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[Maylen Perez Diaz]

Date

**Serotonin 5-HT_{2C} Receptor Modulation of Compulsive and Addictive Behavior
in Female Rhesus Macaques**

By

Maylen Perez Diaz

Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences

Neuroscience

Leonard L. Howell, Ph.D.
Advisor

Mark E. Wilson, Ph.D.
Advisor

Jocelyne Bachevalier, Ph.D.
Committee Member

Michael Owens, Ph.D.
Committee Member

Jonathon Nye, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

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B.S., University of California, Los Angeles, 2011

Advisor: Leonard L. Howell, Ph.D.

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Abstract

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Addiction is a significant public health issue, yet there are limited FDA-approved treatments for certain addictive disorders and none for addiction in general. Identifying the core behavioral and neurochemical processes contributing to an addictive phenotype can inform the development of novel therapeutics to treat multiple addictions. Compulsivity has been highly implicated in the etiology of addiction and reported to be higher among addicted individuals compared to controls. Despite this evidence, the effects of long-term engagement in addictive behaviors on compulsivity have not been tested. Although studies provide evidence that serotonin 5-HT_{2C} receptors are involved in the expression of both compulsive and addictive behaviors, the extent to which these behaviors are regulated in parallel is unknown and the exact effects of 5-HT_{2C} receptors on compulsivity remain unclear. Therefore, the following set of studies employed two types of reinforcers, a high caloric diet (HCD) and methamphetamine (METH), to investigate the effects of long-term intake of these reinforcers on general compulsive behavior in female rhesus macaques. In addition, the present study evaluated the effects of a 5-HT_{2C} receptor agonist, WAY163909 on baseline compulsivity, compulsivity following long-term reinforcer intake, drug intake and drug-seeking of METH, and intake of two different diets. Extended intake of METH or a HCD increased general compulsive behavior. Baseline compulsive behavior was not predictive of future METH or HCD intake, but intake of these reinforcers was predictive of post-intake increased compulsivity. In one study, WAY163909 decreased compulsivity (at baseline and after long-term reinforcer intake) and intake of a HCD. These effects were blocked by the 5-HT_{2C} receptor antagonist SB 242084, demonstrating that the 5-HT_{2C} receptor is necessary for the effects of WAY163909. In the final study, WAY163909 dose-dependently decreased METH intake and METH-induced reinstatement, as well as METH-induced dopamine overflow in the nucleus accumbens. These findings are important because they provide evidence that (1) long-term intake of a drug or food reinforcer increases compulsivity, with the amount of reinforcer intake being predictive of the increase in compulsivity, and (2) 5-HT_{2C}Rs play a crucial role in both compulsivity and reinforcer intake, as activation of these receptors decreases both measures, regardless of the reinforcer or whether compulsivity is measured at baseline or after prolonged intake of reinforcers. The results suggest that agonists at the 5-HT_{2C}R may represent a promising new avenue for treatment of addiction in general.

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Chapter 1. Introduction

1.1 Public Health Relevance of Addiction

Addiction is a significant public health issue affecting hundreds of millions worldwide and costing the U.S. over \$600 billion yearly (Harwood, 2000, 2004; Office of National Drug Control Policy, 2004; Wickizer, 2013). The accumulated costs to the individual, the family, and the community are staggering and arise because of many direct and indirect effects, including compromised physical and mental health, increased spread of infectious disease, loss of productivity, reduced quality of life, increased crime and violence, health care costs, and death (Office of National Drug Control Policy, 2004). Despite the need for effective therapeutics, there is currently no FDA-approved treatment for addiction in general. Twelve Step Programs have had limited success, with relapse rates remaining as high as 50-90% (Carnes, 1983; Brownell, 1986; Schaffer & Zimmerman, 1990; Hodgins & el-Guebaly, 2004; Abdollahi et al., 2014).

Growing evidence supports the existence of an addictive phenotype (Blum et al., 2012). However, most FDA-approved treatments for addictive disorders treat these independently by addressing the symptoms that emerge from abusing the specific substance, but not the underlying factors contributing to the disorder (Shaffer et al., 2004; Richter & Foster, 2014). This approach has hindered advancement in the field because it does not account for co-morbidity between diverse types of addictions (Hatterer, 1982; Carnes, 1983; Schaffer & Zimmerman, 1990; Shaffer et al., 2004; Black, 2007; Blum et al., 2012) and ignores their shared neurobiology (Volkow et al., 2013b). People can become addicted to different reinforcers, including drugs of abuse and palatable, high caloric foods (Shaffer et al., 2004), as well as behaviors that are reinforced, such as gambling (Karim & Chaudhri, 2012) and sex (Blum et al., 2012), often simultaneously. When one type of addiction is treated in isolation, or inadequately, it tends to be replaced by another

(Shaffer et al., 2004; Blum et al., 2012). Thus, novel treatment strategies that prevent replacement and address comorbid addictions would be greatly beneficial. Identifying and understanding the core processes contributing to an addictive phenotype, rather than the symptomatic expression of those processes, which vary across individuals and even within individuals across time, can inform the development of novel therapeutics to treat multiple addictions.

1.2 The Addictive Phenotype

1.2.1 Reinforcement and Factors Implicated in Addiction

Although no single factor, either at the individual, family, or community level, can determine whether an individual will develop an addictive disorder, there are numerous factors that are thought to confer vulnerability to these conditions and have been shown to contribute to both the onset and progression of addiction. The most important factor that contributes to the risk of developing an addiction is exposure to a reinforcer or engagement in a reinforced behavior (Office of National Drug Control Policy, 2004). In humans, this can be any substance or experience that an individual finds rewarding within a given context. Food consumption, for instance, can be pleasurable in some contexts (ex. when one is hungry or when the food is highly palatable) because certain macronutrients, such as sugar, can cause release of dopamine (DA) in the striatum. Individuals may then increase intake of such macronutrients in the future in response to, or with the purpose of attaining, the pleasurable feeling associated with this DA release. Many reinforcing substances and reinforced behaviors are related to biological processes important to

the survival of the organism (Ferster & Skinner, 1957), such as food and reproduction. However, the circuitry mediating reinforcement can be activated by substances or behaviors unimportant for survival or an individual's evolutionary fitness, such as drugs of abuse and gambling (Volkow et al., 2011b, 2013b). Moreover, an individual can excessively engage in behaviors or intake of substances that are important for survival, leading to imbalance within the circuitry that mediates reinforcement and increasing that individual's risk of developing an addictive disorder (Volkow et al., 2013b). Thus, a common feature of addiction in general is that the reinforcing substance or reinforced behavior can cause neurochemical changes that positively affect mood (Schaffer & Zimmerman, 1990; Izquierdo & Jentsch, 2011). Extended intake of these reinforcers or engagement in reinforced behaviors can sometimes lead to withdrawal symptoms following short-term abstinence, as is seen with nicotine and, more severely, with opioid addicts (Carnes, 1983; Schaffer & Zimmerman, 1990). In preclinical animal models, the term reinforcer has a much more specific definition; specifically, it is any stimulus that increases the probability that a given response or behavior will occur in the future (Ferster & Skinner, 1957).

Stress is considered to be one of the most significant factors contributing to an individual's risk for developing addiction (Cadet, 2016). Early life stress and stressor exposure in adolescence or adulthood are common risk factors for both onset and relapse to addiction (Hatterer, 1982; Schaffer & Zimmerman, 1990). Early life stressors can include physical, emotional, and sexual abuse; neglect, household instability (Dube et al., 2003), and poverty (Najavits et al., 2017). Epidemiological studies have shown that patients with a history of trauma, especially during childhood and adolescence, have a higher prevalence of addictive disorders than controls (Breslau, 2002; Messina et al., 2008; Kendler et al., 2014; Patchev et al., 2014). Interestingly, there

is a relationship between the severity and length of early life stress and future drug dependency (Luyten et al., 2008; Enoch, 2011) and food addiction (Mason et al., 2014). Further, evidence from preclinical studies supports the notion that acute and chronic exposure to stressors increases initiation and escalation of drug use (for extensive review, see Sinha 2008) and food intake of a palatable, high caloric diet (Michopoulos et al., 2012b, 2016). The increased risk of addiction due to stressful events may be mediated by the effects of these experiences on brain structure and function (Cadet, 2016).

There is convincing evidence that both acute and chronic stress can negatively impact the epigenome in rodents by altering expression of brain-derived neurotrophic factor (BDNF) and histone acetylation of BDNF and other factors associated with neuronal cell structure stability and formation (Fuchikami et al., 2009; Chakravarty et al., 2014; Kenworthy et al., 2014). These factors, when altered in brain regions contributing to either reinforcement or decision making, can have detrimental effects on behavior and drive it toward an addictive phenotype (Cadet, 2016). Stress exposure also changes signaling within brain areas implicated in addiction that are responsible for reinforcement, decision-making, and motivation (Grant et al., 1998; Ichise et al., 2006; Michopoulos et al., 2016).

In addition to stress, other environmental and genetic factors have been identified that are associated with addiction. Experimenting with substances of abuse during adolescence increases the risk of developing a substance use disorder later in life, and the earlier the exposure, the greater the risk (Hanson et al., 2011). Approximately 74% of 18- to 30-year olds admitted to treatment programs began using substances at the age of 17 or younger (Substance Abuse and Mental Health Services Administration, 2014). Adolescence is a critical vulnerability period for

development of addiction because the brain undergoes significant changes during this life stage that make it particularly vulnerable to substance exposure (Giedd et al., 1999). Specifically, the prefrontal cortex (PFC) does not fully develop until the early to mid-20s, and research shows that substance use during adolescence affects development of this area critical for decision-making and behavioral inhibition (Squeglia et al., 2015).

Not all adolescents who experiment with substances of abuse go on to develop a substance use disorder, but evidence suggests that those who do progress to more harmful abuse may have pre-existing conditions that increase their risk of addiction (US Department of Health and Human Services, 2016). For instance, individuals with a variety of mental health disorders are at a higher risk of developing a substance use disorder (Grant et al., 2004a, 2004b). Moreover, genetic factors are thought to account for 40-70% of individual differences in addiction risk (Prescott & Kendler, 1999; Schuckit et al., 2001). Some of these genetic factors are associated with metabolism of certain substances, such as alcohol or cocaine, or involve receptors and other proteins associated with key neurotransmitters involved in all parts of the addiction cycle, whereas others are involved in strengthening the connections between neurons and forming new drug memories (Drgonova et al., 2015; Zhong et al., 2015). As with other chronic health conditions, substance use disorders are influenced by a complex interplay between genes and environment and additional study on how these factors interact and their underlying mechanisms would provide insight into how addictive disorders develop and how they can be prevented and treated.

1.2.2 Neurocircuitry and Neurochemistry Implicated in Addiction

Decades of research have yielded a robust understanding of the circuits and signaling molecules in the brain that play key roles in addiction. Though much of this work has focused on drug addiction, recent evidence suggests that this neurocircuitry and neurochemistry may be involved in addiction in general (Volkow et al., 2011c). Evidence from preclinical and clinical studies has demonstrated that changes in mesolimbic dopaminergic neurocircuitry, connecting the ventral tegmental area (VTA), ventral striatum, and PFC, underlie several addictive disorders (Ito et al., 2004; Wise, 2004; Sesack & Grace, 2010; Everitt & Robbins, 2013; Koehler et al., 2013; Volkow et al., 2013b).

Support for the PFC as one of the major brain regions mediating the addiction process has been provided by imaging studies (Volkow & Fowler, 2000). These studies paint a complex picture of the role that various PFC regions play in cognition as it relates to addictive behaviors. The PFC is involved in executive function, including inhibitory control, decision making, emotional regulation, purposefulness, motivation, and salience attribution, among others, and disruption of this region can have profound consequences on this wide range of behaviors (Volkow et al., 2006). Corticostriatal networks modulated by DA allow the PFC to work in tandem with the striatum to affect behaviors relevant to addiction (Haber, 2003). Corroborating preclinical studies of chronic drug self-administration, PET studies in drug addicted humans have found significant reductions in dopamine D2 receptor availability in the striatum (Volkow et al., 2009), and these are associated with decreases in the activity of several PFC regions, including the orbital PFC (oPFC), which is involved in salience attribution and goal-directed behaviors, the anterior cingulate cortex (ACC), which regulates inhibitory control and awareness, and the dorsolateral PFC (dlPFC), involved in higher cognitive operations and decision making (Volkow et al., 1993,

2001; 2007). It is hypothesized that improper DA modulation of these PFC regions in addicted subjects underlies the addict's loss of control over engagement in the addictive behavior or intake of the addictive substance (Volkow & Fowler, 2000).

Palatable foods, high in sugar and fat, activate the reward pathway similarly to drugs of abuse (Liu et al., 2010; Davis et al., 2011). Specifically, anticipation and ingestion of highly palatable foods increase extracellular DA levels in the nucleus accumbens (NAcc), a ventral region of the striatum (Hernandez & Hoebel, 1988; Rada & Hoebel, 2000), as do drugs of abuse (Manvich et al., 2012a; Murnane, 2013; Volkow et al., 2013b). Moreover, DA plays a vital role in the reinforcement of behaviors such as sex (Ikemoto & Panksepp, 1999; Becker et al., 2001; Hull et al., 2004) and gambling (Steeves et al., 2009; Voon et al., 2010). Chronic exposure to reinforcers or engagement in reinforcing behaviors can lead to impairments in the DA system, including a reduction in baseline levels of DA function and an attenuation of the typical increases in DA neurotransmission elicited by normal rewarding stimuli (Koob & Le Moal, 2001; Wise, 2004). Availability of D2 receptors, which are well known to mediate the reinforcing properties of DA, is reduced within the striatum of obese individuals (Wang et al., 2001), as well as drug addicts. These adaptations can induce a hypodopaminergic state, contributing to ongoing reinforcer intake, or engagement in reinforced behaviors, and relapse, as evidenced by behavioral and imaging studies (Volkow et al., 2013b).

Serotonin (5-HT) signaling can regulate dopaminergic neurotransmission and thus has also been highly implicated in addiction (Howell & Cunningham, 2015). Serotonergic cell bodies originating in the raphe nuclei synapse directly onto both DA and non-DA neurons in the ventral tegmental area (Herve et al., 1987). They also project to brain areas where DA is released, such

as the NAcc, dorsal striatum (caudate and putamen), and PFC, all of which are involved in addiction (Parent et al., 1981; Halliday & Tork, 1989; Di Matteo et al., 2008). Through these direct and indirect interactions with the DA system, the 5-HT system can influence the effects of reinforcers or behaviors that are reinforced. However, 5-HT's effects on DA neurotransmission depend on both the brain region and the 5-HT receptor subtype involved (Alex & Pehek, 2007; Hayes & Greenshaw, 2011).

Activation of 5-HT_{2C}R reduces both drug (Pentkowski et al., 2010) and food consumption (Hess & Cross, 2013; Hoy, 2013). 5-HT_{2C}Rs are poised to increase inhibitory signals in the ventral striatum as they are widely expressed by GABAergic interneurons in the VTA and the NAcc. They are also highly expressed on both glutamatergic and inhibitory interneurons in the PFC. Thus, activation of these receptors should lead to inhibition of DA signaling, decreasing reinforcer intake. Overconsumption of palatable foods can also decrease 5-HT neurotransmission, as cerebral spinal fluid (CSF) levels of 5-HT and its metabolites are lower in obese compared to lean women (Strombom et al., 1996) and subcortical 5-HT transporter (SERT) availability in humans was negatively correlated to body-mass index (BMI) (Erritzoe et al., 2010). Psychostimulants can have similar effects, with amphetamine-based drugs reducing 5-HT metabolite levels in rodents (Roger-Sanchez et al., 2013). Due to its ability to modulate DA signaling, changes in 5-HT could disrupt an already dysfunctional reward circuit, further contributing to addiction. It is important that more studies focus on the role that 5-HT and its receptors play in the development of addictive behaviors and their underlying neurochemistry.

1.3 Overview of Compulsivity

1.3.1 Compulsivity in Addiction

Compulsive actions represent a lack of inhibition that can lead to maladaptive behavioral strategies. These behaviors, however, are not uniformly dysfunctional and have evolved because in some situations they may be advantageous. Although compulsive behaviors can be beneficial in certain situations, in other situations they can become maladaptive and lead to negative consequences or disadvantageous outcomes. In many cases, behaviors that start off as impulsive or goal-directed, such as experimenting with drugs to feel euphoria, can become compulsive after prolonged engagement. In fact, one of the hallmarks of addiction is compulsive engagement in an activity or intake of a substance, despite increasing insensitivity to the positive subjective effects and compounding negative consequences (Everitt & Robbins, 2005; 2016; Torregrossa et al., 2011). Although compulsivity can manifest itself within the context of addiction, compulsive behaviors are a natural part of our behavioral repertoire. There are individual differences in general compulsivity that are unrelated to addiction, and manifest in the context of normal experiences and activities. However, in the following discussions, the focus will be on compulsivity within the context of addiction.

It has been shown in humans that compulsivity and addictive behaviors (American Society of Addiction Medicine, 2011) are related, with high compulsivity being reported in cocaine-dependent (Fernandez-Serrano et al., 2011) and obese individuals (Cserjesi et al., 2007), as well as pathological gamblers (Leeman & Potenza, 2012). Additionally, compulsivity is considered an etiological factor in the development and maintenance of addiction, being the final stage in a transition from recreational use of reinforcers, through habits, and finally to compulsive use (for a detailed review see Everitt & Robbins, 2005; 2016; Torregrossa et al., 2011). A transition from

top-down control by the PFC to bottom-up control by the striatum over decision-making and behavior is hypothesized to underlie this progression (Belin et al., 2013).

1.3.2 Defining Compulsivity

Compulsivity may be defined as a tendency to act persistently and repetitively without it leading to an actual reinforcer. This pattern of behavior has been referred to as a “perseverative syndrome” (Butter, 1969), involving an inability to alter established behavioral strategies despite changes in response-reinforcer contingencies (Schultz & Searleman, 2002), and is now described in terms of compulsive behavior (Torregrossa et al., 2008). Compulsivity has been thought to emerge as a means of harm-avoidance, to prevent negative consequences, in addition to being a means of obtaining a reinforcer (Fattore & Melis, 2016). Some examples of these harm-avoidance compulsive behaviors present in patients with obsessive-compulsive disorder (OCD) are repetitious talking or actions such as cleaning, hoarding, or nail biting. Patients with eating disorders, especially binge-eating disorder, and trichotillomania, in which people compulsively pull out their hair, also exhibit greater compulsive tendencies than the general population (Fattore & Melis, 2016). These behaviors are not specifically associated with positive reinforcement, but continue to be expressed repetitively. They may serve to reduce anxiety, however, not all compulsive behaviors are related to harm-avoidance.

Here, the focus is on one such specific component, contingency-related cognitive inflexibility, which entails impaired adaptation of behavior after negative feedback (Fineberg et al., 2014). In other words, individuals exhibit perseverative (persistent and repetitive) responding despite evidence that their responses are no longer reinforced or provide some positive outcome

(i.e. these behaviors were once reinforcing, but now there is an absence of reinforcement). Thus, this component of compulsivity is characterized by stimulus-driven instead of goal-directed behaviors. In the rodent literature, this type of behavior is generally referred to as habitual (Yin & Knowlton, 2006; Gourley et al., 2013).

Examples of this type of compulsive behavior can be seen in people that suffer from addiction. Drug addicts often overdose because they take drug compulsively, either despite already experiencing the drug “high” or in an attempt to increase the “high” once it begins to dissipate. At high doses, substances of abuse begin to exhibit aversive subjective and health effects, but addicts continue to use these reinforcers despite these negative consequences, event to the point of overdose. Previous experience and the explicit requirement for an adaptive change to an established behavioral strategy play a key role in this component of compulsivity.

1.3.3 Behavioral Measures of Compulsive Action

Within the construct of compulsivity, the subcomponent focusing on contingency-related cognitive inflexibility is most often demonstrated experimentally using reversal learning tasks that require a subject to alter established response strategies when outcome contingencies change (Fineberg et al., 2014). The most common type of task used to measure perseverative responding in both humans and laboratory animals is known as a Discrimination Reversal Learning Task (DRLT) (Fig. 1). This task can be administered using a Wisconsin General Testing Apparatus (WGTA) or a touch screen, but regardless of its execution, it is designed to assess a subject’s ability to adapt to changes in response-reinforcer contingencies. There are many

iterations of this task, but the most basic form employs a WGTA (Rogers et al., 2000; Izquierdo & Jentsch, 2012).

For the DRLT, subjects are presented with two objects, each of which is covering a well in which a treat can be hidden. During the first stage of testing, the acquisition stage, one of the objects will be paired with the reinforcer. Acquisition of the discrimination continues until the subject reaches some performance criterion. After meeting these criteria, they undergo a reversal in which the treat becomes associated with the other previously un-reinforced object. The primary measure of compulsivity is the number of perseverative errors (when the subject chooses the previously reinforced object) made during reversal. If three or more objects are used for this task, measures of random errors can also be taken that can be used as controls for determining the specificity of an effect (due to some disorder, environmental change, lesion or pharmacological intervention) on perseverative responding. For instance, if a drug treatment decreases perseverative errors, but not random errors, this would suggest that the treatment specifically affects compulsivity and is not generally decreasing behavior or inhibiting nonspecific discrimination learning.

The contingency-related cognitive inflexibility component of compulsivity can also be tested using a common task for measuring habitual behavior in rodents known as Contingency Degradation (CD). For contingency-degradation (Fig. 2), subjects are trained to respond (usually on nose-poke apertures) for a food reinforcer. They are trained to respond on both nose-pokes for treats until they acquire the behavior. Then, one of the nose-pokes becomes degraded such that reinforcers appear randomly and are not directly a consequence of responding. Thus, when subjects respond on the degraded nose-poke, their responses are no longer predictive of a

reinforcer. After one of the nose-pokes has been degraded, subjects undergo an extinction test day in which both nose-pokes are available for responding, though no reinforcers are ever presented, and they have a choice of whether to respond on the degraded or non-degraded aperture. Subjects that do not show a preference for the non-degraded aperture are said to display “habitual” stimulus-driven behavior, as opposed to goal-directed behavior. The strength of the “habit” can be increased by increasing the length of initial training on this task.

There are two key differences between contingency degradation and reversal learning tasks. The first is that in CD, during the session where one of the apertures is being degraded, when subjects respond on the degraded aperture, they are still encountering reinforcers despite their responses not being directly predictive of the reinforcer. In reversal learning tasks, during the reversal phase, only when subjects choose the newly rewarded object do they encounter the reinforcer. Secondly, during the extinction test in CD, neither of the apertures is being rewarded, despite responding, whereas in reversal learning tasks, during reversal, the newly rewarded object is reinforced.

1.3.4 Neuroanatomical Components of Compulsivity

Compulsive actions can be divided into multiple dimensions and present with dysfunction in a myriad of neural processes such as perception, attention and coordination of cognitive as well as motor function. Recently, Fineberg et al. (2014) reviewed the current literature regarding neuroanatomical models of compulsivity, outlining the evidence for a distinct cortico-striatal circuit that regulates these actions. They hypothesize that within this circuit, there appears to be a striatal component that drives behavior and a prefrontal component that inhibits behavior.

Thus, increased activity in the striatal component of this circuit and/or decreased activity in the prefrontal component should lead to a heightened tendency to behave compulsively in a specific behavioral context due to increased drive for and/or decreased inhibition of these behavioral tendencies. However, some evidence from behavioral studies discussed below suggests that decreased, rather than increased, activity in the striatal component of this circuit may increase compulsive actions.

Compulsivity is regulated by the circuit comprised of both the ventral and dorsal striatum (caudate/putamen), and oPFC, which exerts inhibitory control. Lesions (Castañe et al., 2010), as well as inactivation using local anesthetic (Ragozzino et al., 2002), of the dorsal striatum in rats produce a significant increase in compulsivity, as they display impairments in reversal learning. The NAcc/ventral striatum plays a role in the expression of compulsive behavior. Ventral striatum lesions caused by electrolysis (a technique in which electric stimulation leads to neuronal death) in rhesus monkeys (Divac et al., 1967) and excitotoxic lesions in marmoset monkeys (Clarke et al., 2008) impair reversal learning on discrimination tasks. The evidence for the oPFC's role in compulsivity spans decades of research and is quite compelling.

Several lines of research have demonstrated that rats, cats, mice, marmosets (Dias et al., 1996), monkeys (Settlage et al., 1948; Butter et al., 1963; Butter, 1969), and humans with oPFC damage are slower to acquire reversals, making more perseverative errors (Bechara et al., 1997; Ferry et al., 2000; Chudasama and Robbins, 2003; Fellows and Farah, 2003; Schoenbaum et al., 2003; Izquierdo et al., 2004; Bissonette et al., 2005). Gourley et al. (2013) found that mice with oPFC-targeted knockdown of brain derived neurotrophic factor (BDNF) or symmetric lesions separating the oPFC from the striatum were insensitive to contingency degradation and exhibited

impaired reversal learning. The dlPFC appears to not play a role in the expression of compulsivity, as humans with bilateral lesions of the dlPFC exhibit no impairments in reversal learning (Hornak et al., 2004). On the other hand, lesions of the ventral medial prefrontal cortex (vmPFC) have been shown to impair reversal learning in monkeys (Iversen & Mishkin, 1970). Thus far, no studies have evaluated the effects of the right inferior frontal gyrus (RIFG) on perseverative responding during a discrimination reversal learning task. There is some evidence, however, suggesting that lesions of thalamic nuclei have no effect on perseveration during reversal learning (Chudasama et al., 2001).

1.3.5 Neurochemistry and Pharmacology of Compulsive Behavior

Altered DA neurotransmission is involved in compulsivity, as it is with addictive behaviors. The specific effects of these alterations appear to depend both on the type of alteration and the brain region involved. For instance, optogenetic excitation of DA neurons in the VTA, a key part of the mesolimbic DA circuit implicated in addiction, improves reversal learning performance in rats (Adamantidis et al., 2011; Rossi et al., 2013). Conversely, prenatal exposure to psychostimulants, which increase DA signaling, in mice (Lloyd et al., 2013) and rats (Son et al., 2013) increases perseverative behavior (i.e. compulsivity). In patients receiving dopaminergic medication for the treatment of Parkinson's Disease, chronic exposure to DA agonists is associated with an increase in compulsive behaviors, including pathologic gambling, compulsive shopping, and compulsive eating, among others (Raja & Bentivoglio, 2012).

5-HT signaling has also been implicated in the expression of compulsive behavior, as inhibition of central 5-HT reuptake using citalopram increases compulsivity in rodents

(Chamberlain et al., 2006). Conversely, selective depletion of 5-HT in the oPFC increases compulsivity as well (Clarke et al., 2004, 2007). Contingency-related flexibility, for instance, is dependent on serotonergic systems (Fineberg et al., 2014, Clarke et al., 2005). 5-HT depletion from the PFC of the marmoset monkey increases perseverative responding during a Serial Discrimination Reversal (SDR) Task (Rygula et al., 2014). Moreover, 5-HT and SERT levels in the rodent oPFC predict individual variation in reversal learning performance (Stolyarova et al., 2014; Barlow et al., 2015). Studies manipulating 5-HT by selectively destroying and depleting PFC neurons (West et al., 2013; Rygula et al., 2014; Ochoa et al., 2015), centrally blocking 5-HT receptors (Boulougouris & Robbins, 2010) or treating with SSRIs (Furr et al., 2012; Wallace et al., 2014) have consistently shown that reduced 5-HT signaling, particularly in the cortex, increases perseverative responding and impairs reversal learning, whereas increased 5-HT generally facilitates reversal learning (Clarke et al., 2004). Additional research using selective 5-HT receptor drugs has produced mixed results. For instance, selective antagonism of 5-HT_{2A} receptors using M100907 in rats impaired, whereas antagonism of 5-HT_{2C} receptors using SB 242084 facilitated, reversal performance (Boulougouris et al., 2008; Boulougouris & Robbins, 2010; Furr et al., 2012; Nilsson et al., 2012). Others have reported opposite results of SB 242084 on reversal learning (Alsio et al., 2015).

Of all the 5-HT receptor subtypes, the 2A and 2C receptors appear to be involved in compulsivity. Selective antagonism of 5-HT_{2A} receptors leads to an increase in compulsivity. Conversely, the effects of the 5-HT_{2C} receptor on compulsivity are not fully understood. These two 5-HT receptor subtypes are differentially distributed throughout the reward pathway. The 5-HT_{2A} receptor is more highly expressed on DA neurons in the ventral tegmental area (VTA) and

its activation leads to a general increase in DA tone, whereas the 5-HT_{2C} receptor is more highly expressed on GABAergic interneurons in the VTA, the NAcc and the PFC and its activation leads to a general decrease in DA tone (Howell & Cunningham, 2015). In the case of the effects of agonists and antagonists at the 5-HT_{2C} receptor, mixed results may be due to differences in the agonists/antagonists used for the studies, as well as operational differences in how the reversal learning tasks were conducted. Given these conflicting reports, further research is necessary to disentangle the role that the 5-HT_{2C} receptor plays in the expression of compulsive behaviors. The evidence presented here suggests that alterations in DA signaling have an important effect on compulsivity (Adamantidis et al., 2011; Wiskerke et al., 2011; Rossi et al., 2013). However, these effects may depend on the neuroanatomical region in which DA signaling is being altered and which DA receptors are being targeted (Van Gaalen et al. 2006; Floresco et al., 2008).

Compulsivity can also be modulated by pharmacological and genetic manipulations of BDNF. As mentioned earlier, mice with oPFC-targeted BDNF knockdown (Gourley et al., 2013), but not mPFC-targeted BDNF knockdown (Gourley et al., 2012), exhibited habitual behavior following contingency degradation and exhibited impaired reversal learning. Overall, the role of BDNF in the expression of compulsive behaviors differs depending on the neuroanatomical structures involved. Compulsivity is not just a risk factor for the development of addiction; it characterizes the repetitive, persistent nature of on-going drug use and relapsing behavior (Dalley et al., 2011). Understanding how compulsivity contributes to the various stages of addiction can be useful in the administration of strategies for treatment at different time points during the disorder.

1.3.6 Importance of Studying Compulsivity in Females

There are distinct differences in compulsivity between men and women. Among addicted individuals, girls and women tend to be more compulsive than their male counterparts (Fattore & Melis, 2016). Given these differences, females were chosen as subjects for the following studies over males because it was predicted that baseline compulsivity would be higher in females. This is beneficial because one of the main goals of these studies was to determine if agonists at the 5-HT_{2C} receptor could decrease baseline compulsivity. The likelihood of having baseline compulsivity measures that were high enough to be decreased was greater if females were used, otherwise a floor effect may have been encountered.

Although men start experimenting with addictive substances and behaviors at a younger age and at higher rates than women, women progress more quickly to psychopathology and have higher rates of escalation of substance intake compared to men (Fattore & Melis, 2016). Females may be more susceptible to the detrimental effects of drugs, as female, but not male, stimulant addicts have lower grey matter volume across the brain and higher dependence symptoms, even after prolonged abstinence (Regner et al., 2015). Moreover, studies in rhesus monkeys have shown that cocaine intake in females during extended access to self-administration is significantly greater than in males (Henry et al., 2009). As with compulsivity, based on the literature, it was predicted that female subjects would exhibit higher levels of METH intake and would escalate their intake more quickly than males. Higher levels of METH intake are beneficial because, again, one of the main goals of these studies was to determine if higher levels of METH intake would be predictive of increased compulsivity compared to baseline. The likelihood of

observing robust increases in compulsivity would be greater if the amount of METH intake could be as high as possible, and females represented a better option for this than males.

With respect to food, women are also more likely to engage in binge-eating behaviors and suffer from obesity than men (Al Lawati et al., 2003; Gu et al., 2005; Kelly et al., 2008). As with METH intake, based on the literature, it was predicted that female subjects would exhibit higher levels of food intake than males. Since another main goal of these studies was to determine if higher levels of food intake would be predictive of increased compulsivity compared to baseline, females again represented the best choice for these experiments.

Despite the epidemiological data discussed above, preclinical studies of the neurobiology of addiction most typically use males. Therefore, focusing on females is important because they represent an understudied and potentially vulnerable patient population. These relationships are highly variable, however, and often depend on the task being used to measure compulsivity, on the age of the individuals being compared, and various other confounding factors. A deeper understanding of compulsive behaviors in females is essential for determining effective intervention and prevention strategies for men and women suffering from behavioral inhibition disorders.

1.4 Overall Goals and Hypotheses

Despite the evidence suggesting that compulsivity is a core behavior feature of an addictive phenotype, the effects of long-term engagement in addictive behavior on compulsivity remain elusive. In addition, although it is clear that 5-HT_{2C} receptors are involved in the expression of both compulsive and addictive behaviors, the extent to which these behaviors are regulated in

parallel is unknown. Moreover, the exact effects of these receptors on compulsivity, specifically, remain unclear.

To answer these questions, the following set of studies employed two different types of reinforcers, palatable high caloric foods and methamphetamine (METH). As discussed above, these reinforcers have been shown to alter signaling across the mesolimbic DA pathway (Hernandez & Hoebel, 1988; Rada & Hoebel, 2000; Manvich et al., 2012a; Murnane, 2013; Volkow et al., 2013b), especially within the ventral striatum, and are thus expected to affect compulsivity, in which DA signaling plays a role. Thus, in Chapter 2, the effects of long-term intake of these reinforcers on general compulsive behavior in female rhesus macaques are discussed. Based on the literature reviewed, the following hypotheses are evaluated: (1) extended intake of reinforcers increases compulsivity compared to baseline, (2) baseline compulsivity, measured prior to any reinforcer exposure, is predictive of a subject's reinforcer intake during a period of extended access to the reinforcer, and (3) reinforcer intake during a period of extended access is predictive of changes in compulsivity compared to baseline, are evaluated.

In Chapter 3, the effects of a 5-HT_{2C} receptor agonist on baseline compulsivity and on compulsivity following extended reinforcer intake (food and METH), as well as on food intake of two different diets, are discussed. This chapter also examines whether the 5-HT_{2C} receptor is necessary for the effects of the agonist. Previous studies have produced confounding evidence regarding the role of these receptors in the expression of compulsive behaviors. However, based on the distribution of 5-HT_{2C} receptor within the VTA, NAcc, and PFC, and the evidence that activation of these receptors should decrease DA signaling across these areas, it was hypothesized that a 5-HT_{2C} receptor agonist would (1) decrease compulsive behavior and (2)

intake of palatable high caloric foods. In Chapter 4, the effects of a 5-HT_{2C} receptor agonist on the behavioral neuropharmacology of METH, including its effects on intake and drug-seeking and on DA neurotransmission within the striatum, are evaluated.

Finally, in Chapter 5, the results presented in Chapters 2-4 are synthesized. This chapter also addresses how the present findings integrate with and inform the existing literature, and explores future productive experiments that would complement, as well as expand upon, the current studies. The major goals of the following studies are to determine, not only the relationship between intake of reinforcers and compulsivity, and the effects of the 5-HT_{2C} receptor on compulsivity, but also to understand whether the effects on reinforcer intake are accompanied by parallel changes in compulsivity. The results of this work will elucidate the relationship between compulsivity and intake of two different types of reinforcers, as well as the ability of 5-HT_{2C}R to regulate these behaviors. These results may provide evidence for the use of 5-HT_{2C}R agonists in treating an addictive phenotype, facilitating the development of comprehensive therapeutics that will ameliorate addiction and improve the health and lives of people who suffer from this disorder.

1.5 Figures

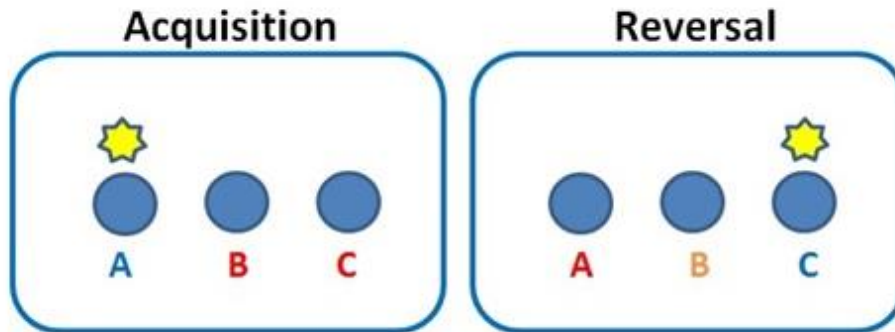


Figure 1.1 Diagram of Discrimination Reversal Learning Task (DRLT)

The two main phases of this task (administered on a WGTA) are shown. During the acquisition phase, subjects must learn to discriminate between three objects (A, B and C) by discovering which is associated with a treat (yellow star). Once a performance criterion has been met, typically the subject must choose the correct object (A) 90% of the time, they move onto the reversal phase. During reversals, the treat becomes associated with one of the two previously unrewarded objects (C). If a subject chooses object A during reversal, this is a perseverative error and is considered a primary measure of compulsivity. If the subject chooses C, this is a correct response and if they choose B, an object that has never been associated with a reward, this is a random error. For subsequent rounds of acquisition and reversal, different sets of objects are used.

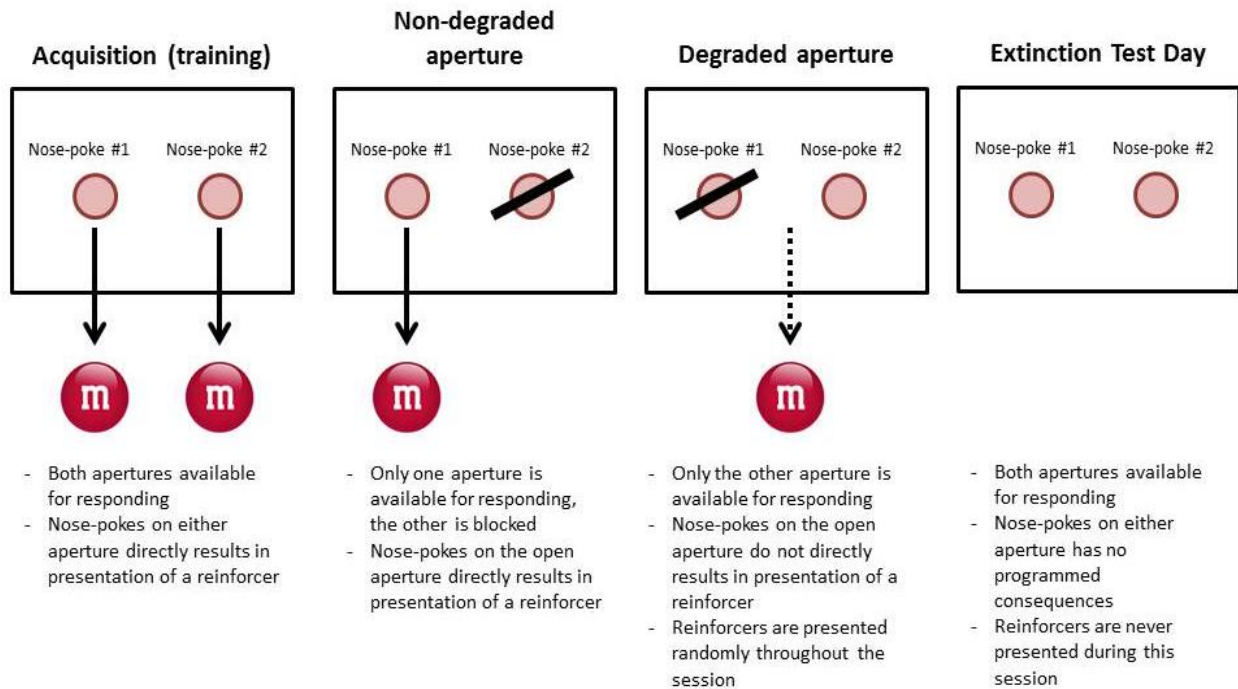


Figure 1.2 Schematic of Contingency Degradation Task (CDT)

During the acquisition (training) phase of this task, subjects are trained to respond (nose-poke) on one of two apertures. Responding on either aperture directly results in presentation of some reinforcer, typically a food pellet or treat of some sort. After subjects have acquired this behavior, one of the apertures becomes degraded such that responding on that aperture no longer leads to programmed consequences (i.e. presentation of the reinforcer). Reinforcers are still presented, but randomly or at regular time intervals. The other aperture is blocked off during this time and is not available for responding. After one of the apertures has been degraded, subjects undergo an extinction test in which both apertures are available for responding, but responding on either of them has no programmed consequences. No reinforcers are presented during this test.

Task	Domain	Behavior Tested
		<ul style="list-style-type: none"> - Inhibition of persistent, repetitive responses that are not reinforced
Discrimination	Reversal	
Learning Task	Contingency-related cognitive inflexibility	<ul style="list-style-type: none"> - Adaptation of motor strategies after negative feedback - Stimulus- vs goal-driven responses - Inhibition of persistent, repetitive responses that are not reinforced
Contingency	Degradation	
Task	Contingency-related cognitive inflexibility	<ul style="list-style-type: none"> - Adaptation of motor strategies after negative feedback - Stimulus- vs goal-driven responses

Table 1.1 Summary of Preclinical Tasks for Measuring Compulsive Behaviors

The following table lists the preclinical tasks used to measure compulsive and habitual behavior.

**Chapter 2. Effects of Long-Term Methamphetamine or High Caloric Diet Intake on
Compulsivity in Female Rhesus Macaques**

2.1 Abstract

Rationale There are many genetic and environmental factors thought to contribute to an individual's likelihood of developing addiction. Similarly, engaging in addictive behavior is thought to have robust effects on decision-making strategies. A deeper understanding of these relationships may lead to better predictive tools and models for the development of future therapeutics. Evidence suggests that compulsivity, which can be defined as a general inability to alter behavior with changing reinforcement contingencies, is a common behavioral feature of addiction in humans.

Objectives The aims of this study were to evaluate (1) the effects of long-term intake of either a drug (methamphetamine) or food (high caloric diet) reinforcer on compulsivity, (2) whether baseline compulsivity was predictive of future reinforcer intake, and (3) whether long-term reinforcer intake levels were predictive of post-reinforcer changes in compulsivity.

Methods Baseline compulsivity was measured in all subjects using a DRL task, after which half self-administered methamphetamine and half consumed a high caloric diet for a period of 6 months. Following this reinforcer period, compulsivity was measured again.

Results Long-term intake of methamphetamine or a high caloric diet significantly increased perseverative responding (i.e. compulsivity). Moreover, reinforcer intake levels were positively correlated with post-reinforcer, but not baseline, compulsivity.

Conclusions The present findings indicate that an individual's level of compulsivity in a naïve state may not be predictive of their future engagement in addictive behaviors. However, an individual's level of reinforcer intake was predictive of increased compulsivity, regardless of whether the reinforcer was a drug of abuse or palatable, high caloric food.

2.2 Introduction

Every year, the U.S. spends over \$600 billion on addiction-related costs, including medical and legal damages (Harwood, 2000, 2004; Office of National Drug Control Policy, 2004; Wickizer, 2013). Presently, there are few FDA-approved treatments available for the millions of people affected by addictive disorders, and many of the available treatments have low efficacy. Given the high rates of relapse (50-90% across all addictive disorders; see Brownell, 1986; Schaffer & Zimmerman, 1990; Hodgins & el-Guebaly, 2004; Block JJ, 2008; Abdollahi et al., 2014), the discovery of effective treatments is imperative.

Historically, addictions have been treated in isolation, with a goal of reducing the main addictive behavior. However, this approach has produced no effective treatments for addiction in general and few for specific addictions. Further, there is evidence supporting the existence of an addictive phenotype. For example, people can become addicted to a wide variety of different reinforcers such as drugs, food (Shaffer et al., 2004), gambling (Karim & Chaudhri, 2012), and sex (Blum et al., 2012), often simultaneously. More importantly, when one type of addiction is treated in isolation or inadequately, it tends to be replaced by another (Shaffer et al., 2004; Blum et al., 2012), suggesting that there are common underlying neurochemical and behavioral processes contributing to addiction that are insensitive to changes in the expression of specific addictive behaviors. By expanding our understanding of how these processes contribute to addictive behaviors, novel therapeutic targets may be revealed and leveraged for the treatment of all addictions.

Compulsivity, which can be defined as a general inability to alter behavior when there has been a change in reinforcement contingencies, has been highly implicated in all types of addictive

disorders (American Society of Addiction Medicine, 2011). Drug-dependent (Fernandez-Serrano et al., 2011) and obese individuals (Cserjesi et al., 2007), as well as pathological gamblers (Leeman & Potenza, 2012), all exhibit higher levels of compulsivity in both self-reports and cognitive testing. Additionally, compulsivity is considered an etiological factor in the development and maintenance of addiction, being the final stage in a transition from recreational use of reinforcers, through habits, and finally to compulsive use (for a detailed review see Everitt & Robbins, 2005, 2016; Torregrossa et al., 2011). A transition from top-down control by the prefrontal cortex to bottom-up control by the striatum over decision-making and behavior is hypothesized to underlie this progression (Belin et al., 2013).

The following study is the first to evaluate the hypothesis that compulsivity is one of the underlying behavioral features of an addictive phenotype. Other studies have evaluated the link between impulsivity and addiction (Dawe & Loxton, 2004; Verdejo-Garcia et al., 2008; Crews & Boettiger, 2009), but compulsivity has been understudied. Impulsivity describes behaviors that are poorly conceived, premature or inappropriate, whereas compulsivity refers to inappropriate behaviors specifically within the context of past training and changing reward contingencies. Both are thought to contribute to the development of addiction, yet the specific role of compulsivity is not fully understood.

Nonhuman primates (NHPs) are an ideal model for exploring the relationship between compulsivity and addictive behaviors because they share a common physiology and neurobiology with humans (Gould et al., 2012) and exhibit complex decision-making and reinforcement contingency learning (Everitt & Robbins, 2016). In addition, studies with NHPs allows for extensive environmental control and experimental manipulations that would be unethical to do

in humans. To determine the nature of the relationship between compulsivity and reinforcer intake in NHPs, and to demonstrate that it is not dependent on the specific reinforcer used, this study examined two different reinforcers: methamphetamine (METH) and palatable, high caloric food. The following key hypotheses were addressed: (1) long-term intake of reinforcers increases compulsivity, (2) higher baseline compulsivity predicts higher levels of reinforcer intake, and (3) greater reinforcer intake predicts increases in compulsivity.

The first model, for the evaluation of METH intake measures, was drug self-administration (SA), which is the most widely used technique for modeling drug addiction (Gardner, 2000). The self-administration paradigm provides a measure of ongoing drug-taking behavior and allows for the evaluation of the reinforcing properties of drugs of abuse (Howell & Wilcox, 2002). This paradigm can also be modified to examine other aspects of the drug addiction process. For example, extinction is a useful model of the efficiency with which subjects can inhibit responding that was previously reinforced by drug, but is no longer being reinforced. Reinstatement, on the other hand, is thought to model relapse to drugs, specifically the degree to which subjects will seek drug when exposed to either a non-contingent drug prime or drug-associated cues, or both (Katz & Higgins, 2003; Epstein et al., 2006). All three models were employed to probe the relationship between compulsivity and drug intake (self-administration), inhibition of behavior previously reinforced by drug (extinction), and drug-seeking behavior (reinstatement).

The second model of reinforcer intake, consumption of a palatable, high caloric diet (HCD) containing higher concentrations of fat and sugar than laboratory chow, models some key aspects of “food addiction” or binge-eating disorder, such as excessive caloric intake (uncoupled from metabolic needs) and increased weight gain (Michopoulos et al., 2012b). Although there is

considerable debate regarding the existence of “food addiction”, there is evidence to support that consumption of some diets or foods can produce maladaptive behaviors and neurochemical changes that include addiction-like components. For instance, palatable, high caloric foods activate the reward pathway similarly to drugs of abuse (Liu et al., 2010; Davis et al., 2011; Manvich et al., 2012; Murnane, 2013; Volkow et al., 2013b), increasing extracellular dopamine (DA) levels in the nucleus accumbens (Hernandez & Hoebel, 1988; Rada & Hoebel, 2000). Moreover, repeated intake of these reinforcers can lead to DA depletion and an eventual downregulation of DA receptors, which can induce a hypodopaminergic state. Evidence from behavioral and imaging studies in humans (Wang et al., 2001; Volkow et al., 2013b) suggests that this process contributes to ongoing addiction and relapse in many types of addictive disorders.

2.3 Methods

2.3.1 Experimental Design

An outline of the experimental design is shown in Fig. 2.1. At the onset of this study, all subjects were experimentally naïve and baseline measures of compulsivity were taken. Subsequently, one group of subjects self-administered METH for 6 months (METH group), while the other group consumed a HCD for 6 months (HCD group). Measures of drug and food intake were collected for the 6-month period. After this period of reinforcer intake, subjects were re-tested for compulsivity.

2.3.2 Subjects

Adult female rhesus macaques (*Macaca mulatta*) weighing 7-14kg served as subjects for this study ($N=10$). All subjects were experimentally naïve at the onset and had never been given access to drugs of abuse or palatable, high caloric foods. All procedures strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Eighth Edition, revised in 2010), and were approved by the Institutional Animal Care and Use Committee of Emory University.

METH Group

Half of the subjects ($N=5$) were pair-housed in indoor stainless-steel home cages at the Yerkes National Primate Research Center (YNPRC) Main Station and were assigned to the METH group. They were fed Purina monkey chow (Ralston Purina, St. Louis, MO, USA) supplemented with fruits and vegetables and had water continually available in the colony, which was maintained at an ambient temperature of $22\pm 2^{\circ}\text{C}$ at 45-50% humidity and a 12-hour light/dark cycle (lights on at hour 0700; lights off at hour 1900). Environmental enrichment was provided on a regular rotating basis. Subjects were fitted with collars (Primate Products®, Immokalee, FL, USA) used for accesses.

HCD Group

The other half of the subjects ($N=5$) were housed in indoor—outdoor 144 ft² (12 x 12 ft.) pens at the YNPRC Field Station as a part of groups of 5 to 6 females and were assigned to the HCD group. Because of the enclosures, monkeys were exposed to seasonal day lengths and temperatures. As with the METH group, environmental enrichment was provided on a regular rotating basis for the HCD group subjects. Because socially housed female rhesus monkeys form a dominance hierarchy with subordinates showing a distinct behavioral and physiological

phenotype (Michopoulos et al., 2012a), middle ranking (ranks 3 and 4) animals only were chosen for this study. Ranks were determined based on the outcome of dyadic agonistic interactions (as described by Michopoulos et al., 2016).

2.3.3 Compulsivity Measurements

All subjects ($N=10$) were trained on the Discrimination Reversal Learning (DRL) task, a commonly used compulsivity measure (Rogers et al., 2000; Izquierdo & Jentsch, 2011). First, subjects were habituated to the rolling cage and the Wisconsin General Test Apparatus (WGTA). The apparatus was fitted with a stimulus tray containing 3 wells with which the subject could interact. DRL sessions began after subjects were habituated to the apparatus and were conducted once/day (Monday-Friday at the Yerkes Field Station and Monday-Sunday at the Yerkes Main Station). Each session consisted of 30 trials separated by 15 second intervals. The positions of objects during each trial were randomized to control for any preference in location.

Acquisition

During acquisition of the DRL task, one of 3 distinct objects was paired with a hidden reward (M&Ms, Skittles, or other small treats that could fit into the wells of the WGTA response panel). If the subject's first response during a trial was to choose the rewarded object, then the trial would end, they would undergo a 15 second time-out, and the next trial would begin. However, if the subject's first response was to choose one of the unrewarded objects, there would be a 15 second time-out, followed by a trial with a forced correction, in which the incorrect object would be moved to reveal the empty well underneath. Subjects could make an unlimited number of erroneous responses until they chose the rewarded object and moved to the next

trial. Acquisition continued until the subject reached a performance criterion of 90% correct responses (the rewarded object was chosen first) during a session. After meeting these criteria, they underwent a reversal the following day.

Reversal

During reversal, the reward was hidden under one of the two previously un-rewarded objects. Reversal lasted for only one session consisting of 30 trials and subjects were not required to meet any performance criterion. Reversal trials were conducted identically to the acquisition trials, with subjects retrying each trial, with unlimited forced corrections, until the newly rewarded object was chosen (correct response). Possible errors included choosing the previously rewarded object (perseverative error) or selecting the third object that had never been paired with the reward (random error). The number of perseverative errors made during reversal was the primary measure of compulsivity.

Compulsivity was measured twice, before and after intake of METH or HCD for 6 months. Two rounds of the DRL task were conducted for both baseline and post-reinforcer compulsivity and a new set of 3 objects was used for each round.

2.3.4 Intravenous Drug Self-Administration, Extinction, and Reinstatement

Following baseline compulsivity testing, subjects ($N=5$) in the drug group were surgically prepared with a chronic indwelling venous catheter under sterile conditions. The apparatus and self-administration procedure are described in detail by Howell and Wilcox (2001b). Briefly, animals were trained to respond under a fixed-ratio (FR) 20 schedule of drug delivery, after which they self-administered METH during 60-min sessions once daily, in the morning (starting between

0700 and 1000 hours) Monday-Friday. (\pm) Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC, USA). The drug was dissolved in 0.9% sterile physiological saline and administered intravenously.

Before the 6-month SA block began, dose-effect curves for METH (0.003mg/kg, 0.01mg/kg and 0.03mg/kg) were determined, as well as each subject's peak dose (the dose that engendered the highest response rates during SA). For the dose-effect curves, METH doses were presented in a counter-balanced order and the response rate for each dose was measured as an average of 3 consecutive sessions during which responding was stable. Subjects were considered stable on SA once they met stability criteria (the subject responded for 3 consecutive sessions at rates that did not differ from the mean of those sessions by 30% or more). For all subjects, the peak dose of METH was 0.01mg/kg, therefore this was the dose chosen for the 6-month METH SA block.

During the SA block, subjects self-administered METH for 168 consecutive days. Subjects were placed in a primate chair (Primate Products[®], Immokalee, FL, USA) and taken to a sound-attenuating experimental chamber for the duration of the session. During the test session, the behavioral chamber was illuminated with a white light that served as a discriminative stimulus. Completion of an FR 20 response requirement resulted in a change in the stimulus light from white to red (conditioned stimulus) for 15 seconds and a METH infusion (0.01 mg/kg in 0.5 ml infused over 3 seconds), followed by a 60 second timeout, during which the stimulus light was turned off and responding had no programmed consequences. At the end of the time-out, the white light was presented again to signal the opportunity to complete another FR and earn another infusion. Average response rate (responses/second) and average and total METH intake

(mg/kg/day and mg/kg, respectively) were measured throughout the 6-month SA block. Response rates were calculated as the total number of lever presses during the active period divided by the active time throughout the session.

Following the 6-month METH SA block (and subsequent DRL task re-testing), subjects underwent extinction and reinstatement experiments. Behavior was maintained by the unit dose of 0.01mg/kg/infusion METH. Once subjects reached stability criteria on maintenance, behavior was extinguished by substituting saline for METH in the syringe, and was operationally defined as a response rate that was <20% of the 3-day mean response rate during the maintenance of METH SA. Extinction sessions also differed from maintenance sessions in that the conditioned stimulus (CS) was not illuminated. On the day after subjects met extinction criteria, a reinstatement test was conducted. Reinstatement sessions consisted of an experimenter-administered priming dose of METH given through the vascular access port 5 minutes before the start of the session. During these sessions, saline was substituted for METH in the syringe, but the CS light was illuminated once again following successful completion of the FR 20 response requirement, thus these sessions were drug- and cue-induced reinstatement tests. Dose-effect curves for METH prime doses (0.03mg/kg, 0.1mg/kg, 0.3mg/kg, and 1.0mg/kg) were determined, as well as each subject's peak reinstatement dose (the dose that engendered the highest response rates during reinstatement). For the dose-effect curves, METH prime doses were presented in a counter-balanced order. After each reinstatement test, subjects were returned to METH SA maintenance conditions until stability criteria were met again, then a new block of extinction and reinstatement began.

2.3.5 Food Intake

Prior to and during baseline compulsivity testing, subjects ($N=5$) in the HCD group had *ad libitum* access to standard monkey chow (LCD; 3.45 kcal/g, Purina 5038) only via previously validated automated feeders that allowed for constitutive quantification of caloric intake (Michopoulos et al., 2012b). Briefly, activation of a radio-frequency (RF) antenna via a RF identification chip within each subject's wrist signaled a computer to dispense a single pellet of food. The caloric composition of the low caloric diet (LCD) was 12% fat, 18% protein, 4.14% sugar carbohydrate, and 65.9% fiber carbohydrate. Following baseline compulsivity testing, subjects in the HCD group had access to both the LCD and a HCD (4.47 kcal/g, D07091204S Research Diets, New Brunswick NJ) with a caloric composition of 36% fat, 18% protein, 16.4% sugar carbohydrate and 29.6% fiber—starch carbohydrate) for 6-months.

Monkeys significantly prefer the HCD (Michopoulos et al., 2012b; Moore et al., 2013) and availability of diet choice sustains excess calorie intake and promotes obesity compared to availability of only a HCD (Rolls et al., 1983; la Fleur et al., 2014). Body weights were measured each week throughout the study. Monkeys typically ate all the food they accessed and there was no evidence that food intake was influenced by other members of the group (Wilson et al., 2008). Average and total food intake (kcal/week and kcal, respectively) of both the LCD and the HCD was measured.

2.3.6 Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 7. Paired t-tests and repeated measures analysis of variance (RM ANOVA) with Dunnett's *post hoc* test were used

when appropriate. Pearson's correlation analyses were used to determine the relationships between baseline compulsivity measures, reinforcer intake during the 6-month periods, and post-reinforcer compulsivity. The correlation coefficient r calculated using this analysis serves as the effect size index for the given correlation between two continuous variables. The closer the correlation coefficient is to 1 (on a scale from zero to 1), the larger the effect size.

2.4 Results

2.4.1 Baseline Compulsivity

Baseline DRL task measures were collected using a WGTA, replicated twice, and are reported here as mean \pm SEM. The means for all these measures were very similar between the two groups, especially for correct responses. During reversal sessions, the METH group ($N=5$) made 13.3 ± 2.48 correct responses, 23.3 ± 4.90 perseverative errors and 6.6 ± 1.21 random errors. In addition, subjects took approximately 4.8 ± 0.60 days to acquire each baseline discrimination before moving on to reversal. The HCD group ($N=5$) made 13.7 ± 2.64 correct responses, 24.3 ± 4.33 perseverative errors, 7.5 ± 2.38 random errors, and took 3.3 ± 0.41 days to acquire the object discriminations.

2.4.2 Drug Self-administration

METH Dose-effect Curves

Following baseline compulsivity measures, subjects in the METH group were trained to i.v. self-administer METH. Training ended as soon as they reached terminal performance criteria

for SA, which consisted of a 60-minute session duration with 5 minutes start delay, 60-second time out between infusions of METH, and FR20 response requirement. On average, subjects took 51 ± 15.24 days to reach terminal SA parameters. There was a wide distribution of SA acquisition latencies, with 18 being the least number of days to reach performance criteria and 106 being the most. Post training, dose-effect curves for METH unit doses were determined for each subject (Fig. 2.2A). The peak METH unit dose, the dose that produced the highest rates of responding, for all subjects was 0.01mg/kg/infusion. Subjects emitted 0.214 ± 0.128 responses/second for 0.003mg/kg METH, 0.515 ± 0.317 responses/second for 0.01mg/kg METH, and 0.211 ± 0.118 responses/second for 0.03g/kg METH. Although most of the subjects in this group had comparable response rates across METH doses, Pz13 consistently responded at much higher rates than the rest and Tr13 had the flattest dose-effect curve of the group. METH intake across these doses (0.003, 0.01, and 0.03 mg/kg) was 0.051 ± 0.019 , 0.266 ± 0.057 , and 0.482 ± 0.175 mg/kg/day, respectively.

6-month METH SA Block

Once dose-effects curves were established, subjects began their 6-month (24 week) METH SA block. Subjects self-administered the peak dose of 0.01mg/kg/infusion METH at the terminal SA parameters. Response rates (Fig. 2.2B) and daily METH intake (Fig. 2.2D) both typically increased throughout the 6-month SA block for all subjects. Total cumulative METH intake (Fig. 2.2C) over the entire 24-week period was 48.01 ± 9.335 mg/kg. Pz13 had the greatest cumulative intake of 83.65mg/kg METH, whereas Fn14 had the lowest intake of 30.72 mg/kg.

Cue- and METH-induced Reinstatement

After completing the 6-month METH SA block, subjects were re-tested on the DRL task and then participated in reinstatement experiments in which the following priming doses of METH were tested: 0.03, 0.1, 0.3 and 1.0mg/kg (Fig. 2.2E). Under maintenance conditions, subjects made 1.03 ± 0.637 responses/second, 0.136 ± 0.09 responses/second under extinction conditions, and 0.317 ± 0.163 , 0.722 ± 0.483 , 0.858 ± 0.524 , and 0.364 ± 0.394 responses/second under 0.03, 0.1, 0.3, and 1.0mg/kg priming conditions, respectively. On average, subjects took 5.6 ± 0.678 days to reach extinction criteria the first time they were extinguished, with Pz13 taking the longest (8 days) and Tr13 taking the shortest (2 days). Days to reach extinction criteria significantly decreased over time as subjects underwent further rounds of extinction [$F(3,5) = 14.36$, $p = 0.0057$] (Fig. 2.2F).

2.4.3 Food Intake

Baseline Food Intake

Prior to baseline compulsivity measures, baseline food intake and weight were measured for the HCD group. Under baseline conditions, only a LCD was available and subjects consumed an average of 1833.73 ± 223.90 kcal/week (Fig. 2.3A,C) and weighed approximately 10.25 ± 0.488 kg (Fig. 2.3B,D).

6-month HCD Consumption Block

Following baseline compulsivity measures, subjects in this group were given access to both a LCD and a HCD for a period of 6 months. Food intake was significantly affected by this period of consumption of the HCD [$F(4,5) = 41.41$, $p < 0.0001$]. *Post hoc* analysis with Dunnett's test revealed that average consumption of the LCD significantly decreased to 685.63 ± 119.94

kcal/week ($p = 0.0006$), whereas average total consumption (LCD + HCD) increased to 3060.65 ± 287.74 kcal/week ($p = 0.0004$), during the 6-month HCD consumption block compared to LCD intake at baseline (Fig. 2.3A). Subjects in this group demonstrated a preference for the HCD over the LCD, as most of their weekly caloric intake consisted of HCD (Fig. 2.3C). In addition, there was a significant, albeit small effect of HCD consumption on weight (Fig. 2.3B), with subjects' weighing 10.88 ± 0.550 kg after 6-month consumption the HCD [$t(4) = 2.348$, $p = 0.0393$].

2.4.4 Post-Reinforcer Compulsivity

All subjects ($N=10$) were re-tested on the DRL task after completion of their 6-month reinforcer intake blocks. Measures were again collected using a WGTA and replicated twice. Perseverative errors were significantly increased compared to baseline after either extended self-administration of METH [$t(4) = 3.493$, $p = 0.0125$] (Fig. 2.4B) or consumption of the HCD [$t(4) = 2.259$, $p = 0.0434$] (Fig. 2.5B). Post-reinforcer correct responses trended toward a decrease compared to baseline in the METH (Fig. 2.4A), but not the HCD group (Fig. 2.5A), however this difference was not significant ($p = 0.0611$). Random errors (Fig. 2.4C & 2.5C) and acquisition latency (Fig. 2.4D & 2.5D) were not affected by extended reinforcer intake in either group.

2.4.5 Correlations between Compulsivity and Reinforcer Intake

Baseline Compulsivity and Reinforcer Intake

Pearson's correlation analyses for the METH group did not reveal a significant relationship between baseline perseveration and response rate [correlation coefficient/effect size index (r) = -0.1618 ; $R^2 = 0.0262$, $p > 0.05$] (Fig. 2.6A) during the 6-month METH SA block, or between baseline

perseveration and cumulative METH intake [correlation coefficient/effect size index (r) = -0.1933; R^2 = 0.0374, $p > 0.05$] (Fig. 2.6B). On the other hand, for the HCD group, trends toward significant relationships were found between baseline perseveration and HCD only intake [correlation coefficient/effect size index (r) = 0.6623; R^2 = 0.4386, $p > 0.05$] (Fig. 2.7A) during the 6-month HCD consumption block, and between baseline perseveration and total food intake [correlation coefficient/effect size index (r) = 0.5694; R^2 = 0.3242, $p > 0.05$] (Fig. 2.7B). However, these trends did not reach statistical significance.

Reinforcer Intake and Post-Reinforcer Compulsivity

For the METH group, a significant positive relationship between average response rate during the METH SA block and post-reinforcer perseveration [correlation coefficient/effect size index (r) = 0.899; R^2 = 0.8082, $p = 0.0190$] (Fig. 2.6C), as well as between cumulative METH intake during the block and post-reinforcer perseveration [correlation coefficient/effect size index (r) = 0.8986; R^2 = 0.8075, $p = 0.0191$] was found (Fig. 2.6D). In addition, a significant positive relationship between post-reinforcer perseveration and first extinction latency [correlation coefficient/effect size index (r) = 0.9114; R^2 = 0.8306, $p = 0.0156$] (Fig. 2.6F), but not between post-reinforcer perseveration and peak reinstatement response rate [correlation coefficient/effect size index (r) = -0.5499; R^2 = 0.3024, $p = 0.1685$] (Fig. 2.6E), was detected. Pearson's correlation analysis for the HCD group also revealed a significant positive relationship between HCD only intake during the HCD consumption block and post-reinforcer perseveration [correlation coefficient/effect size index (r) = 0.8676; R^2 = 0.7527, $p = 0.0283$] (Fig. 2.7C), as well as between total food intake and post-reinforcer perseveration [correlation coefficient/effect size index (r) = 0.8263; R^2 = 0.6827, $p = 0.0423$] (Fig. 2.7D).

2.5 Discussion

2.5.1 Overview

The study examined the relationship between compulsivity and intake of two different reinforcers: METH and a palatable HCD. General compulsivity was initially characterized in nonhuman primates using a DRL task, after which half of the subjects either self-administered METH or consumed a HCD. Post-reinforcer compulsivity was then determined to assess whether this extended reinforcer intake period influenced compulsivity. The findings reveal that extended intake of METH and the HCD increased compulsivity compared to baseline, and that the amount of reinforcer intake was predictive of this increase. However, the results demonstrated that baseline compulsivity was not predictive of future intake of either METH or the HCD.

2.5.2 Reinforcer Intake During the 6-month Period

During the 6-month period, an increase of reinforcer intake was seen in both the METH and HCD groups. METH group subjects responded at higher rates and self-administered greater amounts of METH at the end of the reinforcer intake period than at the beginning. This increase in drug-taking is a well-documented effect of prolonged access to drugs of abuse and has been demonstrated in rats (Ahmed & Koob, 1998) and monkeys (Carroll et al., 2005). As discussed in the results, there was considerable individual variation in response rates for and cumulative intake of METH. One subject, Pz13, exhibited much higher response rates and METH intake compared with the rest of her group, while another subject, Tr13, had the lowest response rates and intake levels. Moreover, Tr13's responses to different unit doses of METH were comparable,

suggesting that she may be relatively insensitive to METH dose and perhaps less sensitive to the reinforcing properties of METH compared to the rest of her group. These individual differences could be explained by individual differences in receptor neurochemistry, specifically with respect to the D2 receptor. Indeed, previous studies in both humans (Dalley et al., 2007) and monkeys (Nader et al., 2006) have demonstrated that D2 receptor levels play a crucial role in the reinforcing properties of cocaine.

HCD group subjects weighed more and consumed a significantly greater number of calories at the end of the reinforcer intake period than they did at baseline, when only the LCD was available. Furthermore, subjects demonstrated a distinct preference for the HCD over the LCD, with most of their caloric intake consisting of the palatable, high caloric food option. These findings from the HCD group are consistent with previous work in rhesus monkeys (Arce et al., 2010; Michopoulos et al., 2012b, 2016).

The length of 6 months was chosen for the reinforcer intake period in this study to allow ample time for long-term changes in neurochemistry and behavior to occur in response to intake of METH and the HCD. Previous work using the same HCD feeding model employed here has demonstrated that important behavioral changes can be seen after this length of time (Michopoulos et al., 2016).

2.5.3 Effects of Long-term Reinforcer Intake on Compulsivity

Several studies support the hypothesis that long-term intake of reinforcers and engagement in addictive behaviors increases compulsivity and negatively impacts the flexibility of decision-making strategies. For instance, drug-dependent (Simon et al., 2002; Fernandez-

Serrano et al., 2011), obese (Cserjesi et al., 2007), and individuals with binge-eating disorder (Banca et al., 2016) all exhibit higher levels of compulsivity in both self-reports and cognitive testing. These results are also corroborated by other studies reporting that repeated, intermittent cocaine administrations increase response perseveration in vervet monkeys (Jentsch et al., 2002) and repeated acute METH administrations increase perseverative behavior in rats (Son et al., 2013). To the best of our knowledge, experiments that specifically measure the effects of consuming palatable, high caloric foods, rich in sugar and fat, on perseveration have not been conducted until this study.

Long-term intake of either METH or a HCD increased compulsivity and this result is consistent across groups and within all subjects. Interestingly, the degree to which subjects engaged in reinforcer intake during the 6-month period was predictive of post-reinforcer compulsivity. Specifically, the more METH subjects took, or food (HCD only and total HCD+LCD) they consumed over the 6-month period, the higher their compulsivity in post-reinforcer testing. However, it is important to note that the correlation between METH intake and increased post-reinforcer compulsivity appears to be driven by one subject, Pz13, whose level of METH intake was far greater than that of the rest of her group. Her exclusion from this analysis would have likely produced a statistically insignificant correlation. Adding further subjects to this experiment in the future would answer the question of whether this correlation represents a real effect. The increase in compulsivity appears to be specific to perseveration, as no effect was seen in other DRL task measures, including correct responses and random errors. In addition, the increase in perseveration was likely not driven by a general disruption of learning because subjects were able to acquire object discriminations after the reinforcer intake period just as quickly as they did

at baseline. Moreover, long-term reinforcer intake did not change acquisition latency, suggesting that reinforcer effects on perseveration were not due to increased training.

2.5.4 Relationship between Baseline Compulsivity and Reinforcer Intake

This study found no evidence of a significant relationship between baseline perseveration (i.e. compulsivity) and eventual intake of METH. However, for the HCD group, a trend toward a significant relationship between baseline perseveration and food intake (both HCD only and total HCD+LCD) was found and the effect size was quite large (as evidenced by the correlation coefficient), which suggests that the feeding studies might have been underpowered. Overall, the results of this study indicate that general compulsivity in a naïve state may not be predictive of future engagement in addictive behavior. Compulsivity, however, is not the only measure of impaired behavioral inhibition that has been highly implicated in addiction. Impulsivity describes behaviors that are poorly conceived, premature or inappropriate, outside the context of previous experience or training. The relationship between impulsivity and addictive behaviors has received much more attention (Dawe & Loxton, 2004; Belin et al., 2008; Verdejo-Garcia et al., 2008; Crews & Boettiger, 2009). It could be that someone's general impulsive tendencies, rather than their compulsive tendencies, are a more robust predictor of future engagement in addictive behaviors, especially under first intake conditions. After extended intake, however, when use of reinforcers eventually becomes compulsive, it may be driven by compulsivity rather than impulsivity (Everitt & Robbins, 2016).

2.5.5 Compulsivity, Extinction and Reinstatement in the METH Group

Extinction experiments in the METH group revealed a significant relationship between post-reinforcer compulsivity and 1st extinction latency, the number of days subjects took to reach extinction criteria the first time their SA behavior was extinguished. This result suggests that an individual's level of compulsivity is predictive of their ability to inhibit behavior previously reinforced by drug delivery when both the drug reinforcer and drug-associated cues are removed for the first time. The extinction paradigm shares many similarities with the reversal paradigm, with both requiring the subject to first learn a response-reinforcement contingency and then adjust behavior following a change in this contingency. One of the main differences specific to this study is that the extinction paradigm tests a subject's ability to inhibit previously reinforced behavior within a drug-related context, whereas the reversal paradigm tests the same ability within a non-drug context. In addition, both paradigms test a subject's ability to relearn the response-reward contingency. Subjects making outcome-based decisions inhibit the previously reinforced responses when they are no longer being reinforced, whereas continued responding despite a lack of reinforcement is considered stimulus-response behavior (Balleine & O'Doherty, 2010). Importantly, when extinction latencies were averaged over several rounds of extinction, the correlation between post-reinforcer compulsivity and extinction latency disappeared. This breakdown in the correlation is likely due to the learning effect mentioned above, as extinction latency decreased over repeated extinction rounds, with subjects eventually taking only a couple of days to reach extinction criteria.

As opposed to extinction, which measures the efficiency with which subjects inhibit responding that was previously reinforced by drug, reinstatement response rates are considered measures of drug- and cue-induced drug-seeking behavior (Katz & Higgins, 2003; Epstein et al.,

2006). There was no significant correlation between post-reinforcer compulsivity and peak response rates during METH- and cue-induced reinstatement tests. Peak response rates during reinstatement were normalized and were expressed as a % of METH SA response rates during the 6-month block to account for the large variability in response rates across subjects. It is possible that subjects with high METH SA response rates were unable to reinstate at higher response rates compared to SA due to a ceiling effect, whereas subjects with lower METH SA response rates could reinstate at much higher response rates compared to SA. However, the results do not support the hypothesis that compulsivity predicts drug- and cue-induced drug-seeking behavior.

2.5.6 Limitations of the Study

It is important to note some key differences between the METH and HCD groups that may confound direct comparisons between the two treatments. First, the METH subjects were pair-housed to minimize the risk of damage from group mates to the indwelling venous catheters used in drug SA experiments. By contrast, the HCD subjects were socially housed in groups of 5-6 females. Because the HCD subjects were housed in a larger social group, social ranks were established, which could have influenced behavior. However, to minimize this risk, only middle ranking subjects from each group were chosen to participate in this study. Finally, this study employed female subjects only and there are currently no studies in the literature that use the same version of the DRL task used here. Thus, it would be difficult to make any comparisons regarding the results of this chapter with males.

2.6 Figures

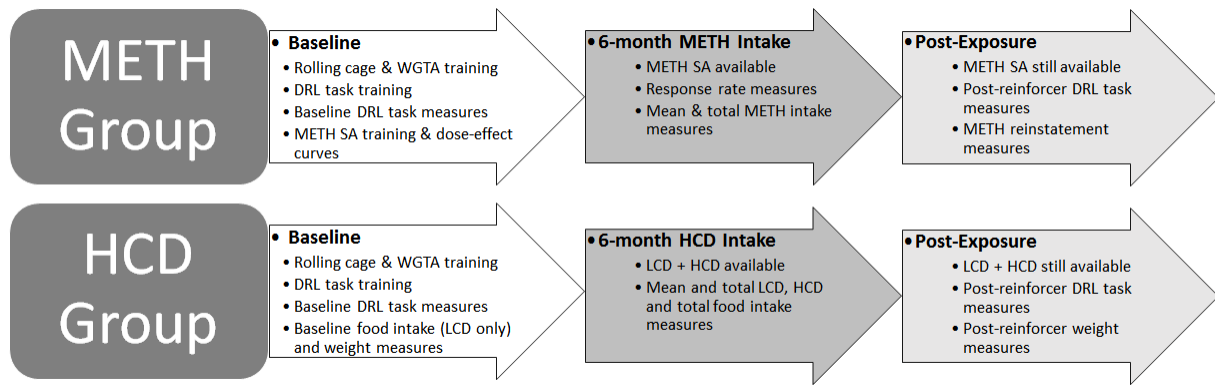


Figure 2.1 Timeline of Experimental Design

The diagram shows the measures collected in this study for both the METH and HCD groups before, during, and after long-term intake of METH or a HCD.

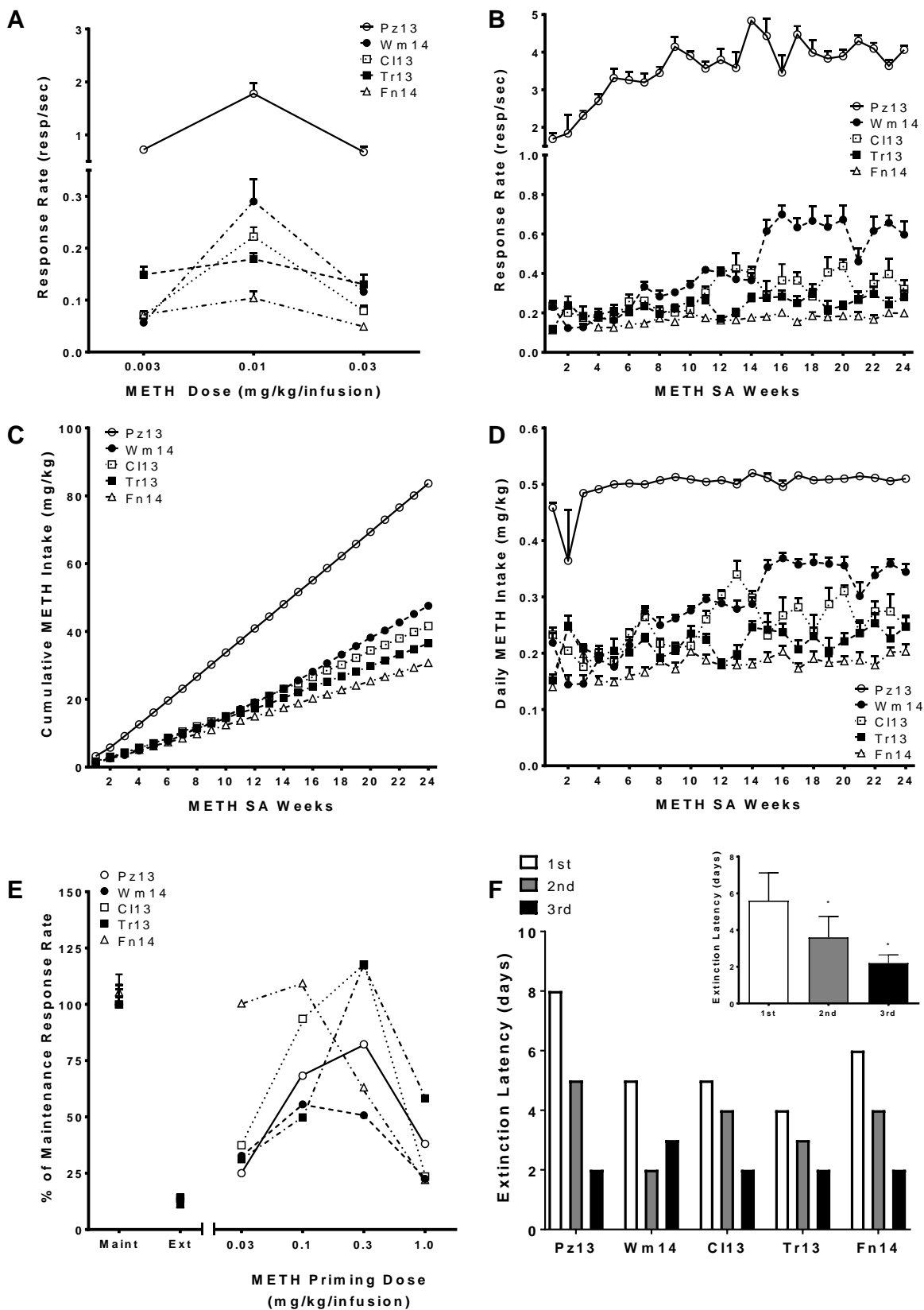


Figure 2.2 Reinforcer Intake During the 6-month Period, Extinction and Reinstatement (METH group)

(A) Dose-effect curves (0.003, 0.01, and 0.03mg/kg/infusion METH) were established for each subject prior to the reinforcer intake period to determine the peak unit dose of METH (0.01mg/kg for all subjects) that engendered the highest response rates. This peak dose was selected as the unit dose for the 6-month SA block. Data for the METH dose-effect curves, response rates (B) and METH intake (D) during the reinforcer intake period, the reinstatement dose-effect curves (E), and extinction latency (F) are presented as the 7-day mean \pm SEM. Data for the total cumulative METH intake throughout the 6-month period (C) are presented as absolute values. * $p < 0.05$ for 2nd and 3rd extinction latencies compared with 1st extinction.

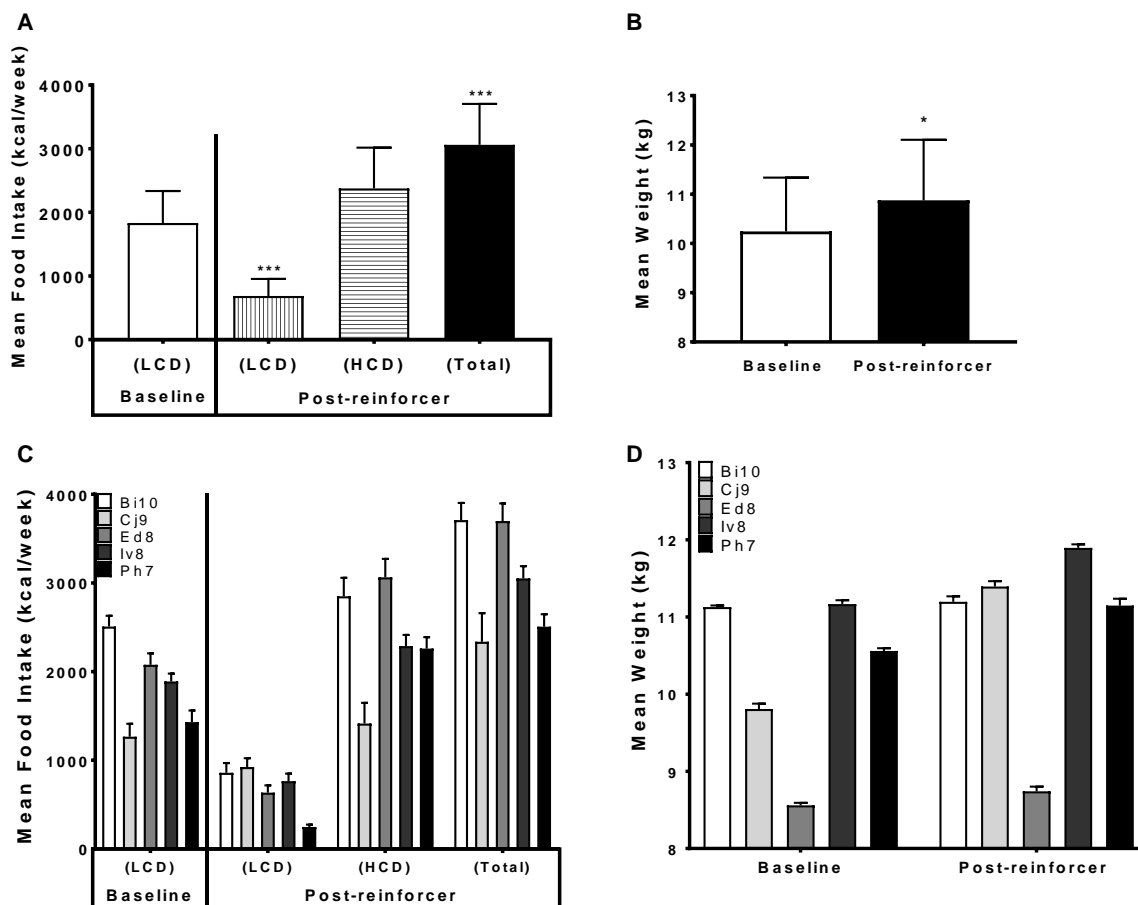


Figure 2.3 Reinforcer Intake During the 6-month Period (HCD group)

Food intake and weight were measured in the HCD group under baseline conditions, when they only had access to a LCD, and after a 6-month period of HCD intake. Data for mean food intake (A) and weight (B) for the group, as well as mean food intake (C) and weight (D) by individual, are presented as the mean \pm SEM. * $p < 0.05$ for post-reinforcer weight compared with baseline weight; *** $p < 0.001$ for post-reinforcer LCD and total food intake compared with baseline LCD.

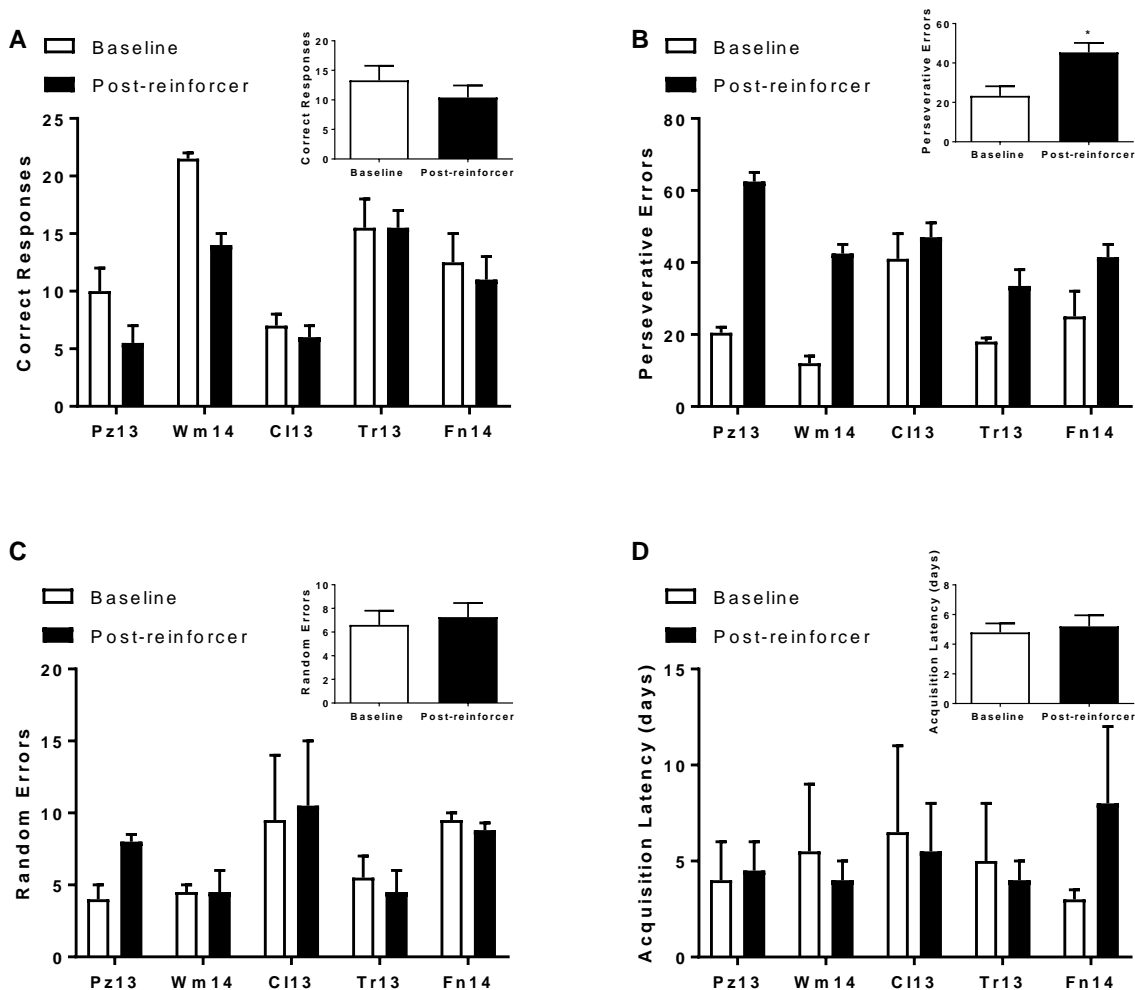


Figure 2.4 Effects of 6-month METH Intake on DRL Task Measures

DRL task measures were taken for the METH group under baseline conditions, when they were experimentally naïve, and after a 6-month period of METH SA. Data for correct responses (A), perseverative errors (B), random errors (C), and acquisition latency (D), prior to and post-METH intake for the group, as well as by individual, are presented as the mean \pm SEM. * $p < 0.05$ for post-reinforcer perseverative errors compared with baseline.

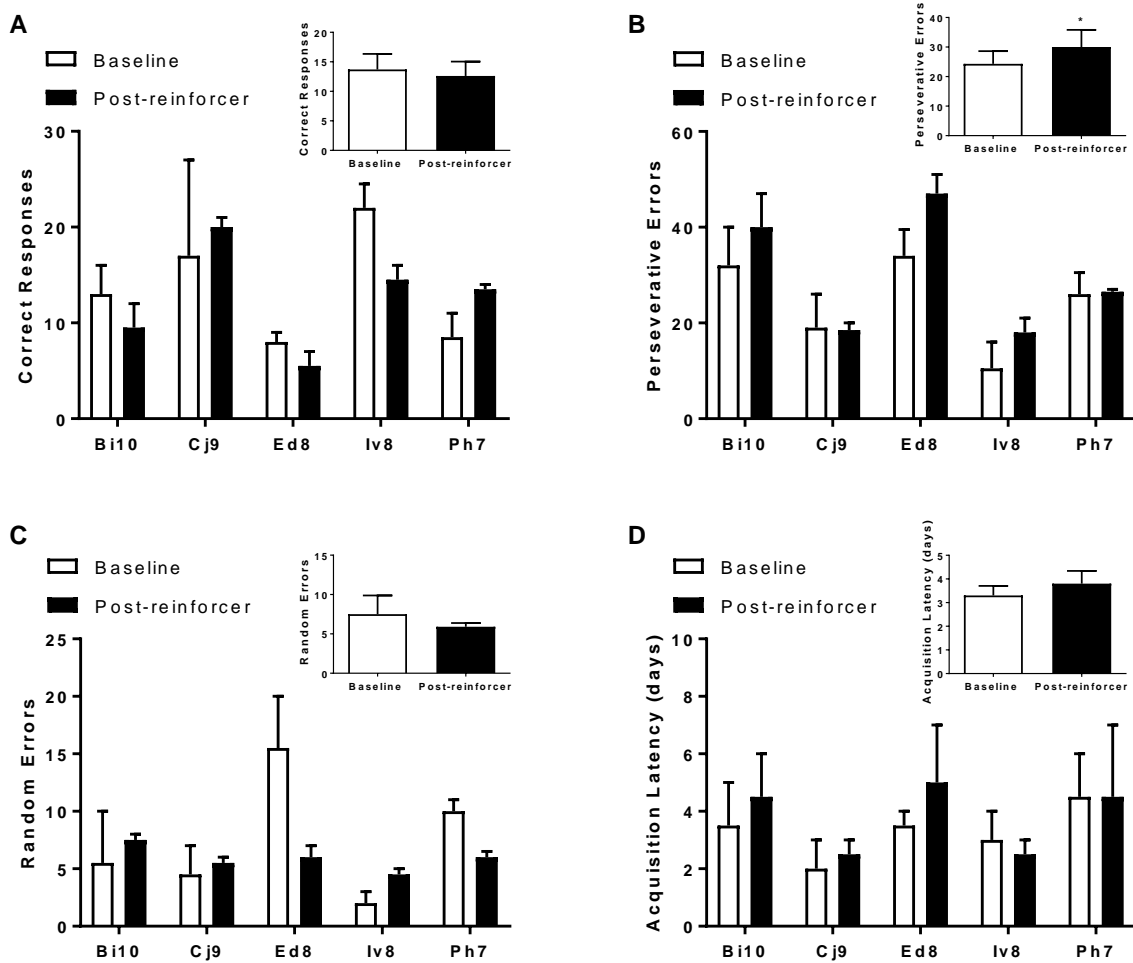


Figure 2.5 Effects of 6-month HCD Intake on DRL Task Measures

DRL task measures were taken for the HCD group under baseline conditions, when they were experimentally naïve, and after a 6-month period of HCD intake. Data for correct responses (A), perseverative errors (B), random errors (C), and acquisition latency (D), prior to and post-HCD intake for the group, as well as by individual, are presented as the mean \pm SEM. * $p < 0.05$ for post-reinforcer perseverative errors compared with baseline.

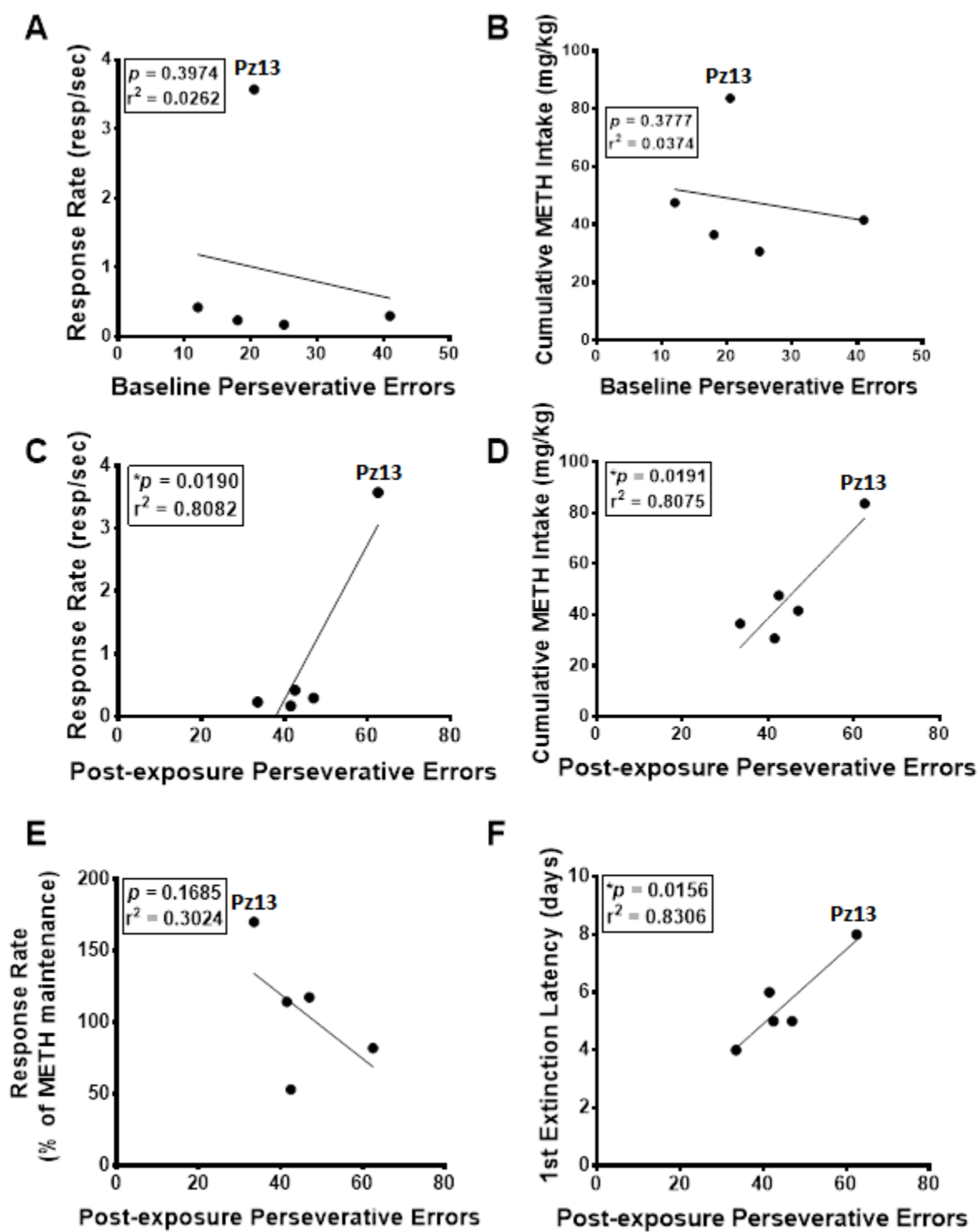


Figure 2.6 METH Group Correlation Analyses

Pearson's correlation analyses were conducted for the METH group to determine the relationship between baseline perseverative errors (PE) and response rates during METH SA (A), between baseline PE and cumulative METH intake (B), response rates during METH SA and post-reinforcer PE (C), cumulative intake and post-reinforcer PE (D), post-reinforcer PE and peak METH reinstatement response rates (E), and between post-reinforcer PE and 1st extinction latency (F). METH group subjects are represented by one data point in each correlation plot; the points representing subject Pz13 are labeled for clarity.

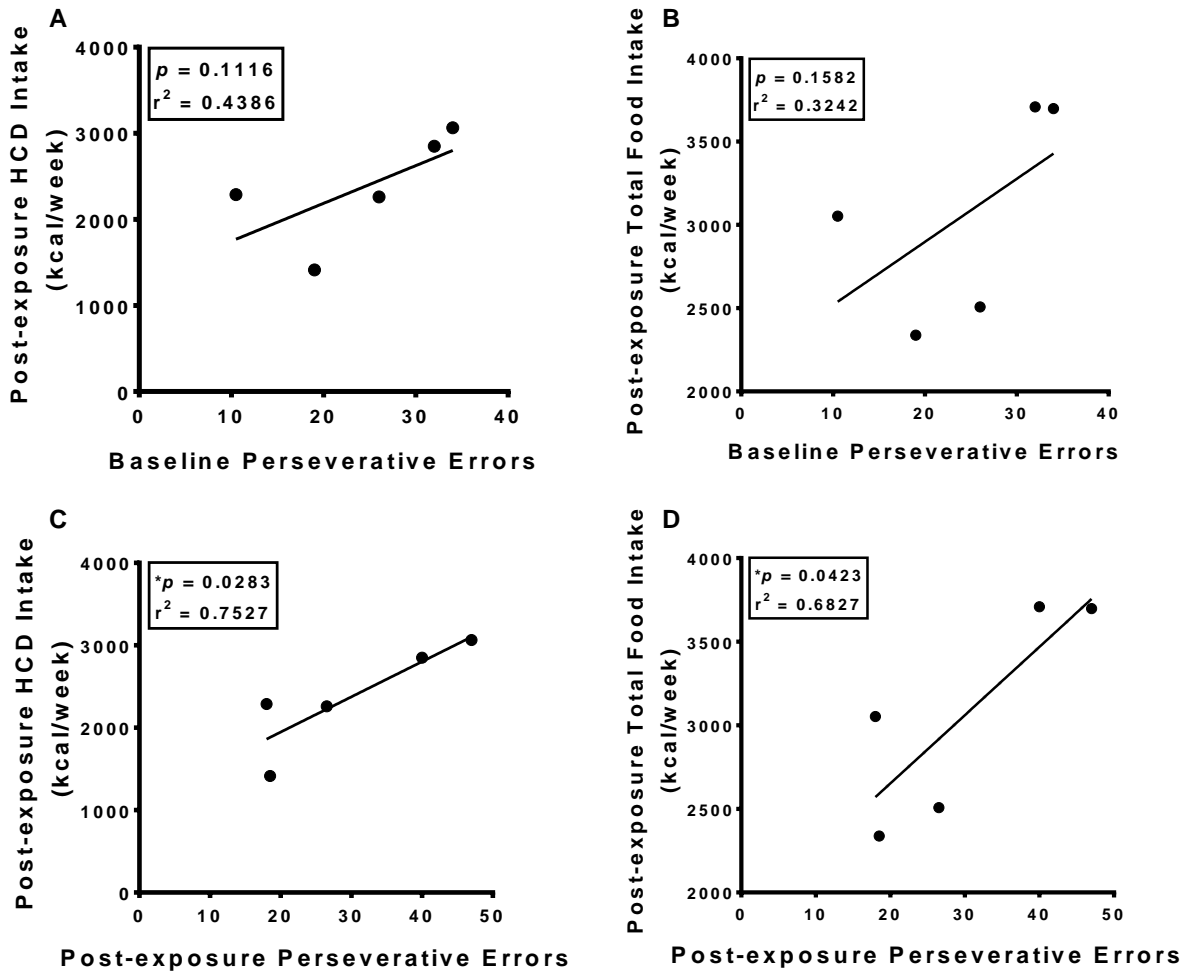


Figure 2.7 HCD Group Correlation Analyses

Pearson's correlation analyses were conducted for the HCD group to determine the relationship between baseline perseverative errors (PE) and post-reinforcer HCD intake only (A), between baseline PE and post-reinforcer total food intake (B), post-reinforcer HCD intake only and post-reinforcer PE (C), and post-reinforcer total food intake and post-reinforcer PE (D). HCD group subjects are represented by one data point in each correlation plot.

Chapter 3. Activation of Serotonin 2C Receptors Reduces Compulsivity and Intake of a Palatable, High Caloric Diet, but not a Low Caloric Diet, in Female Rhesus Macaques

3.1 Abstract

Rationale Activation of serotonin 2C receptors attenuates intake of cocaine and palatable, high caloric foods. However, it is still unknown whether the same doses of a serotonin 2C receptor agonist reduces both reinforcer intake and compulsivity. A deeper understanding of the role of serotonin 2C receptors in the expression of these behaviors will improve our knowledge of the neurochemical processes that regulate flexible decision-making and whether improvements in decision-making are accompanied by parallel decreases in reinforcer intake.

Objectives This study evaluated the effects of the serotonin 2C receptor agonist WAY163909 on (1) baseline compulsivity, (2) on compulsivity following extended reinforcer intake, and (3) on food intake of two different diets in adult female rhesus monkeys. It also examined whether the serotonin 2C receptor is necessary for the effects of WAY163909.

Methods WAY163909 pretreatments were given prior to compulsivity testing at baseline and after extended reinforcer intake (methamphetamine or a palatable, high caloric diet), and prior to measures of food intake under two different dietary conditions. Antagonists challenges were conducted using the serotonin 2C receptor antagonist SB 242084 to block the effects of WAY163909 on these measures.

Results WAY163909 increased correct responses on a discrimination reversal learning task and decreased perseverative errors (i.e. compulsivity), both at baseline and after extended reinforcer intake. Similarly, these treatments decreased consumption of a palatable, high caloric diet, but not a low caloric diet. The effects of WAY163909 on a discrimination reversal learning task and food intake measures were blocked by co-administration with the serotonin 2C antagonist SB 242084.

Conclusions The data indicate that reductions in food intake produced by WAY163909 are associated with parallel improvements in decision-making strategies. WAY163909 decreased compulsivity, irrespective of reinforcer intake history, and decreased food intake of a palatable, high caloric diet, but not a low caloric diet. Finally, it was demonstrated that these effects are all dependent on the serotonin 2C receptor.

3.2 Introduction

The yearly cost of addiction in the U.S. is almost \$600 billion and millions of people across the globe are affected by this disorder (Harwood, 2000, 2004; Office of National Drug Control Policy, 2004; Wickizer, 2013). Addiction has detrimental effects on the lives of addicts and their families, putting a heavy financial, medical, and legal burden on society and making the development of effective treatments vitally important.

Twelve-step programs like Alcoholics Anonymous, Narcotics Anonymous, Overeaters Anonymous, Gamblers Anonymous, Sex Addicts Anonymous, and many others, are commonly used to treat addiction. However, these programs have had limited success (Carnes, 1983) and relapse rates remain as high as 50-90% (Brownell, 1986; Schaffer & Zimmerman, 1990; Hodgins & el-Guebaly, 2004; Abdollahi et al., 2014). Despite the need for adequate therapeutics, FDA-approved treatments for addiction are limited. For specific addictions, limited pharmaceutical treatments have been developed with problematic characteristics. For example, naltrexone and acamprosate, both of which were developed for treatment of alcohol dependence, only decreased relapse rates within one year by 41% and 17%, respectively (Rubio et al., 2001). Treatments for opioid dependence, on the other hand, such as methadone, decrease weekly

heroin use to 31% after 5 years, but presented with a variety of negative side effects, including constipation, respiratory depression, dizziness, sedation, and liver issues (Tetrault & Fiellin, 2012). For patients suffering from many other types of drug addiction (stimulant addicts) and other behavioral addictions (food addicts, sex addicts, gambling addicts, etc.), no pharmaceutical treatments are available. There is currently an FDA-approved treatment for obesity (Fleming et al., 2013; Shukla et al., 2015), the serotonin 2A/2C receptor agonist lorcaserin, which has a relatively high affinity for the serotonin 2C receptor ($K_i = 15\text{nM}$) compared to the serotonin 2A receptor ($K_i = 112\text{nM}$) (for detailed review see Howell & Cunningham, 2015). However, its effects on weight loss and food intake are modest and the underlying mechanisms are poorly understood. Further, the degree to which food addiction or binge-eating disorder are involved in obesity remains controversial (Volkow et al., 2013a) and no one has evaluated whether the decrease in food intake is affected by or dependent on diet type.

A commonality between these treatments is the idea that specific addictive disorders are independent of one another and the goal is generally to reduce the primary addictive behavior. This approach has hindered advancement in the field because it does not account for the comorbidity between different types of addiction (Hatterer, 1982; Carnes, 1983; Schaffer & Zimmerman, 1990; Black, 2007; Blum et al., 2012), ignores their shared neurobiology (Volkow et al., 2013b), and often does not address the underlying causes of the disorder (Shaffer et al., 2004; Richter & Foster, 2014). An alternate approach, which assumes that these disorders are all related, tries to identify the core neurochemical processes underlying addictive behaviors. This approach may be beneficial in the search for novel pharmaceuticals that could potentially treat various addictions.

Dopamine (DA) has been widely associated with addiction in general (Volkow et al., 2011b; Nutt et al., 2015). Increased DA neurotransmission, especially within the striatum, is critical in the reinforcing properties of addictive drugs (Ritz et al., 1987; Woolverton and Kleven, 1988; Veeneman et al., 2012) and palatable, high caloric foods (Small et al., 2003; Norgren et al., 2006; Wise, 2006; Volkow et al., 2011a). Moreover, DA plays a vital role in the reinforcing properties of behaviors that can become addictive, such as sex (Ikemoto & Panksepp, 1999; Becker et al., 2001; Hull et al., 2004) and gambling (Steeves et al., 2009; Voon et al., 2010). Attempts to leverage dopaminergic drugs for treatment of addiction have yielded no medications that lack abuse liability (Bergman et al., 1989; Howell & Byrd, 1991; Howell et al., 2000; Howell & Wilcox, 2001; Howell et al., 2007; Howell & Negus, 2014) and exhibit efficacy to reduce craving, enhance abstinence, and prevent relapse.

Serotonin (5-HT) signaling can regulate dopaminergic neurotransmission and has become a novel target for pharmacotherapies for treatment of addiction (Howell & Cunningham, 2015). Serotonergic cell bodies originating in the raphe nuclei synapse directly onto both DA and non-DA neurons in the ventral tegmental area (Herve et al., 1987). They also project to brain areas where DA is released, such as the nucleus accumbens (NAcc), dorsal striatum, and prefrontal cortex (PFC). These regions are all highly implicated in addiction (Parent et al., 1981; Halliday & Tork, 1989; Di Matteo et al., 2008). Through these direct and indirect interactions with the DA system, the 5-HT system can influence the abuse-related effects of reinforcers. However, 5-HT's effects on DA neurotransmission depend on both the brain region and the 5-HT receptor subtype involved (Alex & Pehek, 2007; Hayes & Greenshaw, 2011).

Of the 14 genetically encoded 5-HT receptors subtypes (Bockaert et al., 2006; Hannon & Hoyer, 2008), the serotonin 2C (5-HT_{2C}) receptor is the most promising therapeutic candidate (Bubar & Cunningham, 2006, 2008; Cunningham & Anastasio, 2014). 5-HT_{2C} receptors are G protein-coupled receptors (GPCRs) that specifically couple to G $\alpha_{q/11}$, which activate phospholipase C (PLC) second messengers, leading to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to diacyl glycerol (GAD) and inositol triphosphate (IP₃), leading to depolarization of the postsynaptic neuron (for review see Howell & Cunningham, 2015). In addition, 5-HT_{2C} receptors are the only GPCRs known to undergo pre-RNA editing such that this receptor can exist in 32 predicted mRNA isoforms that could encode up to 24 different receptor protein isoforms in the human brain (Gurevich et al., 2002). *In situ* hybridization and radioligand binding studies in both rodents and non-human primates (NHPs) showed that 5-HT_{2C} receptors are localized to the PFC and striatal areas, including the ventral tegmental area (VTA), NAcc, caudate and putamen (Pompeiano et al., 1994; Clemett et al., 2000; Lopez-Gimenez et al., 2001). Although 5-HT_{2C} receptors are expressed on both dopaminergic and GABAergic neurons (Eberle-Wang et al., 1997; Bubar & Cunningham, 2007), rodent (Liu et al., 2007) and human (Pasqualetti et al., 1999) studies showed that they are most highly expressed on the latter.

The predominant localization to GABAergic interneurons, especially within the VTA and PFC, suggests that activation of 5-HT_{2C} receptors should inhibit mesolimbic DA signaling (Howell & Cunningham, 2015), and attenuate the abuse-related effects of reinforcers. Indeed, electrophysiological and microdialysis studies provide evidence that 5-HT_{2C} receptor agonists decrease, whereas antagonists increase, DA neuron firing in the VTA as well as DA release within

the NAcc (reviewed by Bubar & Cunningham, 2006, 2008). Similarly, 5-HT_{2C} receptor knock-out mice exhibit increased DA levels in the Nacc and dorsal striatum (Abdallah et al., 2009).

These effects on DA neurotransmission generalize to reinforcer-induced increases in DA. For example, the 5-HT_{2C} receptor agonist Ro 60-0175 attenuated, whereas the 5-HT_{2C} receptor antagonist SB 242084 enhanced, cocaine-induced increases in DA in the NAcc of rats (Navailles et al., 2008). Comparable results were found in squirrel monkeys, where the effective dose of Ro 60-0175 also significantly decreased cocaine self-administration and reinstatement (Manvich et al., 2012a) and the effective dose of SB 242084 maintained self-administration and increased cocaine-seeking during reinstatement (Manvich et al., 2012b). In addition to cocaine, the 5-HT_{2C} receptor agonist lorcaserin decreased food intake in humans (Hess & Cross, 2013; Hoy, 2013) and nicotine self-administration (Levin et al., 2011) and alcohol intake (Rezvani et al., 2014) in rats. Taken together, these data support the hypothesis that 5-HT_{2C} receptors mediate not just the abuse-related effects of drug reinforcers, but likely reinforcers in general. However, whether the reductions in reinforcer intake produced by 5-HT_{2C} receptor agonists are associated with parallel reductions in compulsivity, remains unclear.

Compulsivity, which can be defined as a general inability to alter behavior with changing reinforcement contingencies, has been highly implicated in all types of addictive disorders in humans (American Society of Addiction Medicine, 2011). Specifically, higher levels of compulsive behavior have been found in drug-dependent (Fernandez-Serrano et al., 2011), obese (Cserjesi et al., 2007), and pathological gambling individuals (Leeman & Potenza, 2012) compared to controls. Further, a progression from recreational use of reinforcers, through habits, and finally to compulsive use, is thought to underlie the development and maintenance of addiction (Everitt &

Robbins, 2005; 2016; Torregrossa et al., 2011). A transition from top-down control by the PFC to bottom-up control by the striatum over decision-making and behavior is hypothesized to underlie this progression (Belin et al., 2013).

Reinforcer-induced increases in DA signaling within the striatum could play a crucial role in the balance between PFC and striatal control over decision-making, and thus both compulsivity and intake of reinforcers. Studies demonstrating that prenatal exposure to psychostimulants in mice (Lloyd et al., 2013) and methamphetamine (METH) pretreatment in rats (Son et al., 2013) increases perseverative behavior (i.e. compulsivity) provide evidence in support of this hypothesis. The 5-HT system plays a role in the expression of compulsive behavior, as well as the abuse-related effects of reinforcers, likely through its effects on DA neurotransmission. For instance, selective depletion of 5-HT in the orbital PFC (oPFC) increases perseverative responding in animal models (Clarke et al., 2004; 2007). As discussed above, activation of 5-HT_{2C} receptors, specifically, can inhibit reinforcer-induced increases in DA in the NAcc, and intake of many types of reinforcers. However, studies addressing the effects of 5-HT_{2C} receptors on compulsive behavior in rodents have yielded mixed results. For instance, the 5-HT_{2C} receptor agonists Ro 60-0175 (Agnoli & Carli, 2012) and CP 809.101 (Del'Guidice et al., 2014) both decreased perseverative responding, whereas another study found that the 5-HT_{2C} antagonist SB 242084 had the same effect (Boulougouris & Robbins, 2010). Thus far, there have been no studies on the effects of 5-HT_{2C} receptor agonists or antagonists on compulsive behavior in nonhuman primates (NHPs), or on whether the 5-HT_{2C} receptor is necessary for these effects.

To address these gaps in the field, the following study evaluated whether a selective 5-HT_{2C} receptor agonist decreases perseverative responding (i.e. compulsivity), at baseline and/or after

prolonged intake of two different reinforcers (METH or a palatable, high caloric diet) in adult female rhesus monkeys. In addition, the present study determined whether 5-HT_{2C} receptor activation decreases intake of a low caloric diet (LCD) and/or a choice diet, where both a LCD and a palatable, high caloric diet (HCD) are available. To demonstrate that the 5-HT_{2C} receptor is necessary for these effects, a selective 5-HT_{2C} receptor antagonist was administered to block the effects of the 5-HT_{2C} receptor agonist on both compulsivity and food intake. The effects of a selective 5-HT_{2C} receptor agonist on METH intake will not be discussed in this chapter because they will be presented in detail in Chapter 4.

As discussed in Chapter 2, NHPs are an ideal model for exploring the neurochemical processing contributing to compulsivity and addictive behaviors because they share a common physiology and neurobiology with humans (Gould et al., 2012), their metabolic processing of pharmacological agents is similar to that of humans, and NHPs exhibit complex decision-making and reinforcement contingency learning (Everitt & Robbins, 2016). Importantly, studies with NHPs allow for extensive environmental control and experimental manipulations that are precluded in humans. A clearer understanding of the role that 5-HT_{2C} receptors play in the expression of compulsive behavior and intake of reinforcers, such as palatable, high caloric foods, may inform the search for novel therapeutics for the treatment of addiction.

3.3 Methods

3.3.1 Experimental Design

The experiments for this study were conducted in parallel with those described in Chapter

2. An outline of the experimental design is shown in Fig. 3.2. At the onset of this study, all subjects were experimentally naïve and baseline measures of compulsivity were taken after pretreatment with a 5-HT_{2C} receptor agonist, vehicle, or no pretreatment. Subsequently, one group of subjects self-administered METH for 6 months (METH group), whereas the other group consumed a HCD for 6 months (HCD group). After this period of reinforcer intake, subjects were re-tested for compulsivity after pretreatment with vehicle, a 5-HT_{2C} receptor agonist, or a combination of the agonist and a 5-HT_{2C} receptor antagonist. For the HCD group, baseline measures of consumption of a LCD were taken after pretreatment with a 5-HT_{2C} receptor agonist, vehicle, or no pretreatment. Similarly, after 6 months in a dietary condition where LCD and HCD were both available, food intake was re-determined after pretreatment with vehicle, a 5-HT_{2C} receptor agonist, or a combination of the agonist and a 5-HT_{2C} receptor antagonist.

3.3.2 Subjects

This study utilized the same subjects described in Chapter 2: adult female rhesus macaques (*Macaca mulatta*) weighing 7-14 kg ($N=10$). As mentioned previously, at the onset of this study, all subjects had never self-administered drugs of abuse or consumed palatable, high caloric foods. All procedures strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Eighth Edition, revised in 2010), and were approved by the Institutional Animal Care and Use Committee of Emory University.

METH Group

Half of the subjects ($N=5$) were pair-housed in indoor stainless-steel home cages at the Yerkes National Primate Research Center (YNPRC) Main Station and were assigned to the METH

group. They were fed Purina monkey chow (Ralston Purina, St. Louis, MO, USA) supplemented with fruits and vegetables and had water continually available in the colony, which was maintained at an ambient temperature of $22\pm 2^{\circ}\text{C}$ at 45-50% humidity and a 12-hour light/dark cycle (lights on at hour 0700; lights off at hour 1900). Environmental enrichment was provided on a regular rotating basis. Subjects were fitted with collars (Primate Products) used for accesses.

HCD Group

The other half of the subjects ($N=5$) were housed in indoor—outdoor 144 ft^2 ($12 \times 12\text{ ft.}$) pens at the YNPRC Field Station in groups of 5 to 6 females and were assigned to the HCD group. As with the METH group, environmental enrichment was provided on a regular rotating basis for the HCD group subjects. Because socially housed female rhesus monkeys form a dominance hierarchy with subordinates showing a distinct behavioral and physiological phenotype (Michopoulos et al., 2012a), only middle ranking (ranks 3 and 4) animals were utilized in this study. Ranks were determined based on the outcome of dyadic agonistic interactions (as described by Michopoulos et al., 2016).

3.3.3 Drugs

The selective $5\text{-HT}_{2\text{C}}$ receptor agonist WAY163909 hydrochloride [(7b- R,10a -R)-1,2,3,4,8,9,10,10a -octahydro-7bHcyclopenta[b][1,4] diazepino [6,7,1hi] indole] was acquired as a generous gift from Pfizer Inc.® (New York, NY, USA). WAY163909 was dissolved in 10 mg/mL beta-cyclodextrin (vehicle for WAY) and administered intramuscularly 45 minutes prior to the start of DRL reversal sessions or measures of food intake. WAY163909 (Fig. 3.1) has a very high affinity for the $5\text{-HT}_{2\text{C}}$ receptor ($K_i = 10.5$) (for review see Howell & Cunningham, 2015). The

selective 5-HT_{2C} receptor antagonist SB 242084 [6-chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride hydrate] was purchased from Tocris Bioscience® (Ellisville, MO, USA), dissolved in 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 by using 0.9% saline. All treatments with SB 242084 were administered intramuscularly 15 minutes prior to WAY163909 pretreatments. METH (±) hydrochloride was provided by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC, USA), was dissolved in 0.9% sterile physiological saline, and was administered intravenously.

3.3.4 Compulsivity Measurements

All subjects ($N=10$) were trained on the Discrimination Reversal Learning (DRL) task, a commonly used compulsivity measure (Rogers et al., 2000; Izquierdo & Jentsch, 2011). Compulsivity measurements were collected as described in Chapter 2, section 2.3.3. Briefly, subjects were tested on a Discrimination Reversal Learning (DRL) task using a Wisconsin General Test Apparatus (WGTA) fitted with a stimulus tray containing 3 wells. DRL sessions were conducted once/day (Monday-Friday at the Yerkes Field Station and Monday-Sunday at the Yerkes Main Station). Sessions consisted of 30 trials separated by 15 second intervals and object positions were randomized during trials.

Acquisition

During acquisition, one of 3 distinct objects was paired with a hidden reward until the subject reached a performance criterion of 90% correct responses (the rewarded object was

chosen first) during a session. After meeting these criteria, they underwent a reversal the following day. No pretreatments were given prior to acquisition sessions.

Reversal

During reversal, the reward was hidden under one of the two previously un-rewarded objects. Reversal was conducted identically to acquisition, except it lasted for only one session consisting of 30 trials and subjects were not required to meet any performance criterion. Possible responses included picking the newly rewarded object (correct response), choosing the previously rewarded object (perseverative error), or selecting the third object that had never been paired with the reward (random error). Perseverative errors made during reversal were the primary measure of compulsivity. All pretreatments with vehicle, the 5-HT_{2C} agonist, or the antagonist were given prior to reversal sessions. A new set of 3 objects was used for each subsequent round of acquisition and reversal.

3.3.5 5-HT_{2C} Receptor Agonist and Antagonist Interventions

Following baseline reversal DRL task measures, during which no pretreatments were given, subjects were evaluated following administration of the 5-HT_{2C} receptor agonist WAY163909 or its vehicle. WAY163909 pretreatments (vehicle, 0.1, 0.3, and 1.0 mg/kg) were randomized across subjects in each group. These doses were chosen because they were effective at attenuating the sleep-related effects of METH self-administration in NHPs (Perez Diaz et al., 2017), as well as self-administration of METH and cocaine, METH- and cocaine-primed reinstatement, and METH- and cocaine-induced DA overflow in the NAcc (Berro et al., 2017a). However, for one subject, Iv8, the WAY163909 dose range had to be reduced down because the

highest dose of WAY163909 (1.0 mg/kg) produced behavioral suppression during the DRL task. She was evaluated under the following WAY163909 doses: 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg. Thus, for all HCD group DRL task measure graphs presented here, the dose range of WAY163909 was labeled as low, medium, and high WAY163909 dose (low = 0.03 mg/kg for Iv8, 0.1 mg/kg for the other 4 subjects; medium = 0.1 mg/kg for Iv8, 0.3 mg/kg for the others; high = 0.3 mg/kg for Iv8, 1.0 mg/kg for the others). A second, final baseline reversal DRL task measure was collected for each subject following all other treatments. For the HCD group, baseline food intake of a LCD only, during which no pretreatments were given, was measured. Then, LCD intake was evaluated when WAY163909, or its vehicle, was injected. WAY163909 pretreatments were administered identically to DRL task pretreatments.

After 6 months of METH SA or consumption of the HCD, subjects were re-tested for reversal DRL task measures. During this second round of DRL testing, subjects were first tested under vehicle pretreatment conditions. Then subjects were evaluated when WAY163909 (0.3 mg/kg for Iv8, 1.0 mg/kg for all others), or a combination of WAY163909 and the 5-HT_{2C} receptor antagonist SB 242084 (0.1 mg/kg), was on board. For the HCD group, food intake was measured under no pretreatment conditions. Then, food intake was evaluated when WAY163909 (0.3 mg/kg for Iv8, 1.0 mg/kg for all others), or a combination of WAY163909 and the 5-HT_{2C} receptor antagonist SB 242084 (0.1 mg/kg), was administered. The dose of SB 242084 used in these antagonist challenges was chosen based on previous drug self-administration experiments in squirrel monkeys (Manvich et al., 2012a, 2012b). All pretreatments with WAY163909, or the combination of SB 242084 + WAY163909, were administered as described for DRL task pretreatments.

3.3.6 Intravenous Drug Self-Administration (SA)

Following baseline compulsivity testing, subjects ($N=5$) in the drug group self-administered METH (0.01 mg/kg/infusion i.v.) for a period of 6 months (24 consecutive weeks). Briefly, subjects responded under a fixed-ratio (FR) 20 schedule of drug delivery during 60-min sessions once daily, in the morning (starting between 0700 and 1000 hours) Monday-Friday. Average response rate (responses/second) and average and total METH intake (mg/kg/day and mg/kg, respectively) were measured throughout the 6-month SA block. Response rates were calculated as the total number of lever presses during the active period divided by the active time throughout the session. Detailed description of the METH SA procedure can be found in Chapter 2, section 2.3.4.

3.3.7 Food Intake

Prior to and during baseline compulsivity testing, subjects ($N=5$) in the HCD group had *ad libitum* access to standard monkey chow (LCD; 3.45 kcal/g, Purina 5038) only via previously validated automated feeders that allowed for constitutive quantification of caloric intake (Michopoulos et al., 2012b). Following baseline compulsivity testing, subjects in the HCD group were given access to both the LCD and a HCD (4.47 kcal/g, D07091204S Research Diets, New Brunswick NJ) for 6 months. A detailed description of the food intake procedure is given in Chapter 2, section 2.3.5. Measures of food intake were taken for 24-hrs following vehicle, WAY163909, or SB 242084 + WAY163909 pretreatments.

3.3.8 Statistical Analysis

All statistical analyses were conducted using GraphPad Prism 7. Repeated measures analysis of variance (RM ANOVA) with Dunnett's *post hoc* test were used to determine the effects of pretreatments with vehicle, WAY163909 doses, or SB 242084 + WAY163909, on baseline and post-reinforcer compulsivity in both groups, as well as baseline and post-reinforcer food intake in the HCD group.

3.4 Results

3.4.1 Effects of a 5-HT_{2c} Receptor Agonist on Baseline Compulsivity

Baseline DRL task measures (correct responses, perseverative errors, random errors, and acquisition latency) were collected using a WGTA and are reported here as mean \pm SEM. Subjects were evaluated under the following conditions: 1) baseline, 2) vehicle pretreatment, and 3) WAY163909 pretreatments on reversal days. The order of treatments was randomized.

METH Group

For the METH group ($N=5$), pretreatments with the highest dose of WAY163909 (1.0 mg/kg) significantly increased correct responses (Fig. 3.3A) by $\sim 44\%$ [$F(5,5) = 6.27$, $p = 0.0031$] compared to vehicle. All WAY163909 pretreatments had a significant effect on perseverative errors (Fig. 3.3B), with the 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg pretreatments decreasing this measure by approximately 41%, 56%, and 71%, respectively [$F(5,5) = 12.65$, $p < 0.0001$], compared to vehicle pretreatments. Random errors (Fig. 3.3C) were not affected by WAY163909

[$F(5,5) = 0.6805$, $p = 0.6155$]. Similarly, there was no difference in acquisition latency (Fig. 3.3D) for any of the WAY163909 pretreatments [$F(5,5) = 0.4218$, $p = 0.7906$].

HCD Group

Baseline DRL task measures for the HCD group ($N=5$) were collected under the same conditions as the METH group (baseline, vehicle, and WAY163909 doses, randomized and counterbalanced) and they exhibited similar responses to WAY163909. All doses of WAY163909 had a significant effect on correct responses (Fig. 3.4A), with the low, medium, and high WAY163909 dose pretreatments increasing this measure by approximately 89%, 128%, and 104%, respectively [$F(5,5) = 15.31$, $p < 0.0001$], compared to vehicle. In addition, WAY163909 pretreatments had a significant effect on perseverative errors (Fig. 3.4B), with the low, medium, and high WAY163909 dose pretreatments decreasing perseveration by approximately 49%, 61%, and 67%, respectively [$F(5,5) = 13.09$, $p < 0.0001$], compared to vehicle pretreatments. As with the METH group, random errors for the HCD group (Fig. 3.4C) were not affected by WAY163909 [$F(5,5) = 0.7972$, $p = 0.5441$] and there was no difference in acquisition latency (Fig. 3.4D) for any of the WAY163909 pretreatments [$F(5,5) = 0.7656$, $p = 0.5630$].

3.4.2 Effects of a 5-HT_{2c} Receptor Agonist on Consumption of a LCD

At baseline, subjects in the HCD group ($N=5$) only had access to a LCD. Baseline food intake in this group was measured for 24 hours under the following conditions: 1) vehicle pretreatment and 2) WAY163909 pretreatments. The order of treatments was randomized across subjects and food intake measures are reported here as mean \pm SEM. RM ANOVA revealed no significant

change in 24-hr total food intake (Fig. 3.5) across any of the conditions tested [$F(4,5) = 0.3243$, $p = 0.8078$].

3.4.3 Effects of a 5-HT_{2c} Receptor Agonist and Antagonist on Post-Reinforcer Compulsivity

Following baseline compulsivity testing, subjects in the METH group self-administered METH for a period of 6 months, whereas subjects in the HCD group were given access to both a LCD and a palatable HCD for 6 months. All subjects ($N=10$) were re-tested on the DRL task after their 6-month reinforcer intake blocks. As with baseline, post-reinforcer DRL task measures (correct responses, perseverative errors, random errors, and acquisition latency) were collected using a WGTA and are reported as mean \pm SEM. On reversal days, subjects were evaluated under the following conditions: 1) vehicle pretreatment, 2) high WAY163909 dose pretreatment, and 3) 0.1 mg/kg SB 242084+ high WAY163909 dose pretreatment. The order of treatments was randomized.

METH Group

For the METH group ($N=5$), pretreatments with the highest dose of WAY163909 (1.0 mg/kg) significantly increased correct responses (Fig. 3.6A) by $\sim 92\%$ [$F(3,5) = 30.46$, $p = 0.0002$] compared to vehicle. When a dose of the antagonist SB 242084 (0.1 mg/kg) was given in combination with the high dose of WAY163909, this effect of WAY163909 was blocked. Similarly, the high dose of WAY163909 significantly decreased perseverative errors (Fig. 3.6B) by $\sim 77\%$ [$F(3,5) = 50.22$, $p < 0.0001$], an effect that was blocked by SB 242084. Random errors (Fig. 3.6C) were not affected by pretreatments with either the high dose of WAY163909 or the combined

SB 242084 + WAY163909 pretreatment [$F(3,5) = 3.79, p = 0.0695$] and there was no difference in acquisition latency (Fig. 3.6D) for any of the pretreatments [$F(3,5) = 2.646, p = 0.1312$].

HCD Group

Post-reinforcer measures for the HCD group ($N=5$) were collected under the same conditions as the METH group: vehicle, high WAY163909 dose and SB 242084 dose + high WAY163909 dose. As with the METH group, pretreatments with the high WAY163909 dose significantly increased correct responses (Fig. 3.7A) by ~68% [$F(3,5) = 43.89, p < 0.0001$] in the HCD group compared to vehicle. SB 242084 (0.1 mg/kg), given in combination with WAY163909, blocked this effect. The high dose of WAY163909 also significantly decreased perseverative errors (Fig. 3.7B) by ~71% [$F(3,5) = 15.15, p = 0.0019$], an effect that was blocked by SB 242084. Random errors (Fig. 3.7C) were not affected by any of the tested pretreatments [$F(3,5) = 3.253, p = 0.0925$] and there was no difference in acquisition latency (Fig. 3.7D) across these conditions [$F(3,5) = 2.341, p = 0.1583$].

3.4.4 Effects of a 5-HT_{2c} Receptor Agonist and Antagonist on Consumption of a Palatable HCD

Following baseline compulsivity testing, subjects in the HCD group ($N=5$) consumed a palatable HCD for 6 months. During this time, subjects had access to both the LCD and the HCD. Post-reinforcer food intake in this group was measured for 24 hours under the following conditions: 1) vehicle pretreatment, 2) WAY163909 dose pretreatments, and 3) 0.1 mg/kg SB 242084 + high WAY163909 dose. The order of treatments was randomized across subjects and food intake measures (LCD only, HCD only, and total) are reported here as mean \pm SEM. During this round of re-testing, one of the main goals was to determine whether a 5-HT_{2c} receptor

antagonist could block the effects of WAY163909 on food intake measures. For this purpose, the high dose of WAY163909 again was chosen to be given in combination with a dose of the antagonist SB 242084.

As was observed at baseline, RM ANOVA revealed no significant change in 24-hr LCD calorie intake (Fig. 3.8A) across any of the pretreatments tested [$F(5,5) = 0.8625, p = 0.5073$]. However, WAY163909 did have a significant effect on HCD only intake (Fig. 3.8B), with the medium and high doses decreasing HCD only 24-hr food intake by approximately 45% and 60% [$F(5,5) = 12.63, p < 0.0001$], respectively, compared to vehicle. SB 242084 (0.1 mg/kg), given in combination with WAY163909 (1.0 mg/kg), blocked this effect. WAY163909 also had a significant effect on total food intake (Fig. 3.8C), with the medium and high WAY163909 doses decreasing total 24-hr food intake by approximately 21% and 45% [$F(5,5) = 12.14, p < 0.0001$], respectively, compared to vehicle.

3.5 Discussion

3.5.1 Overview

The present study examined the effects of the highly selective 5-HT_{2C} receptor agonist WAY163909 on both compulsive behavior and food intake. Baseline compulsivity was initially characterized in NHPs using a DRL task. Subjects were given pretreatments of various doses of the 5-HT_{2C} agonist WAY163909 prior to reversal sessions. Results demonstrated that WAY163909 pretreatments decreased compulsivity at baseline. After the effects of WAY163909 on baseline compulsivity were determined, subjects either self-administered METH (METH group) or

consumed a HCD (HCD group) for a period of 6 months, after which the effects of WAY163909 on enhanced, post-reinforcer compulsivity were characterized. Again, WAY163909 pretreatments were effective at reducing post-reinforcer compulsivity. Additionally, the effects of WAY163909 on food intake were also determined under two dietary conditions (LCD only and LCD+HCD). As with compulsivity, WAY163909 pretreatments reduced intake of the HCD, but not the LCD. Moreover, WAY163909 was given in combination with a 5-HT_{2C} receptor antagonist, SB 242084, to determine whether this receptor was necessary for the effects of WAY163909 on both compulsivity and food intake. SB 242084 pretreatments blocked the effects of WAY163909 on both post-reinforcer compulsivity and HCD intake. The effects of WAY163909 on both drug intake and drug seeking of METH were evaluated in a separate experiment (Berro et al., 2017b), the results of which are presented in detail in Chapter 4.

3.5.2 Effects of WAY163909 on Compulsivity

Activation of 5-HT_{2C} receptors decreased baseline compulsivity and decreased the enhanced compulsivity induced by long-term intake of METH or a HCD. Although all subjects experienced a significant increase in compulsivity following extended intake of reinforcers (discussed in detail in Chapter 2), pretreatment with the high WAY163909 dose effectively normalized compulsivity to baseline levels. A similar effect on random errors was not observed, suggesting that the effects of WAY163909 on perseveration were specific. Moreover, there was no difference in acquisition latency across treatment conditions, which indicates that the decrease in perseveration produced by WAY163909 pretreatments was not due to a decrease in object discrimination exposure or training. These results agree with previous findings that the 5-

HT_{2c} receptor agonists Ro 60-0175 (Agnoli & Carli, 2012) and CP 809.101 (Del'Guidice et al., 2014) both decreased perseverative responding, but conflict with another study reporting that the 5-HT_{2c} antagonist SB 242084 had the same effect (Boulougouris & Robbins, 2010). Differences in the compounds used, the specifics of the task, and model organisms may account for these disparate results. For example, Agnoli and Carli (2012) examined the effects of Ro 60-0175 on perseveration induced by blockade of NMDA receptors in the PFC in rats using the five-choice serial reaction time task (5-CSRTT). Del'Guidice and colleagues (2014), on the other hand, determined the effects of CP 809.101 on perseveration induced by a loss of function mutation in the serotonin synthesis enzyme TPH2 in mice using an automated H-maze delayed reversal task. Finally, Boulougouris and Robbins (2010) used rats to probe the effects of SB 242084 on perseveration using a two-lever spatial discrimination task (2-LSDT). They infused SB 242084 into specific brain regions and only found an increase in perseveration when this compound was infused into the oPFC, but not the mPFC or the NAcc (Boulougouris & Robbins, 2010).

Others have shown that subjects can “learn to learn” on the DRL task, and that the number of acquisition days tends to decrease with experience, paralleled by a decrease in perseveration (Clarke et al., 2004, 2007; Lee et al., 2007; Rudebeck et al., 2013), when the task is run with only two objects and when serial reversals are conducted (the same objects are reversed more than once). This general trend was observed in the present study, but cannot be attributed to practice since the order of treatments was randomized and this practice confound was not robust enough to mask the profound effects of WAY163909. In addition, the current study employed a modified version of the DRL task, which included having 3 objects instead of 2, such that subjects had more behavioral options than just correct responses and perseverative errors

during reversal, and no set of objects was ever reversed more than once. These modifications to the task, and the use of forced corrections, may account for the high number of perseverative errors observed, compared to previous studies (Clarke et al., 2004, 2007; Lee et al., 2007; Rudebeck et al., 2013).

In addition to decreasing perseveration, WAY163909 also improved performance on the DRL task during reversals by increasing correct responses at baseline and after prolonged self-administration of METH or consumption of a HCD. Taken together, these results support the hypothesis that WAY163909 improves flexible decision making within the context of changing reinforcement contingencies, regardless of food or drug intake history, by both increasing newly reinforced responses and decreasing responses that are no longer being reinforced.

3.5.3 Effects of WAY163909 on Intake of a Low Caloric Diet vs a Palatable HCD

Activation of 5-HT_{2C} receptors via pretreatments with WAY163909 had no effect on baseline 24-hr consumption of a LCD in the HCD group subjects. Importantly, under the dietary choice condition, when subjects consumed both a LCD and a palatable HCD, WAY163909 dose-dependently reduced total caloric intake by attenuating intake of the HCD, but not the LCD, for a period of 24 hours. Thus, modulation of food intake by activation of 5-HT_{2C} receptors appears to be diet-specific. This specificity is beneficial from a therapeutic viewpoint because it suggests that these effects are not due to general decreases in motor function or appetitive behavior, but instead are specific to consumption of foods that are high in fat and sugar, which are known to be highly reinforcing (Small et al., 2003; Norgren et al., 2006). In agreement with the findings reported here, previous studies using the less selective 5-HT_{2C} receptor agonist lorcaserin found

that this compound effectively reduced intake of a high fat diet and body weight in rats (Thomsen et al., 2008) and humans (Martin et al., 2011). The effects of lorcaserin on behaviors that are reinforced, such as gambling, have not been evaluated.

Agonists at the 5-HT_{2C} receptor exhibit efficacy at decreasing intake of drug reinforcers as well as food reinforcers. For instance, the 5-HT_{2C} receptor agonist Ro 60-0175 reduced cocaine intake in squirrel monkeys (Manvich et al., 2012a), and lorcaserin reduced intake of nicotine (Levin et al., 2011) and alcohol (Rezvani et al., 2014) in rats. In a separate experiment, it was demonstrated that the same doses of the 5-HT_{2C} agonist WAY163909 used in this study effectively reduced both drug intake and drug seeking of METH (Perez Diaz et al., 2017b) in rhesus monkeys. The details of that study are reported at length in Chapter 4. Given that the effects of 5-HT_{2C} agonists are generalizable to both food and drug reinforcers, agonists at this receptor have the potential to decrease intake of other reinforcers as well, including reinforced behaviors like sex and gambling.

Here, the results provide evidence that the same doses of WAY163909 decreased both intake of a HCD and perseverative responding in the subjects. Moreover, the same dose of SB 242084 blocked the effects of WAY163909 on both compulsivity and food intake. Similarly, the same doses of WAY163909 that reduced compulsivity in the current study attenuated drug intake and drug seeking of METH (Perez Diaz, et al., 2017b). Together, these data suggest that the reduction in palatable, high caloric food or METH intake induced by activation of 5-HT_{2C} receptors is accompanied by a parallel decrease in general compulsive behavior. One hypothesis to account for these effects is that activation of 5-HT_{2C} receptors simultaneously modulates both compulsive behavior and intake of reinforcers through related molecular mechanisms. An alternate

hypothesis is that activation of these receptors decreases intake of reinforcers through its effects on compulsivity. Further studies are necessary to clarify the precise mechanisms that underlie these effects.

3.5.4 Effects of SB 242084 on Compulsivity and Food Intake

To determine whether the effects of WAY163909 on compulsivity and food intake were dependent on the 5-HT_{2C} receptor, and not off-target signaling, antagonist challenges were conducted using the 5-HT_{2C} receptor antagonist SB 242084. Combination pretreatments with SB 242084 and the high dose of WAY163909 were given prior to both post-reinforcer compulsivity testing (in all subjects) and food intake measures (in HCD group subjects). The findings support the hypothesis that the 5-HT_{2C} receptor is necessary for the effects of WAY163909, as SB 242084 blocked them. Although the high dose of WAY163909 increased correct responses, and decreased both perseverative responding and food intake of the palatable HCD, these effects were all attenuated when SB 242084 was given in combination with WAY163909. Although these results conflict with a previous study showing that SB 242084 decreases perseveration (Boulougouris & Robbins, 2010) in rodents, they align with other findings demonstrating that 5-HT_{2C} receptor agonists, rather than antagonists, decrease perseverative responding (Agnoli & Carli, 2012; Del'Guidice et al., 2014). Moreover, the present results also agree with studies in NHPs showing that SB 242084 blocks the decreases in cocaine self-administration induced by 5-HT_{2C} receptor agonists (Manvich et al., 2012a) and can itself recapitulate some of the abuse-related effects of psychostimulants (Manvich et al., 2012b).

3.5.5 Limitations of the Study

It should be noted that this study employed female subjects only, and thus the applicability of the results presented here to males will need to be tested. Similarly, antagonist challenges with SB 242084 and WAY163909 were conducted using only one dose of SB 242084, instead of establishing a full dose-effect curve for all measures using SB 242084. This choice was made to limit the number of reversals that subjects underwent in an effort to mitigate the “learning to learn” (practice) effect seen with the DRL task. In addition, previous studies with SB 242084 in nonhuman primates (Manvich et al., 2012a, 2012b) provided a useful guide for choosing a behaviorally relevant dose that proved effective in the behavioral assays used here. Finally, as mentioned in Chapter 2, this study employed female subjects only and there are currently no studies in the literature that use the same version of the DRL task used here. Thus, it would be difficult to make any comparisons regarding the results of this chapter with males.

3.7 Figures

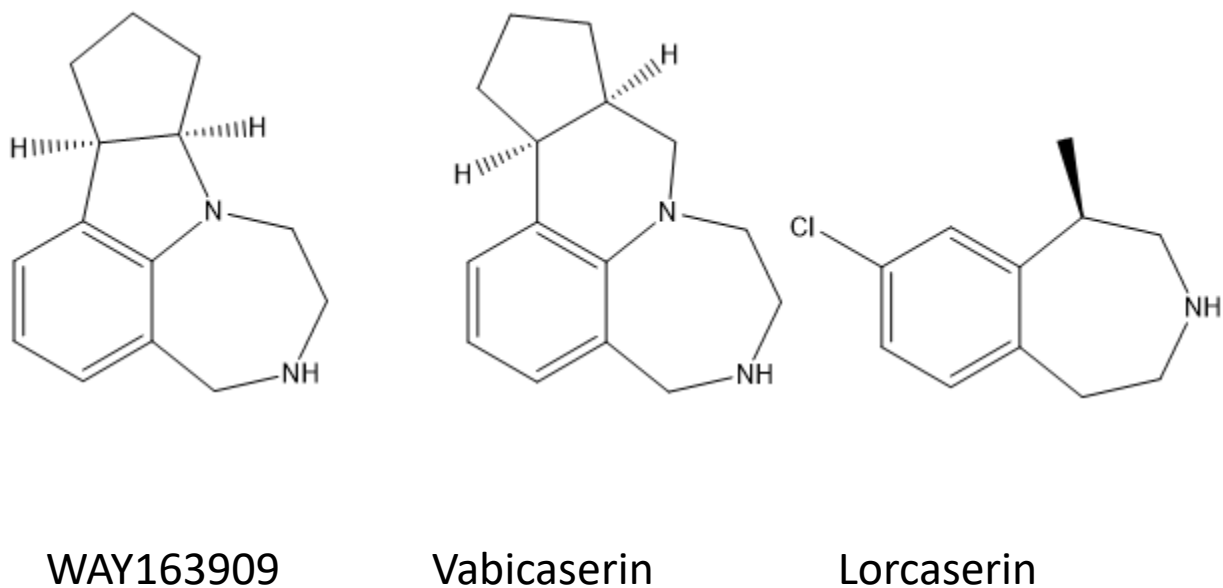


Figure 3.1 Chemical Structures of 5-HT_{2C} Agonists

The chemical structures of three 5-HT_{2C} receptor agonists are shown. WAY163909 is the most highly selective for the 5-HT_{2C} receptor, Vabicaserin is slightly less selective than WAY163909, and Lorcaserin is the least selective of the three.

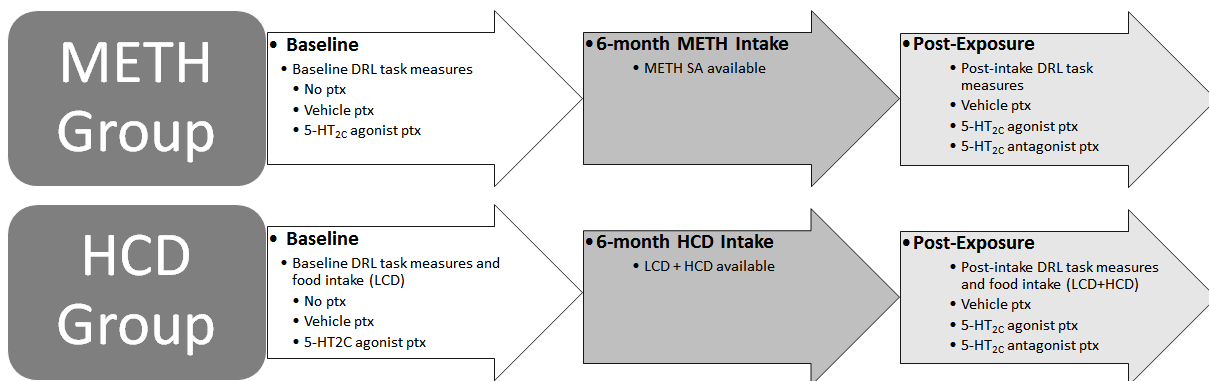


Figure 3.2 Timeline of Experimental Design

The diagram shows the measures collected in this study for both the METH and HCD groups under all experimental conditions, including pretreatments with the 5-HT_{2C} receptor agonist WAY163909 and the antagonist SB 242084.

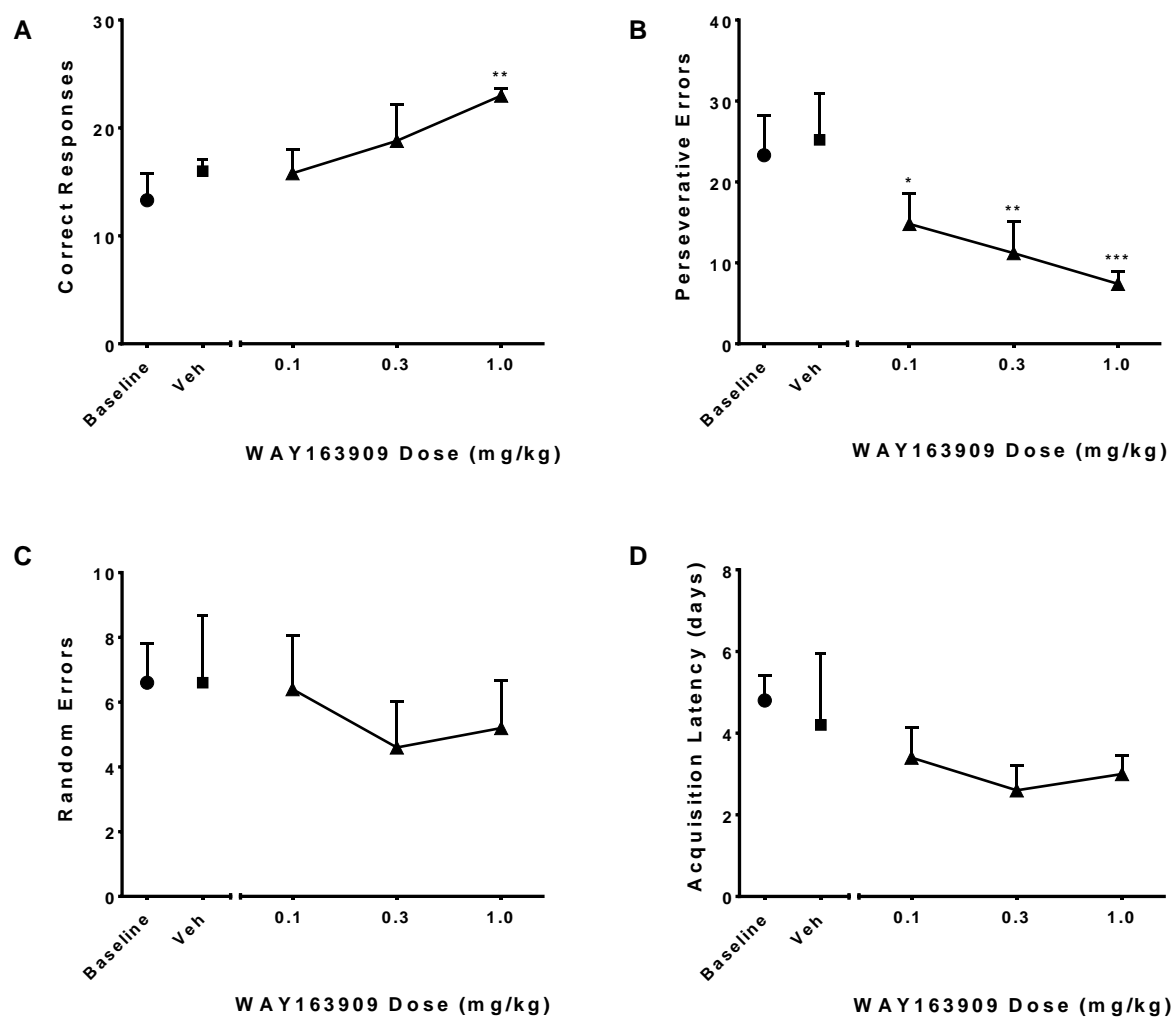


Figure 3.3 Effects of WAY163909 on Baseline DRL Task Measures (METH group)

Subjects participated in a DRL task in which several measures were noted during reversal sessions. Dose-effect curves for WAY163909 (0.1, 0.3, and 1.0 mg/kg) were established for the METH group for the following measures: (A) correct responses, (B) perseverative errors, (C) random errors, and (D) acquisition latency. Data are presented as the group mean \pm SEM for each experimental condition.

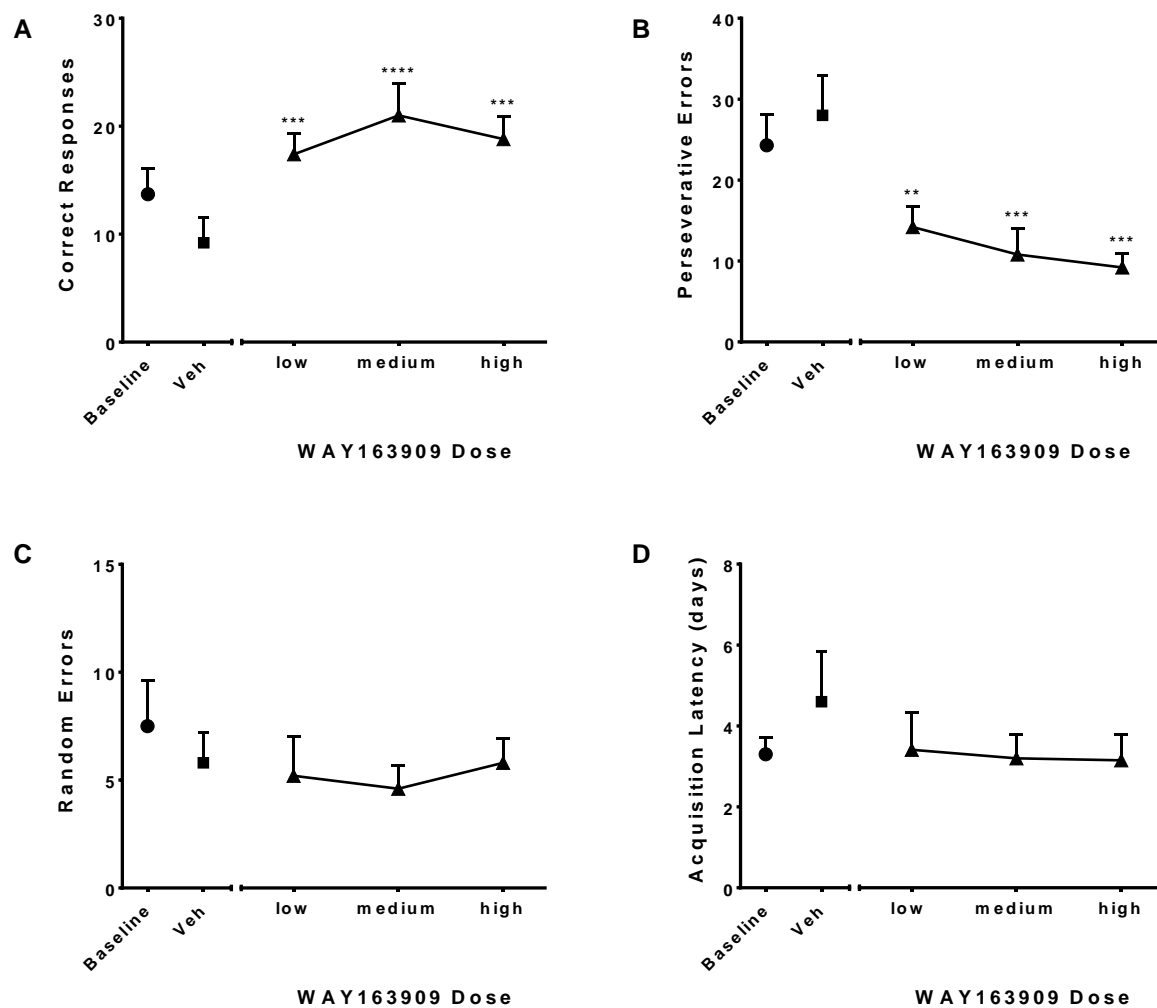


Figure 3.4 Effects of WAY163909 on Baseline DRL Task Measures (HCD group)

Subjects participated in a DRL task in which several measures were noted during reversal sessions. Dose-effect curves for WAY163909 (low, medium, and high doses) were established for the HCD group for the following measures: (A) correct responses, (B) perseverative errors, (C) random errors, and (D) acquisition latency. Data are presented as the group mean \pm SEM for each experimental condition.

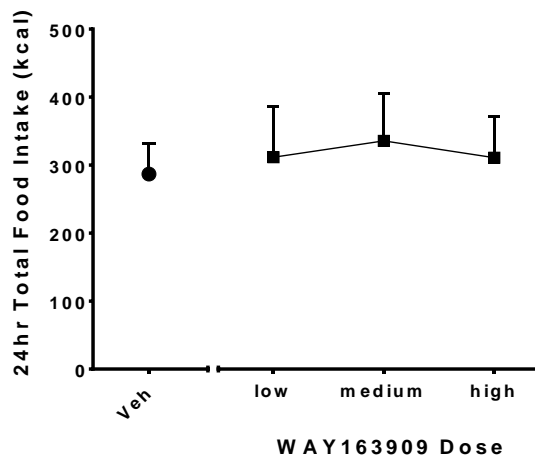


Figure 3.5 Effects of WAY163909 on Food Intake of a Low Caloric Diet (HCD group)

Food intake measures were taken for all subjects at baseline, when they had access only to a LCD.

A dose-effect curve for WAY163909 (low, medium, and high doses) was established for the HCD group for 24-hr total food intake. Data are presented as the group mean \pm SEM for each experimental condition.

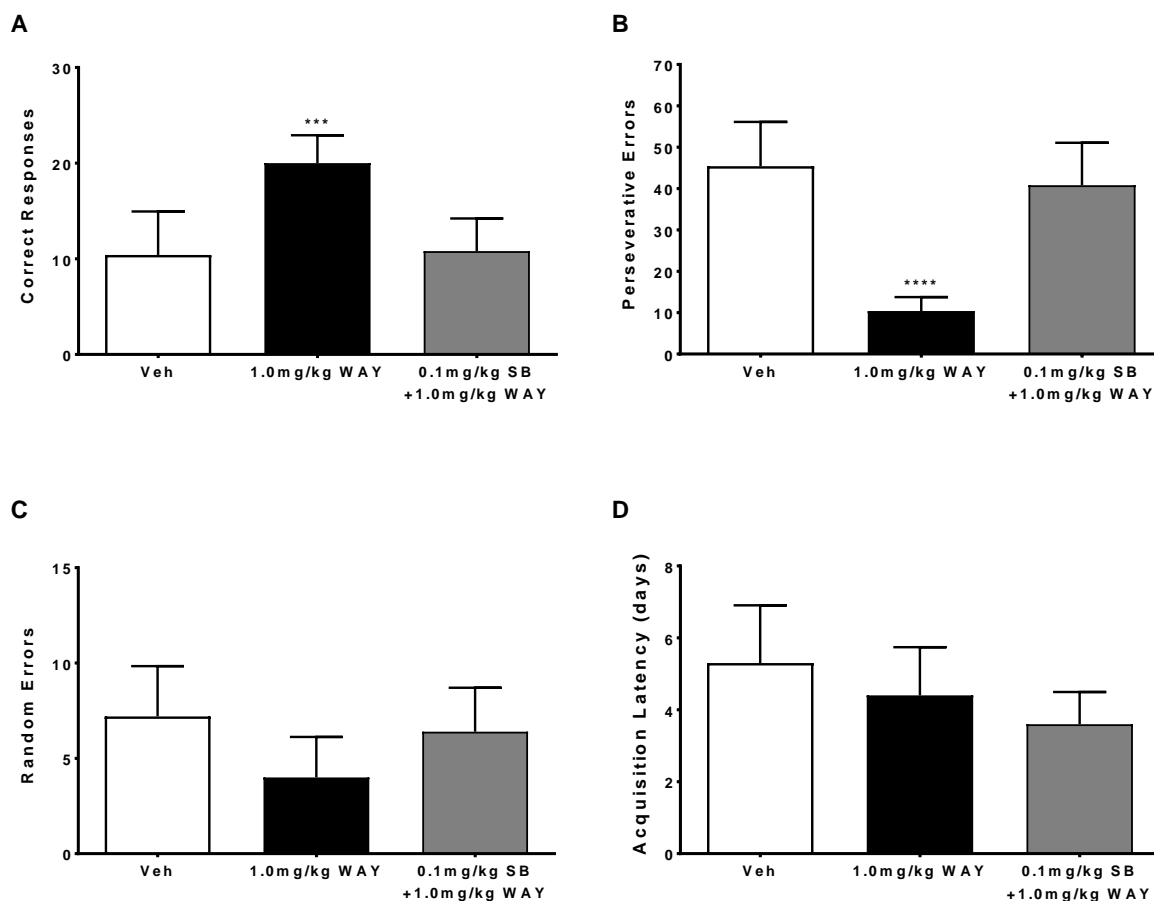


Figure 3.6 Effects of Combined Pretreatment with WAY163909 and SB 242084 on Post-exposure DRL Task Measures (METH group)

Subjects in the METH group self-administered METH for 6 months. Afterward, post-exposure compulsivity was measured using a DRL task under the following experimental conditions: vehicle pretreatment, high dose of WAY163909 (1.0 mg/kg) pretreatment, and high dose of WAY163909 + SB 242084 dose (0.1 mg/kg) combination pretreatment. Effects of these pretreatments on DRL task measures are shown for (A) correct responses, (B) perseverative errors, (C) random errors,

and (D) acquisition latency. Data are presented as the group mean \pm SEM for each experimental condition.

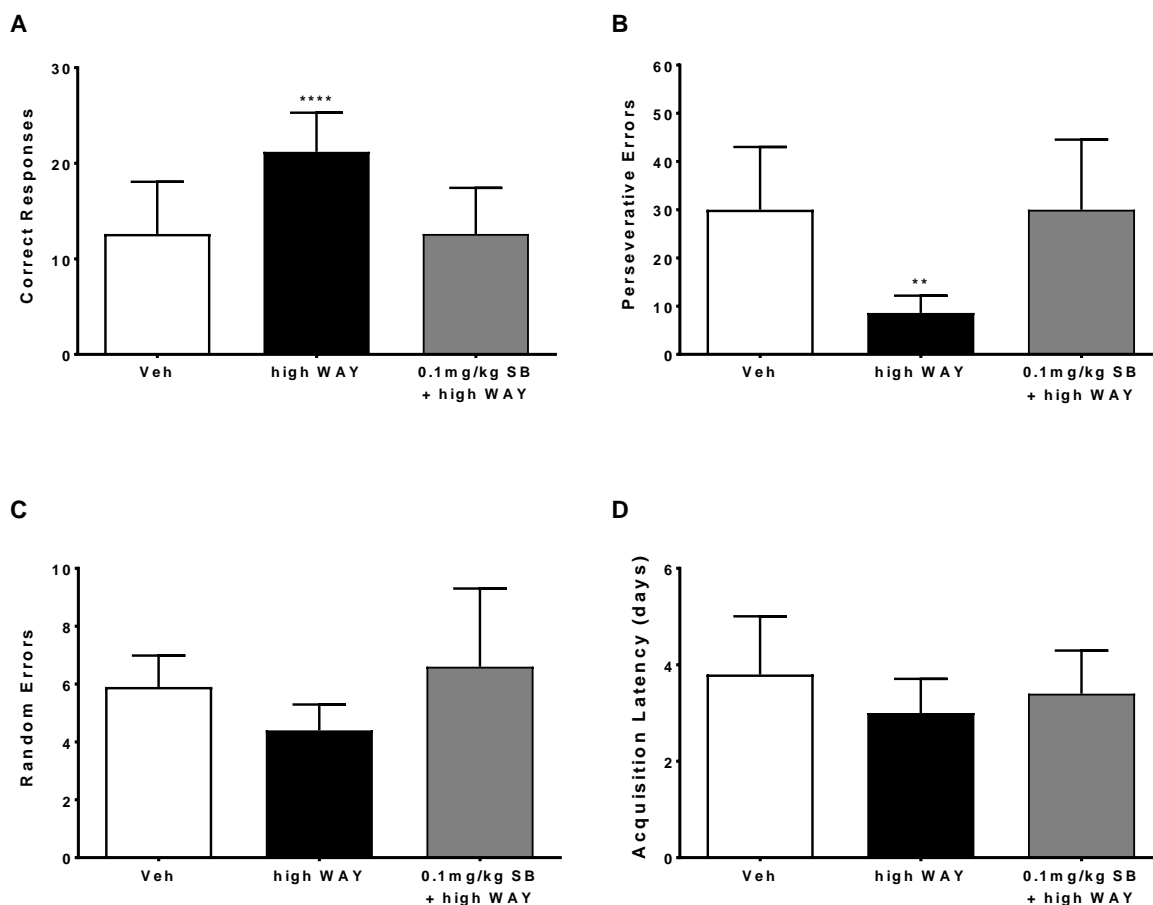


Figure 3.7 Effects of Combined Pretreatment with WAY163909 and SB 242084 on Post-exposure DRL Task Measures (HCD group)

Subjects in the HCD group were given access to both a LCD and a palatable HCD for 6 months. Post-exposure compulsivity was measured under the following conditions: vehicle pretreatment, high dose of WAY163909 pretreatment, and high dose of WAY163909 + SB 242084 dose (0.1 mg/kg) combination pretreatment. Effects of these pretreatments on DRL task measures are shown for (A) correct responses, (B) perseverative errors, (C) random errors, and (D) acquisition latency. Data are presented as the group mean \pm SEM for each experimental condition.

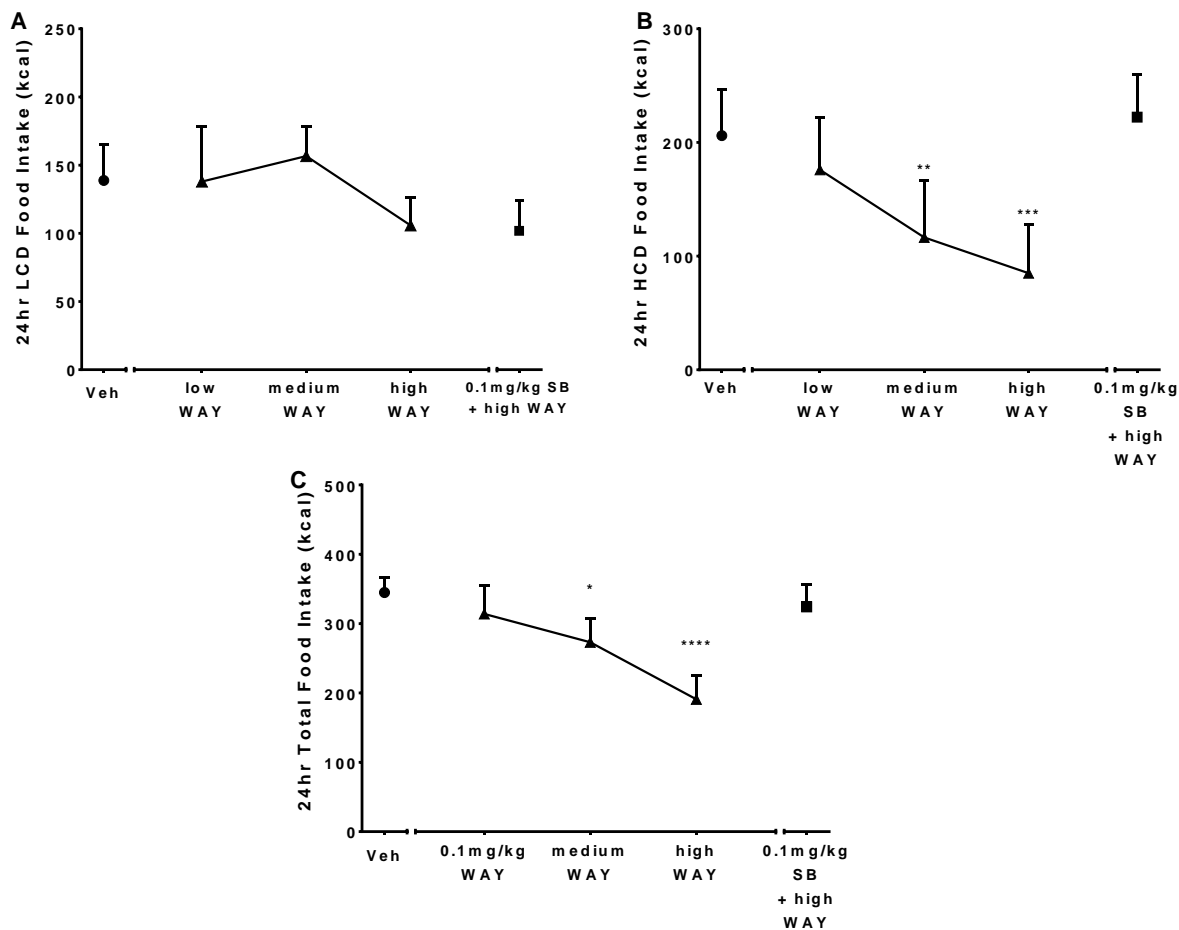


Figure 3.8 Effects of Combined Pretreatment with WAY163909 and SB 242084 on Food Intake of a Palatable Diet (HCD group)

Subjects in the HCD group were given access to both a LCD and a palatable HCD for 6 months. Immediately after the 6-month period, a dose-effect curve for WAY163909 (low, medium, and high doses) was established for the HCD group for 24-hr (A) LCD only food intake, (B) HCD only food intake, and (C) total food intake. Intake was also measured when a high dose of WAY163909

+ SB 242084 dose (0.1 mg/kg) combination pretreatment was given. Data are presented as the group mean \pm SEM for each experimental condition.

Chapter 4. Effects of the Serotonin 2C Receptor Agonist WAY163909 on the Abuse-Related Effects and Mesolimbic Dopamine Neurochemistry Induced by Methamphetamine in Rhesus Monkeys

[All text and figures from (Berro, Perez Diaz, et al., 2017a)]

4.1 Abstract

Rationale Accumulating evidence shows that the serotonergic system plays a major role in psychostimulant abuse through its interactions with the dopaminergic system. Studies indicate that serotonin 5-HT_{2C} receptors are one of the main classes of receptors involved in mediating the influence of serotonin in drug abuse.

Objectives The aim of the present study was to evaluate the effects of the selective serotonin 5-HT_{2C} receptor agonist WAY163909 on the behavioral neuropharmacology of methamphetamine in adult rhesus macaques.

Methods Methamphetamine self-administration and reinstatement were evaluated under a fixed-ratio schedule of reinforcement. Methamphetamine-induced increases in dopamine were assessed through *in vivo* microdialysis targeting the nucleus accumbens.

Results Pretreatment with WAY163909 dose-dependently attenuated methamphetamine self-administration and drug-induced reinstatement of extinguished behavior previously maintained by methamphetamine delivery. In an additional experiment, WAY163909 induced a dose-dependent attenuation of methamphetamine-induced dopamine overflow in the nucleus accumbens.

Conclusions The present data indicate that selective 5-HT_{2C} receptor activation decreases methamphetamine intake and drug-seeking behavior in nonhuman primate models of methamphetamine abuse through neurochemical mechanisms likely involved in the modulation of mesolimbic dopamine.

4.2 Introduction

Psychostimulant abuse is a chronic relapsing disorder characterized by loss of control over drug intake despite harmful consequences (American Psychiatric Association, 2013). Although psychostimulants differ in their pharmacodynamic properties, stimulant drugs exhibit abuse liability as a common feature and exert their reinforcing properties by increasing dopamine levels in the mesolimbic dopaminergic system (Koob & LeMoal, 2006). For instance, the effectiveness of psychostimulants to increase extracellular dopamine levels in the nucleus accumbens has been shown to parallel their stimulant, reinforcing and reinstating effects in nonhuman primates (for review see Howell & Negus, 2014). Thus, dopaminergic neurotransmission has been a primary target for medications development for psychostimulant abuse.

Despite decades of research, there are no approved dopamine-based medications for the treatment of stimulant abuse. This lack of effective medications has prompted researchers to investigate other possible neural systems that contribute to the development and maintenance of drug abuse. In this context, the serotonergic system has gained attention for playing a key role in the addictive cycle, especially through its direct and indirect interactions with the mesolimbic dopaminergic system (Howell & Cunningham, 2015). Several serotonin receptors have been implicated in mediating the effects of serotonin on dopamine neurotransmission. However, studies indicate that the serotonin 5-HT_{2A} and 5-HT_{2C} receptors play a vital role in mediating the influence of serotonin in drug abuse (Bubar & Cunningham, 2006, 2008; Cunningham & Anastasio, 2014).

Serotonin 5-HT_{2A} receptors are highly expressed on dopaminergic neurons in the ventral tegmental area and on glutamatergic neurons in the prefrontal cortex, whereas GABAergic

interneurons in those brain regions predominantly express 5-HT_{2C} receptors (Howell & Cunningham, 2015), suggesting that these receptors may directly modulate dopamine neurotransmission and dopamine levels in the nucleus accumbens. *In vivo* microdialysis studies provide convincing evidence that the reinforcing properties of psychostimulants are mediated primarily by dopamine release in the nucleus accumbens (Schultz et al., 1997; Martin-Soelch et al., 2001; Watanabe et al., 2001; Czoty et al., 2002). Both the self-administration and the reinstatement models provide a measure of drug seeking behavior and have been used increasingly as laboratory models to evaluate the reinforcing properties of drugs of abuse in nonhuman primates (Howell & Wilcox, 2002; Katz & Higgins, 2003; Epstein et al., 2006). Because of the regional distribution of serotonin 5-HT_{2A} and 5-HT_{2C} receptors in the brain, those receptors have been shown to exert opposing effects on cocaine self-administration and cocaine-induced reinstatement in nonhuman primates (Murnane et al., 2013; Manvich et al., 2012b). Particularly, activation of 5-HT_{2C} receptors has been shown to decrease cocaine self-administration, cocaine-induced reinstatement, and dopamine overflow in the nucleus accumbens in both rodents (Grottick et al., 2000; Neisewander & Acosta, 2007; Burbassi & Cervo, 2008; Fletcher et al., 2008; Navailles et al., 2008) and squirrel monkeys (Manvich et al., 2012a; Rüedi-Bettschen et al., 2015). However, the effects of receptor subtype-selective compounds and the extent of those findings to other stimulant drugs have yet to be systematically evaluated.

The aim of the present study, therefore, was to evaluate the effects of the highly selective serotonin 5-HT_{2C} receptor agonist WAY163909 (Dunlop et al., 2005; Neelamegam et al., 2014) on the behavioral neuropharmacology of methamphetamine (METH) in rhesus monkeys. Studies have shown that WAY163909 exhibits excellent binding affinity (K_i of ~10 nM) and functional

selectivity for the human 5-HT_{2C} receptor (Dunlop et al., 2005), being one of the most selective agents identified to date compared with other compounds reported in the literature, including Ro 60–0175 (Martin et al., 1998) and novel compounds such as lorcaserin (Thomsen et al., 2008) and vabicaserin (SCA-136) (Dunlop et al., 2011). WAY163909 presents low intrinsic activity as a partial agonist at 5-HT_{2B} receptors and lacks functional activity at 5-HT_{2A} receptors (Dunlop et al., 2005). The effects of WAY163909 were evaluated on METH self-administration and reinstatement, as well as on METH-induced increases in dopamine in the nucleus accumbens. The present study adds to the literature by evaluating the effects of a highly selective 5-HT_{2C} receptor agonist on self-administration, reinstatement, and neurochemistry induced by METH in a unique translational primate model.

4.3 Methods

4.3.1 Subjects

Five adult rhesus monkeys (2 females and 3 males) weighing 10–16 kg served as subjects in the methamphetamine behavioral studies. *In vivo* microdialysis experiments were conducted in a group of four adult female rhesus monkeys weighing 7–9 kg. Animals were fitted with collars (Primate Products®, Immokalee, FL, USA) prior to the initiation of the studies. Each subject was individually housed in stainless steel home cages and was fed Purina monkey chow (Ralston Purina, St. Louis, MO, USA), supplemented with fruit and vegetables daily. Water was continuously available in the colony. Environmental enrichment was provided on a regular basis. The colony was maintained at an ambient temperature of 22 ± 2 °C at 45–50% humidity, and the

lights were set to a 12-h light/dark cycle (lights on at 7 h; lights off at 19 h). All subjects had a history of exposure to psychostimulants. All protocols and animal care and handling strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, revised 2011) and the recommendations of the American Association for Accreditation of Laboratory Animal Care, and were approved by the Institutional Animal Care and Use Committee of Emory University.

4.3.2 Drugs

Methamphetamine hydrochloride (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% saline and was administered intravenously. The 5-HT_{2C} receptor agonist WAY163909 hydrochloride [(7b–R,10a–R)-1,2,3,4,8,9,10,10a–octahydro-7bHcyclopenta[b][1,4] diazepino [6,7,1hi] indole] (provided as a generous gift from Pfizer Inc.[®], New York, NY, USA), was dissolved in 10 mg/mL beta-cyclodextrin and administered intramuscularly.

4.3.3 Surgery

All surgeries were conducted under aseptic conditions. Animals were initially anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0 mg i.m.) and ketamine HCl (20 mg i.m.), and anesthesia was maintained throughout the procedure with inhaled isoflurane (0.5–1.5%). A major vein (femoral or jugular) was implanted with a chronic indwelling catheter attached to a subcutaneous vascular access port, as previously described (Howell & Wilcox, 2001b). The subjects of the microdialysis experiments were also implanted with bilateral CMA/11

guide cannulae (CMA Microdialysis, Holliston, MA, USA), as previously described (Murnane et al., 2010). To target the area directly above the nucleus accumbens (NAcc), the guides were placed 23 mm anterior to the interaural midpoint in each subject and were placed bilaterally at 4 mm off the midline.

4.3.4 METH Self-Administration

The animals were positioned in a primate chair (Primate Products®, Immokalee, FL, USA) and were placed in a sound-attenuating experimental chamber for the duration of the self-administration (SA) sessions. For the METH experiments, five animals (ROf8, RVm8, RLk4, RZs9, and RJl8) were trained to respond under a FR 20 schedule of drug delivery. The apparatus and SA procedure were previously described in detail by Howell and Wilcox (2001b). Subjects had the opportunity to self-administer methamphetamine during 60-min daily sessions. During the test session, the behavioral chamber was illuminated with a white light positioned over the lever in the operant panel that served as a discriminative stimulus. Completion of the FR 20 response requirement resulted in a change in the stimulus light from white to red (CS) for 15 s and a methamphetamine infusion (0.5 ml infused over 3 s). This infusion was followed by a 60-s timeout during which responding on the lever had no programmed consequences. At the end of the timeout, the white light was presented again to signal the opportunity to complete another FR.

Response rates were calculated as the total number of lever presses during the active period divided by the active time throughout the session. Before the beginning of drug interaction studies, the maximally effective behavioral stimulant dose of methamphetamine (EDMax), i.e., the dose that maintained the highest rates of responding during self-administration

maintenance, was identified for each individual subject. The unit dose of METH (0.001, 0.003, 0.01, or 0.03 mg/kg/infusion) was altered until the EDMax was identified. The order of doses was randomized and counterbalanced across subjects. The EDMax dose of METH was 0.003 mg/kg/infusion in two subjects and 0.01 mg/kg/infusion in the remaining subjects.

For drug-interaction studies, the effects of pretreatment with WAY163909 were tested in combination with two doses of METH: EDMax and one-half log-step unit dose below the EDMax dose (-0.5 EDMax). Stable SA behavior was defined as response rates that varied by <30% over 3 days. Once responding stabilized at a given dose (EDMax or -0.5 EDMax) for each of the animals, vehicle or WAY163909 (0.1, 0.3, or 1.0mg/kg) were then repeated with the other dose (EDMax or -0.5 EDMax) of METH. Between different pretreatments, animals were returned to normal METH SA conditions until stability criteria were met again, then a new pretreatment was administered on the following day. The order of SA doses (EDMax or -0.5 EDMax) and the order of pretreatments (vehicle or WAY163909 doses) were randomized and counterbalanced across subjects.

4.3.5 METH Reinstatement

For the reinstatement experiments, behavior was maintained by the unit dose of 0.03 mg/kg/infusion METH ($N=4$; ROF8, RLk4, RZs9, and RJI8). Behavior was extinguished by substituting saline for methamphetamine, and was operationally defined as a rate of responding over two sequential sessions that was <20% of the 3-day mean rate of responding during the maintenance of METH SA. During the extinction sessions, the CS (red light) was never illuminated. Otherwise, the extinction sessions were identical to the SA maintenance sessions. Starting on the

day after subjects reached extinction criteria, reinstatement test days consisted of an experimenter administered prime of METH given through the vascular access port 5 min before the start of the session. During these sessions, saline was substituted for METH and the CS was illuminated again throughout the session. Therefore, the sessions are described as drug- and cue-induced reinstatement tests. Firstly, the dose of METH (0.01, 0.03, 0.1, 0.3, or 1.0 mg/kg) was altered until the EDMax dose of METH priming was identified for each individual subject. The order of doses was randomized and counterbalanced across subjects. The EDMax for subjects varied from 0.03 to 0.3 mg/kg METH. The subsequent reinstatement experiments were conducted with the EDMax doses of METH prime.

WAY163909 (0.1, 0.3, or 1.0 mg/kg, i.m.) or its vehicle were administered in the home-cage 45 min before the drug prime. Reinstatement sessions with vehicle pretreatment alternated with WAY163909 pretreatment sessions to ensure a reliable reinstatement response. If the reinstatement effect dissipated, animals were returned to METH SA conditions until stability criteria were met again, then a new block of extinction and reinstatement began. The order of treatments or doses was randomized and counterbalanced across subjects.

4.3.6 *In Vivo* Microdialysis

Microdialysis samples were collected and analyzed as previously described (Murnane et al., 2010, 2012). Briefly, all procedures were performed in fully conscious subjects while they sat in a primate chair (Primate Products®, Immokalee, FL, USA) within a sound attenuating testing chamber. After the subject was placed in the chamber, 28-mm stainless steel microdialysis probes with 4 mm membranes (CMA Microdialysis, Holliston, MA, USA) were inserted into the

surgically implanted guide cannulae. Four subjects (RDn8, RHp8, RNb7, and RJs6) underwent the microdialysis protocol six times, such that each subject received vehicle and WAY163909 (0.1 and 1.0 mg/kg, i.m.) pretreatments combined with subsequent saline or METH (1.0 mg/kg, i.v.) treatments. Experiments consisted of a 1-h equilibrium period after which samples were collected every 10 min. Vehicle and WAY163909 were administered 30 min after the sampling began and 30 min before saline or METH administration and samples were collected over the next 2 h. The viability of the sampling site was verified through retrodialysis of a potassium-enriched (100 mM) solution otherwise ionically matched to artificial cerebrospinal fluid (aCSF). Dopamine concentrations within the dialysate were quantified using high-pressure liquid chromatography with electrochemical detection, as previously described (Murnane et al., 2010, 2012). The data were analyzed by comparison with standard concentration curves using Chromeleon 6.8 Chromatography Data System (Thermo Fisher Scientific, Waltham, MA, USA). The doses of METH used in the microdialysis experiments were based on previous studies (Kirkland Henry et al., 2009; Murnane et al., 2013; Berro et al., 2017b). In vivo microdialysis sessions were performed no more frequently than every 2 weeks for each subject. A microdialysis session with METH following saline pretreatment was conducted between blocks of experiments to rule out the effects of non-specific site damage on the results, and data on different sessions were averaged for each animal. The order of vehicle and WAY163909 doses was counterbalanced across subjects.

4.3.7 Statistical Analysis

Data for the self-administration experiments are presented and were analyzed as normalized data (percentage of average response rates during self-administration maintenance) or as raw data (drug intake/session). Data for the reinstatement experiments are presented as normalized data (percentage of average response rates/saline infusions during reinstatement induced by the EDMax dose of METH prime). Behavioral data were analyzed by one- or two-WAY163909 repeated measures (RM) analysis of variance (ANOVA). Microdialysis data were analyzed by two-way RM ANOVA. The three data points immediately preceding the pretreatment administration were averaged to create the baseline. All *post hoc* comparisons were performed using Dunnett's test. All statistical tests were performed using PASW Statistics 18 (SPSS Statistics Software), and significance was arbitrated at a probability of $p < 0.05$.

4.4 Results

4.4.1 METH Self-Administration

The effects of pretreatment with WAY163909 on METH SA are shown in Fig. 4.1A. The mean response rate (\pm S.E.M.) during SA maintenance was 1.03 ± 0.11 responses/s for the EDMax dose of METH, and 0.44 ± 0.13 for the -0.5 EDMax dose. Two-way RM ANOVA corrected for multiple comparisons using Dunnett's *post hoc* test revealed a significant effect of treatment (vehicle vs WAY163909) [$F(2,12) = 63.37, p < 0.0001$]. Pretreatment with WAY163909 dose-dependently decreased response rates during METH SA regardless of METH dose. Pretreatment with the middle ($p < 0.01$) and the high ($p < 0.001$) doses of WAY163909 significantly decreased response rates compared to vehicle at the EDMax dose of METH. Pretreatment with the high

dose of WAY163909 also significantly decreased response rates at the -0.5 EDMax dose of METH ($p < 0.001$). Analysis of METH intake during SA sessions (Fig. 4.1B) also revealed a significant interaction effect between treatment (vehicle vs WAY163909) and METH dose (EDMax vs -0.5 EDMax) (two-way RM ANOVA [$F(2,12) = 7.307, p < 0.01$]), showing that WAY163909 dose-dependently decreased drug intake, with the middle ($p < 0.01$), and the high ($p < 0.001$) doses of WAY163909 significantly decreasing METH intake compared to vehicle at the EDMax dose of METH. The high dose of WAY163909 also significantly decreased drug intake at the -0.5 EDMax dose of METH ($p < 0.001$).

4.4.2 METH Reinstatement

The mean METH SA response rate (\pm S.E.M.) typically declined within two to three sessions during extinction, from 0.32 ± 0.03 during SA maintenance to 0.04 ± 0.01 responses/s during effective extinction conditions. The mean response rate (\pm S.E.M.) engendered by the EDMax prime dose of METH was 0.59 ± 0.17 responses/s (approximately 187% of the rates typically observed during METH SA). The effects of pretreatment with a range of doses of WAY163909 on drug- and cue-induced reinstatement are shown in Fig. 4.2A. One-way RM ANOVA [$F(3,15) = 8.99, p < 0.01$] corrected for multiple comparisons using Dunnett's *post hoc* test showed that pretreatment with WAY163909 dose-dependently decreased response rates during reinstatement. Pretreatment with the middle ($p < 0.05$) and the high ($p < 0.01$) doses significantly decreased response rates compared to vehicle. Analysis of the number of saline infusions during reinstatement sessions (Fig. 4.2B) also showed that WAY163909 dose-dependently decreased the number of infusions (one-way RM ANOVA [$F(3,15) = 12.11, p < 0.01$]),

with the middle ($p < 0.05$) and the high ($p < 0.001$) doses of WAY163909 significantly decreasing the number of saline infusions compared to vehicle.

4.4.3 *In Vivo* Microdialysis

For METH reinstatement experiments, the mean baseline dopamine levels (\pm S.E.M.) varied from 4.35 ± 1.38 nM for sessions preceded by vehicle pretreatments to 5.28 ± 1.51 nM and 4.08 ± 1.83 nM for sessions preceded by 0.1 and 1.0 mg/kg WAY163909 pretreatments, respectively. Two-way RM ANOVA revealed a significant main effect of time [$F(17,102) = 65.55$, $p < 0.0001$], but not pretreatment or interaction for pretreatment with 0.1 mg/kg WAY163909 (Fig. 4.3A). Dunnett's *post hoc* test showed that following vehicle pretreatment, METH significantly elevated extracellular dopamine levels from min 20 through 80 after injection compared with baseline; an effect that was not altered by pretreatment with the lowest dose of WAY163909. For pretreatment with 1.0 mg/kg WAY163909, two-way RM ANOVA revealed a significant interaction effect between time and pretreatment [$F(17,102) = 8.26$, $p < 0.01$] (Fig. 4.3B). Dunnett's *post hoc* test showed that METH was significantly less effective at increasing extracellular dopamine following 1.0 mg/kg WAY163909 pretreatment at times 20 through 80 compared to vehicle. Importantly, 1.0 mg/kg WAY163909 had no effect on baseline dopamine levels *per se* (data not shown). An additional two-way ANOVA conducted with the 3 pretreatment conditions (vehicle, 0.1 mg/kg WAY163909 or 1.0 mg/kg WAY163909) for times 0 through 60 revealed a significant interaction effect between time and pretreatment [$F(12,54) = 1.95$, $p < 0.05$].

4.5 Discussion

4.5.1 Overview

The major finding of the present study was that the highly selective 5-HT_{2C} receptor agonist WAY163909 dose-dependently attenuated METH SA and the reinstatement of extinguished behavior previously maintained by METH delivery in rhesus monkeys. Importantly, in an additional experiment, WAY163909 dose-dependently attenuated METH-induced increases in dopamine in the nucleus accumbens. Moreover, WAY163909 has also been shown to attenuate cocaine self-administration, cocaine-induced reinstatement of behavior previously maintained by cocaine delivery, and cocaine-induced increases in dopamine in the nucleus accumbens (Perez Diaz et al., 2017b). Together, these results indicate that selective 5-HT_{2C} receptor agonist WAY163909 attenuates the behavioral and neurochemical effects of stimulant drugs in rhesus monkeys.

4.5.2 5-HT_{2C} Receptor Agonists Attenuate the Abuse-Related and Dopaminergic Effects of Psychostimulants

The present findings are consistent with previous studies showing that the 5-HT_{2C} receptor agonist Ro 60–0175 attenuated the behavioral and neurochemical effects of cocaine in squirrel monkeys (Manvich et al., 2012a). Pretreatment with Ro 60–0175 dose-dependently attenuated cocaine self-administration and cocaine-induced reinstatement, also decreasing extracellular dopamine levels induced by cocaine in the nucleus accumbens. In a recent study, Rüedi-Battschen and colleagues (2015) also demonstrated attenuation of reinstatement of drug

seeking induced by cocaine priming after pretreatment with Ro 60–0175, even in the absence of the cocaine-paired stimulus. Moreover, antagonism at the serotonin 5-HT_{2c} receptor has been shown to exhibit abuse-related effects typical of stimulants in squirrel monkeys, also interacting with cocaine in an apparently additive manner (Manvich et al., 2012b). Pretreatment with the selective 5-HT_{2c} receptor antagonist SB 242084 in subjects trained to lever-press on a fixed-interval schedule of stimulus termination produced behavioral-stimulant effects *per se* and potentiated the self-administration, drug-induced reinstatement, and dopaminergic effects of low doses of cocaine in squirrel monkeys. The potentiating effects of SB 242084 on cocaine-induced reinstatement, however, were not confirmed in a recent study by others (Rüedi-Battschen et al., 2015).

4.5.3 Schedules of Reinforcement

WAY163909 was effective in decreasing self-administration and drug- and cue-induced reinstatement of METH under a fixed ratio schedule of reinforcement. An important feature of FR schedules is the direct relationship between rate of responding and frequency of drug injection (for review see Spealman & Goldberg, 1978). In addition, the reinstatement procedure also provides a measure of drug-seeking behavior and has been used increasingly as a laboratory model of relapse to drug abuse, although the validity of the model to drug relapse in humans remains to be documented (Katz & Higgins, 2003; Epstein et al., 2006).

Interestingly, a separate experiment found that WAY163909 was also effective at decreasing cocaine self-administration and drug- and cue-induced reinstatement of cocaine under a second-order schedule of reinforcement (Perez et al., 2017b). Second-order schedules

are particularly useful for the study of the ways in which drug-associated stimuli can control drug-seeking behavior that results in a limited number of drug-presentation, minimizing possible interference of drug effects with operant responding (Howell & Fantegrossi, 2009). Thus, the effects of WAY163909 on drug intake and drug-seeking behavior appear to be independent of the schedule of reinforcement used.

Overall, these results demonstrate that the selective 5-HT_{2C} receptor agonist WAY163909 dose-dependently decreased both METH and cocaine intake during SA, but also drug-seeking behavior induced by a METH or cocaine prime and maintained by the presentation of METH- or cocaine-associated cues during reinstatement. Thus, the present data indicate that a selective 5-HT_{2C} receptor agonist has a broad action on the abuse-related effects of stimulant drugs, inhibiting both drug intake and drug-seeking behavior in nonhuman primate models of drug abuse.

4.5.4 WAY163909 Mechanisms of Action

The mechanism of action of 5-HT_{2C} receptors on the behavioral-stimulant effects of METH and other psychostimulants has been proposed to be related to increased GABAergic activity, both in the ventral tegmental area and in the prefrontal cortex (Howell & Cunningham, 2015). Serotonin 5-HT_{2C} receptors are highly expressed on GABAergic interneurons in those brain areas, and activation of 5-HT_{2C} receptors activates those neurons, increasing GABA release. Increased GABAergic activity in the ventral tegmental area and in the prefrontal cortex inhibits dopaminergic and glutamatergic neurons, respectively, ultimately decreasing dopamine levels in the nucleus accumbens. Although the high dose of WAY163909 did not affect baseline dopamine

levels in the nucleus accumbens, it was effective in attenuating the dopaminergic effects of METH. These results were surprising given that METH acts as a releaser of DA and METH-induced DA overflow in the nucleus accumbens should occur independent of DA neuronal firing (Riddle et al., 2006). Therefore, increased GABAergic inhibition of DA neurons in the ventral tegmental area alone, induced by activation of 5-HT_{2C} receptors, cannot account for the effectiveness with which WAY163909 attenuates METH induced DA overflow in the accumbens. Thus, further study of the mechanisms by which 5-HT_{2C} receptors regulate mesolimbic DA neurochemistry are warranted.

4.5.5 Serotonin 5-HT₂ Receptors and Striatal Dopamine Neurotransmission

The present results also shed light on the neurochemical and neuroanatomical mechanisms by which serotonin receptors differentially regulate the abuse-related behavioral effects of psychostimulants. It has been previously demonstrated that the selective serotonin 5-HT_{2A} receptor antagonist M100907 blocks cocaine-induced reinstatement, but does not attenuate cocaine self-administration in rhesus monkeys, an effect that was accompanied by a decrease in cocaine-induced dopamine overflow in the caudate nucleus, but not in the nucleus accumbens (Murnane et al., 2013). Those results suggest that 5-HT_{2A} receptors modulate the nigrostriatal and mesocortical dopamine pathways, and that those pathways are mainly involved in drug-induced reinstatement, but not self-administration, of psychostimulant drugs (Murnane et al., 2013). The present findings, on the other hand, indicate that the influence of 5-HT_{2C} receptors is broader, with 5-HT_{2C} activation blocking METH-induced reinstatement and attenuating drug self-administration. Although the effects of WAY163909 on METH-induced

dopamine overflow in the caudate nucleus were not evaluated, in a previous study conducted in squirrel monkeys, selective 5-HT_{2C} activation attenuated the dopaminergic effects of cocaine in the nucleus accumbens, but not in the caudate nucleus (Manvich et al., 2012a). Thus, 5-HT_{2C} receptors selectively modulate the mesolimbic dopamine pathway, and this pathway appears to be important for several abuse-related effects of psychostimulants, including drug intake and drug-seeking behavior.

4.5.6 Summary

The present study presents new evidence in support of previous studies indicating that serotonin 5-HT_{2C} receptors play a major role in the behavioral and neurochemical effects of psychostimulants. Unlike 5-HT_{2A} receptor antagonists, which are not expected to suppress drug intake, the current data show that activation of 5-HT_{2C} receptors may have a broad effect on the abuse-related behavioral effects of stimulant drugs, inhibiting drug intake and drug-seeking behavior in nonhuman primate models through mechanisms involved in the modulation of the mesolimbic dopamine pathway. Modulation of 5-HT_{2C} receptors may thus represent promising avenues for addiction therapies.

4.6 Figures

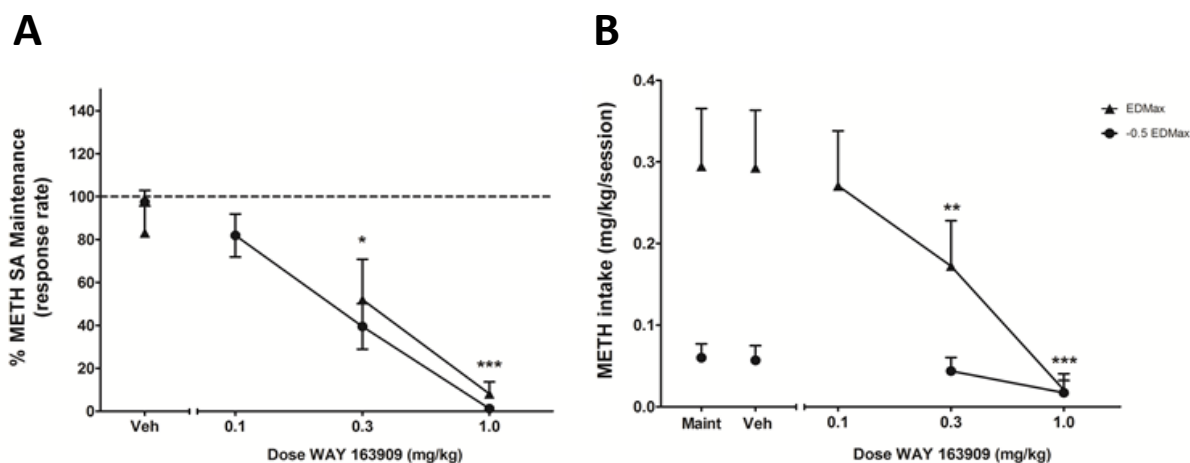


Figure 4.1 Effects of WAY163909 on METH Self-Administration (SA)

Response rates (A) during METH SA (0.01 mg/kg/infusion) and drug intake (B) during METH maintenance or after pretreatment with vehicle or WAY163909 (0.1, 0.3 and 1.0 mg/kg). Data for response rates are presented as normalized data (percentage of average response rates during SA maintenance). Dotted lines represent response rates during SA maintenance (100%). Data are expressed as mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$ for the EDMax dose compared with vehicle pretreatment; *** $p < 0.0001$ for both EDMax and -0.5 EDMax doses compared with vehicle pretreatment.

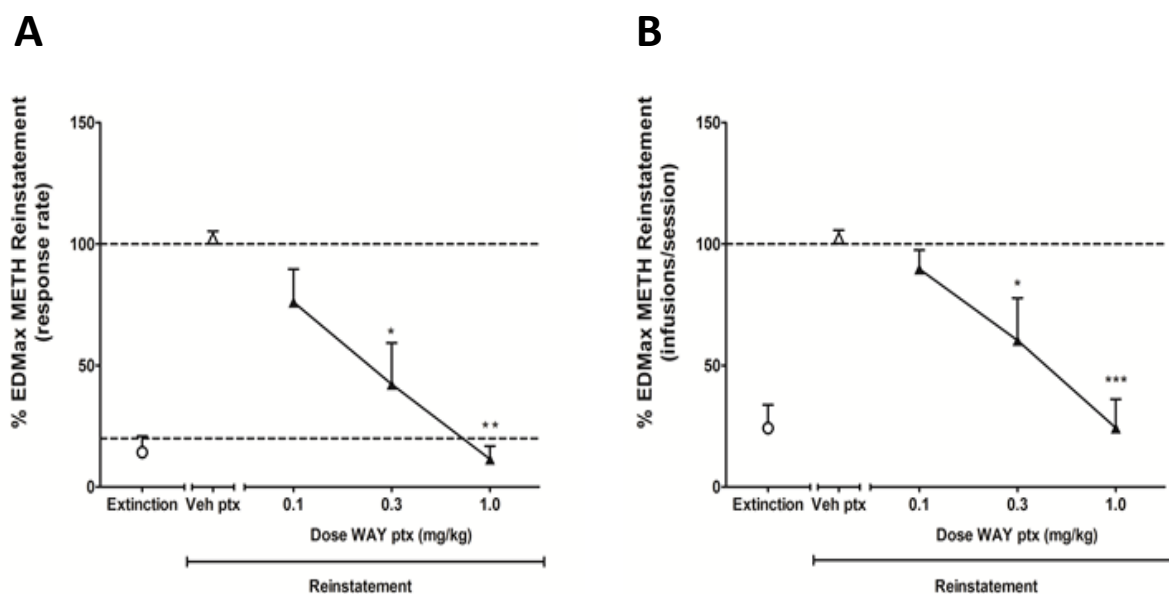


Figure 4.2 Effects of WAY163909 on METH Reinstatement

Response rates (A) during the reinstatement of extinguished behavior previously maintained by METH SA (0.03 mg/kg/infusion) and number of saline infusions/session (B) during METH reinstatement after pretreatment with vehicle or WAY163909 (0.1, 0.3 and 1.0 mg/kg). Data for response rates are presented as normalized data (percentage of average response rates during SA maintenance). (A) Dotted lines represent extinction criteria (20%) and response rates during self-administration maintenance (100%). (B) Dotted lines represent average number of infusions during SA maintenance (~26.1). Data are expressed as mean \pm SEM. * $p < 0.05$, and ** $p < 0.01$, and *** $p < 0.001$ compared with vehicle pretreatment.

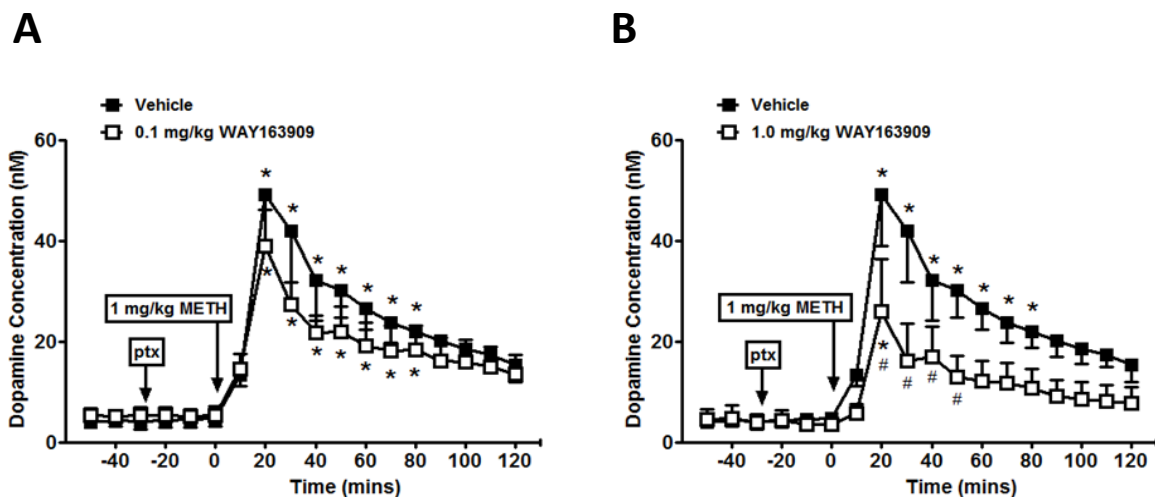


Figure 4.3 Effects of WAY163909 on Dopamine Overflow Induced by METH in the Nucleus

Accumbens

Increases in extracellular dopamine levels induced by METH (1 mg/kg, i.v.) in the nucleus accumbens following pretreatment with vehicle, (A) 0.1 mg/kg or (B) 1.0 mg/kg WAY163909. All data points represent the mean \pm SEM. * $p < 0.05$ compared with baseline. # $p < 0.05$ compared with vehicle pretreatment.

Chapter 5. General Discussion

5.1 Summary of Results

5.1.1 Summary of Chapter 2

The goals described in Chapter 2 were to evaluate (1) the effects of long-term intake of either a drug, methamphetamine (METH), or food, a high caloric diet (HCD), reinforcer on general compulsive behavior, (2) whether baseline compulsive behavior was predictive of future reinforcer intake, and (3) whether long-term reinforcer intake levels were predictive of post-reinforcer changes in general compulsivity. To address these goals, baseline compulsivity (perseverative responding) was measured in naïve adult female rhesus monkeys using a Discrimination Reversal Learning (DRL) task, after which one group of subjects (METH group) self-administered METH and another group (HCD group) consumed a palatable HCD for a period of 6 months. Following this period of reinforcer intake, compulsivity was measured again using the same task.

It was hypothesized that this extended period of reinforcer intake would produce a change in general compulsivity compared to baseline measures. Because higher compulsivity has been reported in both drug-dependent (Fernandez-Serrano et al., 2011) and obese individuals (Cserjesi et al., 2007), it was predicted that this period of prolonged intake of METH or a HCD would increase general compulsive behavior, irrespective of the reinforcer. Compulsivity is considered an etiological factor in the development and maintenance of addiction, being the final stage in a transition from recreational use of reinforcers, through habits, and finally to compulsive use (Everitt & Robbins, 2005, 2016; Torregrossa et al., 2011). Therefore, it was hypothesized that general compulsive behavior in naïve subjects would be predictive of their future level of

reinforcer intake. In addition, previous studies described above found that prenatal exposure to psychostimulants in mice (Lloyd et al., 2013) and METH pretreatment in rats (Son et al., 2013) increased compulsivity. Thus, it was also hypothesized that reinforcer intake during the 6-month period would be predictive of their increased post-intake compulsivity.

A significant impact of long-term reinforcer intake on general compulsivity was found, with 6-month intake of METH or a HCD increasing perseverative responding on the DRL task. Baseline perseveration in the METH group was not predictive of the amount of METH that subjects self-administered during the 6-month intake block. However, in the HCD group, baseline perseveration trended toward predicting the number of calories subjects consumed of both the HCD only and total calories during the 6-month intake block. One of the HCD group subjects appeared to be an outlier for this analysis, suggesting that this experiment may have been underpowered. METH, HCD only, and total food intake levels during the 6-month block were all significantly predictive of post-intake perseverative responding. Subjects that self-administered more METH or consumed more calories made more perseverative errors compared to subjects that made less. These results underscore the idea that an individual's level of general compulsivity in a naïve state may not be predictive of their future engagement in addictive behaviors, but their level of intake over an extended period of time is predictive of increased compulsivity, regardless of whether the reinforcer is a drug of abuse or a palatable, high caloric food.

5.1.2 Summary of Chapter 3

Using the same subjects and experimental conditions described in Chapter 2, the goals of Chapter 3 were to investigate the effects of the serotonin 2C receptor (5-HT_{2c}R) on (1) baseline compulsive behavior, (2) on compulsive behavior following extended reinforcer intake, and (3) on food intake of two different diets. To elucidate these effects, pretreatments with the selective 5-HT_{2c}R agonist WAY163909 were given to adult female rhesus monkeys prior to baseline compulsivity testing on the DRL task and after a 6-month period of METH or palatable HCD intake. Pretreatments with WAY163909 were also given to subjects in the HCD group prior to measures of food intake under a low caloric diet (LCD) only dietary condition and under a choice dietary condition, when subjects had access to both a LCD and a palatable HCD. Finally, to establish that the 5-HT_{2c}R was necessary for the effects of WAY163909, the 5-HT_{2c}R antagonist SB 242084 was administered to block the effects of WAY163909 on these measures.

Although some studies have reported that activation of 5-HT_{2c}Rs with less selective agonists, such as Ro 60-0175 and CP 809.101, decreased perseverative responding in rodents (Agnoli et al., 2012; Del'Guidice et al., 2014), another study found that the antagonist SB 242084 had the same effect (Boulougouris & Robbins, 2010). Although these studies have failed to clarify the role of 5-HT_{2c}Rs on compulsivity, another study evaluating compulsive behavior in rodents found that 5-HT_{2c}Rs knockout mice exhibited more compulsive behaviors than control animals (Chou-Green et al., 2003). Interestingly, Pelloux and colleagues (2012) found that activation of 5-HT_{2c}Rs with the agonist *m*-chlorophenylpiperazine (mCPP) reversed, whereas the antagonist SB 242084 mimicked, compulsive cocaine seeking induced by depletion of forebrain 5-HT in rats with a limited cocaine taking history. Therefore, it was hypothesized that activation of 5-HT_{2c}Rs with WAY163909 would decrease perseverative errors on the DRL task in a dose-dependent

manner, both before and after prolonged intake of reinforcers (METH or a HCD), and that pretreatments with the antagonist SB 242084 would block these effects. Studies have also reported that activation of 5-HT_{2c}Rs with the agonist lorcaserin, which has been approved by the FDA for treatment of obesity, reduced food intake in preclinical models as well as humans (Fleming et al., 2013; Hess & Cross, 2013; Hoy, 2013; Shukla et al., 2015). Given these findings, it was hypothesized that activation of 5-HT_{2c}Rs with WAY163909 would decrease food intake in the HCD group subjects and that pretreatments with SB 242084 would block these effects.

In the present study, WAY163909 decreased perseverative errors (i.e. compulsivity), both at baseline and after 6 months of either METH or HCD intake, while increasing correct responses on the DRL task. Importantly, the same doses of WAY163909 that reduced perseveration also decreased consumption of a palatable HCD, but not a LCD. The effects of WAY163909 on DRL task measures and food intake measures were all blocked by co-administration with SB 242084. These findings highlight that reductions in food intake, specifically of a palatable HCD, are associated with parallel improvements in decision making strategies. In addition, the data presented here provides evidence that the 5-HT_{2c}R is necessary for the effects of WAY163909 on both compulsivity and intake of a food reinforcer.

5.1.3 Summary of Chapter 4

The goals described in Chapter 4 were to investigate the effects of WAY163909 on (1) drug intake and drug-seeking of METH, as well as dopamine (DA) overflow in the nucleus accumbens (NAcc) induced by METH. To determine these effects, METH self-administration (SA) and reinstatement were evaluated under a fixed-ratio 20 schedule of reinforcement in male and

female adult rhesus monkeys. METH-induced increases in DA were assessed through *in vivo* microdialysis targeting the NAcc to probe the downstream mechanism by which activation of 5-HT_{2c}Rs may be affecting METH SA and reinstatement.

Previous studies have demonstrated that activation of 5-HT_{2c}Rs with less selective agonists than WAY163909 decreased cocaine SA and cocaine-induced reinstatement, and DA overflow in the NAcc in rodents (Grottick et al., 2000; Neisewander & Acosta, 2007; Burbassi & Cervo, 2008; Fletcher et al., 2008; Navailles et al., 2008) and squirrel monkeys (Manvich et al., 2012a; Rüedi-Bettschen et al., 2015). In addition, antagonism of these receptors with SB 242084 potentiated the SA, drug-induced reinstatement, and dopaminergic effects of low doses of cocaine in squirrel monkeys (Manvich et al., 2012b). Thus, it was hypothesized that pretreatments with WAY163909 would dose-dependently decrease METH SA and METH-induced reinstatement, and that these effective doses would be the same doses that also reduced compulsivity and intake of a palatable HCD described in Chapter 3. It has also been reported that less selective 5-HT_{2c}R agonists attenuated the dopaminergic effects of cocaine in the NAcc in squirrel monkeys (Manvich et al., 2012a), so it was hypothesized that WAY163909 pretreatments would dose-dependently attenuate DA overflow in the NAcc induced by METH.

Pretreatment with WAY163909 dose-dependently decreased METH SA and drug-induced reinstatement of extinguished behavior previously maintained by METH delivery. In addition, WAY163909 pretreatment dose-dependently blocked METH-induced DA overflow in the NAcc. Taken together, these results support the idea that activation 5-HT_{2c}Rs attenuates both METH intake and drug-seeking behavior in non-human primate (NHP) models of METH abuse through neurochemical mechanisms involved in the modulation of mesolimbic DA. Finally, the same

doses of WAY163909 that reduced compulsivity and intake of a palatable HCD described in Chapter 3 also effectively attenuated METH intake, METH-seeking, and METH-induced DA overflow in the NAcc, suggesting that reductions in all these METH-related measures may also be associated with parallel improvements in decision making strategies, as was seen with the HCD.

5.2 Integration of Findings

5.2.1 Effects of Extended Reinforcer Intake on General Compulsivity

Compulsivity has been highly implicated in a variety of addictive disorders (American Society of Addiction Medicine, 2011) and is considered an etiological factor in the development and maintenance of addiction (Everitt & Robbins, 2005, 2016). Specifically, compulsivity is thought to be the final stage in a transition from recreational use of reinforcers or engagement in behaviors that are reinforced, through habitual use or engagement, and finally to compulsive use or engagement (Torregrossa et al., 2011). However, the relationship between compulsive and addictive behaviors is poorly understood. Therefore, one of the primary goals of this thesis was to investigate whether compulsivity is an underlying behavioral feature of an addictive phenotype. To address this goal, the present study examined whether high baseline compulsivity was predictive of future intake of reinforcers, long-term intake of reinforcers was predictive of increased compulsivity, or both in rhesus monkeys. To establish that this relationship is generalizable to more than one type of addiction, both a drug (METH) and food (HCD) reinforcer were used. Although addiction *per se* cannot be assessed in preclinical laboratory models, such as NHPs, intake of reinforcers, a key feature of addiction, can be readily determined.

The results described in Chapter 2 demonstrated that baseline compulsivity in a naïve state is not predictive of an individual's future intake of METH, but may be predictive of future intake of a palatable HCD. However, further exploration of this is necessary, given that a trend was found in the HCD group that did not reach statistical significance. On the other hand, long-term intake of either a drug or food reinforcer not only increased compulsivity compared to baseline, but the amount of intake was linearly correlated with increased post-intake compulsivity. Similarly, increased post-intake compulsivity was predictive of the amount of time it took subjects to extinguish responding for METH, suggesting that deficits in general decision-making may contribute to an inability to alter behavior following a change in drug-reinforcement contingency. Overall, the data described in Chapter 2 support the idea that compulsivity may be an underlying behavior feature of an addictive phenotype.

5.2.2 Effects of Extended Abstinence from Drugs on General Compulsivity

In addition to understanding how an extended period of reinforcer intake would affect compulsivity, the present study sought to determine how compulsivity in abstinent subjects with a history of drug taking would compare to baseline compulsivity in naïve subjects. To this end, a separate experiment was carried out in which compulsivity was measured using the DRL task in a group ($N=5$) of adult rhesus monkeys with an extensive history of cocaine SA who had been abstinent for >12 months. These measures were then compared to those taken from the METH group at baseline (Fig. 5.2).

No significant differences between the subjects tested in a naïve state and the abstinent subjects were found ($p > 0.05$), suggesting that compulsivity measures are comparable between

drug-naïve subjects and those that have been abstinent from drug self-administration for over a year. Because compulsivity was not measured in the abstinent group prior to cocaine SA or directly before the abstinence period, it is not possible to determine the trajectory of changes in their general compulsivity across these conditions. However, taken together, the results from the abstinent group and the findings described in Chapter 2 support the idea that long-term intake of reinforcers leads to an increase in general compulsive behavior, and an extended period of abstinence from these may normalize compulsivity such that it is no longer increased compared to the naïve condition. These findings suggest that engagement in addictive behaviors may increase general compulsivity, whereas abstaining from these could potentially attenuate this increase. Further, results from these studies also supports the idea that general compulsive behavior is dynamic and can be regulated in response to an individual's engagement in or abstinence from addictive behaviors.

5.2.3 Effects of 5-HT_{2c}Rs on Compulsivity and Reinforcer Intake

The mechanisms underlying the expression of compulsive behaviors and intake of reinforcers have not been fully established. Through its direct and indirect interactions with the DA system, the 5-HT system can influence the abuse-related effects of reinforcers (Howell & Cunningham, 2015). Evidence from the literature suggests that 5-HT_{2c}R agonists can effectively reduce intake of food (Hess & Cross, 2013; Hoy, 2013) and a variety of drugs of abuse (Levin et al., 2011; Manvich et al., 2012a; Rezvani et al., 2014), however, the effects of these compounds on compulsive behavior remain unclear (Boulougouris & Robbins, 2010; Agnoli et al., 2012; Del'Guidice et al., 2014). Moreover, no one has determined if the reductions in reinforcer intake

produced by 5-HT_{2c}R agonists are accompanied by parallel reductions in general compulsivity. Therefore, the other primary goal of this thesis was to determine whether the 5-HT_{2c}R agonist WAY163909 could concurrently decrease intake of reinforcers and compulsivity, and to demonstrate that the 5-HT_{2c}R was necessary for these effects.

In Chapter 3, it was demonstrated that WAY163909 decreased general compulsivity, both before and after extended intake of METH or a HCD. In addition, the same doses of WAY163909 also decreased intake of a HCD, but not a LCD, and these effects were blocked by the 5-HT_{2c}R antagonist SB 242084. In Chapter 4, it is reported that the same doses of WAY163909 that decreased compulsivity and HCD intake also attenuated METH intake and reinstatement of responding previously maintained by METH delivery. Thus, the present findings support a role for 5-HT_{2c}Rs in the expression of both compulsive and addictive behaviors. Namely, agonists at this receptor ameliorate, whereas antagonists exacerbate, behavioral tendencies that may contribute to an addictive phenotype, such as heightened compulsivity and intake of reinforcers in general. Finally, these effects are mediated by 5-HT_{2c}Rs and not off-target signaling.

5.2.4 Mechanisms Underlying Compulsive and Addictive Behaviors

The mechanism of action of 5-HT_{2c}Rs on reinforcer intake is likely related to the effects of this receptor on DA neurotransmission. Increased DA neurotransmission, especially within the striatum, is critical in the reinforcing properties of drugs (Ritz et al., 1987; Woolverton and Kleven, 1988; Veeneman et al., 2012) and palatable, high caloric foods (Small et al., 2003; Norgren et al., 2006; Wise, 2006; Volkow et al., 2011a). 5-HT_{2c}Rs are highly expressed on GABAergic interneurons in the ventral tegmental area (VTA) and the prefrontal cortex (PFC), and their

activation is hypothesized to increase inhibitory GABAergic activity (for review see Howell & Cunningham, 2015). This inhibition should ultimately decrease DA levels in the NAcc. Indeed, in Chapter 4, WAY163909 attenuated METH-induced DA overflow in the NAcc in rhesus monkeys. WAY163909 (Berro et al., 2017a) and other 5-HT_{2c}R agonists (Manvich et al., 2012a) also had similar effects on cocaine-induced DA overflow in the NAcc and the abuse-related effects of cocaine in NHPs. Electrophysiological and microdialysis studies provide corroborating evidence that 5-HT_{2c}R agonists decrease, whereas antagonists increase (Navailles et al., 2008; Manvich et al., 2012b), DA neuron firing in the VTA as well as DA release within the NAcc (reviewed by Bubar & Cunningham, 2006, 2008). For instance, 5-HT_{2c}R knock-out mice exhibit increased DA levels in the Nacc and dorsal striatum (Abdallah et al., 2009). Thus, decreased DA signaling in the NAcc appears to be one of the primary effects of activating 5-HT_{2c}Rs, and likely underlies the WAY163909-induced decreases in reinforcer intake reported in this study.

As with reinforcer intake, regulation of DA neurotransmission by 5-HT_{2c}Rs is a likely mechanism of action by which these receptors modulate compulsivity. Increases in DA signaling within the striatum are thought to play a crucial role in the balance between PFC and striatal control over decision-making, and thus compulsivity. Moreover, a transition from top-down control by the PFC to bottom-up control by the striatum over decision-making and behavior is hypothesized to underlie the progression from recreational use of reinforcers or engagement in behaviors that are reinforced to the compulsive use or engagement seen in addiction (Belin et al., 2013). Results from Chapters 3 and 4 demonstrate that the same doses of WAY163909 that reduced reinforcer intake and METH-induced DA overflow in the NAcc also effectively decreased compulsivity in all subjects. Together, these findings support the idea that activation of 5-HT_{2c}Rs

with WAY163909 decreases DA neurotransmission in the NAcc, which in turn may lead to a reduction in general compulsivity. Studies reporting that prenatal exposure to psychostimulants, which produce robust increases in DA, increases compulsive behavior in rodents (Lloyd et al., 2013; Son et al., 2013) provide further evidence in favor of this hypothesis. It is possible that decreases in DA signaling in the NAcc shift the balance between PFC and striatal control over decision-making toward greater top-down control by the PFC, driving responding away from compulsivity and toward more flexible behavior.

Our understanding of how DA signaling affects intake of reinforcers is relatively clear, however the case for how DA affects compulsivity remains uncertain. Although the findings reported here, along with other studies, support a role for reduced DA signaling in the NAcc decreasing compulsivity, some rodent studies provide evidence to the contrary. For example, studies have reported that optogenetic excitation of DA neurons in the VTA improves reversal learning (i.e. decreases compulsivity) in rodents (Adamantidis et al., 2011; Rossi et al., 2013). In addition, antagonism of 5-HT_{2A}Rs using M100907 (M100) in rats impaired, whereas antagonism of 5-HT_{2C} receptors using SB 242084 facilitated, reversal performance (Boulougouris et al., 2008; Boulougouris & Robbins, 2010; Furr et al., 2012; Nilsson et al., 2012). Antagonists at the 5-HT_{2A}R have been shown to decrease DA neurotransmission within the caudate nucleus, but not the NAcc, in NHPs (Murnane et al., 2013). Thus, although 5-HT_{2C}R agonists and 5-HT_{2A}R antagonists both decrease DA signaling in the striatum, they target different striatal structures based on where these receptors are distributed (Howell & Cunningham, 2015). This difference may account for some of the confounding results of these compounds on compulsivity, however it

cannot explain all the disparate effects reviewed here, and so the exact role of DA neurotransmission on compulsivity remains elusive and requires further study.

5.3 Future Directions

Future studies are necessary to clarify some of the results of the present studies. For instance, baseline compulsivity was not predictive of future intake of either METH or a HCD. This lack of effect may have been due to underpowered experiments, especially with the HCD group, and future studies employing a larger number of subjects may shed light on whether this lack of effect is real. Similarly, METH intake was found to predict increased post-reinforcer compulsivity, but this relationship appeared to be driven by one subject specifically. Again, future studies using a larger number of subjects would clarify the validity of this finding.

Future studies could also expand upon the findings presented here and answer several remaining questions. For instance, long-term intake of METH or a HCD increased compulsivity, but these are only two types of reinforcers. Studies replicating the experiments presented in Chapter 2, using other types of reinforcers (alcohol, nicotine, opioids, etc.) would demonstrate whether this increase in compulsivity is generalizable to other reinforcers, and thus might provide further evidence that compulsivity is a core behavioral feature of an addictive phenotype, and not just of specific types of addiction. Similarly, studies examining the effects of WAY163909 and SB 242084 on intake of other types of reinforcers, would improve our understanding of the degree to which these effects are generalizable to various reinforcers and brain regions. In addition, similar findings with other reinforcers would support the idea that 5-HT_{2c}R agonists might be useful for treatment of multiple addictions, not just those involving stimulants or

palatable, high caloric foods. Indeed, the FDA-approved lorcaserin has been effective at reducing intake of nicotine and alcohol in previous studies (Levin et al., 2011; Rezvani et al., 2014), further supporting the idea that HT_{2c}R agonists could prove useful therapeutics for addiction in general.

An interesting avenue for future studies could include determining how stress interacts with long-term intake of reinforcers to alter compulsivity, as well as how and if it alters the efficacy of WAY163909 to reduce both compulsivity and reinforcer intake. This could easily be addressed by replicating the experiments discussed here using socially housed female rhesus macaques that are either very high-ranking or very low-ranking, as opposed to middle-ranking subjects like those used for the present studies. Low ranking female rhesus monkeys are a useful model of chronic psychosocial stress (Michopoulos et al., 2012a) and social rank has been shown to have a strong impact on stress levels, stress reactivity, and the effects of diet availability on feeding behavior (Michopoulos et al., 2012b). Thus, it is likely that social rank might interact with long-term intake of reinforcers to either attenuate or exacerbate its effects on compulsivity. It is hypothesized that the effects of long-term reinforcer intake on compulsivity would be heightened in chronically stressed subjects (i.e. subordinate or low-ranking females), with these exhibiting an even greater increase in compulsivity than middle-ranking females, and that high-ranking or dominant females would exhibit the smallest increase. Similarly, chronic stress may decrease the efficacy of WAY163909, in which case pretreatments with this compound would produce much smaller decreases in both compulsivity and reinforcer intake in low-ranking females than in middle-ranking females, with the highest efficacy in high-ranking females.

As discussed above, mixed results have been reported regarding how alterations in DA signaling modulate compulsive behaviors. Although some studies (Lloyd et al., 2013; Son et al., 2013), including those presented here, suggest that increasing DA signaling increases compulsivity, others have found opposite results (Adamantidis et al., 2011; Rossi et al., 2013). Because 5-HT_{2c}R agonists have been shown to decrease DA overflow in the NAcc (Manvich et al., 2012a; Berro et al., 2017a), and WAY163909 decreased compulsive behavior, it was hypothesized that decreases in DA signaling in the NAcc decrease compulsivity. However, the effects of WAY163909 on DA signaling in other pertinent brain areas has not been determined, and these effects may aid in the clarification of conflicting reports. In a pilot positron emission tomography (PET) study using the HCD group subjects, DA 2 receptor binding potential (D2R BP) in the orbital PFC (oPFC) and the putamen (another striatal region) was negatively correlated with baseline compulsivity (Table 5.1, Fig. 5.3), suggesting that DA neurotransmission and activity within these areas may also play a regulatory role in compulsive behavior. To elucidate the effects of WAY163909 on DA signaling in these and other key regions, future studies using microdialysis techniques in NHPs could target (1) the putamen, (2) the oPFC, which is involved in salience attribution and goal directed behaviors, (3) the anterior cingulate cortex (ACC), which is involved in regulation of inhibitory control and awareness, and (4) the dorsolateral PFC (dlPFC), which is involved in higher cognitive operations and decision-making. METH-induced DA overflow, as well as the effects of WAY163909 on this overflow, can be measured in all these regions to determine whether activation of 5-HT_{2c}Rs has differential effects on DA neurotransmission in different areas. The results of such a study would confer a greater insight into the effects of 5-HT_{2c}Rs on

DA signaling within the mesolimbic DA pathway, as well as the possible mechanisms by which WAY163909 reduces compulsive behavior (Volkow et al., 1993, 2001; 2007).

To further elucidate the neurochemical mechanisms contributing to the expression of compulsivity and addictive behaviors, another study could be designed to determine the effects of long-term reinforcer intake on activity and functional connectivity within and between the PFC and striatum regions mentioned above. Compulsivity, as well as activity and functional connectivity between these regions, could be measured both before and after long-term intake of METH or a HCD. This experiment would allow for the investigation of which brain regions might be playing a crucial role in the expression of these behaviors under each given condition. Interestingly, a previous study in female rhesus monkeys reported that cocaine administration robustly decreased global functional connectivity and selectively impaired top-down prefrontal circuits that control behavior, and that impaired connectivity between prefrontal and striatal areas during abstinence predicted cocaine intake when subjects resumed cocaine self-administration (Murnane et al., 2015). Therefore, it would not be surprising to find that prior to long-term intake of reinforcers, subjects have greater activity in the PFC regions compared to the striatum, or more robust functional connectivity between these areas, and that after long-term intake they exhibit higher striatum: PFC activity, or less robust connectivity. These findings would support the hypothesis that a transition from top-down control by the PFC to bottom-up control by the striatum over decision-making and behavior (Belin et al., 2013) underlies the progression to increased compulsivity induced by extended reinforcer intake.

5.4 Final Conclusions

Overall, this dissertation provides evidence that (1) long-term intake of a drug (METH) or food (HCD) reinforcer increases compulsivity, with the amount of reinforcer intake being predictive of the increase in compulsivity, and (2) 5-HT_{2c}Rs play a crucial role in both compulsivity and reinforcer intake, as activation of these receptors decreases both measures, regardless of the reinforcer or whether compulsivity is measured at baseline or after prolonged intake of reinforcers (Fig. 5.1). Importantly, decreases in reinforcer intake are associated with parallel decreases in compulsivity and are produced by the same doses of the 5-HT_{2c}R agonist WAY163909 that attenuate METH-induced DA overflow in the NAcc. Figure 5.1 provides a diagram summarizing the integrated findings of this dissertation. As a whole, the results suggest that agonists at the 5-HT_{2c}R may represent a promising new avenue for treatment of addiction in general, especially if these findings can be expanded upon to demonstrate efficacy with other types of reinforcers and reinforced behaviors.

5.5 Figures

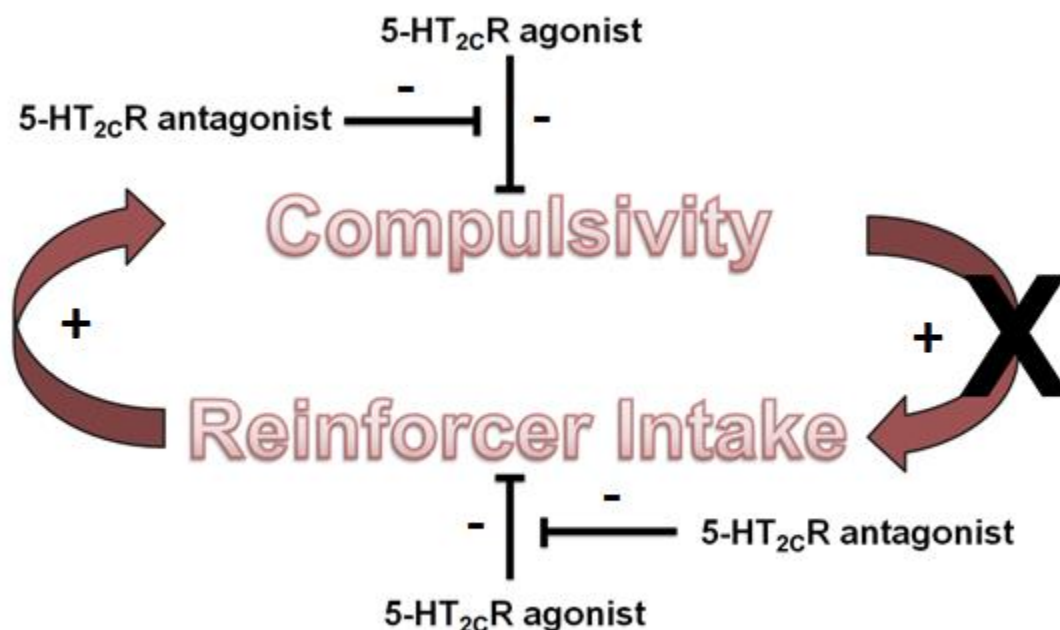


Figure 5.1 Diagram of the Significant Relationships Between Compulsivity, Reinforcer Intake and 5-HT_{2c}Rs

The diagram shows an integrated model of how compulsivity and reinforcer intake relate to one another, as well as the effects of agonists and antagonists at the 5-HT_{2c}Rs on these behaviors. The findings of this thesis indicate that baseline compulsivity is not predictive of future intake of reinforcers, but long-term intake of reinforcers does predict increased compulsivity. Additionally, agonists at the 5-HT_{2c}R decrease compulsivity and intake of reinforcers, and these effects can be blocked by 5-HT_{2c}R antagonists.

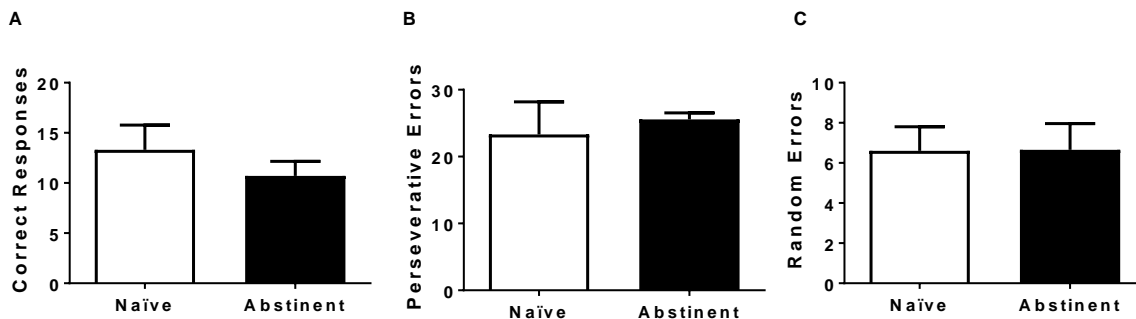


Figure 5.2 Effects of Extended Abstinence from Cocaine on DRL Task Measures

DRL task measures were taken for the METH group ($N=5$) under naïve conditions and for a separate abstinent group ($N=5$) with an extensive history of cocaine SA and >12 months of abstinence. Data for correct responses (A), perseverative errors (B), and random errors (C) are presented as the mean \pm SEM and were compared using t-tests. There was no significant difference between groups for any of the DRL task measures analyzed.

Brain Region	Significant Correlation (Pearson's)	P value	R ² value
ACC	No	0.1112	0.4397
oPFC	**	0.0029	0.9434
mPFC	No	0.0554	0.6263
dIPFC	No	0.0522	0.6395
AMY	No	0.2141	0.2177
HIPP	No	0.4016	0.0241
HYP	No	0.3040	0.0980
Caudate	No	0.3539	0.0537
Putamen	*	0.0125	0.8531
NAcc	No	0.4113	0.0196

Table 5.1 Summary of Pearson's Correlation Analyses of Baseline D2R BP and Baseline Compulsivity (HCD group)

Descriptive statistics and significance of correlation analyses between D2R BP of several brain regions and baseline perseveration (i.e. compulsivity) in the HCD group. All measures of D2R BP were collected at baseline, before subjects were given access to the choice diet condition (LCD+HCD). D2R BP in the orbital PFC and the putamen was significantly negatively correlated with perseverative errors. Abbreviations: ACC = anterior cingulate cortex; oPFC = orbital prefrontal cortex; mPFC = medial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; AMY = amygdala; HIPP = hippocampus; HYP = hypothalamus; NAcc = nucleus accumbens.

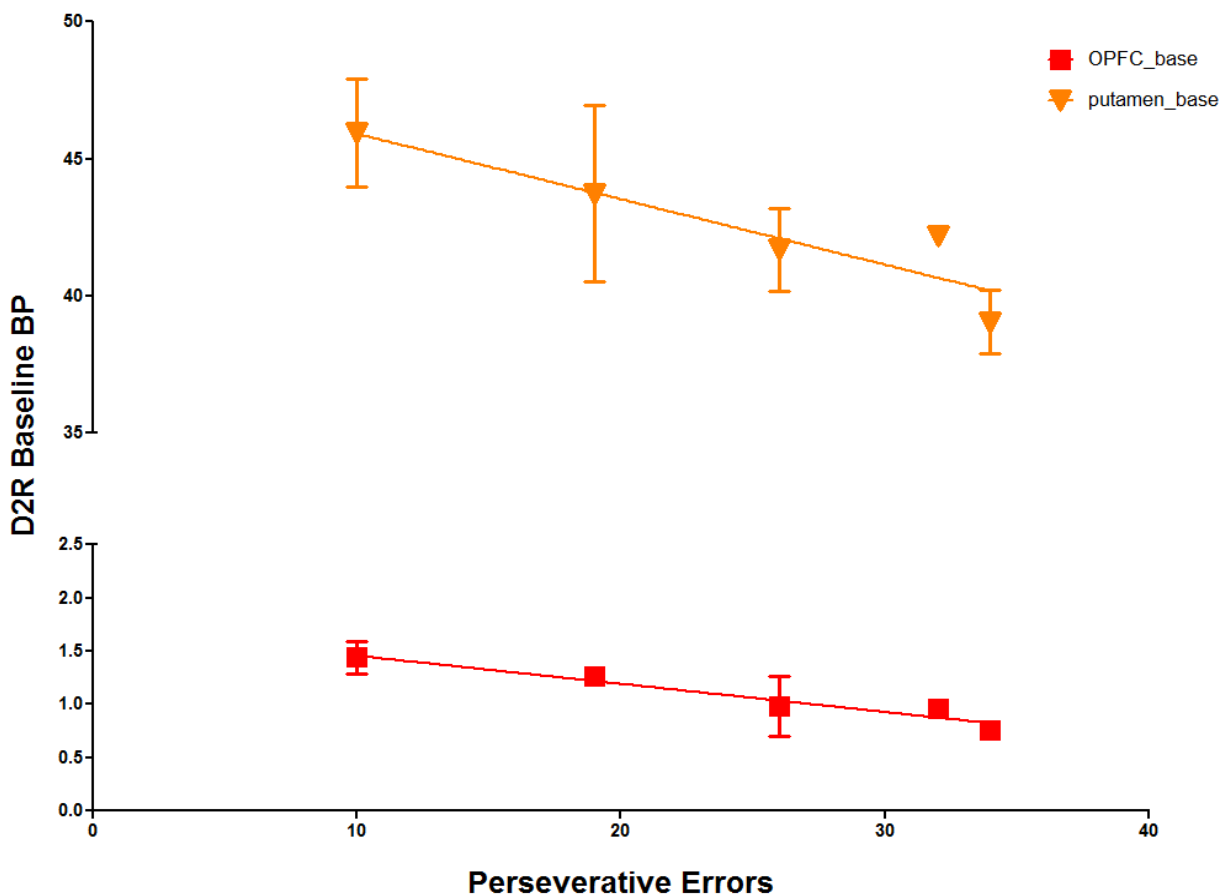


Figure 5.3 Pearson's Correlations of Baseline D2R BP and Baseline Compulsivity (HCD group)

Correlation analyses were conducted between D2R BP of several brain regions and baseline perseveration (i.e. compulsivity) in the HCD group. All measures of D2R BP were collected at baseline, before subjects were given access to the choice diet condition (LCD+HCD). D2R BP in the orbital PFC and the putamen was significantly negatively correlated with perseverative errors. Data are presented as the mean \pm SEM.

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