range for the cocaine prime one-half log-step lower to examine the full ascending limb of the dose-response function. This allowed us to study the interaction between SB 242084 and a dose of cocaine that, when administered alone, did not appreciably induce reinstatement. The effects of pretreatment with SB 242084 on cocaine-induced reinstatement are shown in Figure 13. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.70 ± 0.28 responses/s. Following vehicle SB 242084 pretreatment, priming with the ED_{Peak} dose of cocaine reinstated responding to ~102% relative to response rates during maintenance self-administration sessions. Lowering the dose of the cocaine prime by a full log-step in each subject resulted in a dramatic reduction of the reinstatement effect (~23%). Pretreatment with SB 242084 resulted in an apparent upward-shift of the ascending limb of the cocaine dose-response curve. Two-way repeated-measures ANOVA indicated significant main effects for cocaine dose ($F_{(3,6)} = 13.44$, $p = 0.005$) and SB 242084 dose ($F_{(2,4)} = 15.85$, $p = 0.013$) but not a significant interaction ($F_{(6,12)} = 0.51$, $p = 0.79$). Subsequent one-way repeated-measures ANOVAs within each cocaine dose failed to detect significant differences between doses of SB 242084. However, it is critical to note that the data clearly suggest that pretreatment with SB 242084 potentiated the effects of cocaine. For example, pretreatment with 0.1 mg/kg SB 242084 prior

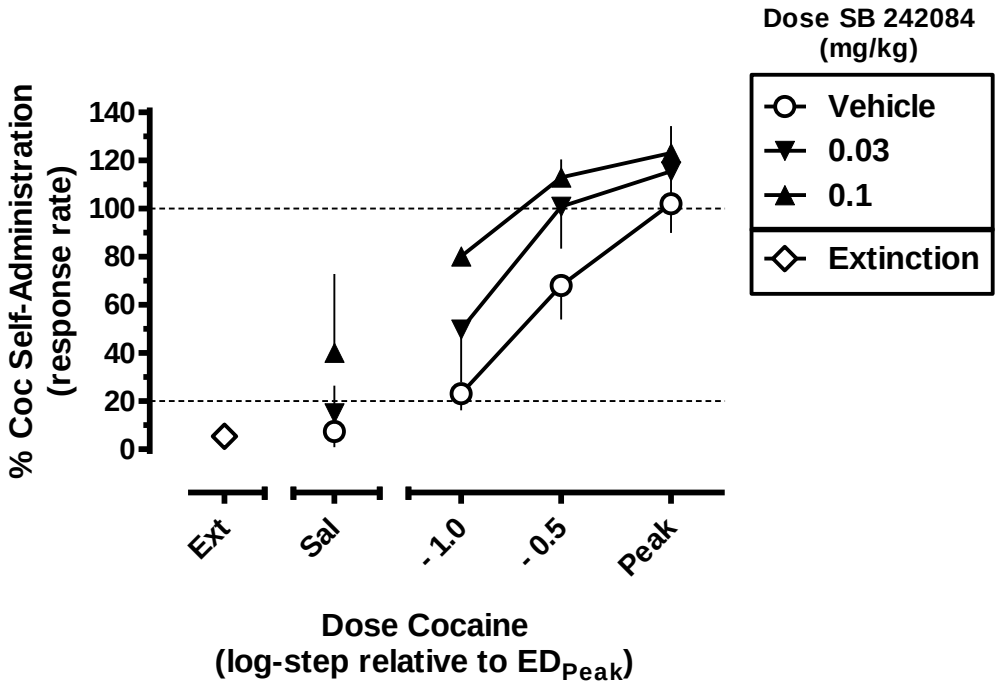

Figure 13. Effects of pretreatment with SB 242084 (veh, 0.03-0.1 mg/kg) on cocaine-induced reinstatement in squirrel monkeys (n=3). Following stable cocaine self-administration behavior, responding was extinguished (open diamond). Presession priming with cocaine reinstated responding in a dose-dependent manner (open circles). Although statistical analyses failed to detect significant effects, SB 242084 appears to have caused a dose-dependent upward shift of the ascending limb of the cocaine dose-response function (filled symbols). Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted lines represent baseline self-administration rate (100%) and extinction criterion (20%). *Abscissa*: dose of cocaine prime. *Ordinate*: normalized response rate.

to the lowest dose of cocaine reinstated responding nearly four times greater than the same dose of cocaine alone (~80% vs. ~23%, respectively). Additionally, the combination of either 0.03 or 0.1 mg/kg SB 242084 with the intermediate dose of cocaine reinstated responding at or above the 100% value, indicative of a full reinstatement effect. It is likely that the failure of the statistical analyses to detect significant differences is due to several factors, including a small sample size (n=3) and an apparent ceiling effect with respect to response rates as the reinstatement effect seems to plateau at approximately 120%. Importantly, our analysis revealed that no doses of SB 242084 tested induced reinstatement alone ($F_{(2,4)} = 1.51$, $p = 0.32$). The large degree of variability following the 0.3 mg/kg SB 242084 dose prior to saline prime occurred because this pretreatment dose induced full reinstatement (~106%) in one subject, but was ineffective in the other two subjects (~5% and ~10%). This effect was dose-dependent as reducing the dose of SB 242084 to 0.03 mg/kg reduced the reinstatement effect within this single subject to ~38%. It is possible that increasing the dose of SB 242084 to 0.3 mg/kg might have induced reinstatement in the other two subjects. However, the administration of this dose of SB 242084 to one subject in a pilot study induced adverse physiological effects, including emesis and prolonged whole-body scratching, which would likely confound the dependent measure of response rate as these adverse effects might disrupt lever-pressing. We therefore did not systematically test doses of SB 242084 above 0.1 mg/kg.

c. 5-HT_{2A}R Antagonist: M100907

The effects of pretreatment with the 5-HT_{2A}R antagonist M100907 on cocaine-induced reinstatement are shown in Figure 14. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.29 ± 0.12 responses/s. Because M100907 failed to alter the behavioral-stimulant effects of cocaine (Chapter II), the effects of M100907 in the

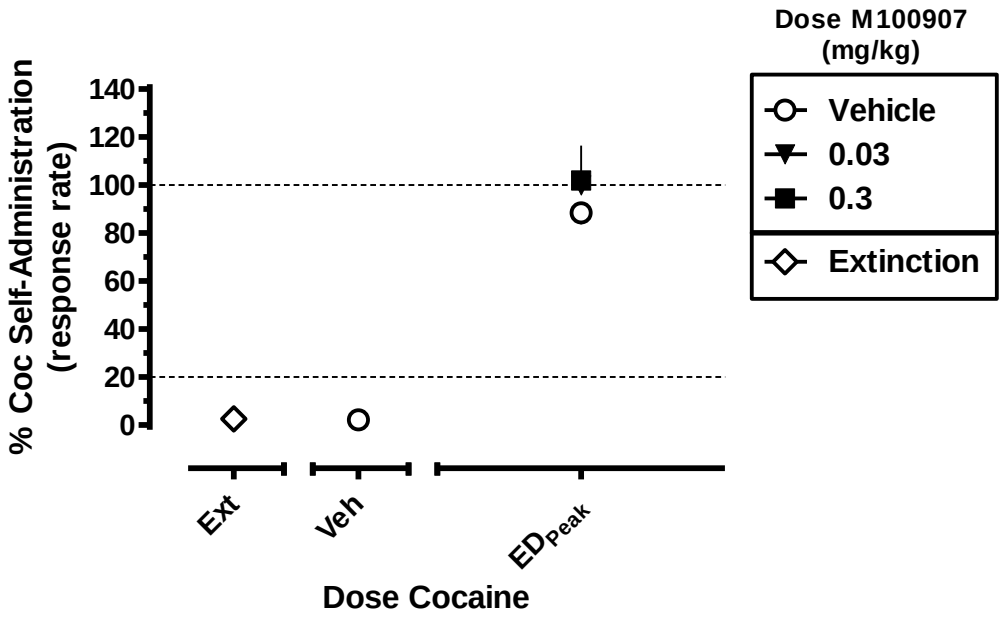

Figure 14. Effects of pretreatment with M100907 (veh, 0.03-0.3 mg/kg) on cocaine-induced reinstatement in squirrel monkeys (n=3). Following stable cocaine self-administration behavior, responding was extinguished (open diamond). Presession priming with ED_{Peak} cocaine reinstated responding to levels near those maintained by cocaine self-administration (open circle, "ED_{Peak}"). M100907 did not alter the reinstatement effect of cocaine (filled symbols). Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. The dotted lines represent baseline self-administration rate (100%) and extinction criterion (20%). *Abscissa*: dose of cocaine prime. *Ordinate*: normalized response rate.

present experiment were initially assessed only prior to the ED_{Peak} cocaine priming dose because this allowed for the maximal opportunity to observe a rate-reducing effect, as was predicted for M100907 pretreatment. Similar to the previous experiments, priming with ED_{Peak} cocaine following vehicle pretreatment resulted in reinstatement of lever-pressing that was near the level of responding during maintenance cocaine self-administration sessions (~89%). However, one-way repeated-measures ANOVA indicated that M100907 did not significantly alter reinstatement induced by the ED_{Peak} cocaine prime ($F_{(2,4)} = 0.49$, $p = 0.65$). Although this was an unexpected result, it was in agreement with the prior finding that M100907 failed to modulate the behavioral-stimulant effects of cocaine (Chapter II). Because we failed to detect an effect of M100907 pretreatment in combination with the ED_{Peak} dose of cocaine, we thought it unlikely that M100907 would modulate the reinstatement induced by other doses of cocaine and therefore terminated the experiment in an effort to conserve and better utilize experimental resources.

2. Cocaine Self-Administration

To determine if 5-HT_{2C}R activation alters the direct reinforcing effects of cocaine, subjects were pretreated with the selective 5-HT_{2C}R agonist Ro 60-0175 prior to maintenance cocaine self-administration sessions (Fig. 15). The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.56 ± 0.41 responses/s. Varying the unit dose of cocaine produced a typical inverted U-shaped dose-response function. Similar to its effects on the behavioral-stimulant and reinstatement effects of cocaine, pretreatment with Ro 60-0175 caused a dose-dependent downward shift of the dose-response function, indicating an attenuation of the direct reinforcing effects of cocaine. Two-way repeated-measures ANOVA indicated significant main effects of cocaine dose ($F_{(2,4)} = 9.21$, $p = 0.032$) and

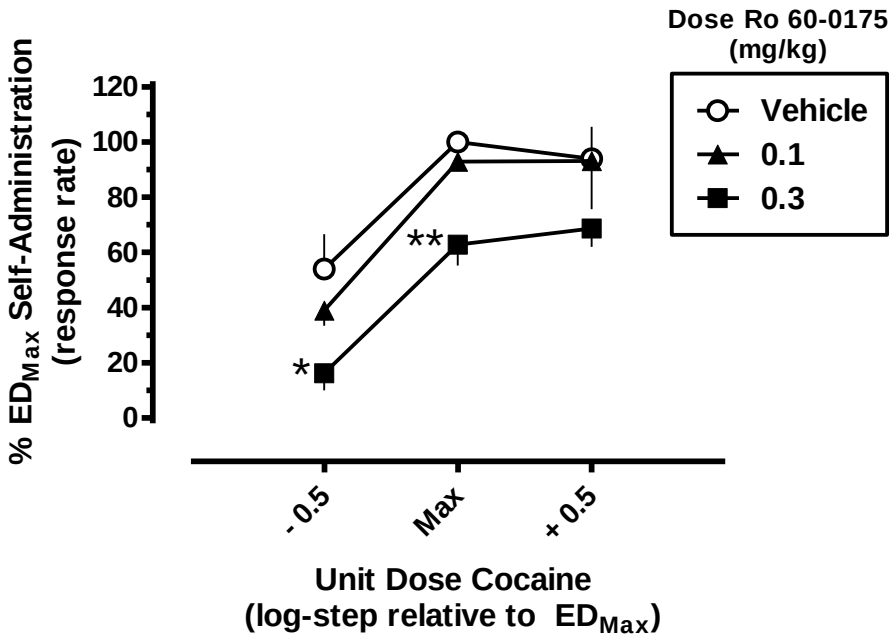

Figure 15. Effects of pretreatment with Ro 60-0175 (veh, 0.1-0.3 mg/kg) on cocaine self-administration in squirrel monkeys (n=3). The ED_{Max} unit dose of cocaine was determined for each subject, identified as the dose which maintained maximal response rates. Varying the unit dose of cocaine available for self-administration produced a typical inverted U-shaped dose-response function (open circles). Pretreatment with Ro 60-0175 caused a dose-dependent downward shift of the cocaine dose-response curve (filled symbols). Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions following "Vehicle" Ro 60-0175 pretreatment. Asterisks indicate significant difference (* $p < 0.05$, ** $p < 0.01$) for a given data point compared to "Vehicle" Ro 60-0175 treatment prior to the same unit dose of cocaine. *Abscissa*: unit dose of cocaine available for self-administration. *Ordinate*: normalized response rate.

Ro 60-0175 dose ($F_{(2,4)}$ = 24.79, p = 0.006) but not a significant interaction ($F_{(4,8)}$ = 1.14, p = 0.41). Subsequent one-way repeated-measures ANOVAs with post hoc analyses indicated that 0.3 mg/kg Ro 60-0175 significantly attenuated response rates maintained by the ED_{Max} cocaine dose ($F_{(2,4)}$ = 27.00, p = 0.005; Tukey's test, p = 0.005 compared to vehicle) and the lower dose of cocaine ($F_{(2,4)}$ = 14.89, p = 0.014; Tukey's test, p = 0.012 compared to vehicle). Specifically, response rates maintained by the ED_{Max} cocaine unit dose were reduced to ~63% compared to baseline, while response rates maintained by the lower dose of cocaine were reduced to levels approximating the extinction criterion of 20%. It should be noted that the effect of Ro 60-0175 pretreatment upon self-administration rates at the higher dose of cocaine just barely failed to meet significance ($F_{(2,4)}$ = 6.473, p = 0.056). The effects of Ro 60-0175 pretreatments emerged on the first day of administration and there was no evidence of tolerance across three consecutive test sessions (data not shown). Additionally, on the cocaine self-administration day immediately following the last pretreatment of each week, response rates typically recovered to near the 100% baseline.

3. SB 242084 Substitution

In the present series of experiments, our results demonstrate that the selective 5-HT_{2C}R antagonist SB 242084 exhibits a behavioral profile indicative of psychostimulant-like effects. Because psychostimulants such as cocaine and amphetamine typically function as robust reinforcers in animals, we first sought to determine whether SB 242084 would function as a reinforcer when substituted for cocaine availability in subjects actively self-administering cocaine. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.59 ± 0.73 responses/s. The effects of SB 242084 substitution upon cocaine-maintained self-administration responding are shown in Figure 16a. When the SB 242084

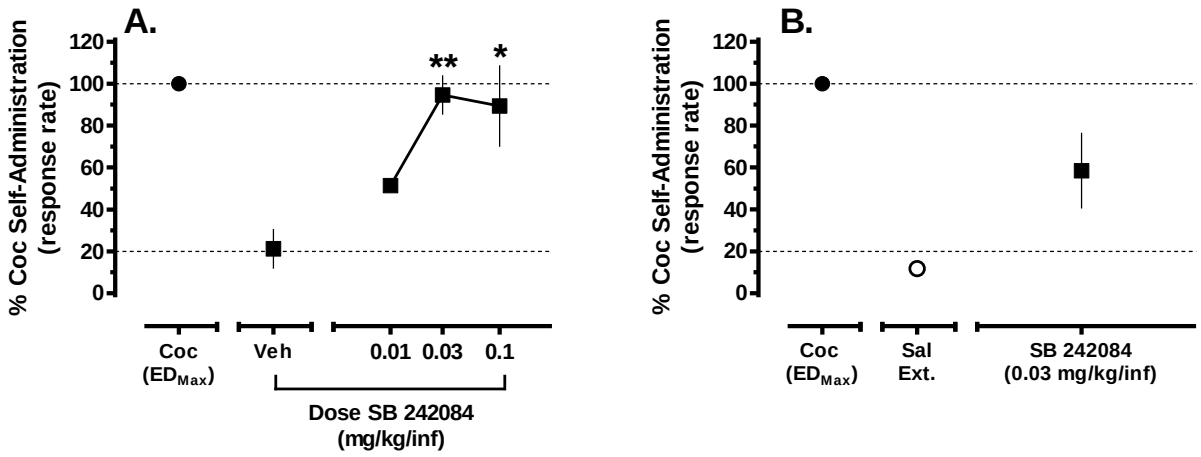

Figure 16. An assessment of the reinforcing effects of SB 242084 (veh, 0.01-0.1 mg/kg/inf) in squirrel monkeys trained to self-administer cocaine on a second-order operant schedule (n=3). (*A*) Substitution of SB 242084 for cocaine availability. Responding was first allowed to stabilize during ED_{Max} cocaine self-administration sessions (filled circle), and then various doses of SB 242084 were substituted for cocaine availability (filled squares). SB 242084 produced an inverted U-shaped dose-response function and maintained maximal rates of responding at levels near those maintained by the ED_{Max} of cocaine. (*B*) Response rates maintained by SB 242084 self-administration immediately following extinction sessions. Following ED_{Max} cocaine self-administration (filled circle), responding was extinguished via saline substitution and absence of conditioned-cue reinforcement (open circle). Once responding had extinguished, SB 242084 was made available and conditioned-cue reinforcement was reintroduced (filled square). Only the maximally-effective dose (0.03 mg/kg/inf) was tested. Data (mean ± SEM) are expressed as the percent of responding maintained during ED_{Max} cocaine self-administration sessions. Asterisks indicate significant difference (* $p < 0.05$, ** $p < 0.01$) for a given data point compared to the rate of responding maintained by "Veh" SB 242084. *Abscissae*: unit dose of drug available for self-administration (A) or session type and drug/dose available (B). *Ordinates*: normalized response rate.

vehicle was substituted for cocaine, responding decreased to below the 20% extinction criterion within 3-4 session in two subjects and to near-extinction levels (~40%) in a third subject, indicating that the level of responding was indeed dependent upon the availability of a pharmacological reinforcer. Substituting SB 242084 (0.01-0.1 mg/kg/infusion) for cocaine availability produced an inverted U-shaped dose-response function. For all doses of SB 242084 tested, response rates stabilized within 3-6 sessions in each individual subject. The number of days required to reach stabilization of response rates for all doses tested did not differ significantly (one-way repeated-measures ANOVA: $F_{(2,4)} = 4.00$, $p = 0.11$). One-way repeated-measures ANOVA followed by post hoc analyses indicated that 0.03 and 0.1 mg/kg/infusion SB 242084 reliably maintained self-administration behavior ($F_{(3,6)} = 11.417$, $p = 0.007$; Tukey's tests, $p = 0.009$ and $p = 0.013$ respectively) relative to responding maintained during vehicle substitution. Importantly, rates of responding during self-administration of these effective doses of SB 242084 were nearly equivalent to those maintained during ED_{max} cocaine self-administration (i.e. 100%).

Following the first session of intravenous self-administration of 0.03 mg/kg/infusion SB 242084, we observed whole-body scratching behavior in one of three subjects (s209). Self-administration of 0.1 mg/kg/infusion SB 242084 also elicited whole-body scratching behavior, but in each of the three subjects tested, and the effect was persistent across all test sessions during which this dose was substituted. In all cases, the scratching behavior appeared to subside within 30-60 min of the end of the self-administration session. Additionally, self-administration of 0.1 mg/kg/infusion was accompanied by emesis in two of three subjects, but this effect was only observed on the first day of substitution.

To further test the reinforcing efficacy of SB 242084, we assessed the capacity of the maximally-effective unit dose of SB 242084 (0.03 mg/kg/inf) to restore and maintain responding following the extinction of cocaine self-administration behavior. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.60 ± 0.44 responses/s. When conditioned reinforcers were withheld and saline was substituted for cocaine availability, responding extinguished below the 20% baseline within 2-7 sessions. The effects of subsequent 0.03 mg/kg/inf SB 242084 availability upon responding are shown in Figure 16b. The mean (± SEM) number of days required for response rates to stabilize was 7.33 ± 2.4 days. Across all subjects, response rates stabilized at ~59% of the ED_{Max} cocaine self-administration rate. Paired t-test indicated that this level of responding did not significantly differ from response rates during saline extinction ($p = 0.11$). It should be noted that responding maintained by SB 242084 under these conditions was highly variable across subjects. SB 242084 maintained high rates of responding in one subject (~92%), moderate response rates in a second subject (~54%), and near-extinction levels of responding in a third subject (~30%).

4. Stimulus Termination

The effects of pretreatment with the preferential 5-HT_{2C}R agonist mCPP on responding maintained by a second-order schedule of stimulus termination are shown in Figure 17a. The mean response rate (± SEM) following vehicle mCPP pretreatment was 0.91 ± 0.06 responses/s. mCPP significantly attenuated response rates in a dose dependent manner (one-way repeated-measures ANOVA; $F_{(2,6)} = 17.37$, $p = 0.003$). Post hoc Tukey's tests indicated that 0.3 mg/kg mCPP significantly reduced response rates to ~53% of baseline ($p = 0.003$).

The effects of pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 on response rates are shown in Figure 17b. The mean response rate (± SEM) following vehicle Ro 60-0175

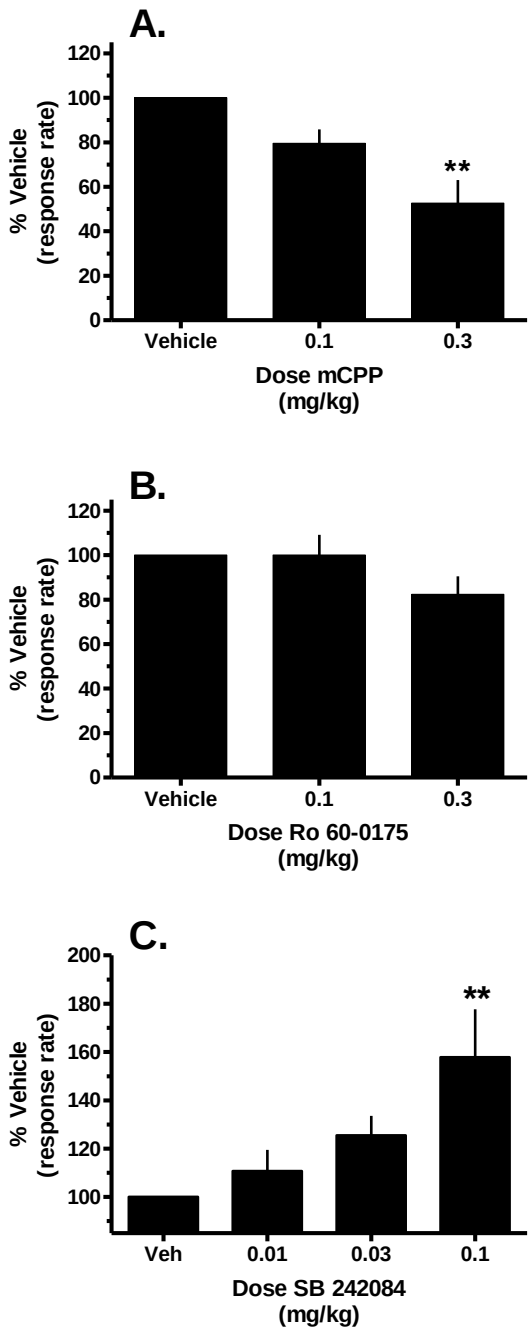

Figure 17. Effects of pretreatment with mCPP (*A*), Ro 60-0175 (*B*), or SB 242084 (*C*) on responding maintained by a second-order schedule of stimulus termination in squirrel monkeys (n=4). Data (mean ± SEM) are expressed as a percent of responding following administration of the drug vehicle. Asterisks indicate significant difference (** $p < 0.01$) for a given data point

compared to “Vehicle” pretreatment. *Abscissae*: dose of pretreatment. *Ordinates*: normalized response rate.

pretreatment was 0.83 ± 0.05 responses/s. In contrast to the effects of mCPP, pretreatment with Ro 60-0175 did not significantly alter responding (one-way repeated-measures ANOVA, $F_{(2,6)} = 2.40$, $p = 0.17$). The highest dose of Ro 60-0175 tested (0.3 mg/kg) had a weak effect upon responding as response rates were ~82% of the baseline.

Pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 dose-dependently increased response rates maintained by the second-order schedule of stimulus termination (Fig. 17c). One-way repeated-measures ANOVA indicated a significant effect of SB 242084 dose ($F_{(3,9)} = 7.71$, $p = 0.007$). Post hoc Tukey's tests revealed that 0.1 mg/kg SB 242084 significantly increased responding to ~158% of the baseline ($p = 0.007$).

D. Discussion

The main purpose of these experiments was to assess the impact of 5-HT_{2C}R-selective and 5-HT_{2A}R-selective compounds upon cocaine-induced reinstatement in nonhuman primates, a behavioral measure that has been described as a preclinical model of relapse to drug use. The results described here demonstrate for the first time that pretreatment with the 5-HT_{2C}R agonists mCPP and Ro 60-0175 attenuated, whereas the 5-HT_{2C}R antagonist SB 242084 enhanced, cocaine-induced reinstatement in squirrel monkeys, while the 5-HT_{2A}R antagonist M100907 was ineffective.

The modulatory impact of 5-HT_{2C}R activation and antagonism upon cocaine-induced reinstatement of extinguished cocaine-maintained responding has previously been reported in rodents (Grottick et al 2000; Neisewander and Acosta 2007; Pentkowski et al 2010). However, a detailed analysis of the pretreatment effects in these studies was limited by the use of a single cocaine dose to reinstate operant responding. In the present study, we evaluated the effect of all 5-HT_{2C}R-selective pretreatments across several cocaine priming doses to determine the

nature of the shift in the dose-response function. Our results demonstrated that both 5-HT_{2C}R agonists (mCPP and Ro 60-0175) induced dose-dependent downward shifts in the cocaine dose-response function, indicating an insurmountable attenuation of the cocaine-induced reinstatement effect. Similar 5-HT_{2C}R agonist-induced downward shifts were previously observed with respect to the behavioral-stimulant effects of cocaine (Chapter II). Also in agreement with our previous experiments, the present data indicated that mCPP was slightly more potent and slightly more effective at reducing the effects of cocaine as compared to Ro 60-0175. For example, 0.1 mg/kg mCPP significantly attenuated reinstatement following both the high and low cocaine priming doses, whereas 0.1 mg/kg Ro 60-0175 was ineffective at altering the effects of any dose of cocaine. It could again be postulated, as it was previously, that the effects of mCPP in the present study were mediated by a combination of 5-HT_{2C}R activation and 5-HT release (Chapter II, Discussion). However, the effects of maximally-effective doses of both mCPP and Ro 60-0175 were completely blocked by pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 at a dose which had no effect alone upon responding, confirming that the observed reductions in reinstatement behavior by both Ro 60-0175 and mCPP were indeed mediated by actions at the 5-HT_{2C}R. It is of course possible that increasing the dose of Ro 60-0175 might have produced a stronger shift of the cocaine dose-response function. However, we previously noted that 1.0 mg/kg Ro 60-0175 significantly suppressed responding in squirrel monkeys lever-pressing on a fixed-interval 300-sec schedule of stimulus termination (Chapter II), and thus chose not to administer this dose as nonspecific disruptions of operant behavior were likely to occur.

To rule out the possibility that the effects of doses of mCPP or Ro 60-0175 used presently could be attributed to nonspecific disruptions of operant behavior rather than modulation of cocaine effects per se, both compounds were administered to a separate group

of squirrel monkeys responding on an identical schedule of reinforcement, but maintained by a nondrug reinforcer (i.e. stimulus termination). The results were strikingly similar to those obtained previously (Chapter II), as the highest dose of mCPP (0.3 mg/kg) significantly suppressed responding to ~50% of baseline, whereas Ro 60-0175 did not significantly alter responding at any dose tested. However, it is important to note that 0.1 mg/kg mCPP, a dose which did produce significant reductions in cocaine-primed reinstatement, did not significantly alter nondrug-maintained responding. Taken together, the results indicate that both mCPP and Ro 60-0175 significantly reduced cocaine-induced reinstatement at doses which did not appreciably affect nondrug-maintained responding, but mCPP was more potent than Ro 60-0175 in this regard. It therefore appears that 5-HT_{2C}R agonists exhibit a small but measurable degree of behavioral selectivity when comparing effects upon cocaine-maintained vs. nondrug-maintained behaviors in nonhuman primates.

The neurobiological mechanisms underlying cocaine-induced reinstatement are not fully understood, but there is a clear role of DA signaling (Spealman et al 1999). Studies in rats have demonstrated that 5-HT_{2C}R agonists attenuate cocaine-induced increases in extracellular DA levels (Di Matteo et al 1999; Di Giovanni et al 2000), and it has been argued that this neurochemical consequence mediates their capacity to alter the reinforcing and reinstatement effects of cocaine in this species. It is therefore possible that 5-HT_{2C}R activation also attenuates cocaine-induced reinstatement in monkeys by suppressing cocaine-induced elevations in DA. The final series of experiments in this report (Chapter IV) employed *in vivo* microdialysis techniques to more directly address this issue.

It should be noted that in our behavioral procedure, reinstatement of responding could be a product of both the cocaine prime and the reintroduction of response-contingent cocaine-

associated visual cues. Such cues, when presented in the absence of a pharmacological prime, are capable of inducing reinstatement in rodents and nonhuman primates (Spealman et al 1999; Shaham et al 2003). It is therefore impossible for us to conclude whether 5-HT_{2C}R activation in the present studies is affecting the reinstatement effects of the cocaine prime, the cocaine-associated cues, or both. This might be an important consideration given that DA signaling within different brain regions mediates cue-induced vs. cocaine-induced reinstatement. For example, cocaine-induced reinstatement has been found to rely upon DA signaling within the striatum and PFC (for review, Shaham et al 2003), whereas cue-induced reinstatement involves DA signaling within the amygdala (See et al 2001). Nevertheless, 5-HT_{2C}R agonists have been found to attenuate both cue-induced reinstatement (Neisewander and Acosta 2007; Burbassi and Cervo 2008; Fletcher et al 2008; Pentkowski et al 2010) and cocaine-induced reinstatement (Grottick et al 2000; Neisewander and Acosta 2007; Pentkowski et al 2010) in rats, and it is therefore likely that both modalities were similarly affected in the present studies.

Given that the reinforcing effects of cocaine are also highly dependent upon dopaminergic mechanisms, it is not surprising that studies in rodents have demonstrated that ongoing cocaine self-administration is also attenuated by pretreatment with 5-HT_{2C}R agonists (Grottick et al 2000; Fletcher et al 2008). Our current results now extend these findings to nonhuman primates, as pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175, at a dose which did not alter nondrug-maintained responding, reduced cocaine self-administration across a range of cocaine doses. The dose-response function again shifted downward, in agreement with the insurmountable effects of Ro 60-0175 upon other cocaine-mediated behaviors we have observed thus far. These data suggest that pretreatment with a 5-HT_{2C}R agonist attenuates the direct reinforcing effects of cocaine in nonhuman primates. It should be noted that mCPP was

not tested against ongoing cocaine self-administration because we wanted to assess the effects of the more 5-HT_{2C}R-selective compound.

In opposition to the effects of 5-HT_{2C}R agonists, cocaine-induced reinstatement was enhanced following pretreatment with the selective 5-HT_{2C}R antagonist SB 242084. This result is in agreement with a previous study demonstrating a dose-dependent potentiation of cocaine-induced reinstatement following SB 242084 pretreatment in rats (Fletcher et al 2002) and is likely mediated via an enhancement of cocaine-induced elevations of DA levels within the mesolimbic and/or nigrostriatal systems, an effect which has been observed in rodents (Navailles et al 2004) but has yet to be corroborated in nonhuman primates. The findings are also consistent with previous studies by us in nonhuman primates (Chapter II) and others in rodents (Fletcher et al 2002; Fletcher et al 2006) indicating that pretreatment with SB 242084 enhanced the behavioral-stimulant effects of cocaine. Interestingly, one prior study found that 5-HT_{2C}R antagonism alone was insufficient to induce reinstatement of cocaine-seeking behavior in rats (Fletcher et al 2002), yet in the present study, SB 242084 administration alone did induce reinstatement at the highest dose tested in one subject. This discrepancy may be accounted for by highlighting the tested dose range within each experiment. For example, the dose of SB 242084 used for reinstatement experiments in the previous rodent study (0.5 mg/kg) also failed to induce significant locomotor effects (Fletcher et al 2002). However, increasing the dose of SB 242084 to 1.0 mg/kg did produce a modest but significant effect upon locomotor activity in a separate study (Zaniewska et al 2009). In our experiments, SB 242084 induced reinstatement in one subject, but only at a dose that also reliably engendered significant behavioral-stimulant effects in a separate group of animals (0.1 mg/kg). Furthermore, decreasing the dose of SB 242084 to 0.03 mg/kg prior to priming with saline resulted in the absence of any appreciable reinstatement effect in all subjects. It is therefore possible that 5-HT_{2C}R antagonism may have

been capable of inducing reinstatement in the previous rodent study, but the dose range tested may have been insufficient to reveal such an effect. Accordingly, it is possible that increasing the dose in the present experiments may have had more robust reinstatement effects across all subjects. However, we were hesitant to perform such tests due to the emergence of adverse physiological side effects following administration of the 0.1 mg/kg dose.

Thus far, our results indicate that SB 242084 enhances the behavioral-stimulant and reinstatement-inducing effects of cocaine in nonhuman primates, and that these interactions are believed to be mediated through converging elevations of DA within the striatum. Given that the reinforcing effects of nearly all drugs of abuse are thought to be mediated by similar increases in DA signaling, we were interested in determining whether SB 242084 might function as a positive reinforcer under an operant schedule of self-administration. Indeed, squirrel monkeys trained to self-administer cocaine continued to lever-press when cocaine availability was substituted with intravenous infusions of SB 242084. Importantly, SB 242084-maintained responding occurred at levels that were similar to those maintained by the ED_{Max} unit dose of cocaine, indicative of relatively strong reinforcing effects. To our knowledge, this is the first study reporting the direct reinforcing effects of a 5-HT_{2C}R antagonist.

Second-order drug self-administration studies in nonhuman primates have been employed previously to examine the reinforcing properties of several drug classes, including psychostimulants, opiates, ethanol, and nicotine, among others (Schindler et al 2002). These studies typically result in an inverted U-shape curve when the response rate is plotted as a function of the unit dose available for self-administration. The ascending limb of the curve reflects the intermediate reinforcing strength of the drug at lower doses compared to the peak response rates maintained by higher doses. In contrast, the decrease in response rates observed

at higher doses, on the descending limb of the dose-response function, are generally thought to reflect the emergence of behavioral effects that disrupt the animal's ability to reliably manipulate the operandum or that disrupt stimulus control over behavior, rather than a decrease in the reinforcing strength of the drug per se. In the case of the highest dose of SB 242084 tested (0.1 mg/kg/infusion), we observed incidences of emesis and whole-body scratching that likely disrupted operant performance, thus producing a modest decrease in responding and yielding the descending limb of the dose-response function. Yet, it is important to note that despite these observed behavioral effects, animals continued to lever-press at high rates of responding. In fact, rates of responding maintained by both 0.03 and 0.1 mg/kg/infusion SB 242084 resulted in levels of responding that were nearly identical to those produced when each individual subjects' ED_{max} unit dose of cocaine was available for self-administration (i.e. 100%). This finding suggests that the reinforcing effectiveness of SB 242084 is similar in magnitude to the reinforcing effectivenes of the ED_{max} cocaine unit dose when substituted for cocaine availability.

One may speculate that responding during SB 242084 self-administration sessions were not due to reinforcing properties of SB 242084 per se, but instead represented resistance to extinction following the removal of cocaine. However, substitution with vehicle reduced response rates to near-extinction levels (~20%), suggesting that the animals were indeed sensitive to the presence of a pharmacological reinforcer. Taking into account the high rates of responding maintained by the highest doses of SB 242084, as well as the extinction of responding following absence of both cocaine and SB 242084, we conclude that SB 242084 functioned as a reinforcer with a maximum effectiveness similar to that of cocaine when substituted for recent cocaine availability. There are however some important limitations to consider. First, the substitution test period was of a relatively short duration. Although each

dose was tested until responding stabilized across three consecutive sessions, it is possible that allowing for a longer substitution duration may have resulted in the loss of self-administration responding. Second, the subjects used in the present study had extensive histories of cocaine self-administration. Prior studies have found that some drugs, e.g. MDMA, will maintain self-administration when substituted in monkeys trained to self-administer cocaine (Fantegrossi et al 2002), but fail to entrain self-administration responding in drug-naïve counterparts (Dr. William Fantegrossi and Dr. Leonard Howell, personal communications). Future studies may be designed to address each of these concerns.

To at least partially characterize the reinforcing effects of SB 242084 under different conditions, we chose to test whether its availability could restore and maintain behavior in subjects which had their cocaine self-administration behavior extinguished immediately prior to testing. Under these conditions, 0.03 mg/kg/infusion SB 242084 did seemingly maintain response rates above extinction levels (~60% vs. ~15%), but self-administration was clearly less robust than when this same dose was substituted off of a cocaine baseline (~95%). Furthermore, SB 242084-maintained behavior required a high number of sessions to stabilize when the self-administration sessions followed extinction training, whereas cocaine typically restores responding to 100% within 1-2 sessions under similar conditions (personal observations). Taken together, these findings suggest that SB 242084 can function as a robust reinforcer in monkeys with a recent history of cocaine self-administration, but that its reinforcing effects can vary based upon experimental conditions. Future studies should determine the reinforcing effects of SB 2420824 and other 5-HT_{2C}R antagonists and inverse agonists in both drug-experienced and drug-naïve subjects under varying conditions to provide a more comprehensive index of the abuse liability of these compounds.

Previous studies have demonstrated that selective pharmacological antagonism of the 5-HT_{2A}R attenuated cue-induced and cocaine-induced reinstatement in rodents (Fletcher et al 2002; Nic Dhonnchadha et al 2009; Pockros et al 2011). In the present study, we found that pretreatment with the selective 5-HT_{2A}R antagonist M100907 had no effect upon reinstatement induced by the maximally-effective priming dose of cocaine in squirrel monkeys. Although this result is in contrast to those obtained in rodent studies, it is consistent with our previous finding that M100907 was ineffective at modulating the behavioral-stimulant effects of cocaine (Chapter II). Given that both the reinstatement and behavioral-stimulant effects of cocaine are strongly linked to actions upon the DA system, our data collectively suggest that 5-HT_{2A}R antagonism does not functionally influence the DA-mediated effects of cocaine in nonhuman primates.

In summary, the present experiments clearly demonstrate that pharmacological activation or antagonism of the 5-HT_{2C}R attenuate and enhance, respectively, the abuse-related effects of cocaine in nonhuman primates, whereas 5-HT_{2A}R antagonism had no effect. We speculate that the effects observed with 5-HT_{2C}R-selective compounds are mediated via modulation of DA signaling. As DA systems are understood to mediate the reinforcing and abuse-related effects of most drugs of abuse, it is therefore possible that the capacity for 5-HT_{2C}R agonists and antagonists to alter the effects of other drugs will not be limited to cocaine alone. Interestingly, based upon the present results, we would argue that both 5-HT_{2C}R agonists and antagonists could be considered as potential pharmacotherapeutics for the treatment of cocaine abuse, but with opposing mechanistic approaches. Whereas 5-HT_{2C}R agonists attenuate the behavioral effects of cocaine and could therefore be developed as functional antagonists (e.g. naltrexone for opiate addiction), 5-HT_{2C}R antagonists share a behavioral profile similar to cocaine and could be investigated as a novel substitute agonist therapy (e.g. methadone to

opiates). However, the novel finding that SB 242084 maintains intravenous self-administration is indicative of a potential abuse liability in humans and suggests that the clinical development and use of 5-HT_{2C}R-selective antagonists and/or inverse agonists may require careful caution and consideration.

Chapter IV. 5-HT_{2C} Receptors: Modulation of Cocaine Neurochemical Effects in Caudate Nucleus and Nucleus Accumbens

A. Introduction

Cocaine is a relatively nonselective inhibitor of the monoaminergic transporter proteins, and its administration produces increased synaptic levels of DA, NE, and 5-HT via disrupted neurotransmitter reuptake (Reith et al 1986; Madras et al 1989; Ritz et al 1990). However, abundant evidence indicates that the abuse-related effects of cocaine are specifically mediated via actions upon brain DA systems. For example, the potencies of various cocaine-like drugs to bind to the DAT or displace [^{3}H]cocaine from striatal tissue strongly correlated with their potencies to maintain self-administration behavior (Ritz et al 1987; Bergman et al 1989). Furthermore, self-administration behavior in animals is maintained by DAT-selective inhibitors (Howell and Byrd 1991; Roberts 1993; Howell et al 2000; Lindsey et al 2004), but not by compounds selective for the NET or SERT (Tessel and Woods 1975; Woolverton 1987; Howell and Byrd 1995). Dopaminergic systems have also been strongly implicated in mediating the behavioral-stimulant effects (Spealman et al 1989) and reinstatement effects of cocaine (Spealman et al 1999; Shaham et al 2003), behavioral effects that have been associated with abuse liability. The importance of cocaine DAT binding extends to humans as well, as the self-reported “high” resulting from cocaine administration was highly correlated with DAT occupancy in the striatum of experienced cocaine users (Volkow et al 1997).

Among the various DA brain systems that have been theorized to play a role in the abuse-related effects of cocaine, the mesolimbic system has received the most attention. The mesolimbic system consists of DA-producing neurons localized within the VTA that send axonal projections to several terminal regions within the limbic system, including PFC, amygdala, and

the NAcc within the anteroventral portion of the striatum (Moore and Bloom 1978; Haber and McFarland 1999). Early studies demonstrated that lesions of the VTA or the NAcc dramatically reduced the hyperlocomotive effects (Kelly and Iversen 1976) and reinforcing effects (Roberts et al 1977; Roberts et al 1980; Roberts and Koob 1982) of cocaine in rodents. Furthermore, local administration of DA receptor antagonists into the NAcc attenuated cocaine self-administration (Robledo et al 1992). These studies together serve to highlight the importance of mesolimbic DA signaling for the abuse-related effects of cocaine in rodents.

Despite a clear role for mesolimbic DA neurotransmission in the behavioral effects of cocaine, more recent evidence suggests that the mesolimbic DA system is especially critical for the acquisition of cocaine self-administration behavior, whereas the transition from goal-directed behavior to a more habit-based control over responding may be dependent upon DA signaling within increasingly dorsolateral aspects of the striatum that receive DA input from the SNpc, i.e. the nigrostriatal system (Wise 2009). For example, Porrino et al (2004) studied the effects of varying exposures to cocaine self-administration upon the metabolic effects of self-administered cocaine in the nonhuman primate brain. In subjects with a limited history (5 days) of cocaine self-administration, cocaine-induced glucose utilization mapping was evident in the ventral aspects of the striatum, but was absent in the more dorsolateral regions of the caudate and putamen. However, as self-administration exposure was increased to 100 days, cocaine activated not only the ventral striatum, but also incorporated larger expanses of the striatum which included more dorsal and caudal aspects, areas that are commonly associated with sensorimotor processing and habitual behaviors (Haber et al 2000; Yin et al 2004; Faure et al 2005). The relationship between cocaine intake and dorsal striatal function has also been confirmed in human cocaine abusers, as exposure to drug-associated visual stimuli increased subjective reports of craving that were strongly correlated with decreased binding of the

radiolabeled D_2-like receptor antagonist [^{11}C]raclopride within dorsal, but not ventral, regions of the striatum, an effect that is believed to reflect endogenous DA release in the former region (Volkow et al 2006).

The pattern of interconnectivity among the various striatal territories and midbrain dopaminergic afferents provides a putative neurobiological mechanism through which extended exposure to cocaine incorporates progressively broader expanses of the striatum. Tract-tracing studies have revealed that the midbrain DA-producing cells and striatal subterritories are linked via a series of intricate and complex reciprocal connections. Specifically, the dorsal tier of the dopaminergic midbrain (VTA and dorsal SNpc) projects to ventromedial striatum, including NAcc, which in turn projects back to a more ventral portion of the mibrain region. This pattern of information flow continues on to form an ascending spiral of connectivity, whereby ventral aspects of the striatum may influence increasingly dorsal regions of the striatum through actions upon progressively ventral portions of the midbrain (Haber et al 2000). It has been proposed that this spiraling network may indeed mediate the transition of the pattern of drug use from goal-directed to habitual in nature (Porrino et al 2004). Therefore, it is likely that the abuse-related effects of cocaine in subjects with extensive histories of drug use are mediated by DA neurotransmission in both ventral and dorsal aspects of the striatum.

Mounting evidence indicates that the 5-HT system exerts a modulatory influence over the behavioral effects of cocaine. For example, administration of indirect 5-HT agonists attenuated the behavioral-stimulant effects of cocaine in nonhuman primates (Howell and Byrd 1995). Similar pretreatments also attenuated the reinforcing effects (Kleven and Woolverton 1993; Glowa et al 1997; Czoty et al 2002; Negus et al 2007) and reinstatement effects (Ruedi-Bettschen et al 2010) of cocaine in monkeys. More relevant to the present study is the finding

that 5-HT exerts an inhibitory influence on cocaine-induced increases in extracellular DA. For example, we have previously demonstrated that pretreatment with the SERT inhibitor alaproclate attenuated cocaine-induced elevations of DA levels in squirrel monkeys (Czoty et al 2002). Additionally, studies in which administration of monoamine releasers that induced the release of DA equipotently *in vitro*, but varied in their potency to release 5-HT *in vitro*, identified an inverse relationship between potency to release 5-HT and capacity to increase DA *in vivo* in the caudate nucleus of squirrel monkeys (Kimmel et al 2009). Importantly, this inhibitory influence of 5-HT upon drug-induced increases in DA correlated with reduced behavioral-stimulant effects (Kimmel et al 2009) and lower rates of self-administration (Wee et al 2005; Rothman et al 2005). Taken together, these results suggest that enhanced 5-HT signaling attenuates both the neurochemical and abuse-related effects of cocaine.

Although the inhibitory influence of 5-HT upon cocaine-induced behavioral and neurochemical effects is well-established, the specific 5-HT receptors mediating these interactions have yet to be identified. However, recent evidence derived from rodent studies suggests that the 5-HT_{2C}R exerts a modulatory influence over cocaine-induced increases in DA within the ventral striatum. Specifically, activation of the 5-HT_{2C}R inhibited, while blockade of the 5-HT_{2C}R enhanced, cocaine-induced elevations of DA in the NAcc of rats (Di Matteo et al 1999; Di Giovanni et al 2000; Navailles et al 2004; Navailles et al 2008). These modulatory effects of 5-HT_{2C}R agonists and antagonists upon the neurochemical effects of cocaine correlated with subsequent alterations of the behavioral effects of cocaine, including its reinforcing and reinstatement effects (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008).

We have previously demonstrated for the first time that systemic pretreatment with the 5-HT_{2C}R agonists mCPP or Ro 60-0175 attenuates the behavioral-stimulant, reinforcing, and reinstatement effects of cocaine in nonhuman primates (Chapter II, Chapter III). Furthermore, we have also shown that pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced the behavioral-stimulant and reinstatement effects of cocaine in monkeys, and this compound also maintained self-administration behavior under a second-order schedule of reinforcement. However, the mechanisms by which these compounds mediate these observed effects remains to be determined. In rodents, studies have revealed that 5-HT_{2C}Rs are situated predominantly on GABAergic interneurons within the VTA (Eberle-Wang et al 1997; Bubar and Cunningham 2007), where their activation increases GABA-mediated inhibition upon DA-producing cells within the midbrain, thus functionally inhibiting the capacity of the DAT inhibitor cocaine to increase extracellular levels of DA (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008). Although it is possible that a similar scenario exists in the nonhuman primate brain, an alternative explanation could be that 5-HT_{2C}Rs may exert their behavioral effects by acting downstream of DA-receiving neurons within striatal and/or cortical terminal regions, and thus would not be expected to directly alter the DA-increasing effects of cocaine. Given that some differences exist between rodents and nonhuman primates regarding the distribution of 5-HT_2Rs in DA pathways (see Chapter I, sections 2a and 3a), an assessment of the impact of 5-HT_{2C}R activation or antagonism upon the neurochemical effects of cocaine in nonhuman primates is clearly warranted.

In the present studies, we employed *in vivo* microdialysis techniques in awake squirrel monkeys to assess cocaine-induced changes in DA within the caudate nucleus and NAcc following pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 or the selective 5-HT_{2C}R antagonist SB 242084. As described earlier, the abuse-related effects of cocaine appear to be

mediated by DA signaling in both dorsal and ventral aspects of the striatum. However, our previous data have suggested that 5-HT_{2C}R agonists and antagonists may differentially alter the effects of cocaine within these striatal territories in squirrel monkeys (Chapter II). The experimental design used here allows us to determine whether such regional specifity of effects occurs. The results from these experiments provide a clearer understanding of the mechanisms by which 5-HT_{2C}R-selective compounds modulate the behavioral effects of cocaine in nonhuman primates.

B. Methods

1. Subjects

Seven adult male squirrel monkeys (*Saimiri sciureus*) weighing 800 – 1350g served as subjects. Between experimental sessions, animals were individually housed in a climate-controlled room and fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal) with ad libitum access to water. Daily enrichment was provided via access to foraging devices, toys, and climbing/swing devices. Each animal had served in previous behavioral studies involving administration of compounds acting upon monoaminergic, and/or glutamatergic systems (Ginsburg et al 2005; Kimmel et al 2005; Kimmel et al 2007; Banks et al 2009; Bauzo et al 2009; Fantegrossi et al 2009; Kimmel et al 2009). All studies were conducted in strict accordance with the National Institutes of Health's "Guide for Care and Use of Laboratory Animals", the American Association for Accreditation of Laboratory Animal Care (AAALAC), and were approved by the Institutional Animal Care and Use Committee of Emory University.

2. Apparatus

During experimental sessions, animals were comfortably seated in a commercially-available plexiglass chair within a ventilated, sound-attenuating chamber (Med Associates Inc., St. Albans, VT) supplemented with an adjustable Lexan barrier that was situated slightly above the level of the animal's shoulders to prevent disturbance to microdialysis probes and connective tubing. A motor-drive syringe pump (Model 11Plus Dual-Syringe, Harvard Apparatus, Holliston, MA) was mounted on top of the operant chamber for automated delivery of microinfused solutions.

3. Surgeries

Subjects were implanted with bilateral guide cannulae (CMA/11; CMA/Microdialysis, Acton, MA) using stereotaxic techniques under aseptic conditions as described previously (Czoty et al 2000). Subjects were initially anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0mg) and ketamine HCl (20mg) and maintained with inhaled isoflurane anesthesia (0.5-1.5%) to effect. Guide cannulae targeted the caudate nucleus and nucleus accumbens within the same dorsal-ventral plane using the following coordinates from the earbar: anterior/posterior + 15.0, medial/lateral +/- 3.0.When not in use, stainless-steel stylets were situated within the cannulae to maintain the integrity and sterility of the tissue site. Subjects were allowed one month of recovery before microdialysis experiments commenced. For all surgical procedures, preoperative and postoperative antibiotics (ceftriaxone) and postoperative analgesics (meloxicam or flunixin) were administered by veterinary staff who closely monitored the animals.

4. *In Vivo* Microdialysis

The microdialysis protocols used in the present study were similar to those described previously (Czoty et al 2000; Czoty et al 2002; Kimmel et al 2005; Kimmel et al 2007; Bauzo et al 2009). CMA/11 dialysis probes (CMA Microdialysis, North Chelmsford, MA) with a shaft length of 14 mm (caudate nucleus access) or 20mm (nucleus accumbens access) and active dialysis membrane measuring 4 x 0.24 mm (caudate nucleus) or 2 x 0.24 mm (nucleus accumbens) were used for all studies. Separate groups of animals were used to assess drug effects in either the caudate nucleus (n=4) or nucleus accumbens (n=3). The probe inlet was connected via FEP Teflon tubing to a microinfusion syringe mounted on a motor-driven syringe pump. Probes were flushed with artificial cerebrospinal fluid (aCSF; 1.0 mM Na_2HPO_4, 150 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.15 mM ascorbic acid) for 30 min prior to insertion into guide cannulae. FEP Teflon tubing was connected to the probe outlet and terminated outside the experimental chamber to allow for sample collection within microcentrifuge tubes.

During experiments, aCSF was perfused through the probe at a flow rate of 0.2 µl/min. Once probes were inserted into the guide cannula, a 60-min equilibration period was followed by acquisition of three baseline samples collected at 10-min intervals prior to drug treatment for determination of basal DA concentrations. Following baseline sample collection, additional 10-min samples were taken following drug administration according to the following conditions: Ro 60-0175 (veh, 0.3 mg/kg) administered 15-min prior to cocaine (1.0 mg/kg), or SB 242084 (veh, 0.3 mg/kg) administered 30-min prior to cocaine (0.3 mg/kg). Following cocaine administration, additional 10-min samples were collected for a total duration of 2 hours. The interval between pretreatments and cocaine administration and the doses of all drugs were chosen based on results from previous behavioral studies. All samples were refrigerated or frozen until

immediately prior to analysis. Probes were tested *in vitro* both prior to and immediately after each session to determine probe viability and percent-recovery. To confirm integrity of the site, the KCl concentration within the perfused aCSF was increased to 100 mM after the final experimental samples had been collected within each session to induce voltage-dependent DA release, and a final 10-min sample was collected. A robust increase in extracellular DA levels confirmed site viability. We have previously demonstrated the validity of repeated microdialysis accesses without a resultant loss of site viability (Czoty et al 2000). Each experimental session was conducted in a single brain hemisphere. For each subject, all drug combinations within a given experiment were acquired from the same ipsilateral hemisphere. Accesses at each brain site were separated by at least two weeks. The order of drug dose combinations was randomized within subjects.

Levels of DA were quantified within each sample using high-performance liquid chromatography with electrochemical detection as described previously (Czoty et al 2000; Kimmel et al 2005; Bauzo et al 2009). The HPLC system consisted of a small-bore (3 mm i.d. x 100 mm) column (5 µm C_{18} stationary phase; Thermo Hyperssil, Keystone Scientific Operations, Bellefonte, PA) with a commercially-available mobile phase (ESA, Chelmsford, MA). Microcentrifuge vials containing experimental samples (20 µl) were loaded into a refrigerated CMA/200 autosampler. Each sample was mixed with 3 µl of ascorbate oxidase, and 5 µl of the mixture was injected into the HPLC system via an ESA 582 solvent delivery pump at a flow rate of 0.6 ml/min. Electrochemical analyses were performed using an ESA dual-channel analytical cell (model 5040) and guard cell (model 5020) and an ESA Coulochem II detector. Potentials were set as follows: channel 1, -150 mV (oxidation); channel 2, +275 mV (reduction); guard cell, 350 mV. EZChrome Elite v. 3.1 software (Scientific Software, Pleasanton, CA) was used to generate chromatograms for each sample analyzed. A set of DA standards containing

experimenter-prepared concentrations of DA (0.5-25 nM) were analyzed before and after each set of experimental samples. Area under the curve (AUC) was calculated for each standard and used to generate a standard plot (AUC x estimated DA concentration) from which the estimated DA concentration for each experimental sample could be extrapolated.

5. Drugs

Cocaine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC) and Ro 60-0175 (Tocris Bioscience, Ellisville, MO) were dissolved in 0.9% saline. SB 24204 (Tocris Bioscience, Ellisville, MO) was initially dissolved at a concentration of 1.0 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO) , and 0.9% saline, and further diluted to appropriate concentrations using 0.9% saline. All drug solutions were passed through a 0.2 µm-pore polysulfone filter prior to use. Doses were calculated from the salt weights. All drugs were administered via the intramuscular route into the thigh muscle.

6. Data Analysis

Because the effects of cocaine typically returned to near-baseline levels within 60-min post cocaine administration, samples collected after this timepoint were excluded from analyses. For each subject, DA levels within each test session were normalized as the percent of the mean of three baseline values acquired prior to drug administration. Data were analyzed using a two-way repeated-measures ANOVA. Tukey's post hoc tests corrected with a Bonferroni adjustment then determined at each timepoint whether DA levels were affected by pretreatment with a 5-HT_{2C}R-selective compound compared to vehicle pretreatment. Data were graphically plotted using GraphPad v. 5.01 (GraphPad Software Inc., La Jolla, CA) and analyzed using SigmaStat v. 3.0 software (Systat Software Inc., San Jose, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

C. Results

1. 5-HT_{2C}R Agonist Ro 60-0175

a. Caudate Nucleus

Mean (± SEM) basal DA levels uncorrected for probe recovery were 4.90 ± 0.98 nM. As reported previously, 1.0 mg/kg cocaine following vehicle (saline) pretreatment increased extracellular DA in the caudate nucleus to ~230% of basal DA levels within 20-min after cocaine administration that returned to near-baseline levels within 60-min post drug injection (Czoty et al 2000; Czoty et al 2002). Pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 15-min prior to cocaine did not alter the subsequent timecourse or maximal increase in DA levels within the caudate nucleus (Fig. 18a). Two-way ANOVA revealed a significant main effect of time ($F_{(10,30)}$ = 28.57, $p < 0.001$), but not for the main effect of Ro 60-0175 pretreatment ($F_{(1,3)}$ = 0.40, p = 0.57) or their interaction ($F_{(10,29)}$ = 0.14, p = 1.0).

b. Nucleus Accumbens

Mean (± SEM) basal DA levels uncorrected for probe recovery in the nucleus accumbens were 2.68 ± 1.35 nM. Administration of 1.0 mg/kg cocaine produced a peak increase of ~324% of basal DA levels at 20-min after cocaine administration (Fig. 18b). The timecourse of cocaine-induced changes in DA levels were similar to those observed within the caudate nucleus as the effect peaked at 20-min after cocaine administration and returned to near-baseline levels within 60-min. In contrast to a lack of effect in the caudate nucleus, pretreatment with Ro 60-0175 attenuated the effects of cocaine on extracellular DA levels. Two-way repeated-measures ANOVA revealed significant main effects of time ($F_{(10,20)}$ = 61.75, $p < 0.001$) and pretreatment with Ro 60-0175 ($F_{(1,2)}$ = 17.96, p = 0.05) and a significant interaction ($F_{(10,20)}$ = 2.52, p = 0.038).

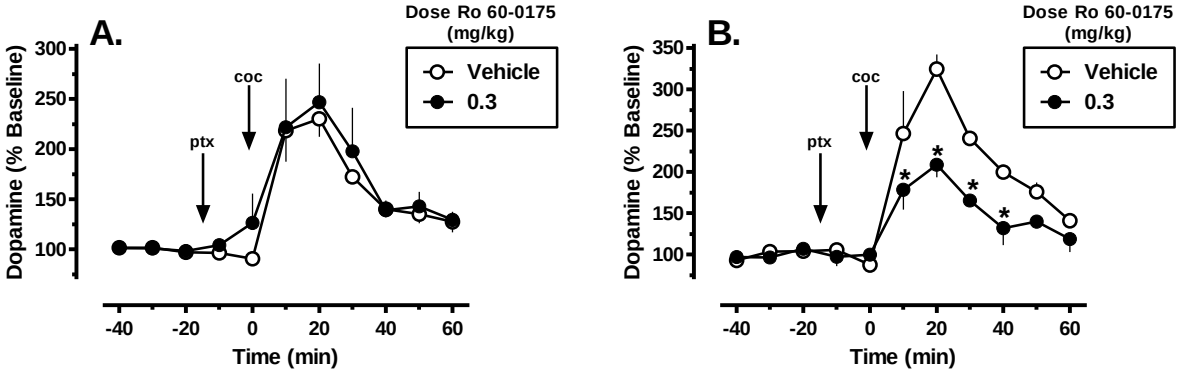

Figure 18. Effects of cocaine (1.0 mg/kg) on extracellular levels of DA in the caudate nucleus (A; n=4) or nucleus accumbens (B; n=3) following pretreatment with 0.3 mg/kg Ro 60-0175 (filled circles) or its vehicle (open circles) in squirrel monkeys. Data points (mean ± SEM) are expressed as the percent of DA levels prior to drug administration. Asterisks indicate significant difference (* $p < 0.05$) for a given data point compared to "Vehicle" pretreatment within the same timepoint. *Abscissae*: time relative to cocaine administration. *Ordinates*: normalized DA concentration.

Subsequent post hoc analyses indicated that although Ro 60-0175 had no effect upon DA levels in the interval preceding cocaine administration, the effects of cocaine were significantly attenuated by Ro 60-0175 pretreatment at each sampling interval within the 40-min time period immediately following cocaine injection ($p < 0.05$).

2. 5-HT_{2C}R Antagonist SB 242084

a. Caudate Nucleus

Mean (± SEM) basal DA levels uncorrected for probe recovery were 5.00 ± 1.48 nM. Because we expected that 5-HT_{2C}R blockade would produce an enhancement, rather than an attenuation, of cocaine effects, we reduced the dose of cocaine to 0.3 mg/kg to induce a more modest effect upon DA levels and pretreated with a relatively high dose of the selective 5-HT_{2C}R antagonist SB 242084 (0.3 mg/kg) to allow for a greater possibility of observing a potentiation following combined drug administration. The 0.3 mg/kg dose of SB 242084 was chosen based on our previous findings that administration of a slightly lower dose (0.1 mg/kg) produced modest behavioral-stimulant effects (Chapter II) and induced reinstatement of previously-extinguished cocaine-maintained behavior in 1/3 subjects tested (Chapter III). This dose was not administered in behavioral studies due to an expected nonspecific disruption on operant responding.

0.3 mg/kg cocaine following vehicle pretreatment increased extracellular DA in the caudate nucleus to ~190% of basal DA levels within 20-min after cocaine administration that returned to near-baseline levels within 60-min. The effects of pretreatment with 0.3 mg/kg SB 242084 on cocaine-induced elevations of DA in the caudate nucleus are shown in Figure 19a. Two-way repeated-measures ANOVA indicated a significant main effect of time ($F_{(11,22)} = 13.34$, $p < 0.001$) but not for the main effect of SB 242084 pretreatment ($F_{(1,2)} = 2.03$, $p = 0.29$) or their interaction ($F_{(11,20)} = 1.09$, $p = 0.42$). Although visual inspection of the data suggest that

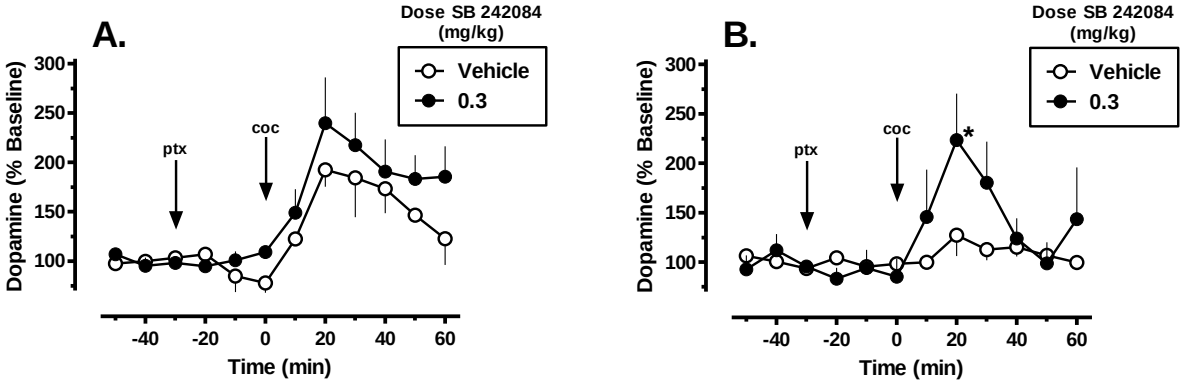

Figure 19. Effects of cocaine (0.3 mg/kg) on extracellular levels of DA in the caudate nucleus (A; n=4) or nucleus accumbens (B; n=3) following pretreatment with 0.3 mg/kg SB 242084 (filled circles) or its vehicle (open circles) in squirrel monkeys. Data points (mean ± SEM) are expressed as the percent of DA levels prior to drug administration. Asterisks indicate significant difference (* $p < 0.05$) for a given data point compared to "Vehicle" pretreatment within the same timepoint. *Abscissae*: time relative to cocaine administration. *Ordinates*: normalized DA concentration.

pretreatment with SB 242084 may have prolonged the duration of the cocaine-induced increase in DA levels, post hoc analyses failed to detect significance at any timepoint.

b. Nucleus Accumbens

Mean (± SEM) basal DA levels uncorrected for probe recovery in the nucleus accumbens were 2.83 ± 1.40 nM. In the nucleus accumbens, administration of 0.3 mg/kg cocaine produced a modest increase in extracellular DA which peaked at ~130% of basal DA levels at 20-min after cocaine administration. DA levels subsequently returned to baseline levels soon after the onset of the peak change (Fig. 19b). Combined administration of SB 242084 and cocaine produced a peak increase of ~223% of DA levels within 20-min after cocaine administration that returned to baseline within 60-min. Two-way repeated-measures ANOVA revealed a significant main effect of time ($F_{(11,22)} = 3.00$, $p = 0.014$) but not for the main effect of SB 242084 pretreatment ($F_{(1,2)} = 1.01$, $p = 0.42$). However, the time x SB 242084 interaction was significant ($F_{(11,22)} = 2.99$, $p = 0.014$). Post hoc analyses indicated that although SB 242084 did not affect DA levels during the interval preceding cocaine administration, the peak increase of DA levels following combined SB 242084 and cocaine administration was significantly higher as compared to the increase following cocaine and vehicle SB 24284 pretreatment ($p < 0.05$).

D. Discussion

The purpose of the present studies was to examine the impact of pretreatment with a selective 5-HT_{2C}R agonist (Ro 60-0175) or antagonist (SB 242084) on cocaine-induced increases in extracellular DA within the caudate nucleus and NAcc of the awake squirrel monkey. Our results suggest that activation of the 5-HT_{2C}R attenuated, whereas 5-HT_{2C}R antagonism enhanced, the neurochemical effects of cocaine in the NAcc, while neither pharmacological pretreatment altered cocaine-induced elevations of DA within the more dorsal caudate nucleus.

Several studies have indicated that the mesolimbic DA system, comprised of DA-producing cells within the VTA that project to the NAcc and associated limbic regions, is modulated by signaling at the 5-HT_{2C}R. For example, systemic administration of 5-HT_{2C}R agonists reduced both the basal firing rate of VTA dopaminergic neurons and basal DA levels within the NAcc (Di Matteo et al 1999; Di Giovanni et al 2000; Gobert et al 2000; De Deurwaerdere et al 2004; Marquis et al 2007). Both the 5-HT_{2C}R mRNA and protein have been described to localize predominantly on GABAergic interneurons within the rodent VTA (Eberle-Wang et al 1997; Bubar and Cunningham 2007), and local administration of the preferential 5-HT_{2C}R agonist mCPP increased the firing rate of these cells (Di Giovanni et al 2001). It therefore seems likely that 5-HT_{2C}R activation within the VTA functionally inhibits DA release within the mesolimbic terminal regions by stimulating local GABA release onto DA-releasing neurons. Because the neurochemical effects of cocaine are dependent upon impulse-stimulated release of DA from presynaptic terminals, one might postulate that the DA-increasing effects of cocaine would be attenuated by pretreatment with a 5-HT_{2C}R agonist, as the site of action for the latter (i.e. VTA) is upstream of the primary site of action for cocaine (i.e. mesolimbic terminal regions). Consistent with this hypothesis, Navailles et al (2008) demonstrated that intra-VTA administration of the 5-HT_{2C}R agonist Ro 60-0175 attenuated the DA-increasing effects of systemically-administered cocaine in anesthetized rats.

Accordingly, our present results indicate that systemic administration of the 5-HT_{2C}R-selective agonist Ro 60-0175 significantly attenuated cocaine-induced increases in DA levels within the NAcc of nonhuman primates. The dose of Ro 60-0175 (0.3 mg/kg) we used was chosen based on previous experiments which demonstrated selective reductions in cocaine-induced behavioral-stimulant effects (Chapter II), as well as reinforcing and reinstatement effects (Chapter III) in squirrel monkeys. The 1.0 mg/kg cocaine dose was chosen because it was

the maximally-effective dose for producing behavioral-stimulant effects (Chapter II), and prior studies demonstrated that it induces robust and long-lasting increases in DA levels within the caudate nucleus of awake monkeys (Czoty et al 2000; Czoty et al 2002; Bauzo et al 2009).

Interestingly, Ro 60-0175 pretreatment did not affect basal DA levels in the 15-min period preceding cocaine administration, although basal DA levels were reduced by systemic administration of 5-HT_{2C}R agonists in rodents (Di Matteo et al 1999; Di Giovanni et al 2000). The reason for this discrepancy is unclear, but several possible factors may be responsible. First, the time required for the onset of Ro 60-0175 effects has not been determined in nonhuman primates. We chose the 15-min pretreatment time in the present experiments because this was found to be effective in our previous behavioral studies involving interactions with cocaine. However, the DA-reducing effects of 5-HT_{2C}R agonists within the rodent NAcc do not emerge until approximately 40-60 min after i.p. drug administration (Di Matteo et al 1999; Di Giovanni et al 2000). Therefore, reductions in basal DA levels might have occurred after the 15-min pretreatment time had elapsed in the present study. Alternatively, Ro 60-0175 at the dose used presently may have selectively attenuated cocaine-induced elevations in DA without affecting basal levels in nonhuman primates, as has been shown previously with administration of the indirect serotonin agonist alaproclate, the nonselective serotonin agonist quipazine, and the group II metabotropic glutamate receptor agonist LY 379268 (Czoty et al 2002; Bauzo et al 2009). Future studies examining changes in DA levels within the NAcc of squirrel monkeys following administration of multiple doses of Ro 60-0175 alone would aid in clarifying this issue.

The Ro 60-0175-mediated attenuation of cocaine-induced DA increases within the NAcc suggests that 5-HT_{2C}R activation acted upstream of the site of cocaine action. Based on aforementioned evidence derived from rodent studies, one plausible mechanism underlying this

effect could be 5-HT_{2C}R-mediated activation of GABAergic interneurons within the VTA, which in turn would inhibit the activity of neighboring mesolimbic dopaminergic neurons. However, other sites of action cannot be ruled out. For example, 5-HT_{2C}Rs are also found in the rodent PFC where they are predominantly localized to GABAergic interneurons (Liu et al 2007). Given that PFC pyramidal projection neurons send excitatory efferent projections to the VTA (Heidbreder and Groenewegen 2003; Frankle et al 2006), 5-HT_{2C}R activation of PFC interneurons could result in reduced excitatory drive onto VTA DA neurons, thus reducing their activity. Consistent with this hypothesis, intra-mPFC infusion of selective 5-HT_{2C}R agonists attenuated cocaine-induced and cue-induced reinstatement of cocaine-maintained behavior in rats (Pentkowski et al 2010). Accordingly, the 5-HT_{2C}R is also found in the PFC of nonhuman primates, where it appears to colocalize not with pyramidal neurons, but rather with neurons that are morphologically consistent with GABAergic interneurons (Lopez-Gimenez et al 2001a). Because Ro 60-0175 was administered systemically in the present study, the primary sites of its inhibitory influence upon cocaine-induced DA effects cannot be identified and currently remain speculative.

In contrast to the effects of Ro 60-0175, we found that pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 in monkeys potentiated the DA increase within the NAcc following administration of a modest dose of cocaine. This finding is consistent with a previous study demonstrating that systemic administration of the selective 5-HT_{2C}R antagonist SB 242084 enhanced cocaine-induced increases in DA levels within the NAcc in rats (Navailles et al 2004). Interestingly, SB 242084 administration did not alter basal DA levels during the 30-min period preceding cocaine administration, and this lack of effect was mirrored by the 5-HT_{2C}R agonist Ro 60-0175. This finding is in opposition to studies in rodents demonstrating that administration of a 5-HT_{2C}R antagonist or inverse agonist increases basal firing rates of VTA DA neurons and elevates NAcc DA levels (Di Giovanni et al 1999; Di Matteo et al 1999). As stated earlier with

regard to Ro 60-0175, it is possible that the effects of SB 242084 on basal DA levels do not emerge until after the 30-min pretreatment window elapsed. However, whereas the effects of Ro 60-0175 upon basal DA levels were slow to emerge in rodents, the effects with SB 242084 displayed a faster onset, as increased basal DA levels were evident within 20-min of i.p. administration (Di Matteo et al 1999). Furthermore, it should be noted that a pilot study in a single squirrel monkey did not show any significant effect of 0.1 or 0.3 mg/kg SB 242084 pretreatment on basal DA levels over a 150-min sampling period (data not shown). Future studies are needed to clarify the impact of 5-HT_{2C}R blockade upon NAcc DA levels in nonhuman primates. However, the present data suggest that SB 242084 selectively enhanced cocaine-induced elevations of extracellular DA.

This raises the intriguing possibility that it is not the blockade of endogenous 5-HT tone at the 5-HT_{2C}R per se that alters DA levels, but rather that cocaine-induced elevations of 5-HT, through concurrent inhibition of the SERT, provides increased 5-HT tone on 5-HT_{2C}Rs which functionally antagonizes increases in DA, and this effect may in turn be relieved via SB 242084-mediated 5-HT_{2C}R antagonism. The self-limiting impact of increased 5-HT levels upon simultaneous increases in DA levels has been demonstrated with amphetamine analogs that share similar *in vitro* potencies for releasing DA but vary in their *in vitro* potency to release 5-HT. Indeed, compounds with lower DA/5-HT ratios for neurotransmitter release demonstrated markedly lower increases in DA as measured by *in vivo* microdialysis in rats and squirrel monkeys (Rothman et al 2005; Kimmel et al 2009). We therefore propose that cocaine and related psychostimulants which increase levels of both DA and 5-HT may self-limit their dopaminergic effects via increased serotonergic signaling at the 5-HT_{2C}R, and that greater dopaminergic effects may be unmasked in the presence of 5-HT_{2C}R blockade. Further support

for this hypothesis comes from genetic mutant mice lacking the 5-HT_{2C}R, which displayed potentiated behavioral and neurochemical responses to cocaine (Rocha et al 2002).

Studies investigating the impact of 5-HT_{2C}R agonism or antagonism upon basal and cocaine-induced levels of DA within dorsal aspects of the striatum in rodents have yielded conflicting results. For example, several studies have indicated that administration of selective 5-HT_{2C}R agonists attenuated, whereas 5-HT_{2C}R antagonists and inverse agonists enhanced, basal firing rates of SNpc DA neurons and consequent basal DA levels within the dorsal striatum (Di Giovanni et al 1999; Gobert et al 2000; De Deurwaerdere et al 2004; Alex et al 2005), as well as potentiated cocaine-induced DA increases in this brain region (Navailles et al 2004). However, other studies have failed to demonstrate such effects following similar pretreatments (Di Giovanni et al 2000; Di Matteo et al 1999; Marquis et al 2007). The reasons for these discrepancies are unknown, but may be related to differences in drug preparation, dosing, route of drug administration, electrophysiological and neurochemical methodologies, probe/electrode placements, or rodent species, among other variables.

Nevertheless, our data indicate that neither pretreatment with the 5-HT_{2C}R agonist Ro 60-0175 nor the 5-HT_{2C}R antagonist SB 242084 significantly altered the DA-increasing effects of cocaine within the caudate nucleus of squirrel monkeys. These results suggest that, in contrast to the mesolimbic DA system, the nigrostriatal pathway seems to be unaffected by signaling through the 5-HT_{2C}R in nonhuman primates. This result was surprising for two reasons. First, Ro 60-0175 pretreatment was previously demonstrated to attenuate responding maintained by a second-order schedule of cocaine self-administration in a separate group of squirrel monkeys (Chapter III). Second-order schedules of reinforcement are unique in that responding is often maintained for long periods of time by response-contingent presentation of conditioned

reinforcers, rather than by frequent presentation of the primary reinforcer (e.g. drug), and studies in rats have clearly demonstrated an important role for DA signaling within the dorsal striatum in mediating cocaine self-administration behavior under a second-order schedule of reinforcement (Ito et al 2000; Ito et al 2002; Vanderschuren et al 2005). The importance of DA within the dorsal striatum under these conditions has been argued to reflect the habitual nature of behavior inherent to second-order schedules of reinforcement. Second, the subjects used in our aforementioned self-administration experiments with Ro 60-0175 pretreatments had extensive histories of second-order cocaine self-administration behavior, and previous studies have indicated that prolonged exposure to cocaine self-administration results in cocaine-induced metabolic activation of progressively dorsolateral aspects of the striatum in nonhuman primates (Porrino et al 2004). In considering both of these factors, we postulated that the Ro 60-0175-induced reductions in cocaine self-administration we observed previously (Chapter III) might correlate with concurrent reductions in DA signaling within the caudate nucleus. Although such findings were not observed in the present microdialysis experiments, our results are consistent with a previous receptor localization study in which the 5-HT_{2C}R mRNA was found within the VTA and NAcc, but not the SNpc or dorsolateral aspects of the striatum, of nonhuman primates (Lopez-Gimenez et al 2001a).

However, it must be noted that the subjects used in the microdialysis experiments were not actively self-administering cocaine. We and others have previously demonstrated that contingent vs. noncontingent administration of cocaine produces markedly different effects upon extracellular DA (Hemby et al 1997; Porrino et al 2004; Kimmel et al 2005). Thus, Ro 60-0175 may selectively alter the DA-increasing effects of self-administered cocaine, but not of noncontingently-administered cocaine, in the caudate nucleus of squirrel monkeys. The mechanism for a selective modulation of cocaine-induced increases in DA within the caudate

could be explained by the striatonigrostriatal spiraling network of connectivity described by Haber and colleagues (2000). Our present results suggest that the mesolimbic, but not nigrostriatal, DA system is modulated by 5-HT_{2C}R activation or antagonism. However, if chronic exposure to cocaine self-administration produces adaptations in the strength of neural connectivity between ventral-to-dorsal striatal territories via the striatonigrostriatal spiraling pathway, then it is plausible that 5-HT_{2C}R signaling within the mesolimbic system (e.g. VTA) could ultimately modulate the activity of DA-producing neurons within the SNpc. More simply stated, it is possible that 5-HT_{2C}Rs within the VTA may modulate the activity of the nigrostriatal DA system in subjects with histories of chronic cocaine self-administration. Although this hypothesis remains purely speculative, future studies could employ *in vivo* microdialysis techniques concurrent with cocaine self-administration to determine whether DA increases in the caudate nucleus, consequent to response-contingent cocaine self-administration, are altered following pretreatment with a selective 5-HT_{2C}R agonist. Although such studies are technically difficult to undertake, our laboratory has previously demonstrated their feasibility (Kimmel et al 2005).

Finally, it should also be noted that the lack of nigrostriatal modulation by 5-HT_{2C}R activation or antagonism were in accordance with our earlier conclusions regarding cocaine-induced behavioral-stimulant effects (Chapter II). In those studies, we found that 5-HT_{2C}R activation and antagonism affected the ascending limb, but not the descending limb, of the cocaine inverted U-shaped dose-response function. The stimulant-like effects of cocaine in rodents are typically assessed using measures of locomotor activity, and cocaine also produces an inverted U-shaped dose-response curve in these assays. Interestingly, the initial increase in hyperlocomotion is commonly attributed to DA signaling within the mesolimbic pathway, whereas higher doses of cocaine induce behavioral stereotypies that produce the descending

limb, an effect which is dependent upon nigrostriatal DA signaling (Chapter II). If these neuroanatomical principles translate into our nonhuman primate model of stimulant-like effects, it could be argued that the 5-HT_{2C}R-selective pretreatments were selectively modulating cocaine actions within the mesolimbic, but not nigrostriatal, DA pathways, and the present microdialysis results with acute cocaine administration support this conclusion.

In summary, these studies are the first to demonstrate a modulatory role for the 5-HT_{2C}R upon the neurochemical effects of cocaine in nonhuman primates. Specifically, pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 attenuated, whereas pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced, increased DA levels within the NAcc following acute cocaine administration. In contrast, neither pretreatment affected the neurochemical effects of cocaine within the more dorsal caudate nucleus. These findings suggest that the inhibitory influence of indirect serotonin agonists upon the neurochemical effects of cocaine may be at least partially mediated through increased serotonergic signaling at the 5-HT_{2C}R. Furthermore, these data indicate that the serotonergic effects of cocaine and other nonselective monoamine reuptake inhibitors or releasers may serve to counteract their own dopaminergic effects via increased signaling through the 5-HT_{2C}R. Finally, these results provide a possible neurochemical mechanism by which the behavioral effects of cocaine are modulated following 5-HT_{2C}R activation or antagonism.

Chapter V. General Discussion

One therapeutic strategy for the treatment of cocaine abuse is to identify medications which act to functionally antagonize the pharmacological actions of cocaine, thus reducing its subjective and reinforcing effects in animals and humans. Early preclinical studies investigated the impact of D_1-like, D_2-like, and nonselective DA receptor antagonists on the abuse-related effects of cocaine. For example, pretreatment with the nonselective DA receptor antagonist flupenthixol reduced the reinforcing effects of cocaine in both rats and monkeys (Richardson et al 1994; Negus et al 1996) and cocaine-induced reinstatement in squirrel monkeys (Khroyan et al 2000). Similar results have been reported following pretreatment with D_1-like antagonists and D_2-like antagonists (Woolverton 1986; Bergman et al 1990; Caine and Koob 1994; Khroyan et al 2000). Indeed, DA receptor antagonism attenuates many of the behavioral effects of cocaine in rodents and nonhuman primates, including its stimulant-like and discriminative-stimulus effects (for review, Platt et al 2002). Accordingly, administration of flupenthixol and haloperidol attenuated the subjective effects of cocaine in human cocaine users (Sherer et al 1989; Gawin et al 1993; Berger et al 1996; Romach et al 1999). Taken together, these findings suggest that functional antagonism of the dopaminergic effects of cocaine may disrupt cocaine-taking in human drug abusers by preventing the subjective and reinforcing effects of cocaine from emerging. However, the clinical utility of such compounds are drastically limited due to their propensity to induce undesirable side effects in animals, such as extrapyramidal motoric disruptions, nonspecific reductions of operant responding, and catalepsy, which would likely result in poor compliance rates clinically or could even potentially function as a stressful stimulus to promote drug relapse (Woolverton 1986; Woolverton and Virus 1989; Bergman et al 1991; Rosenzweig-Lipson and Bergman 1994; Negus et al 1996). Nevertheless, there is good concordance between preclinical studies and the effects of DA receptor antagonists in humans

to suggest that functional antagonists which lack aversive side effects may be useful in the treatment of cocaine abuse.

Results from preclinical studies have suggested that the 5-HT system also exerts an inhibitory influence over the behavioral effects of cocaine. For example, indirect 5-HT agonists such as SERT inhibitors or 5-HT releasers have been found to attenuate the behavioral-stimulant effects (Spealman 1993; Howell and Byrd 1995), reinforcing effects (Kleven and Woolverton 1993; Glowa et al 1997; Czoty et al 2002; Negus et al 2007), and reinstatement effects (Ruedi-Bettschen et al 2010) of cocaine in nonhuman primates. Furthermore, studies employing *in vivo* microdialysis techniques in awake squirrel monkeys revealed that the altered behavioral effects of cocaine following serotonergic pretreatments correlated with reductions in cocaine-induced DA increases within the striatum (Czoty et al 2002). Taken together, these studies identified the 5-HT system as a source of novel pharmacotherapeutic targets for the treatment of cocaine abuse. This was a particularly exciting possibility because SERT inhibitors have been utilized clinically for several decades to treat a variety of psychiatric disorders, and were known to be safe and well-tolerated. However, several SERT inhibitors unexpectedly failed to reduce cocaine use in clinical trials (Grabowski et al 1995; Batki et al 1996; Ciraulo et al 2005; Winhusen et al 2005; but see Moeller et al 2007). Although the reasons for the clinical lack of efficacy with SERT inhibitors remain speculative, one possible explanation is that the indirect activation of all 5-HT receptor subtypes was not an ideal pharmacotherapeutic strategy, and thus, studies began to explore the therapeutic potential of more specific protein targets within the 5-HT system.

Among the 5-HT receptor subtypes investigated to date, the 5-HT_2 family of receptors has received significant attention. Studies have indeed confirmed that pharmacological activation of the 5-HT_{2C}R recapitulated the effects of 5-HT indirect agonists in rodent models of

cocaine use and relapse (for review, Bubar and Cunningham 2006; Bubar and Cunningham 2008). Our present results extend these findings to nonhuman primates for the first time by demonstrating that pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 insurmountably reduced the behavioral-stimulant effects of cocaine, the reinforcing effects of cocaine, and the reinstatement effects of cocaine, each of which was reversed by pretreatment with the selective 5-HT_{2C}R antagonist SB 242084. Furthermore, by comparing the effects of a preferential agonist (mCPP) with those of a more selective agonist (Ro 60-0175) upon nondrug-maintained operant responding, our results suggest that increased pharmacological selectivity for the 5-HT_{2C}R confers a greater index of behavioral specificity, as Ro 60-0175 more potently altered cocaine-maintained vs. nondrug-maintained behavior.

The mechanisms by which 5-HT_{2C}R agonists attenuate the neurochemical and behavioral consequences of cocaine administration in nonhuman primates remain to be elucidated, but microinjection studies in rodents suggest that the VTA is likely the primary site of action. Within the rat VTA, 5-HT_{2C}Rs localize predominantly to GABAergic neurons (Eberle-Wang et al 1997; Bubar and Cunningham 2007) and increase the activity of these cells when they are pharmacologically activated (Di Giovanni et al 2001), ultimately providing local inhibition on neighboring DA-producing neurons. Because cocaine is a reuptake transporter inhibitor, its capacity to increase levels of DA is dependent upon stimulated DA release from presynaptic nerve terminals. Therefore, the neurochemical effects of cocaine may be attenuated by 5-HT_{2C}R activation via GABA-mediated inhibition of DA neurons within the VTA. In support of this theory, 5-HT_{2C}R mRNA has been localized within the VTA of macaques (Lopez-Gimenez et al 2001a), although its colocalization with biochemical markers for DA and/or GABA in nonhuman primates remains to be elucidated. Interestingly, we did not observe reductions of basal DA levels within the NAcc following administration of a 5-HT_{2C}R agonist, as might be expected based on previous

results in rodents (Di Matteo et al 1999; Di Giovanni et al 2000; Navailles et al 2004; Navailles et al 2008). However, limitations of our study were discussed in detail in Chapter IV, and future studies are needed to clarify whether our results truly reflect a selective reduction of cocaine-induced DA increases by Ro 60-0175, or if they are the product of reduced basal activity of VTA DA neurons.

Interestingly, neither pretreatment with Ro 60-0175 nor SB 242084 had any significant effect upon basal DA levels nor cocaine-induced DA increases within the more dorsally-located caudate nucleus of the striatum. The portion of the caudate nucleus targeted in our microdialysis experiments is part of the central/medial striatum that receives dopaminergic input primarily from the SNpc (Haber et al 2000). Our neurochemical data indicate that although acute cocaine increases DA levels within this striatal territory, the activity of SNpc DA neurons is not susceptible to 5-HT_{2C}R-mediated modulation. Further support for this conclusion was obtained in our experiments in Chapter II where 5-HT_{2C}R-selective pretreatments altered the ascending limb, but not the descending limb, of the cocaine behavioral-stimulant dose-response function, as studies in rodents have indicated that locomotor activation is dependent upon mesolimbic DA activation, whereas the emergence of behaviors which disrupt locomotion (e.g. stereotypy) at higher doses of cocaine administration are dependent upon nigrostriatal DA pathways (Kelly et al 1975; Wise and Bozarth 1987). Thus, our behavioral data also suggest that 5-HT_{2C}R activation or antagonism may have selectively modulated mesolimbic DA neurotransmission. These findings are consistent with some studies in rodents showing that 5-HT_{2C}R agonism or antagonism do not affect the firing activity of SNpc DA neurons, nor do they alter basal DA levels within the dorsal striatum (Di Giovanni et al 2000; Di Matteo et al 1999; Marquis et al 2007), although other studies have reported the existence of such effects (Di Giovanni et al 1999; Gobert et al 2000; De Deurwaerdere et al 2004; Navailles et al 2004; Alex et

al 2005). As mentioned in Chapter IV, our findings are consistent with the reported lack of appreciable 5-HT_{2C}R mRNA within the SNpc or dorsolateral aspect of the striatum in nonhuman primates (Lopez-Gimenez et al 2001a). However, 5-HT_{2C}R mRNA and protein were clearly detected within progressively ventral aspects of the striatum, including NAcc. Given that our caudate nucleus experiments were conducted in a region intermediate to the extremes of dorsolateral and anteroventral striatal territoties, there is likely some presence of 5-HT_{2C}Rs within the central aspect of the striatum where our microdialysis studies were conducted, but their cellular localization remains unknown. Because cocaine-induced DA increases were unaffected by either pretreatment, the receptors are likely not present on presynaptic dopaminergic terminals. However, we cannot rule out the possibility that the receptors may be located postsynaptically on medium spiny neurons, where they would be situated to alter the behavioral effects of cocaine at sites upstream of cocaine's primary site of action within the synapse.

In complete opposition to the behavioral effects of 5-HT_{2C}R agonist administration, our studies demonstrate that pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 induced modest behavioral-stimulant effects when administered alone, and potentiated the behavioral-stimulant effects of cocaine in an apparently-additive manner (Chapter II). These results are consistent with the effects of 5-HT_{2C}R antagonist administration in rodents, as they have been found to potentiate cocaine-induced hyperlocomotion, cocaine self-administration, and cocaine-induced reinstatement (Fletcher et al 2002; Fletcher et al 2006). In the present study, SB 242084 pretreatment induced reinstatement of cocaine-maintained behavior in one subject at the highest dose tested, and enhanced the reinstatement effects of cocaine across all subjects (Chapter III). One notable difference between previous studies in rodents and our present results was that SB 242084 pretreatment alone did not induce reinstatement of

cocaine-maintained responding in rats (Fletcher et al 2002). However, that study used a single-dose experimental design with respect to SB 242084 pretreatment (0.5 mg/kg), and thus may have failed to detect reinstatement effects due to an inadequate range of doses. Indeed, 0.5 mg/kg SB 242084 fails to induce significant hyperlocomotion, but increasing the dose to 1.0 mg/kg elicits a significant increase of this behavioral measure (Zaniewska et al 2009). It is therefore possible that higher doses of SB 242084 might have induced reinstatement effects in rodents, which would more accurately mirror our current findings.

Although the exact mechanisms underlying the reinstatement of previously-extinguished drug-maintained operant behavior are not fully understood, one theory posits that cocaine priming induces reinstatement by providing the animal with a discriminative stimulus, i.e. the interoceptive effects of cocaine itself, which is predictive of the subsequent availability of cocaine infusions during the test session (de Wit and Stewart 1981; Spealman et al 1999). In support of this theory, many drugs that generalize to the discriminative-stimulus effects of cocaine also induce reinstatement in animals trained to self-administer cocaine (for review, Spealman et al 1999). Given that 5-HT_{2C}R antagonism enhances the DA-increasing effects of cocaine, we propose that SB 242084 may potentiate cocaine-induced reinstatement by enhancing the discriminative-stimulus effects of the pre-session cocaine prime, which would in turn strengthen the predictive salience of the cocaine prime and ultimately elicit high levels of cocaine-maintained behavior. Likewise, pretreatment with a 5-HT_{2C}R agonist may reduce cocaine-induced reinstatement by attenuating the discriminative-stimulus effects of the cocaine prime. In agreement with this theory, 5-HT_{2C}R agonism and antagonism have been shown to attenuate and enhance, respectively, the discriminative-stimulus effects of cocaine in rats (Callahan and Cunningham 1995; Filip and Cunningham 2003; Frankel and Cunningham 2004; Filip et al 2006). Unfortunately, studies investigating the effects of selective 5-HT_{2C}R compounds

upon the discriminative-stimulus effects of cocaine in nonhuman primates have yet to be conducted.

The potentiation of cocaine effects following pretreatment with the 5-HT_{2C}R antagonist SB 242084 is likely mediated by an enhancement of cocaine-induced DA increases within the NAcc, as was observed using *in vivo* microdialysis (Chapter IV). Similar enhancements of the neurochemical effects of cocaine within the NAcc have been reported in rodents (Navailles et al 2004). There are two possible mechanisms, which are not mutually exclusive, that may explain how 5-HT_{2C}R blockade enhances the neurochemical effects of cocaine in the present studies. First, SB 242084 may exert its effects by competing with endogenous 5-HT at 5-HT_{2C}Rs located on GABAergic interneurons within the VTA, thereby reducing the normal activation of these cells and functionally disinhibiting neighboring DA neurons. If this were true, then one would predict that pretreatment with SB 242084 alone might be capable of enhancing DA levels. Some of our behavioral data supports this conclusion, as SB 242084 did induce significant behavioral-stimulant effects in squirrel monkeys and maintained self-administration behavior, effects that are typically attributed to increased DA signaling. Additionally, electrophysiological and microdialysis studies in rodents have shown that 5-HT_{2C}R antagonism increases the firing rate of VTA DA neurons and increases DA levels within the NAcc (Di Giovanni et al 1999; Di Matteo et al 1999). However, in the present study we failed to see any significant effects of SB 242084 pretreatment on basal DA levels within the NAcc during the 30-min prior to cocaine administration. Future studies are needed to more systematically evaluate the effects of SB 242084 pretreatments on DA levels within the NAcc of nonhuman primates in the absence of other pharmacological manipulations. The second possible mechanism by which SB 242084 enhances the behavioral and neurochemical effects of cocaine relates to the theory that cocaine may self-limit its own DA-elevating effects by increasing 5-HT tone at the 5-HT_{2C}R, and that this

self-inhibiting effect is blocked by pretreatment with a 5-HT_{2C}R antagonist. Although still a matter of debate, this theory could be tested by assessing the effects of SB 242084 pretreatment upon the DA-increasing effects of a DAT-selective reuptake inhibitor, such as GBR 12909 or RTI-177, which lack appreciable affinity at the SERT and should therefore not be self-limiting through 5-HT_{2C}R activation. Interestingly, pretreatment with the nonselective 5-HT_2R antagonist ritanserin enhanced the behavioral-stimulant and reinforcing effects of GBR 12909, an effect which may attributable to its actions at the 5-HT_{2C}R. However, the lack of adequate receptor subtype specificity with ritanserin prevents a clear understanding of the pharmacological mechanisms involved in those effects. Therefore, if the theory of self-inhibition is valid, pretreatment with a selective 5-HT_{2C}R antagonist, such as SB 242084, should enhance the DA neurochemical effects of cocaine more effectively than those of DAT-selective inhibitors. These are important studies that we hope to conduct in the near future.

The 5-HT_{2C}R antagonist SB 242084 appears to share a behavioral profile similar to other psychostimulants when its observed effects upon responding maintained by operant schedules of stimulus termination and its enhancement of the behavioral and neurochemical effects of cocaine are taken into consideration. Because most psychostimulants function as reinforcers in self-administration procedures, we were curious to see whether intravenous infusions of SB 242084 would maintain responding in squirrel monkeys when substituted for cocaine self-administration. To our knowledge, these studies are the first to assess the direct reinforcing effects of a 5-HT_{2C}R-selective antagonist in any species. Consistent with its suggested behavioral profile, SB 242084 fully substituted for cocaine availability in all subjects tested, maintaining maximal stable rates of responding across three consecutive test sessions that were nearly identical to those maintained by the maximally-effective dose of cocaine (Chapter III). One obvious implication from these results is that 5-HT_{2C}R antagonists may display some degree of

abuse liability in humans. However, additional studies are needed to better understand the reinforcing effects of 5-HT_{2C}R antagonists under a variety of experimental conditions before conclusions about its reinforcing strength and abuse liability are drawn. For example, we only tested the reinforcing effects of SB 242084 in the present study, but the effects observed should be replicated with other 5-HT_{2C}R antagonists and/or inverse agonists to confirm the specific pharmacological mechanism of action. Additionally, we halted SB 242084 self-administration once responding had stabilized across three days, yet it is possible that responding would have eventually extinguished if the exposure were prolonged. Therefore, future studies will need to investigate the reinforcing effects of 5-HT_{2C}R antagonists across longer experimental durations. Furthermore, the subjects used in the present study each had extensive histories of cocaine self-administration behavior, and it therefore remains to be determined whether drug-naïve subjects will acquire self-administration responding via 5-HT_{2C}R antagonist availability. We did obtain some evidence to suggest that SB 242084 may not be as robust a reinforcer as compared to cocaine. When subjects' responding is extinguished, reintroducing cocaine availability typically restores response rates to baseline levels within 1-2 sessions. However, following exposure to extinction sessions, availability of SB 242084 did not result in rapid stabilization of responding, and response rates peaked at only ~60% compared to the maximally-effective unit dose of cocaine. These results indicate that the reinforcing effects of SB 242084 may vary across experimental conditions and highlights the need for further study.

Thus far, we have discussed the pharmacotherapeutic potential only of 5-HT_{2C}R agonists, as pretreatment with Ro 60-0175 functionally antagonized the abuse-related effects of cocaine. However, there is a second, fundamentally-opposed approach to the treatment of cocaine abuse which is relevant in the present discussion. This strategy is commonly referred to as "substitute agonist" therapy, and is based upon the premise that compounds which share the

pharmacological and behavioral profile of cocaine will attenuate rates of cocaine intake in both animals and humans. Similar strategies have proven successful clinically to curtail opiate use (e.g. methadone, buprenorphine) and tobacco use (nicotine replacement). Because the reinforcing effects of cocaine are mediated by indirect elevations of DA via reuptake inhibition, research investigating candidate substitute agonists for the treatment of cocaine abuse have targeted two main pharmacological mechanisms of action. Some studies have proposed the use of direct-acting DA-receptor agonists, which would mimic cocaine-mediated indirect activation of postsynaptic DA receptors, whereas others have proposed the use of monomaine reuptake inhibitors or releasers, which more closely approximate the pharmacological action of cocaine to indirectly elevate monoamine levels. Regardless of pharmacological mechanism, a substitute agonist would be expected to 1) share some of the behavioral and neurochemical effects of cocaine, and 2) produce selective, prolonged reductions of cocaine intake (Howell and Wilcox 2001; Grabowski et al 2004). Previous studies have demonstrated that rationally-designed agonist medications can selectively attenuate cocaine-maintained responding in animals (Negus 2003; Negus and Mello 2003a; Negus and Mello 2003b; Rothman et al 2005; Howell et al 2007; Negus et al 2007) and also reduce cocaine intake in human subjects (for review, Grabowski et al 2004). Perhaps the most obvious disadvantage to using substitute agonist medications however is their abuse liability, due to their inherent effects upon dopaminergic signaling within mesolimbic and/or nigrostriatal pathways. Furthermore, these compounds could have an additive or synergistic effect if combined with cocaine, therefore enhancing the adverse cardiovascular and psychological (e.g. psychotomimetic) effects of cocaine and possibly cause severe health consequences. Nevertheless, to reduce drug-seeking in human cocaine abusers is beneficial as these behaviors are often accompanied by equally-deleterious consequences, such as physiological illness and malnutrition, disruption of social relationships, disease transmission,

exhaustion of monetary resources, or imprisonment, and therefore substitute agonist replacement therapy should be considered as a viable option.

SB 242084 appears to satisfy the first criterion for a candidate agonist therapy, as it modestly mimicked the behavioral-stimulant effects of cocaine. Furthermore, pretreatment with SB 242084 enhanced cocaine-induced reinstatement, a finding which may be indicative of shared discriminative-stimulus effects with cocaine. Whether chronic treatment with SB 242084 or related 5-HT_{2C}R antagonists would reduce cocaine self-administration, however, remains to be determined. Given that 5-HT_{2C}R antagonism enhanced all other behavioral effects of cocaine in the present study, it is in fact possible that SB 242084 might actually enhance, rather than attenuate, the reinforcing effects of cocaine. Indeed, the potentiation of the reinforcing effects of cocaine by SB 242084 pretreatment has been described previously in rodents, although it should be noted that these effects followed acute, rather than chronic, administration of SB 242084 (Fletcher et al 2002). Additionally, SB 242084 demonstrated some degree of abuse liability in the present studies as it substituted for cocaine self-administration behavior. However, differences between the reinforcing effects of cocaine and SB 242084 were revealed when the latter was substituted for cocaine following a brief period of extinction training, suggesting that the reinforcing effects of 5-HT_{2C}R antagonists may be slightly weaker than cocaine under conditions when the subjects did not have recent exposure to cocaine self-administration. Despite the possibility of its abuse liability, our results suggest that an evaluation of 5-HT_{2C}R antagonists as candidate substitute agonist therapies for cocaine abuse is warranted.

In addition to their investigation as novel targets for the treatment of substance abuse, 5-HT_{2C}Rs have also recently been indicated as a pharmacotherapeutic target for several other psychiatric disorders (Lee et al 2010). For example, in preclinical rodent studies, 5-HT_{2C}R-

selective compounds have been evaluated as possible medications for obesity, schizophrenia, affective disorders, and neurodegenerative diseases such as Alzheimer's and Parkinson's Diseases (for review, Lee et al 2010). 5-HT_{2C}R-selective antagonists in particular have demonstrated antidepressant-like effects (Dekeyne et al 2008), enhanced the antidepressant-like effects of SSRIs (Cremers et al 2004), and displayed an anxiolytic-like array of behavioral effects (Dekeyne et al 2008; Burghardt et al 2007; Harada et al 2006; Kantor et al 2005). Given that 5-HT_{2C}R antagonists are being investigated as novel medications for the treatment of depression and anxiety disorders in humans, it is critical to note that these conditions may often present as comorbid with substance abuse, which can complicate both diagnosis and treatment (Regier et al 1990; Brady & Verduin 2005). One might therefore contemplate a deleterious situation in which 5-HT_{2C}R antagonists are utilized clinically to treat one or more affective disorders, but simultaneously exacerbate a concurrent condition of drug abuse or dependence. In contrast, if 5-HT_{2C}R antagonists can function as effective substitute agonist therapeutics, they may represent a novel medication that could actually be used to treat both affective and substance abuse disorders. Clearly further research is needed to clarify these issues, but our present results suggest that the preclinical evaluation of 5-HT_{2C}R antagonists should proceed cautiously given the possible abuse liability and cocaine-enhancing effects of these compounds.

In addition to the clear modulatory role of the 5-HT_{2C}R, studies have demonstrated that blockade of the 5-HT_{2A}R functionally antagonizes some behavioral effects of cocaine in rodents, including cocaine-induced hyperlocomotion and cocaine-induced reinstatement (McMahon and Cunningham 2001; Fletcher et al 2002; Filip et al 2004; Nic Dhonnchadha et al 2009), effects which appear to be mediated by 5-HT_{2A}Rs within the VTA (McMahon et al 2001). However, in the present studies, pretreatment with the selective 5-HT_{2A}R antagonist M100907 failed to alter the behavioral-stimulant or reinstatement effects of cocaine in squirrel monkeys at doses which

were previously shown to be behaviorally active in nonhuman primates (Fantegrossi et al 2002; Fantegrossi et al 2009). These results likely indicate that 5-HT_{2A}R blockade does not appreciably modulate DA activity within the mesolimbic system. In fact, M100907 pretreatment showed a weak trend of enhancing the behavioral-stimulant effects of cocaine (Chapter II). Although these effects were not significant, they may reflect a pharmacological action of 5-HT_{2C}R antagonism by M100907, rather than 5-HT_{2A}R antagonism, in the present study. A similar upward trend was apparent in our reinstatement study (Chapter III). Although M100907 is nearly 100-fold more selective for the 5-HT_{2A}R vs. the 5-HT_{2C}R, its administration in our studies produced mild enhancements of two behavioral effects of cocaine that were consistent with the effects of a selective 5-HT_{2C}R antagonist. We therefore propose that, with regard to cocaine-mediated effects in nonhuman primates, the modulatory impact of 5-HT_{2A}R antagonism is negligible. One possible explanation for this result, described in detail in Chapter II, is an apparent species difference with respect to 5-HT_{2A}R distribution within DA systems of rodents and nonhuman primates (Lopez-Gimenez et al 1999; Hall et al 2000; Ikemoto et al 2000; Lopez-Gimenez et al 2001b). Taken together, our findings do not support the use of selective 5-HT_{2A}R antagonists as pharmacotherapeutics for cocaine abuse.

In previous studies, our laboratory investigated the behavioral and neurochemical effects of cocaine in squirrel monkeys following pretreatment with nonselective 5-HT_2R agonists and antagonists. For example, administration of the nonselective 5-HT_2R agonist quipazine insurmountably attenuated the behavioral-stimulant effects of cocaine (Howell and Byrd 1995) and the DAT-selective inhibitor GBR 12909 (Howell et al 1997), and also reduced the reinforcing and neurochemical effects of cocaine (Czoty et al 2002). In contrast, administration of the nonselective 5-HT_2R antagonists ritanserin or ketanserin enhanced the behavioral-stimulant and reinforcing effects of cocaine (Howell and Byrd 1995) and GBR 12909 (Howell et al 1997). The

results from our present studies have helped to clarify the specific receptors mediating those previously-observed effects. For example, pretreatment with the selective 5-HT_{2C}R agonist Ro 60-0175 mimicked the effects of the nonselective agonist quipazine, suggesting that the effects of quipazine were mediated via actions at the 5-HT_{2C}R. Accordingly, the effects of the nonselective 5-HT_2R antagonists in our previous studies were replicated by pretreatment with the selective 5-HT_{2C}R antagonist SB 242084, but not by the selective 5-HT_{2A}R antagonist M100907. This is consistent with the hypothesis that 5-HT_{2A}Rs in squirrel monkeys are not effective modulators of DA signaling or the dopaminergic effects of cocaine and related psychostimulants. Therefore, our present results reflect a high degree of intra-laboratory consistency across 15 years of research with serotonergic compounds and their effects upon psychostimulant-induced behavioral and neurochemical effects.

In summary, the overall purpose of the experiments described presently was to assess for the first time the impact of selective 5-HT_{2C}R and 5-HT_{2A}R compounds upon the behavioral and neurochemical effects of cocaine in nonhuman primates. Our results indicate that pretreatment with the preferential 5-HT_{2C}R agonist mCPP or the selective agonist Ro 60-0175 functionally antagonized the abuse-related behavioral effects of cocaine in squirrel monkeys and selectively reduced cocaine-induced DA increases within the mesolimbic system, and greater receptor-subtype selectivity resulted in greater behavioral specificity of effects. Accordingly, pretreatment with the selective 5-HT_{2C}R antagonist SB 242084 enhanced the behavioral effects of cocaine and selectively potentiated cocaine-induced DA increases within the mesolimbic system. Furthermore, SB 242084 produced modest behavioral-stimulant effects when administered alone, and we have now demonstrated for the first time that a 5-HT_{2C}R antagonist substituted for cocaine self-administration, indicative of a potential for abuse liability in humans. Other results indicated that 5-HT_{2A}R antagonism does not modulate the behavioral effects of

cocaine in squirrel monkeys. Based upon these findings, we suggest that 5-HT_{2C}R agonists may be useful pharmacotherapeutics for the treatment of cocaine abuse as these compounds functionally antagonize the behavioral and neurochemical effects of cocaine, while 5-HT_{2C}R antagonists/inverse agonists may warrant consideration as candidate substitute agonist therapies for cocaine abuse.

Chapter VI. References

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