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Hannah Shields March 26, 2018

An examination of the role of serotonin 2C ($5\text{-}HT_{2C}$) receptors in psychostimulant self-administration in rhesus macaques.

by

Hannah Shields

Mar Sanchez, Ph.D.
Adviser

Department of Neuroscience and Behavioral Biology

Mar Sanchez, Ph.D.

Adviser

Leah Roesch, Ph.D.

Committee Member

Shannon Gourley, Ph.D.

Committee Member

Alison Wakeford, Ph.D.

Committee Member

2018

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Hannah Shields

Mar Sanchez, Ph.D.

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Neuroscience and Behavioral Biology

2018

Abstract

An examination of the role of serotonin 2C (5-HT_{2C}) receptors in psychostimulant self-administration in rhesus macaques.

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Psychostimulants like cocaine (COC) and amphetamines are some of the most commonly abused drugs, however no effective treatments have been developed for psychostimulant abuse. Additionally, some populations are at increased risk for drug abuse due to early life stress (ELS) experiences and other factors such as gender or drug initiation during adolescence. The 5-HT_{2C} receptor has been of interest as a target for the treatment of psychostimulant addiction due to its modulation of dopaminergic activity in the nucleus accumbens. The aim of the present study was to investigate the effects of a selective 5-HT_{2C} receptor agonist WAY 163909 (WAY) on COC self-administration (SA) in adolescent rhesus monkeys that were hypothesized to be at greater risk for COC abuse due to ELS. Subjects were 8 adolescent rhesus monkeys. Six subjects experienced ELS in the form of maternal maltreatment as infants (MALT) and 2 did not (Control). Mother-infant pairs were studied from birth through 3 months postpartum to examine the maternal care received, and infant maltreatment was operationally defined as co-occurrence of physical abuse and infant rejection. In adolescence (at approximately 4-5 years of age), subjects began COC SA studies, including acquisition of SA, peak dose (ED_{Max}) determination, Limited Access (LA) sessions, and WAY pretreatments before COC SA to examine its effects on SA. Outcome measures were number of infusions and response rate. Rate of acquisition of cocaine SA was not significantly different between Control and MALT groups, however mean ED_{Max} was significantly lower in MALT subjects. Baseline number of infusions, but not response rate, was significantly greater in MALT subjects. WAY pretreatments significantly decreased responding in all subjects at the highest dose, however there were no significant differences in responding after WAY between groups. This suggests that potential therapeutic treatments for psychostimulant abuse that target the 5-HT_{2C} receptor may be effective at reducing drug intake with minimal side effects in multiple different populations. Future studies are warranted in order to make stronger conclusions regarding the relationship between ELS and serotonergic regulation of the mesolimbic system.

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Acknowledgements

I would like to thank Dr. Alison Wakeford for all of her guidance and support throughout this entire project. I would also like to thank Dr. Mar Sanchez for taking me on as an honors student, and members of the Sanchez Lab, including Erin Siebert, Sara Bramlett, and Sarah Katznelson, for their help and assistance in the lab, as well as prior members of the Sanchez lab (in particular Brittany Howell, Anne Glenn, Christine Marsteller, Dora Guzman and Elyse Morin) for the generation and characterization of the early life stress macaque model studied here. Finally, I would like to thank Dr. Leonard Howell and Dr. Laís Berro for giving me the opportunity to work at Yerkes and for sharing their passion for research throughout my time working with them. This research was supported by NIH grants DA 038588, DA 010344 (LLH), DA 031246 (LLH), and P51OD11132 (Yerkes National Primate Research Center).

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Introduction & Background

Drug addiction is a worldwide public health concern. In 2015, over 70,000 people in North America reported using one or more types of drug (Drugs and Crime 2016). Of that, 17% used psychostimulants such as cocaine or amphetamines. Psychostimulants are some of the most commonly abused drugs in the United States, with the two most commonly abused stimulants being cocaine and amphetamines. Importantly, up to 15% of psychostimulant users become dependent (Ciccarone 2011), however zero FDA-approved medications have been developed for psychostimulant abuse (Ciccarone 2011; Grabowski et al. 2004; Howell and Cunningham 2015).

Psychostimulants such as cocaine (COC) and methamphetamine (METH) render their euphoric and reinforcing effects through the activation of the monoaminergic system to increase levels of neurotransmitters such as dopamine (DA), serotonin (5-HT), and norepinephrine (NE) in the synapse (Bradberry et al. 2000; Ciccarone 2011; Drevets et al. 2001; Howell et al. 2007; Koob and Bloom 1988; Romanelli and Smith 2006; Volkow et al. 1997). Specifically, self-reports of euphoria and the 'high' that drug users feel when taking drugs of abuse are directly related to increases in extracellular DA levels in brain areas such as the striatum (Drevets et al. 2001; Volkow et al. 1997; Volkow et al. 2007). These effects occur rapidly after psychostimulant ingestion, accentuating the reinforcing effects of the drug and leading to a greater abuse liability. Both psychostimulants block monoamine transporters, inhibiting monoamine reuptake and consequently leading to an increase in DA levels in the synapse (Howell et al. 2007; Koob and Bloom 1988; Volkow et al. 1997; Volkow et al. 2007; Volkow et al. 2009). METH also acts inside the presynaptic terminal by directly inducing a release of DA from the vesicles into the synapse. The neurochemical consequences of COC and METH occur in the ventral striatum, an area of the brain relevant to drug addiction (Czoty et al. 2002; Martin-Soelch et al. 2001; Schultz

et al. 1997; Watanabe et al. 2001). Within the ventral striatum, DA cell bodies from the ventral tegmental area (VTA) project to other brain areas such as the nucleus accumbens (NA) and prefrontal cortex (PFC), creating a dynamic circuit termed the mesocorticolimbic DA system that is of extreme interest to drug abuse researchers due to its relationship to the reinforcing effects of these drugs (Everitt et al. 2008; Howell and Wilcox 2001; Koob and Bloom 1988; Koob and Volkow 2016; Martin-Soelch et al. 2001). Increased mesocorticolimbic transmission causes an increase in DA release in the NA and PFC and therefore increased levels of DA available to bind to receptors in these areas (Ciccarone 2011; Howell et al. 2007). This occurs in drug-naive individuals after the presentation of a reinforcing stimulus (Martin-Soelch et al. 2001), suggesting that activation of this pathway is related to reward. The monoamine reuptake inhibition caused by psychostimulant ingestion also increases DA levels in this pathway by blocking removal of DA from the synapse, thereby leaving more available extracellular DA (Volkow et al. 2009). Importantly, blocking DA reuptake in the mesocorticolimbic pathway, specifically in the NA, has been directly linked to the behavioral and reinforcing effects of psychostimulants (Ciccarone 2011; Howell and Wilcox 2001; Koob and Bloom 1988). This evidence indicates that increased DA in the mesocorticolimbic DA pathway is crucial for experiencing rewarding stimuli, and overstimulation of this pathway contributes to the highly addictive nature of drugs of abuse like COC and METH. As drug addiction represents a complex set of behaviors that is controlled by multiple interconnected circuits within the brain, the effective treatment of psychostimulant drug abuse requires pharmacological specificity not only at the level of the receptor, but also within areas of the brain that are related to the reinforcing effects of drugs of abuse.

The need for reliable and valid models of drug abuse that exert tight experimental control is critical to the study of drug abuse. Animal models, such as those using rodents or nonhuman primates (NHPs), allow scientists to investigate the effects of abused drugs across a wide-range of experimental variables (such as dose and reinforcement schedule) on behavior, as well as their neurobiological effects (Ator and Griffiths 2003; Banks et al. 2017; Howell and Wilcox 2001; Weerts et al. 2007; Yokel 1987). NHP models, in particular, provide translational evidence for the neurobiological effects of drugs of abuse. Rhesus macaques (*Macaca mulatta),* for example, are highly social Old World monkeys with strong social connections and matrilineal hierarchies within their social groups. They have long life spans (up to 20 years), providing the opportunity of performing longitudinal studies in a species that is evolutionarily closer to humans than rodents with very similar central nervous system organization and neurochemistry, making them an ideal model for drug abuse (Ator and Griffiths 2003; Weerts et al. 2007). While different procedures exist in drug abuse research, drug self-administration (SA) in NHPs remains one of the best translational models to the human condition. SA studies utilize operant conditioning in an effort to closely examine how stimuli come to elicit drug-taking behavior (Banks et al. 2017; Brady et al. 1987; Haney and Spealman 2008; Yokel 1987). Lights can be used to signal the availability of a reinforcing stimulus (such as an intravenous bolus infusion of a drug), whereby pressing a lever results in a switch from one stimulus light to another to signal a drug infusion. *Acquisition* of the task using a fixed-ratio schedule of reinforcement, which requires subjects to press the lever a pre-determined number of times before receiving a drug infusion, is related to the initiation of drug use, whereas *maintenance* of drug-taking behavior requires stable responding over consecutive SA sessions, modeling ongoing drug abuse. *Extinction*, during which lights are turned off and drug infusions are replaced with saline, is used to examine the

rate at which a subject stops responding for a reinforcing stimulus, and may be a proxy measure for abstinence of drug use, though some researchers question the validity of this model of drug abstinence (Reichel and Bevins 2009; Self et al. 2004). *Reinstatement*, whereby a pretreatment injection of the drug used in the study occurs before the onset of a session where a subject is re-exposed to all of the original drug-paired cues but still only receives infusions of saline, is related to drug-induced or cue-induced relapse (Katz and Higgins 2003; Reichel and Bevins 2009). Through the use of these carefully operationalized measures, researchers are able to examine the effects of drugs of abuse on well-defined behavioral outcome measures.

The utility of the SA procedure also allows researchers to test the effects of compounds that may modulate responding and therefore act as potential therapeutic treatments. As most drugs of abuse cause activation of the mesolimbic DA system, blocking this activation may hypothetically decrease the reinforcing effects of the drugs. However, studies that involve DA-antagonists have not been successful (Ciccarone 2011; Howell et al. 2007; Kishi et al. 2013), leading scientists to investigate other systems that modulate dopaminergic transmission. As the dopaminergic system is strongly modulated by the serotonergic system (Howell and Cunningham 2015), researchers have turned to studying certain aspects of the serotonergic system as potential treatment targets. Studies involving drugs that increase 5-HT, such as selective-serotonin-reuptake-inhibitors (SSRIs) or 5-HT direct agonists, have shown to attenuate COC-maintained responding in NHPs (Czoty et al. 2002; Howell and Byrd 1995; Kleven and Woolverton 1993; Spealman 1993). Both mechanisms work to increase serotonergic transmission, supporting the role of 5-HT in regulation of DA transmission in circuits critical to drug use (Howell and Byrd 1995; Howell et al. 2007; Ichikawa and Meltzer 1995; Spealman 1993).

The 5-HT_{2C} receptor has been of particular interest as a target for pharmacotherapeutic treatment of psychostimulant addiction due to its modulation of dopaminergic activity in the ventral striatum (Bubar and Cunningham 2007; Howell and Cunningham 2015). The 5-HT_{2C} receptor, which is primarily postsynaptic, is widely expressed throughout the brain, including on DA and γ-aminobutyric acid (GABA) neurons in the VTA. The 5-HT_{2C} receptor is also expressed in the PFC, bed nucleus of the stria terminalis, and the hypothalamus (Filip et al. 2012; Fletcher et al. 2008; Heisler et al. 2007), the first component in the hypothalamic-pituitary-adrenal (HPA) axis that leads to cortisol release during the stress response. Studies have shown that within the VTA, the 5-HT_{2C} receptor is more concentrated on GABA interneurons, suggesting that activation would lead to net inhibitory regulation of mesolimbic transmission and therefore a decrease in NA DA release (Bubar and Cunningham 2007). Evidence from rodents and NHP studies supports the role of the 5-HT_{2C} receptor in inhibitory mesolimbic transmission. Specifically, the 5-HT_{2C} agonist Ro 60-0175 dose-dependently decreased COC-maintained SA in rodents, and the 5-HT_{2C} antagonist SB 242084 reversed this attenuation (Fletcher et al. 2008; Grottick et al. 2000). The preferential 5-HT_{2C} agonist meta-Chlorophenylpiperazine (mCPP) attenuated COC SA in squirrel monkeys (Manvich et al. 2012), and this effect was blocked by SB 242084 (Manvich et al. 2012). SB 242084 also maintained SA behavior on its own and in combination with COC in squirrel monkeys and maintained a similar response rate on its own compared to COC alone. A more recent study showed that pretreatment with WAY 163909 (WAY), a selective 5-HT_{2C} agonist, dose-dependently decreased both COC and METH SA, and these effects were directly related to decreases in DA levels in the NA (Berro et al. 2017). These studies support an important role of 5-HT, and specifically the 5-HT_{2C} receptor, in the behavioral pharmacology of psychostimulants, and suggest that drugs that target 5-HT_{2C} receptors may be

potential therapeutic candidates for psychostimulant addiction. As the synthesis of compounds with greater specificity to different receptor subtypes increases, the potential to find efficacious treatments for drug abuse with minimal side effects becomes greater; these compounds have become increasingly more available in the last decades, showing promising potential for drug abuse therapeutics and for other addictive disorders, such as overeating (Higgins et al. 2017; Volkow et al. 2017).

With the development of novel serotonergic agonists for the treatment of psychostimulant addiction, the tolerance and efficacy of these treatments in populations that may be more vulnerable to drug abuse remains to be properly examined. For example, age, sex, and early life experience all play critical roles in determining unique susceptibilities to drug abuse (Douglas et al. 2010; Hyman et al. 2006; Hyman et al. 2007; Hyman et al. 2008; MacMillan et al. 2001; Munro et al. 2006; Simpson and Miller 2002; Weber et al. 2008). As areas of the brain continue to develop and undergo remodeling throughout adolescence, which is typically the age of first drug use, perturbations to neurodevelopment may increase vulnerability to drug addiction. This is especially true for alterations in typical development of areas important for decision-making and cognitive control, such as the PFC. Studies have shown that abnormal brain development during sensitive periods lead to an increased likelihood in developing a psychiatric illness in adulthood (MacMillan et al. 2001).

Of the myriad of environmental insults that can contribute to altered brain development, early life stress (ELS), including alterations in the mother-infant relationship such as child maltreatment, has a strong impact on proper development of the brain and the HPA axis (Cicchetti and Rogosch 2001; 2001 a; Cicchetti and Toth 2005; Sinha 2008; Weber et al. 2008) and leads to a strong risk for drug abuse (Cicchetti and Rogosch 2001; 2001 a; Cicchetti and

Toth 2005; Douglas et al. 2010; Hyman et al. 2008; MacMillan et al. 2001; Sinha 2008; 2009). Disruptions in the HPA axis circuit at any point can lead to impaired HPA axis function and improper responses to stress later in life. In particular, experiencing ELS can alter behavioral, neuroendocrine, and emotional regulation systems in the brain that are intimately related to drug use and abuse (Cicchetti and Rogosch 2001; 2001 a; Cicchetti and Toth 2005; Douglas et al. 2010; Hyman et al. 2008; MacMillan et al. 2001; Sinha 2008; 2009). While ELS can be defined in many ways, childhood maltreatment represents a specific form of ELS with debilitating consequences to the victim. In humans, childhood maltreatment can take the form of physical, emotional, or sexual abuse, is highly comorbid with neglect, and is a major risk factor for the development of psychopathologies, including anxiety, depression, and drug abuse (Edwards et al. 2003; MacMillan et al. 2001). In 2016, the US Department of Health and Human Services estimated that 676,000 children were victims to at least one form of maltreatment (Health and Services 2018). Childhood maltreatment typically occurs in multiple co-morbid forms and is more likely to occur before the child reaches puberty. Notably, maltreatment during early developmental periods has the greatest negative consequences on brain and behavior. Early childhood and pre-pubertal stress is strongly linked with adult psychiatric problems, and significantly more patients with psychiatric disorders who experienced ELS experienced it before puberty (Hyman et al. 2006; Hyman et al. 2008; Weber et al. 2008). However, the strength of this association varies between disorders and the type of maltreatment (Weber et al. 2008), and different types of maltreatment have different effects on the neuroendocrine system and manifest in different pathologies (Cicchetti and Rogosch 2001; 2001 a; Hyman et al. 2006; Weber et al. 2008). Studies in humans have shown that levels of cortisol, the outcome stress hormone that is used to examine potential dysregulation of the HPA axis, are not significantly

different between morning and afternoon in maltreated children as a whole. However, when broken into subtypes of abuse, children that were both sexually and physically abused had significantly higher morning cortisol levels than those who had been emotionally or physically abused, neglected, or not abused at all (Cicchetti and Rogosch 2001 a). This pattern of hypercortisolism is linked with internalizing pathologies such as depression or anxiety, whereas the hypocortisolism that is more seen in physically abused children is linked with externalizing pathologies such as aggression (Cicchetti and Rogosch 2001).

Additionally, timing and chronicity of maltreatment play a role in how the brain develops and reacts to stressors later in life. Maltreatment in infancy or childhood has a larger impact on brain development and leads to increased risk of developing an adult psychiatric disorder compared to individuals who did not experience child maltreatment (MacMillan et al. 2001). In tests measuring cognitive control, children that were maltreated during infancy had significantly lower inhibitory control/working memory scores than non-maltreated individuals or children who had been abused at later developmental points (Cowell et al. 2015). Another study using PET imaging found a significant association between increased bilateral DA release (as measured by decreased levels of D2 receptor binding potential in the NA) in response to a stress-induced task and individuals who reported poor maternal care during childhood, compared to individuals with high quality maternal care (Pruessner et al. 2004). Other brain areas such as the amygdala, a critical structure for emotional and stress responses, are also recruited more often during acute stressors if an individual has experienced chronic stress. In one study, childhood emotional abuse and neglect was associated with hyperresponsiveness of the amygdala during a paradigm involving presentation of threatening faces (Dannlowski et al. 2012). These results suggest that ELS in the form of childhood maltreatment affects the development of brain

networks involved in emotional and stress regulation, as well as reward and goal-directed behavior, resulting in alterations in stress neuroendocrine systems, emotional regulation, as well as potential increased risk for drug abuse.

Importantly, ELS has been linked to drug abuse later in life, making individuals who experienced ELS increasingly vulnerable to the reinforcing effects of psychostimulants like COC (Cicchetti and Toth 2005; Hyman et al. 2006; Hyman et al. 2007; Hyman et al. 2008; Sinha 2001; 2008). Childhood maltreatment has been associated with severity of substance use, age of onset of COC use, and COC dependence (Douglas et al. 2010; Hyman et al. 2006; Hyman et al. 2007; Hyman et al. 2008). Additionally, children commonly experience multiple forms of maltreatment (Edwards et al. 2003), and the number of types of abuse is correlated with the likelihood of developing a substance disorder (Douglas et al. 2010). The relationship between ELS and drug abuse may be in part caused by alterations in the development of stress and emotion regulation, as well as reward and goal-directed circuits during childhood and adolescence; however the mechanisms underlying increased vulnerability from ELS are still poorly understood.

Just as NHPs are used to examine the reinforcing effectiveness of drugs of abuse, it is also important to note that ELS also occurs in NHPs in the form of infant maltreatment, in different species, both in captivity and in the wild. One example are rhesus macaque mothers, which are typically very dedicated mothers and remain in close contact with offspring during the first year after birth. This leads to a strong relationship between mother and offspring and creates a steady support system for the offspring during infancy, the juvenile period, and adolescence (Bayart et al. 1990; Hansen 1966; Maestripieri 1994; Maestripieri and Carroll 1998; Maestripieri 1999; Sanchez et al. 2001; Sanchez 2006). The first six months in particular is a period during

which the mother provides warmth, nutrition, security and social cues, as well as acts as a buffer for any stress reactions (Sanchez et al. 2001; Sanchez et al. 2015). However, infant maltreatment has been reported and studied in this and other species of macaques, including at the Yerkes National Primate Research Center (YNPRC) Field Station breeding colony, where approximately 2,000 rhesus monkeys are socially housed. This represents a unique opportunity for the study of long-term effects associated with infant maltreatment that occur naturalistically (i.e., not experimenter induced).

Infant maltreatment in macaques, like in humans, results in heightened emotional reactivity, anxiety, and alterations in the development of the HPA axis function that leads to higher cortisol levels and stress responses later in life (Drury et al. 2017; Koch et al. 2014; McCormack et al. 2006; McCormack et al. 2009; Sanchez et al. 2010). In particular, infant abuse by the mother causes physical pain to the infant and includes behaviors such as crushing, dragging, and hitting (Maestripieri 2005). Abuse is highly comorbid with infant rejection and low maternal responsiveness (Sanchez 2006), all of which are exhibited spontaneously within the first few months of life by approximately 2-5% of the population. Studies investigating the developmental impact of infant maltreatment on rhesus macaques have found that abusive mothers also show higher rejection of infants, less ventral contact, and more breaking of contact (McCormack et al. 2006). These maltreated infants show social developmental delays, including delayed independence from their mothers, spending more time on mothers' ventrum and less time exploring or breaking contact (Howell et al. 2017), and they exhibit behavioral signs of distress, such as high rates of screams and tantrums, and spend more time in solitary than social play (Maestripieri et al. 2006; McCormack et al. 2006). Infant maltreatment also leads to overall hyperfunctionality of the HPA axis. For example, 6-month-old abused rhesus macaques showed

significantly lower adrenocorticotropin-releasing hormone (ACTH) levels following a stress challenge, as well as significantly higher levels of cortisol, indicating a hyperactivity of the HPA axis starting at the paraventricular nucleus of the hypothalamus. These changes in HPA axis function persisted across three years, showing that the effects of ELS are long-lasting (Sanchez et al. 2010). Areas of the brain related to emotional regulation of stress are also affected by infant maltreatment. These areas include the amygdala, which has a larger volume in maltreated animals that is predicted by the higher rate of abuse received during infancy and associated with lower exploration, higher anxiety, and increased aggression and vigilance in the maltreated animals during the juvenile period (Howell et al. 2014).

Monoaminergic systems are also affected by the occurrence of infant maltreatment. Studies investigating changes in the monoamine systems after ELS have indicated an overall decrease in serotonergic and dopaminergic function (Maestripieri et al. 2006; Maestripieri et al. 2006; McCormack et al. 2009; Sanchez et al. 2007). Decreased levels of 5-HT and DA metabolites were found in cerebrospinal fluid (CSF) of rhesus macaques who experienced maltreatment in the first 6 postnatal months, and these reduced levels were associated with increased anxiety and remained constant over time (Maestripieri et al. 2006; Sanchez et al. 2007). Low levels of 5-HT metabolites such as 5-HIAA have been linked to higher levels of maternal rejection in the first 6 months of life and increased probability of perpetuating abuse with their own offspring. Lower CSF 5-HIAA has also been reported to be connected to impulsivity, risk-taking behavior, and aggression (Maestripieri et al. 2006).

Taken together, the impact of ELS on the development of neuroendocrine and monoaminergic systems, emotional and HPA axis regulation, and behavior later in life suggests that ELS poses a risk factor for increased drug-seeking behavior in adolescence due to

heightened anxiety, dysregulation of HPA axis function, and decreases in brain dopaminergic and serotonergic systems. Specifically, animals that experience ELS typically have HPA axis systems characterised by increased cortisol secretion during infancy (Drury et al. 2017; Howell et al. 2013), and the elevated levels of cortisol can affect the brain's 5-HT system (Howell et al. 2016), leading to the develoment of a hyposerotonergic system. Serotonergic regulation over the DA system contributes to how an individual is affected by rewarding stimuli, which suggests that decreased serotonergic regulation may lead an individual to be more susceptible to the reinforcing effects of psychostimulant drugs. However, treatment with a 5-HT_{2C} receptor agonist such as WAY could increase serotonergic regulation over the mesolimbic system in all subjects, and potentially even normalize the decreased regulation in maltreated subjects. Furthermore, this is (to the author's knowledge) the first examination of drug-maintained responding using an NHP infant maltreatment model, as well as of changes in drug-maintained responding in response to a pharmacological intervention. Thus, the aim of the present study is to investigate the effects of a selective 5-HT_{2C} receptor agonist WAY on COC SA in adolescent rhesus monkeys that are at greater risk for COC abuse due to infant maltreatment.

Hypothesis

Subjects who experienced ELS will have a greater sensitivity to the abuse-related effects of COC than controls. Therefore, I expect that they will require lower doses of COC to maintain stable responses. Based on previous data from Dr. Howell's lab (Berro et al. 2017), activation of the 5-HT_{2C} receptor has previously shown to dose-dependently decrease COC-maintained responding; therefore, we expect that pretreatment with the WAY compound will attenuate COC-maintained responding, even in the maltreatment group.

Methods

Subjects, Housing and Rearing Experience

Subjects were 8 adolescent rhesus monkeys (*Macaca mulatta*), 4 male and 4 female, between the ages of 4.5-5.5 years old. Six subjects experienced ELS in the form of maternal maltreatment as infants (MALT, n=6) and 2 received competent maternal care (Control, n=2); see **Table 2** for the experimental groups breakdown. Although previous studies in rhesus macaques have shown that early experiences play a large role in the development of behavioral and neurochemical reactions to stress (Maestripieri et al. 2006), in this study all subjects were cross-fostered at birth and randomized to control or MALT care groups in order to disentangle the effect of postnatal experience from potential hereditary factors that can influence our measures. Infants were separated from their biological mothers and placed with their foster mothers within 5 minutes of initial separation. All animals were successfully adopted by their foster mothers who were selected from different matrilines so that mother-infant pairs would be unrelated. These 8 subjects were part of a bigger longitudinal study of developmental outcomes of infant maltreatment (Drury et al. 2017; Howell et al. 2017). Animals were born and housed at the YNPRC Field Station until 4-5 years of age, where they lived with their mothers in large social groups housed in outdoor compounds, with access to climate-controlled indoor areas. Standard high fiber, low fat monkey chow (Purina Mills Int., Lab Diets, St. Louis, MO) and seasonal fruits and vegetables were provided twice daily, in addition to enrichment items. Water was available *ad libitum*.

Mother-infant pairs were studied from birth through 3 months postpartum in their natal social groups to examine the maternal care received. Infant maltreatment was operationally defined as co-occurrence of physical abuse and infant rejection, where physical abuse was

defined as violent behaviors of the mother that produce pain and distress in the infants, such as dragging, hitting, and sitting on the infant, while rejection was defined as breaking contact with and pushing the infant away, which also lead to infant distress (Howell et al. 2017; Maestripieri 1998; Maestripieri and Carroll 1998; McCormack et al. 2006; McCormack et al. 2009; Sanchez 2006). Behavioral observations were collected using established ethograms and procedures where the infant was the focal animal (Altmann 1962; Howell et al. 2017). Each observation lasted 30 minutes and was conducted on separate days, five times per week in the first month postpartum (20 observations), twice per week in the second month (8 observations), and once per week in the third month (4 observations), resulting in 32 total observations (a total of 16 hours) per mother-infant pair. This observation schedule was chosen based on previous published protocols documenting optimal observation frequency based on the ability to capture early maternal care received by the infant (Maestripieri and Carroll 1998; Maestripieri 1998; McCormack et al. 2006; McCormack et al. 2009).

At approximately 4-5 years of age, subjects were transferred to the Main Research Center for the COC SA studies. Upon arrival, subjects were pair-housed, fed Purina monkey chow (Ralston Purina, St. Louis, MO, USA), supplemented with fruit and vegetables daily, and water was available *ad libitum* in their home cages. Environmental enrichment was provided on a regular basis. Ambient conditions within the colony were maintained at a temperature of 22 ± 2°C and at 45-50% humidity. The room lighting was set to a 12 h light/dark cycle, with the light period from 7:00 A.M. to 7:00 P.M. All subjects were naive to psychostimulants as well as the drug SA procedure. Following a period of acclimation to the new housing environment, the animals underwent several other tasks as part of a larger study that are not included here. Subsequently, animals were fitted with primate collars, chair-trained to sit comfortably in chairs

designed for NHPs (Primate Products, Miami, FL, USA), and implanted with intravenous (i.v.) catheters for the COC SA procedures described below, at approximately 5-6 years of age. All protocols and animal care and handling strictly follow 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 Emory University Animal Care and Use Committee (IACUC).

Psychostimulant and 5-HT_{2C} Receptor Agonist Drugs

Cocaine hydrochloride (National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% sterile saline and administered intravenously. WAY 163909 (WAY) hydrochloride (provided as a generous gift from Pfizer Inc®, New York, NY, USA) is a selective 5-HT_{2C} receptor agonist. WAY was dissolved in 10mg/mL beta-cyclodextrin and administered intramuscularly (0.1, 0.3, or 1.0 mg/kg). The doses of each drug were calculated and expressed as the salt form.

COC SA

Acquisition

As shown in the experimental design diagram depicted in **Figure 1**, subjects began SA through acquisition training, during which they received 0.1 mg/kg/infusion i.v. of COC on a fixed-ratio (FR) 1 schedule of reinforcement followed by a 10 s timeout (TO), during which no drug was available. Lights were used to signal drug availability. Each session lasted up to 1 hr, with a maximum of 5 infusions per session. Completion of three consecutive sessions whereby subjects received 4 or 5 infusions qualified an increase of the FR (1, 2, 4, 8, 16, 20) until FR20 was reached. When the FR20 was met, the TO was extended to 30 s, and subjects had to again receive 4 to 5 infusions for three consecutive sessions before the dose of COC was dropped to

0.03 mg/kg/infusion. Subjects were then allowed 20 maximum infusions followed by 30 s TO per each 1 hr session until 19-20 infusions were received for 5 consecutive sessions. Days to acquire SA was used as the primary dependent measure.

ED_{Max}

Following Acquisition, a dose-response curve was created for each subject by administering a range of COC doses (0.01, 0.03, 0.1 mg/kg/inf.). Doses were administered randomly and each subject was moved to the next dose following acquisition of a stable response rate (variation <25% across three consecutive sessions). Once each subject had a determined peak dose (the dose that engendered the highest response rate: ED_{Max}), they moved on to the Limited Access stage. Number of infusions and response rate (average number of lever presses/second/session) were the dependent measures, and ED_{Max} dose of COC was used as a proxy measure for sensitivity to the reinforcing effects of COC. The ED_{Max} dose of COC for each individual animal was used in all of the following COC SA experiments.

Limited Access

Subjects had 1-hr Limited Access (LA) sessions during which they could administer up to 60 infusions at their ED_{Max} dose of COC under identical parameters as listed above. LA sessions continued until the same criteria listed under acquisition and ED_{Max} were met, whereby subjects proceeded to the WAY pretreatment stage.

WAY Pretreatment

Three doses of the WAY compound (0.1, 0.3, 1.0 mg/kg intramuscular; i.m.) or vehicle were administered three days a week for six weeks. The first and last SA session of each week included a LA session with no pretreatment. The three sessions in the middle of the week were preceded by an i.m. injection of the same dose of WAY or saline 45 min. before the beginning of

the session. Weeks with WAY pretreatment alternated with weeks with vehicle pretreatment until all doses of WAY had been tested. Baseline responding was determined on each Monday session prior to treatment with either vehicle or WAY. That is, baseline sessions did not include any pretreatments before the session began.

Data Analysis

Due to the small sample size, the study is underpowered to examine the effect of sex as a factor; therefore, sex was not included as a factor in the statistical models. If the assumption of variance was violated according to Levene's Test of Equal Variances (Independent samples t-test) or Mauchly's Test of Sphericity (Repeated Measures Analysis of Variance; RMANOVA) data was reported with equal variances not assumed using the Greenhouse-Geisser correction.

Number of days to acquire the FR task was averaged for MALT and control animals and compared using an Independent samples t-test. ED_{Max} doses of COC as determined during the dose-response function were averaged within MALT and control groups and compared using an Independent samples t-test.

Average baseline responding was compared using Independent samples t-test to examine whether mean number of infusions and mean response rates differed significantly between MALT and control groups on the first session of each treatment week. Data used in analyses represent the average of all 6 baseline sessions, whereby no pretreatments were given, prior to pretreatment with WAY or vehicle.

Number of infusions and response rates were normalized as percent to drug-maintained responding compared to baseline responding. Analyses for WAY pretreatment data were conducted using a RM ANOVA to compare differences in mean number of infusions and response rates after vehicle and WAY pretreatments, with within-subject factor being dose

(vehicle, 0.1, 0.3, 1.0 mg/kg WAY) and between-subject factor being group (Control, MALT). Independent samples t-tests with Bonferroni corrections for multiple comparisons were used as post-hoc pairwise comparisons of the means when significant group by WAY dose interaction effects were detected. Analyses were conducted for both data normalized as percent compared to baseline and percent compared to vehicle, however results from these analyses were similar to data presented here, therefore, only data normalized as percent compared to baseline are shown.

All analyses were conducted using IBM SPSS 24 and graphs were made using GraphPad Prism. Significance was defined as $p \leq 0.05$ with the exception of the post-hoc pairwise comparisons of the means for group by WAY pretreatment significant interaction effects, which was set at $p \leq 0.010$ for Bonferroni corrections for multiple comparisons, and data are presented as mean ± SEM unless otherwise stated.

Results

Acquisition

Differences in time to acquire SA between MALT and control subjects are presented in **Figure 2**. Independent samples t-test revealed no significant difference between the rate of acquisition of MALT subjects (38.8333 ± 2.5745) compared to control subjects (37.5000 ± 1.5000) [$t(6) = -0.281$, $p=0.789$].

ED_{Max}

Figure 3 demonstrates the ED_{Max} dose of COC (the dose at which the subject maintained the highest level of responding) averaged between MALT (0.0200 ± 0.00447) and control animals (0.100 ± 0). Independent samples t-test revealed a significant difference in the ED_{Max} dose of COC [$t(5) = 17.889$, $p<0.001$], in that the ED_{Max} dose of all MALT subjects (0.01 or 0.03

mg/kg) is at least one half-log unit dose lower than that of control subjects (0.1 mg/kg). These data are also reflective of leftward shifts in individual dose effect curves, as presented in **Figure 4.**

Baseline Responding

Average number of infusions and response rates for all subjects are shown in **Table 1** and **Table 2**, respectively. **Figure 5** demonstrates average baseline number of infusions and **Figure 6** demonstrates average baseline response rates of MALT and control animals. Independent samples t-tests revealed that the MALT group received significantly more infusions than the control group (MALT: 49.5556 ± 3.5517, Control: 23.6548 ± 3.5119; t(6) = -3.870, p=0.008), however baseline response rates were not significantly different between the MALT group (1.0556 ± 0.3427) and the control group (0.1916 ± 0.0402; t(6) = -1.380, p=0.217).

WAY Pretreatments

Average number of infusions and average response rates for all subjects tested with vehicle and WAY are shown in **Tables 3** and **4**, respectively. To analyze whether dose and group had an effect on COC-maintained responding, we ran a RM ANOVA with dose and group as factors and responding (number of infusions or response rate) as the dependent measure. Results from analyses on average number of infusions for all subjects showed a significant effect of dose [F(1.327, 7.964) = 94.499, p<0.001, $\eta^2_{partial}$ = 0.940], but no significant effect of group [F(1, 6) = 0.268, p=0.623, $\eta^2_{partial}$ = 0.043] or dose by group interaction effect [F(1.327, 7.964) = 2.617, p=0.142, $\eta^2_{partial}$ =0.304]. Bonferroni post-hoc tests corrected for multiple comparisons demonstrated that WAY decreased COC-maintained responding, and that number of infusions earned following 1.0 mg/kg WAY was significantly lower in comparison to infusions earned at vehicle (p<0.001), 0.1 mg/kg WAY (p<0.001), and 0.3 mg/kg WAY (p<0.001). Results are

shown in **Figure 7**.

Results from the RM ANOVA testing differences in average response rates between groups also demonstrated a significant effect of dose [$F(3, 18) = 119.279$, $p<0.001$, $\eta^2_{partial} = 0.952$], a trend towards effect of group [$F(1, 6) = 4.176$, $p=0.087$, $\eta^2_{partial} = 0.410$], and a significant dose by group interaction [$F(3, 18) = 5.385$, $p < 0.01$, $\eta^2_{partial} =0.473$]. Bonferroni post-hoc tests corrected for multiple comparisons demonstrated that WAY decreased COC-maintained responding, and that the response rates at 1.0 mg/kg WAY were significantly lower compared to vehicle ($p<0.001$), low dose ($p<0.001$), and middle dose ($p<0.001$). Independent samples t-tests failed to detect a significant interaction between MALT and control animals at vehicle ($t(6) = 0.982$, $p=0.364$), 0.1 mg/kg WAY ($t(6) = 1.811$, $p=0.120$), 0.3 mg/kg WAY ($t(6) = 3.578$, $p=0.159$), and 1.0 mg/kg WAY ($t(6) = -0.278$, $p=0.791$). Results are shown in **Figure 8**.

Discussion

The present study investigated the effects of a selective 5-HT_{2C} receptor agonist WAY on COC SA in control adolescent rhesus monkeys and in comparison to animals that experienced ELS in the form of infant maltreatment. In support to the hypotheses proposed, the findings reported here suggest that MALT subjects show increased sensitivity to the reinforcing effects of COC indicated by lower peak doses of responding. In addition, pretreatment with the WAY compound resulted in significantly decreased responding in all subjects, including those with ELS.

Our results generally support previous findings in the literature that investigate the effect of ELS on sensitivity to substance abuse (Huggins et al. 2012; Sanchez et al. 2001; Sinha 2001). Increased sensitivity to the reinforcing effects of COC can be measured by comparing peak

response rates between two groups (Ator and Griffiths 2003; Spealman and Goldberg 1978), whereby a lower ED_{Max} is indicative of greater sensitivity in that an individual requires less COC to maintain peak responding. Our study found that the ED_{Max} of MALT subjects was significantly lower than control subjects and showed a significant leftward shift in dose-response curves of MALT subjects by at least one-half log unit dose of COC, suggesting that MALT subjects show greater sensitivity to the reinforcing effects during COC SA. These findings, while important, are in contrast to one study by Corcoran and Howell that found no significant difference in COC ED_{Max} between monkeys exposed to repeated maternal separations as infants (a different model of ELS in NHPs) and controls (Corcoran and Howell 2010). Additionally, maternally-separated monkeys showed decreased response rates during maintenance of COC SA, whereas results from our study indicate higher response rates, albeit not significantly, in subjects who experienced ELS. It is possible that the inconsistent findings between the two studies is a result of different ELS paradigms, as well as differences in the schedule of reinforcement employed. Whereas the Corcoran and Howell study utilized a maternal separation paradigm, our study observed naturalistic infant maltreatment that was not experimenter-induced. Although the maternal separation paradigm has also shown increased cortisol release in response to these acute mother-infant separations (Sanchez et al., 2005), the ability of the mother to buffer stress induced from separation upon reunion may have blunted the effects of stress, as previously demonstrated in this species (Sanchez et al. 2015), and therefore have less of an impact on the reinforcing effectiveness of a drug later on in life (Howell et al. 2017; Sánchez et al. 2005). This may suggest that differences in the ELS paradigm employed may have stark differences in behavioral outcomes related to sensitivity to drug reinforcement. Furthermore, the use of a second-order schedule of reinforcement may have contributed to different findings, as second-order schedules

of reinforcement have been linked to higher responding due to presentation of drug-associated cues (Spealman and Goldberg 1978).

Before looking at the effects of WAY on COC SA, we compared the baseline responding of MALT and control subjects in order to determine whether the two groups responded differently to COC directly prior to WAY pretreatments. We found that number of infusions, but not response rate, was significantly greater in MALT animals compared to control. Although the difference in number of infusions between the two groups was significant, lack of significant differences in response rates suggests that drug-taking behavior between MALT and control subjects did not differ directly before administration of WAY pretreatments. Whereas response rate under these conditions is a more sensitive measure of reinforcing effects (Howell and Wilcox 2001; Spealman and Goldberg 1978), having a maximum number of infusions per session, as utilized in this study, may limit the ability to compare the effect of number of infusions between groups. The pattern of psychostimulant intake at FR schedules of reinforcement is such that subjects respond in waves throughout the SA session, rapidly responding until an infusion is achieved, followed by less frequent responding, potentially to stabilize drug levels in the brain (Haney and Spealman 2008; Spealman and Goldberg 1978; Yokel 1987). This pattern of responding demonstrates a highly regulated SA of the drug. Our FR20 schedule of reinforcement followed by a 30 s timeout allowed for a relatively consistent pattern of COC responding over the hour-long sessions, leading to response rates that are linked directly to the reinforcing effects of COC (Spealman and Goldberg 1978). Furthermore, the considerable inter-individual variation in responding may also account for the lack of significant differences in response rates. We expect that when the number of subjects in both our control and MALT groups are increased to the full sample size (n=25, 11 controls, 14 MALT), we may

detect a significant effect on response rate, given the general trend in higher response rates seen in MALT animals in comparison to control animals (**Table 2** and **Figure 6**).

WAY pretreatments decreased COC-maintained responding, however these decreases were not a function of ELS. Specifically, the highest dose of WAY (1.0 mg/kg) decreased both the number of infusions and the average response rate in comparison to every other dose tested. Our findings contribute to other recent literature examining the effects of WAY on COC SA, including a recent study by our group (Berro et al. 2017) that found a dose-dependent decrease in COC SA after WAY pretreatments. One difference between the results of our study and that of Berro and colleagues is the dose-dependent effect of WAY on COC SA in control subjects. Whereas control subjects in our study showed only a significant effect of WAY at the highest dose (1.0 mg/kg/inf.), the other study showed a dose-dependent decrease in response rate at both 0.3 and 1.0 mg/kg/inf. doses of WAY. Importantly, anecdotal evidence of motoric impairment following this high dose during our studies, as well as previous data showing that the high dose of WAY decreases daytime locomotor activity in adult monkeys with a history of psychostimulant SA (Diaz et al. 2017), suggests decreases in responding at this high dose may be due to non-specific responding, and therefore may not be clinically significant for the treatment of ongoing psychostimulant drug abuse in either adolescents or adults. Interestingly, when the results from our experiments are compared to those of Berro and colleagues, it appears as if the MALT animals show a similar pattern of decreases in COC-maintained responding, whereas control animals seem to show a resistance towards the effects of the WAY pretreatments. One potential reason for this may be that the subjects in our study were adolescent rhesus monkeys naive to psychostimulants, whereas the Berro et al. study used adult rhesus monkeys that had previously self-administered psychostimulants. More highly developed regulation of behaviors

as well as altered neurochemistry from prior psychostimulant use may have contributed to the greater serotonergic regulation over psychostimulant SA seen in the study by Berro and colleagues. Additionally, as noted above, the utilization of a second-order schedule of reinforcement of COC SA in the Berro et al. study may also have led to a greater ability to detect differences in responding after pretreatments of WAY, and this may have led to a greater range of responding that ultimately led to an increase in differences in responding after different pretreatments of WAY.

As mentioned before, there were no significant differences in the effect of WAY between MALT and control groups, despite apparent differences at the intermediate dose of 0.3 mg/kg WAY. This suggests a potential increased sensitivity to this dose of WAY in MALT subjects compared to control subjects and highlights the clinical applications of this compound for human populations with ELS. The connection between infant maltreatment and low 5-HT or neuroendocrine activity may contribute to these results (Maestripieri et al. 2006; Maestripieri et al. 2006; McCormack et al. 2009; Sanchez et al. 2007; Sanchez et al. 2010). One possibility includes a hyposerotonergic system caused by ELS in the MALT group causing changes in function or expression of the 5-HT_{2C} receptor, such as upregulation. While constitutive serotonergic control over mesolimbic transmission during baseline COC SA may have been inadequate in regulating responding in MALT animals, as shown in increased number of infusions, increased activation of 5-HT_{2C} receptors by the WAY compound may have normalized serotonergic regulation over mesolimbic transmission, thereby leading to the lack of significant differences in responding after WAY pretreatments between the two groups. The modulatory effects of the 5-HT_{2C} receptor on mesolimbic transmission are complex, and further investigations into the effect of ELS on 5-HT_{2C} receptor expression and function in relation to

psychostimulant SA are warranted. For example, chronic dosing regimens are necessary to test long-term effects of WAY on COC SA before proposing WAY for pharmacotherapeutic use in humans.

The effect of WAY on COC SA in NHPs suggests that therapeutic treatments targeting the 5-HT_{2C} receptor may be effective in treating individuals with psychostimulant abuse due to the indirect modulation of the 5-HT_{2C} receptor on mesolimbic DA transmission via GABA interneurons (Howell and Cunningham 2015). Increased receptor specificity allows for more targeted effects of potential therapeutic agents with minimal side effects (Manvich et al. 2012), and the selective 5-HT_{2C} receptor compound WAY allows for specific serotonergic regulation over mesolimbic transmission. Importantly, this effect was seen specifically in MALT subjects that showed dose-dependent decreases in responding, indicating that WAY may be effective in treating individuals with increased sensitivity to psychostimulant abuse from abnormal brain development caused by ELS. Studies in humans examining the effects of ELS indicated that altered HPA axis function and cognitive control due to child maltreatment was associated with increased risk for developing psychiatric disorders in adulthood (Cicchetti and Rogosch 2001; 2001 a; Cicchetti and Toth 2005; Cowell et al. 2015; Douglas et al. 2010; Hyman et al. 2008; MacMillan et al. 2001; Sinha 2008). Due to the highly translational value of NHP research, in particular studies that utilize rhesus macaque models (Ator and Griffiths 2003; Weerts et al. 2007) such as the current one, the results from this study provide evidence that compounds specifically targeting the 5-HT_{2C} receptor may be promising therapeutic agents to combat psychostimulant abuse.

Some major limitations in this study may have affected our interpretation of these results. This study was part of a greater longitudinal study investigating the effects of ELS on drug abuse

and potential therapeutic treatment for COC addiction. Due to the limited time frame of data collection, only one cohort of animals completed all stages of the experiment and therefore the sample size for this particular study was too small to make strong conclusions. However, partial effect sizes from WAY pretreatments indicate a strong relationship between COC SA and WAY attenuation. We must be cautious while interpreting effect size based on the partial eta squared value as opposed to the eta squared value, as it may be an overestimate of effect size caused by inclusion of more than one independent variable. Therefore, it also important to mention that the small partial effect sizes of dependent measures between groups alone and after WAY pretreatments may not be representative of that of the population, however further interpretations are beyond the scope of this study due to the small sample size.

Related to small sample size is the unequal distribution of sex and group in this sample. In particular, the lack of male control subjects did not allow for inclusion of sex as a variable in analyses. Studies in humans and NHPs show that sex is an additional risk factor for development of psychopathologies such as substance abuse (Hyman et al. 2006; Hyman et al. 2008; MacMillan et al. 2001; Munro et al. 2006; Simpson and Miller 2002). The addition of male controls, as well as females in both MALT and control groups, would enable future analyses that may unveil sex differences in responding during COC SA with and without WAY pretreatment.

Conclusion

In summary, this study found that the selective 5-HT_{2C} receptor agonist WAY 163909 attenuated COC SA in control subjects as well as subjects that experienced ELS in the form of infant maltreatment. This suggests that potential therapeutic treatments for psychostimulant abuse that target the 5-HT_{2C} receptor may be effective at reducing drug intake with minimal side

effects in populations exposed to ELS. Future studies are warranted to make greater conclusions regarding the relationship between ELS, COC SA, and the 5-HT_{2C} receptor, however the present study contributes to current literature indicating that ELS increases sensitivity to psychostimulant abuse and that the 5-HT_{2C} receptor indirectly modulates mesolimbic DA transmission in order to decrease COC SA.

Tables & Figures

Table 1. Average number of infusions for all subjects during baseline sessions and with vehicle or WAY pretreatments. Each data point represents the mean from 6 baseline sessions and 2-3 WAY or vehicle sessions. Data are presented as mean ± SD.

Subject	Group	Sex	Baseline	Vehicle	WAY 0.1 mg/kg	WAY 0.3 mg/kg	WAY 1.0 mg/kg
Dw13	MALT	M	47.67 ± 8.09	42.22 ± 10.60	38.67 ± 4.04	34.67 ± 5.51	0.00 ± 0.00
Py13	MALT	M	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00	20.67 ± 20.03
Pv13	MALT	M	58.67 ± 1.51	58.56 ± 2.40	57.67 ± 4.04	51.00 ± 2.00	37.67 ± 3.21
Lc14	MALT	M	36.83 ± 6.77	27.00 ± 4.93	24.00 ± 5.29	26.00 ± 3.00	0.00 ± 0.00
Im13	MALT	F	45.00 ± 2.00	40.38 ± 4.31	38.00 ± 3.61	33.67 ± 5.86	0.00 ± 0.00
Rv13	MALT	F	49.17 ± 2.93	45.38 ± 4.78	47.33 ± 2.52	39.00 ± 3.61	0.00 ± 0.00
Nc14	Control	F	20.14 ± 1.68	19.63 ± 1.06	21.00 ± 2.00	21.00 ± 3.61	0.00 ± 0.00
Bf13	Control	F	27.17 ± 3.92	26.44 ± 3.78	26.33 ± 2.89	24.33 ± 1.15	1.00 ± 1.00

Table 2. Average response rate for all subjects during baseline sessions and with vehicle or WAY pretreatments. Each data point represents the mean from 6 baseline sessions and 2-3 WAY or vehicle sessions. Data are presented as mean ± SD.

Subject	Group	Sex	Baseline	Vehicle	WAY 0.1 mg/kg	WAY 0.3 mg/kg	WAY 1.0 mg/kg
Dw13	MALT	M	0.71 ± 0.25	0.55 ± 0.25	0.42 ± 0.09	0.35 ± 0.10	0.00 ± 0.00
Py13	MALT	M	2.66 ± 0.55	2.32 ± 0.39	2.34 ± 0.73	1.68 ± 0.24	0.06 ± 0.22
Pv13	MALT	M	1.29 ± 0.23	1.46 ± 0.45	1.22 ± 0.31	0.78 ± 0.09	0.40 ± 0.06
Lc14	MALT	M	0.34 ± 0.12	0.23 ± 0.07	0.19 ± 0.06	0.25 ± 0.04	0.00 ± 0.00
Im13	MALT	F	0.57 ± 0.06	0.46 ± 0.09	0.40 ± 0.07	0.33 ± 0.09	0.00 ± 0.00
Rv13	MALT	F	0.71 ± 0.11	0.60 ± 0.15	0.64 ± 0.08	0.43 ± 0.07	0.00 ± 0.00
Nc14	Control	F	0.15 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.16 ± 0.04	0.00 ± 0.00
Bf13	Control	F	0.23 ± 0.05	0.22 ± 0.05	0.22 ± 0.03	0.20 ± 0.02	0.01 ± 0.01

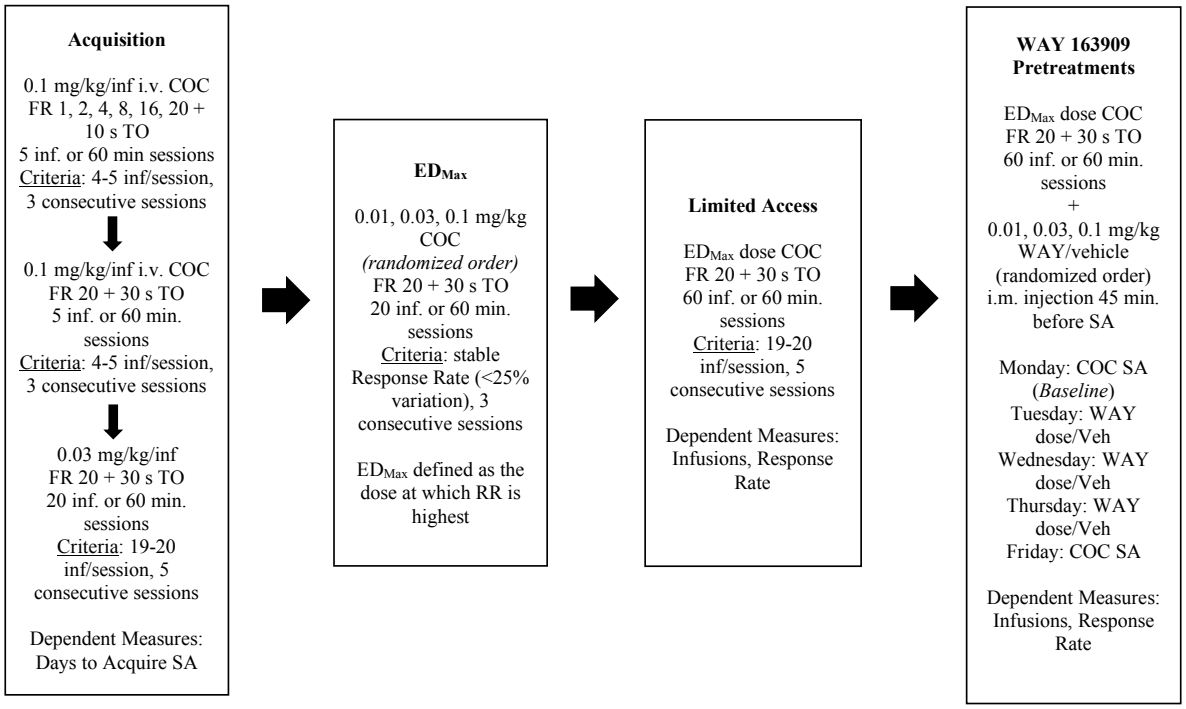

Figure 1. Study design. Subjects started with Acquisition phase, and upon completion, moved to the ED_{Max} determination. Once their ED_{Max} was determined, they went through a LA phase, and then WAY pretreatments.

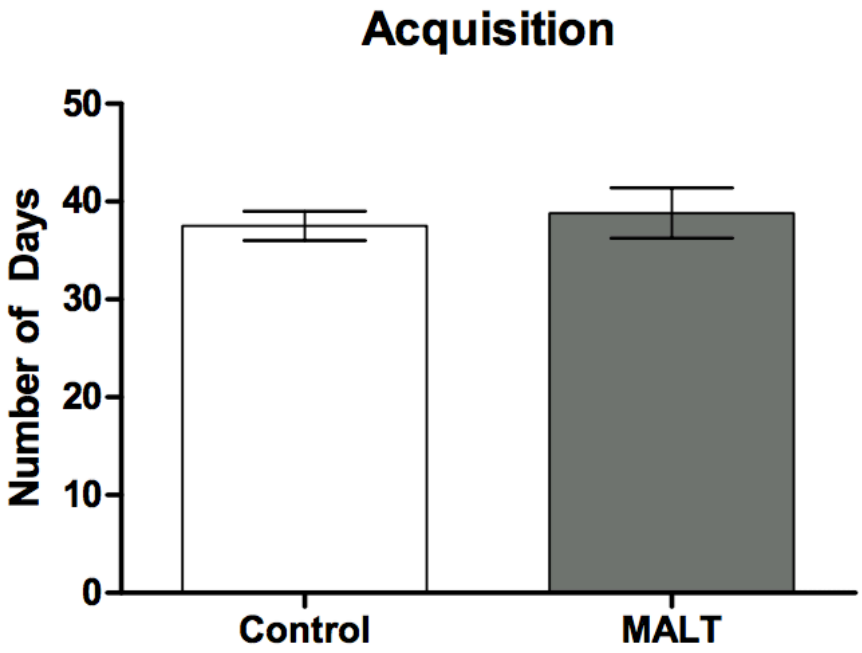

Figure 2. Average number of SA sessions (days) for Control (n=2) and MALT (n=6) subjects to acquire the SA task and receive 19-20 infusions for 5 consecutive sessions at a FR20 schedule of reinforcement with a 30 s timeout. Difference in rate of acquisition was not statistically significant between MALT and control subjects. Data are presented as mean (±SEM).

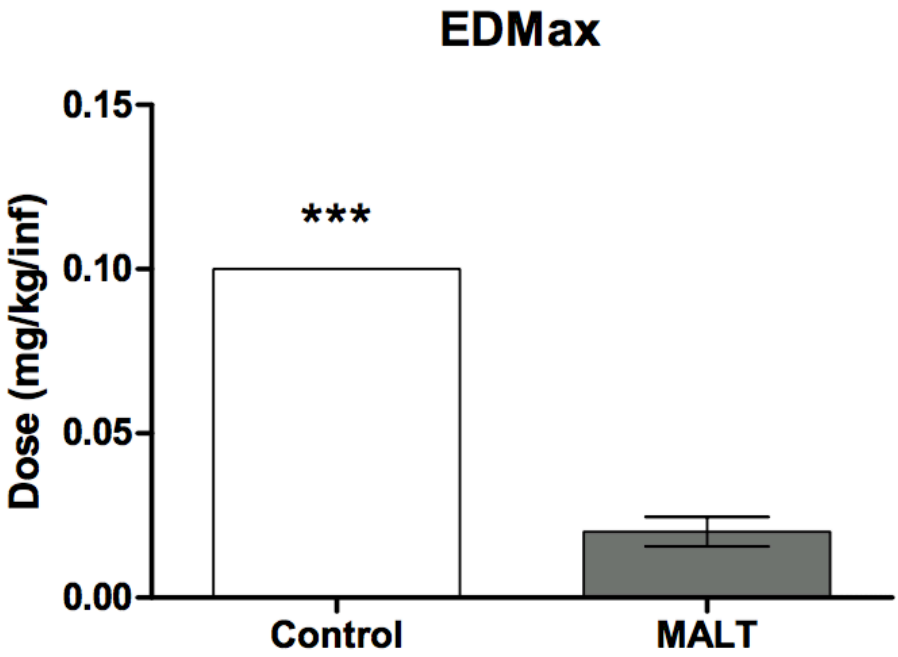

Figure 3. Average peak dose (ED_{Max}) of COC between Control (n=2) and MALT (n=6) subjects. Control subjects had significantly higher (***$p<0.001$) ED_{Max} than MALT subjects. Data are presented as mean (±SEM).

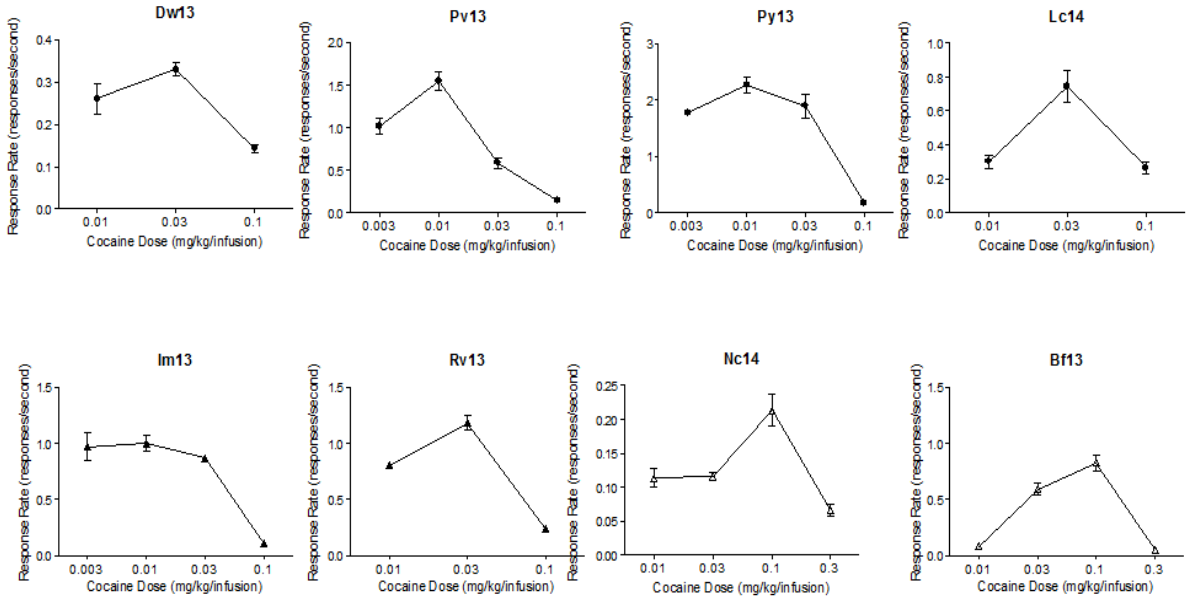

Figure 4. Individual dose-response curves for MALT (Dw13, Pv13, Py13, Lc14, Im13, Rv13) and control (Nc14 and Bf13) subjects during i.v. SA of different unit doses of COC. Doses increased by a half log and were administered in a random order. Closed circle symbols represent MALT males, closed triangles represent MALT females, and open triangles represent control females. The dependent measure is response rate (responses per second) and data are presented as the mean response rate over three consecutive sessions at that dose ± SEM. ED_{Max} was defined as the unit dose of COC at which each subject maintained the highest response rate.

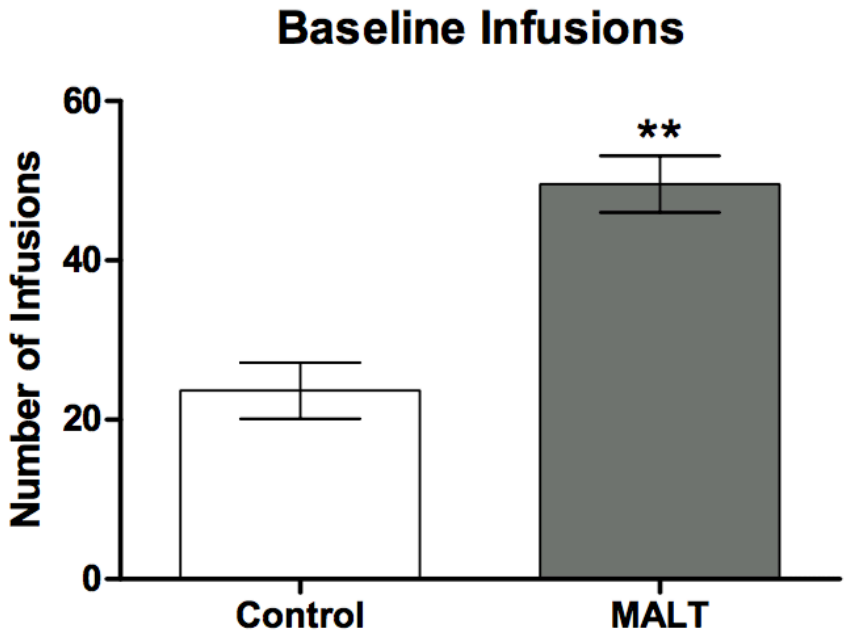

Figure 5. Average number of infusions during maintenance sessions for Control (n=2) and MALT (n=6) subjects at their ED_{Max} dose of COC (as determined in **Figure 4**). The MALT subjects received significantly more infusions than the control group (**$p<0.01$). Data are presented as mean (±SEM).

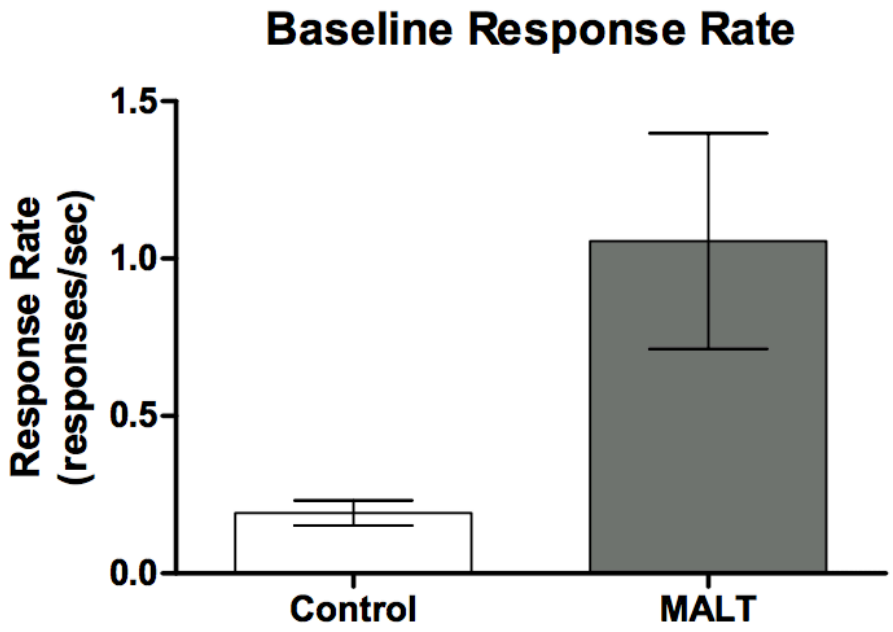

Figure 6. Average response rates during maintenance sessions for Control (n=2) and MALT (n=6) subjects at their ED_{Max} dose of COC (as determined in **Figure 4**). Although the response rates seem higher in the MALT than Control group, there were no statistically significant differences between groups ($p>0.05$). Data are presented as mean (±SEM).

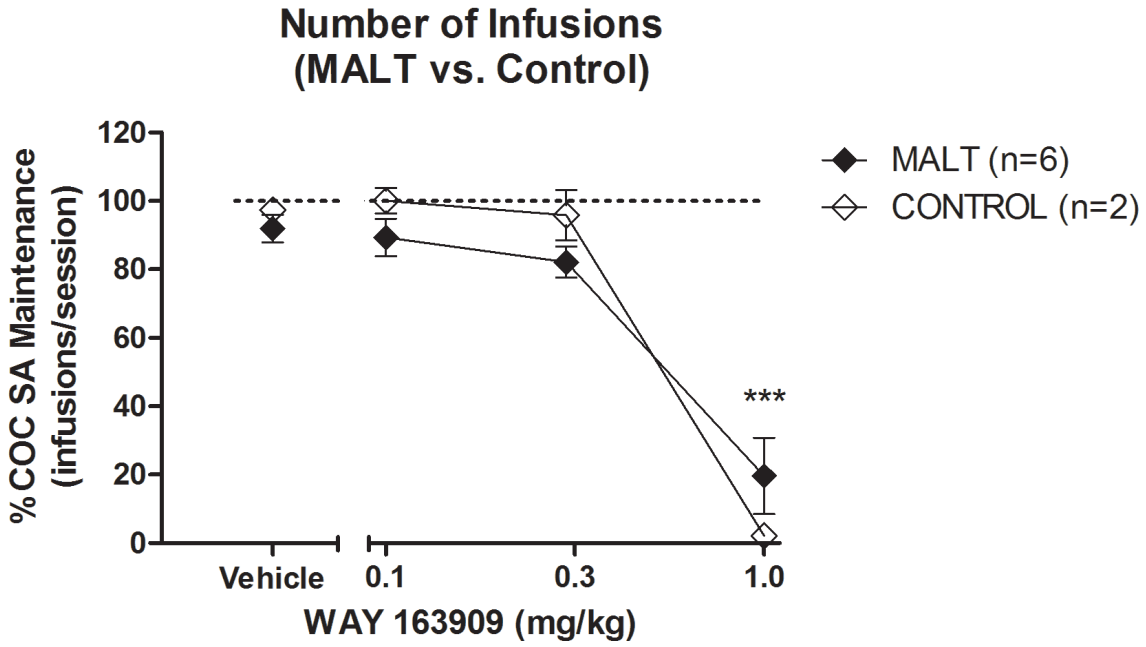

Figure 7. Effect of three doses of WAY pretreatments (0.1, 0.3, 1.0 mg/kg/inf) on COC SA of 8 subjects (6 MALT, 2 Control) at their ED_{Max} dose of COC. Number of infusions per session are normalized and presented as percent to drug-maintained responding compared to baseline responding (dotted line). WAY decreased number of infusions in all subjects at the highest dose (***$p<0.001$). There was no dose by group interaction ($p>0.05$). Data points represent mean response rates collected over the 2-3 testing sessions for each dose (±SEM).

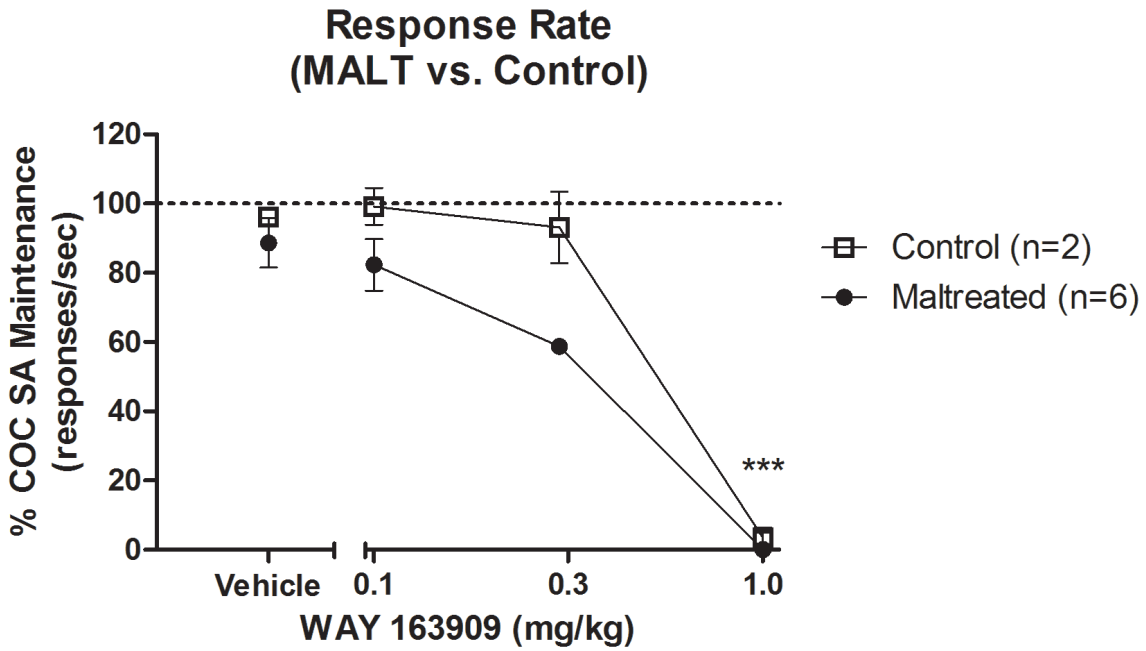

Figure 8. Effect of three doses of WAY pretreatments (0.1, 0.3, 1.0 mg/kg/inf) on COC SA of 8 subjects (6 MALT, 2 Control) at their EDMax dose of COC. Response rates (responses per second) are normalized and presented as percent to drug-maintained responding compared to baseline responding (dotted line). WAY decreased response rate in all subjects at the highest dose (***$p<0.001$). Data points represent mean response rates collected over the 2-3 testing sessions for each dose (±SEM).

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