ROLE OF α_{2A} ADRENERGIC RECEPTORS IN EXTINCTION OF POSITIVE AND NEGATIVE VALENCE LEARNED BEHAVIORS

By

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To Mom
Who gave me my first cup of tea

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

8-hydroxy-2-(di-n-propylamino) tetralin	8-OH-DPAT
Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid	AMPA
Adrenergic receptor.	AR
Artificial cerebral spinal fluid	ACSF
Basolateral amygdala	BLA
Bed nucleus of the stria terminalis	BNST
Dorsal BNST	dBNST
Central Nervous System	CNS
Cocaine and amphetamine regulated transcript	CART
Conditioned response	CR
Conditioned stimulus	CS
Conditioned place aversion	CPA
Conditioned place preference	CPP
Dopamine like 1 receptor	D1
Dopamine like 2 receptor	D2
Dopamine	DA
Dopamine transporter	DAT
Dimethyl Sulfoxide	DMSO
Lateral Hypothalmus	LH
Long term potentiation	LTP
Intracranial self stimulation	ICSS

Knockout	KO
N-methyl *D*-aspartate	NMDA
Norepinephrine transporter	NET
Nucleus accumbens	NAc
Posttraumatic stress disorder	PTSD
Prefrontal cortex	PFC
Self administration	SA
Serotonin	5-HT
Serotonin transporter	5-HTT
Ventral tegmental area	VTA
Wild type	WT

CHAPTER I

INTRODUCTION

Addiction

Addiction is a complex debilitating chronic disease that affects many people throughout the world without regard to such variables as educational or even socioeconomic status. Addiction, for the purpose of this body of work, is defined as seeking a drug of abuse regardless of known adverse consequences, or a loss of control over the use of drugs (Caine and Koob, 1994; Nestler, 2001; Kalivas and Volkow, 2005; Hyman et al., 2006). A powerful example of loss of control was shown in a sub-human study that revealed unlimited access to cocaine can cause death (Johanson et al., 1976). Much of the work on addiction has focused on the dopamine (DA) system, which is discussed in more detail in a later chapter. Even with decades worth of research and a greater understanding of addiction and its effect on the central nervous system, mechanisms and behavioral outputs due to addiction are not fully elucidated.

Impact on Society

Drug abuse is a costly disease due to its impact on healthcare, premature death of babies delivered from drug abusers, vehicle accidents, compensation for victims of drug-related crimes, incarceration, loss of employment, etc. It was estimated that drug abuse cost the United States 180.9 billion dollars in 2006, according to the National Drug Intelligence Center (http://www.usdoj.gov/ndic/pubs11/18862/impact.htm). In 2006, 6 million Americans abused cocaine (National Institute on Drug abuse (NIDA)), a

commonly abused psychostimulant. Treatment is often unsuccessful for addictive drugs such as cocaine and further there is no effective pharmacological therapy (Baumann et al., 1995; Karila et al., 2008). Thus, abusing drugs effects not only family members but also has far reaching effects on society emphasizing a need for a better understanding of the disease and potential therapeutic targets. Further, finding a successful treatment for individuals suffering from addiction with the use of behavioral therapy as well as pharmaceutical agents is needed.

Definition of Reward/Addiction

It is believed that the reinforcing effect of cocaine and other drugs of abuse is to activate the reward system by usurping mechanisms that are in place for natural rewards (Caine and Koob, 1994; Nestler, 2001; Kalivas and Volkow, 2005; Hyman et al., 2006). Thus, addiction can be considered a pathological adaptation of a circuit intended to reinforce natural behaviors. Additionally, it is likely drugs of abuse activate learning and memory mechanisms to a greater degree than that of natural reinforcers. One of the primary pathways that is stimulated by both natural reinforcers and drugs of abuse is termed the mesolimbic dopamine system (Olds and Milner, 1954; Carboni et al., 1989; Wise, 2002). The mesolimbic dopamine system consists of the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) and prefrontal cortex (PFC), which when activated by a reward, natural or a drug causes the release of DA in the NAc. Additionally, it has been suggested that drugs of abuse alter the structure of brain components (Missale et al., 1998; Nestler, 2001; Grogan et al., 2002; Borgland et al.,

2006; Schank et al., 2006). Not only may these changes may contribute to the development of addiction but they may play a role in later stages of addiction as well.

Stages of Addiction

There are three broad stages of addiction: 1) acute drug effects, 2) transition to dependence and 3) end stage addiction (Kalivas and Volkow, 2005). The acute drug effect, also known as initiation, is when an individual takes the drug of abuse for the first time. The transition to dependence phase occurs with repeated administration of the drug of abuse. Lastly, end stage addiction includes withdrawal, abstinence and relapse. The focus of the work presented here is on the abstinence phase. This is a time when addicts may actively seek treatment or try to refrain from taking illicit drugs and thus it is an important phase to investigate in order to shed light on behavioral and pathophysiological changes. If an individual seeks treatment, they are likely to encounter pharmaceuticals or a behavioral modification strategy such as exposure therapy. The treatment is geared to help with withdrawal symptoms. The focus of the present work is on the combination of both pharmacology and exposure therapy due to the concept that this unique combination of drugs and exposure therapy has the potential to prevent relapse (Baumann et al., 1995; Ressler et al., 2004).

Psychostimulants

The drugs of abuse that are the focus of this work are the psychostimulants, cocaine and amphetamine. Cocaine, which is also known as crack, blow, and many other names, is a versatile drug in that it can be injected, smoked or snorted. Not only does it

cause pleasurable effects, it also increases anxiety which may be partially mediated by norepinephrine (Schank et al., 2006).

Cocaine exerts its affects by blocking the dopamine transporter (DAT), serotonin transporter (5-HTT) and the norepinephrine transporter (NET) (Nestler, 2001) (Figure 1) with similar efficacy (Baumann et al., 1995). In support of the idea that psychostimulants are positively reinforcing substances, animals will emit behavior to gain access to the drugs. Lastly, cocaine exerts its effect through the reward circuitry.

Reward Circuitry

As briefly mentioned, the primary reward circuitry consists of the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) and prefrontal cortex (PFC), which when activated by a reward, causes the release of DA in the NAc (Wise, 2006). Over the years, additional brain areas have been implicated as being part of the reward circuitry. As with each different phase of addiction, different brain regions are thought to play different roles in each phase. One brain region of interest for the work presented here is the bed nucleus of the stria terminalis (BNST). Many of the initial studies related to addiction in the BNST involve relapse models. The BNST is implicated in the relapse aspect of addiction as measured by behavioral models (Erb et al., 2000; Leri et al., 2002; Wang et al., 2002). Additionally, it has been shown that injections of a β adrenergic antagonist into the BNST blocks amphetamine induced behavioral sensitization (Colussi-Mas et al., 2005).

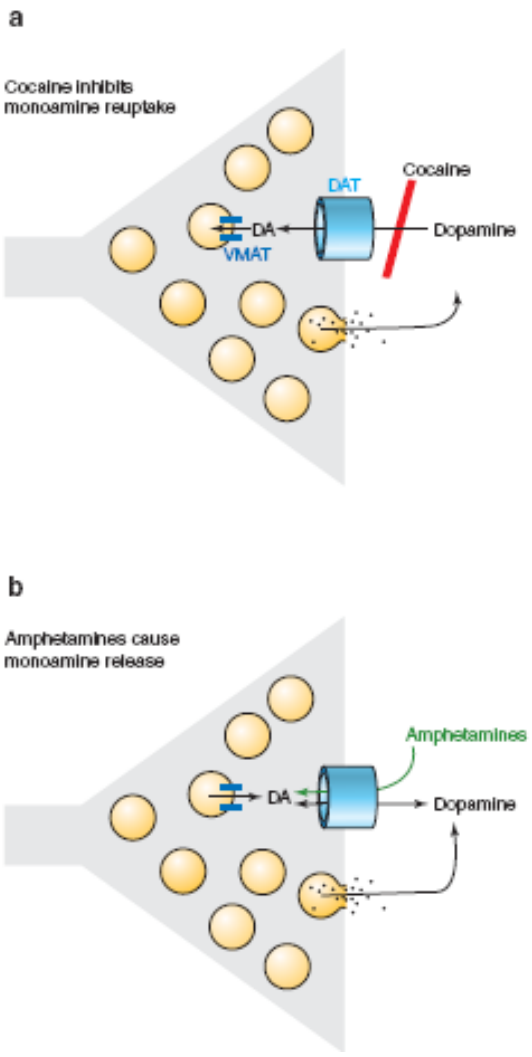


Figure 1. Mechanism of psychostimulants (Hyman et al., 2006).

Anxiety and Addiction

Addiction is a disorder that is rarely singular and is often coupled with other affective disorders. There is a high level of co-morbidity of anxiety disorders (e.g. posttraumatic stress disorder) and addiction (Caine and Koob, 1994; Brady and Clary, 2003; Conway et al., 2006). A recent review (Peters et al., 2009) suggests the prefrontal cortex (PFC) is a major player in the overlap of anxiety and addictive disorders. There are likely other brain regions that are involved in both addiction and anxiety behaviors.

Even with the high degree of overlap in affective disorders, few studies investigate whether agents that facilitate extinction of anxiety measured by behavioral models (e.g. fear) also have the ability to extinguish reward related behaviors (e.g. place preference). Human data has shown that addicts have increased anxiety during the abstinence phase (Kampman et al., 1998; Sinha et al., 1999; Kampman et al., 2001). Recently, it has been shown that d-cycloserine (DCS), an partial *N*-methyl *D*-aspartate (NMDA) receptor agonist, enhanced extinction of individuals suffering from acrophobia (Ressler et al., 2004; Davis et al., 2008). However, the effect may depend on when the drug is administered (Guastella et al., 2008). Overall, the addition of pharmaceutical agents to enhance extinction therapies has the potential to curb relapse of maladaptive behaviors such as addiction.

Intracranial self stimulation

Intracranial self stimulation (ICSS) is a technique that shed light on brain regions involved in reward. It was found that animals would press a lever for electrical stimulation when the electrode was placed in certain areas (Olds and Milner, 1954). It

was presumed that the areas were rewarding. Further, it is believed that the brain regions aforementioned are involved in natural rewards such as food or sex. This helped lead to the discovery of the mesolimbic reward circuitry which was mentioned previously. In further support of the reward pathway, lesions of different parts of the mesolimbic system interfere with drug related behaviors (Isaac et al., 1989; Bernstein et al., 1992; Wang et al., 2002). Additionally, it was found that drugs of abuse alter the ICSS threshold by decreasing the threshold suggesting they act via the natural reward system (Crow, 1970; Thanos et al., 2009). This leftward shift in threshold suggests psychostimulants are positively reinforcing.

Role of Dopamine in reward

Dopamine (DA) is the primary catecholamine in the central nervous system (CNS) and has been implicated in positive reinforcement, locomotor activity, food intake and cognition (Wise, 2006). Dopamine, once released, exerts its actions through its receptors, D1 like (D1 and D5) and D2 like (D2, D3 and D4), and is inactivated by reuptake through the DAT (Missale et al., 1998). Many initial drug abuse studies focused on this catecholamine. For example, dopamine depletion in the NAc by a 6-hydroxydopmaine (6-OHDA) lesion blocks place preference (Pierce et al., 1990; Bernstein et al., 1992) and self administration (Roberts et al., 1977; Pettit et al., 1984; Caine and Koob, 1994) in rats. Additionally, the D1 receptor agonist SKF-82958 decreased the ICSS threshold, consistent with being rewarding (Gilliss et al., 2002).

Pharmacological blockade of Dopaminergic system

Dopamine exerts its actions through its receptors, D1 like (D1 and D5) and D2 like (D2, D3 and D4), and is inactivated by reuptake through the DAT. As mentioned earlier, cocaine blocks DAT and allows for DA to exert a prolonged effect at DA receptors is due to extended availability. When cocaine is administered systemically through an intraperitoneal (i.p.) injection, it produces a reinforcing effect as measured by multiple behavioral paradigms (Mucha et al., 1982) suggesting dopamine plays a role in reward behavior. Paradoxically, knockout (KO) mice lacking DAT (DAT KO), have been shown to acquire conditioned place preference (CPP) (Rocha et al., 1998; Sora et al., 1998) suggesting targets other than DAT plays a vital role in this behavior. However, with the use of a mouse with a cocaine insensitive but otherwise functional DAT, it was found that the animals did not obtain cocaine CPP (Egli et al., 2005) suggesting DAT does play a role in reward.

D1 like receptors have been examined by both genetic and pharmacological methods. Mice lacking D1 receptors are able to acquire cocaine CPP (Miner et al., 1995) but do not acquire cocaine SA (Caine and Koob, 1994) or sensation seeking (Schramm-Sapyta et al., 2006). Mice lacking the D5 receptor developed normal cocaine CPP (Karlsson et al., 2008). These data suggest that either the D1 or D5 receptors do not play a critical role in the reinforcing effect of cocaine or that a molecular compensation occurs. In support of the latter hypothesis, pharmacological blockade of the receptors with a D1R/D5R antagonist SCH23390 does, indeed, block cocaine CPP (Cervo and Samanin, 1995; Baker et al., 1998; Liao et al., 1998). The D3 receptor, when genetically removed, had no influence on cocaine CPP (Karasinska et al., 2005). Interestingly, it has

recently been suggested with the use of D4 receptor deficient mice, the receptor may not play a major role as there was no effect on cocaine CPP but had an effect on the low dose amphetamine CPP (Thanos et al., 2009).

Role of norepinephrine in aspects of addictive behaviors

Although it is clear that DA plays a significant role in reward, other neurotransmitters are shown to play a vital role as well. For instance, norepinephrine (NE) which is most often associated with fight or flight syndrome which is a physiological response to stressful situations (Graeff et al., 1993) is also shown to be involved in drug related behaviors. Additionally, early studies that utilized ICSS to find rewarding areas found cells groups that give rise to NE in the CNS to increase responding, thus they were positively reinforcing brain areas (Crow, 1970; Ritter and Stein, 1973).

In addition to initial ICSS studies, animal models have provided information regarding the role of NE in addictive behaviors. It has been shown that selective NET inhibitors have no effect on self administration (Gu et al., 2002). However, NE does seem to play a significant role in sensitization, a model that examines lasting changes following repeated drug administration, via the α_1-ARs (discussed in more detail below). Prazosin, an α_1-AR antagonist, administered systemically blocks cocaine sensitization (Wellman et al., 2002).

There are several studies investigating the role of NE in CPP and conditioned place aversion (CPA). Mice lacking NET show enhanced cocaine conditioned place preference (CPP), a model of drug reward and are hypersensitive to cocaine (Wang et al.,

1999). Also, NE removal by 6-OHDA lesion in PFC impairs cocaine CPP (Schank et al., 2006). Mice lacking the enzyme dopamine beta hydroxylase (DBH) to convert DA to NE and therefore lacking NE, acquire CPP and are hypersensitive to cocaine (Schank et al., 2006). It is thought the hypersensitivity is due to altered dopaminergic signaling in brain areas key to addictive behaviors. Additionally, it has been shown that mice lacking DBH are resistant to the anxiogenic effects of cocaine which sheds light on acquisition of CPP (Schank et al., 2006). With the use of genetic models, it can be suggested that NE plays a role in CPP as well as other reward behaviors.

There are also pharmacological studies that suggest the noradrenergic system plays a significant role in reward behavior, place preference and aversion. Systemic administration of yohimbine, an α_{2A}- adrenergic receptor (AR) antagonist produces place aversion in rats (File, 1986). Conversely, administration of an α_{2A} -AR agonist produces a place preference (Asin and Wirtshafter, 1985).

Role of serotonin in aspects of addictive behaviors

In addition to DA and NE playing a role in addictive behaviors, there is evidence serotonin (5-HT) plays a role in reward but there is much more research needed in this area. An early study showed that lesion of the primary central nervous system input of serotonin, the dorsal raphe, increased cocaine self administration (Morrow and Roth, 1996). Another example from an early study with mice lacking the serotonin transporter (5-HTT) (Sora et al., 1998) showed that mice lacking the transporter acquire CPP, suggesting that this transporter is not solely responsible for this measure of addictive behavior. Further, it has been shown that mice lacking both DAT and 5-HTT are

insensitive to the rewarding properties of cocaine (Sora et al., 1998). It has been shown that CPP in dopamine deficient mice is not blocked by a D1 receptor antagonist but is blocked by the 5-HTT with fluoxetine, a selective serotonin reuptake inhibitor. In addition, fluoxetine produced CPP in mice (Subhan et al., 2000). This suggests that 5-HTT plays a role in the dopamine response to cocaine and the behavior measured, CPP (Hnasko et al., 2007).

Emerging role of peptides in reward

Although much research has focused on the involvement of catecholamine neurotransmitters in addiction related behaviors, emerging evidence suggests a role for neuropeptides in addiction behaviors (DiLeone et al., 2003; Boutrel et al., 2005; Couceyro et al., 2005; Borgland et al., 2006; Sharf et al., 2008; Aston-Jones et al., 2009). Peptides differ from "classical" transmitters, such as DA, NE and 5-HT, in a variety of ways. Peptide release in not restricted to synaptic areas and thus can modulate a variety of responses throughout the CNS. Further, peptides are inactivated by either enzyme degradation, peptidases, or by diffusion. Lastly, when peptides are inactivated, often the products created are biologically active whereas with "classical" transmitters, this rarely occurs.

One example of a neuropeptide suspected of being involved in behavioral effect of cocaine is the cocaine and amphetamine regulated transcript (CART). CART shows an upregulation of cDNA only in the striatum after acute treatment (Douglass et al., 1995). The CART KO mouse shows attenuated responses to both cocaine and amphetamine

(Couceyro et al., 2005) suggesting this peptide is involved in the behavioral response to psychostimulants.

We examined the neuropeptide, orexin, also known as hypocretin which is discussed in more detail later. Briefly, there are two known orexin peptides, orexin-A and orexin-B, of which both are produced in the lateral hypothalamus (LH) (de Lecea et al., 1998; Sakurai et al., 1998). Orexin, although initially investigated for arousal and sleep behavior, it is suggested to be involved in reward related behaviors (Harris et al., 2005; Borgland et al., 2006; Sharf et al., 2008; Aston-Jones et al., 2009).

Interaction of NE and DA

DA is clearly important in addictive related behaviors. Thus, understanding how NE modulates DA is prudent to investigate. The source of central norepinephrine arises from two projections: the ventral noradrenergic bundle (VNAB) and the dorsal noradrenergic bundle (DNAB). The VNAB arises from the nucleus of the solitary tract while the DNAB arises from the locus coeruleus (Aston-Jones et al., 1999). The VNAB projections terminate in the lateral hypothalamus and two nuclei of the extended amygdala that include the central nucleus of the amygdala (CeA) and BNST (Aston-Jones et al., 1999). The DNAB projects to the forebrain, subcortical structures and the cerebellum and in comparison to the VNAB, has a broader range of innervations. The wide range of brain regions innervated by the DNAB and VNAB implicate the role of NE in manifestation of a plethora of behaviors. Stimulation of the locus coeruleus (LC) increases the activity of dopamine neurons in the VTA (Lategan et al., 1990) and a lesion of the LC causes a decrease of DA release in the NAc (Grenhoff et al., 1993).

NET, which takes up NE, also has the ability to take DA (Raiteri et al., 1977). This is important in the PFC where DA is released but there is little DAT. Additionally, NET and DAT overlap in expression and it is possible that some behavioral effects are mediated by NET and not DAT. To further suggest an interaction between DA and NE, in the striatum, α_{2C}-ARs (discussed in more detail below) are expressed (Uhlen et al., 1997) but there is little noradrenergic innervation to this area.

Further, noradrenergic neurons innervate the VTA (Liprando et al., 2004), which suggests that NE modulates the action of DA in this region critical to drug reward. Additionally the ventral noradrenergic bundle (VNAB) projects to the NAc (Delfs et al., 1998). Both of these studies suggest there is an anatomical connection for reward between the noradrenergic and dopaminergic systems. Indeed, when NE is depleted in the prefrontal cortex, cocaine CPP is abolished (Schank et al., 2006).

Summary

Addiction is a complex chronic disease with many different phases. A lot of work has examined DA in addictive behaviors but other systems are being investigated more heavily. The focus of the work presented is abstinence and the contribution of NE. With the interaction of the noradrenergic and dopaminergic systems, it is likely NE contributes to many behaviors attributed solely to DA.

Behavioral Neuroscience

The use of behavior models to study the pathophysiology of disorders has advanced the understanding of many diseases. In addition, the use of genetically modified mice has advanced biomedical research. Animal models can be loosely categorized although one behavior measured with a model likely overlaps with another model. The behavioral tests listed below are non-exhaustive.

With any behavior measured, it is wise to understand the basic properties of the animals in use. Thus, mice need to undergo basic physiological tests in order to gain a broad understanding of their neurological reflexes. Additionally, these basic behavioral measures help to prevent false result in behavioral measures. Some of the basic observations included in basic physiological test 1) general appearance, 2) body weight/temperature, 3) gait, 4) and vision. An example of such a battery of reflexes has been performed for four commonly used strains of inbred mice. It was found that rotorod performance, a test of motor performance, was showed impairment by decreased time on the rotorod in BALB/c and 129/Ola strains (Royle et al., 1999).

For motor performance, commonly used tasks are the open field, rotorod, balance beam test, vertical pole test, wire hang and stereotypies.

As for sensory abilities, tasks include tests for olfaction, vision, taste, touch, pain sensitivity and auditory ability. For example, a task that tests whether a mouse can hear or not is the acoustic startle test (Paylor and Crawley, 1997). A loud noise is produced which causes the animal to flinch.

A few of the behavioral paradigms that test learning and memory include the Morris water maze (Kolb et al., 1983), cued and contextual fear conditioning (Rogan et

al., 1997), passive and active avoidance (Crawley, 2008), mazes (t-maze, barnes maze, etc.), and conditioned taste aversion (Welzl et al., 2001). Fear conditioning, which is extensively used in extinction studies, is a task that requires the animal to learn that a particular context and tone is associated with a shock (Rogan et al., 1997; Hagan et al., 1999).

There are also models that try to mimic human emotional states, such as fear, depression and even hallucinations. Since one cannot ask a mouse how it is feeling at any particular moment, we must observe the behavioral and physiological response in response to stimuli. A few of the behavioral tests used for fear related behavior is fear conditioning (Rogan et al., 1997), fear potentiated startle (Davis et al., 2008), and ultrasonic vocalization (Blanchard et al., 2001; Covington and Miczek, 2003; Scattoni et al., 2009). Some tests for anxiety related behaviors include the light/dark test (Hascoet et al., 2001; Dere et al., 2002) and the elevated plus/zero maze (Cook et al., 2001). For depression, there is the forced swim test (Porsolt et al., 1977; Cryan and Mombereau, 2004), learned helplessness (Drugan et al., 1985; Hagan et al., 1999) and tail suspension (Cryan and Mombereau, 2004; Crawley, 2008).

The focus of the work presented here are reward models which include cocaine conditioned place preference, psychostimulant sensitization and self administration, all of which are discussed in more detail below.

Lastly, it is important for animal models to be predictive of drug efficacy in humans. Further, human behavior is very complicated and of course, relies on neural processes. To gain insight in to human behavior, animal behavioral models are very useful since animals can be genetically modified (Crawley, 2008).

Examples include bedding material (Potgieter and Wilke, 1997), maternal phenotype (Zupan and Toth, 2008), handling (Campbell et al., 2000; Bechtholt et al., 2004; Vazquez et al., 2006), background strain (Panksepp et al., 2007; Karlsson et al., 2008) and others variables. One important factor when doing behavioral work is consistency and when publishing, to include all of the details. Unfortunately, many behavioral studies published leave out such relevant information. Another important factor is to perform appropriate controls for the behavior measured. If an animal has poor vision naturally or because it was genetically modified, a task that relies on vision may yield false results because the experimenter never tested vision.

Reward Models

There are models that explore anxiety, aspects of learning and memory, and motor learning. Models measuring reward are the focus of this work and these models fall under learning and memory models. The three primary models in the literature are 1) sensitization, 2) self administration and 3) conditioned place preference.

Psychostimulant sensitization is a task that is used widely in the drug addiction field. An animal is given a psychostimulant or another drug in a context repeatedly which causes an increase of locomotor activity from one day to the next and is a sign of central activation. Although it is not entirely clear what sensitization models, it has been suggested sensitization may contribute to relapse (Nestler, 2001). In support of this, sensitization can last to up one year after the last dose of the psychostimulant (Paulson et al., 1991).

Self administration (SA) is a behavioral task that has been used in many species of animals. It requires the subject to press a lever or perform a nose poke in an operant chamber in order to receive a reinforcer. Rats (Bergman et al., 1990) and mice (Schramm-Sapyta et al., 2006) will self administer cocaine. Additionally, it has been shown that SA is strain dependent in mice (Grahame and Cunningham, 1995).

Another measure of rewarding behavior is conditioned place preference, the primary task used in this body of work (Figure 1). It is a type of associative learning, is widely used (Tzschentke, 2007) and has been obtained in a variety of species: rats (O'Dell et al., 1996), mice (Schramm-Sapyta et al., 2006), and even zebra fish (Lau et al., 2006). Although the methodology differs between each laboratory, the reward or aversive stimulus is paired with a context. The motivational property of the drug is the unconditioned stimulus (US) that is paired with a side of the chamber that consists of neutral cues. After multiple pairings, the US now is a conditioned stimulus (CS) and elicits a behavior, i.e. spending more or less time on the side paired for the drug. CPP has been validated for many drugs of abuse and can be replicated between various laboratories (Tzschentke, 2007). Although there is variation of the actual chambers used in the task (two- and three- chambered apparati are the most common), the behavior measured is the same. All of my studies utilize the two-chamber set up that has a black/smooth floor one side and the other with a white/textured floor (Figure 2).

CPP has been obtained for many different stimuli including natural rewards such as food (Lepore et al., 1995; Chaperon et al., 1998), copulation (Mehrara and Baum, 1990), and even novel environments (Isaac et al., 1989; Laviola et al., 1992). CPP typically measures a rewarding drug in that animals will spend more time on the

chamber/context associated with the drug. CPP has been verified for cocaine as well as other drugs of abuse. CPP has been very useful in measuring how reward is altered in genetically modified mice (Sora et al., 1998; Cunningham et al., 2003; Juhila et al., 2005; Schank et al., 2006) which lends insight to neural substrates as well as various receptors, etc., involved in certain types of CPP.

Drugs and stimuli that are able to acquire CPP are often able to obtain SA. Examples include cocaine (Nomikos and Spyraki, 1988; Caine and Koob, 1994), amphetamine (Yokel and Wise, 1976; Nomikos and Spyraki, 1988) and ethanol (Reid et al., 1985; Quirk et al., 2000). This is not always true, however, as mice lacking the D1 receptor acquire cocaine CPP (Miner et al., 1995) but not cocaine SA (Caine and Koob, 1994).

In some respects, CPP is advantageous to SA because no surgery is required and little training is needed. However, CPP does not measure motivation in the same way as SA because the drug is administered passively. Additionally, animals are tested in a drug free state in CPP whereas in SA, drug is on board during testing. There is evidence that CPP and SA involved different neural substrates in rats. Systemic administration of D2 receptor antagonists does not alter cocaine induced CPP (Cervo and Samanin, 1996) but attenuates SA (Caine and Koob, 1994).

Limitations of genetic mouse models

The use of genetically modified mice has provided the field with a great deal of information about many neurological disorders. It allows for an experimenter to ask whether a particular gene is involved in a particular behavior. However, there are

disadvantages to utilization of this type of model. One disadvantage is the removal of the genetic target may cause developmental changes to compensate for the removed gene. For example, removal of NET caused an increase in the amount of NE and an upregulation of some of the targets of NE such the adrenergic receptors (AR) α_{2A} and α_{2C} (Gilsbach et al., 2006). Thus, this complicates the interpretation of the behaviors measured. Another disadvantage is that the background strain the genetically modified animal is made may influence the behavior. As discussed previously, the background strain alone can have an effect on the behavior measured (Gerlai, 1996; Lominska et al., 2001; Dominguez-Salazar et al., 2004; Knight et al., 2004; Wohr et al., 2008; Caldwell and Young, 2009). Thus, it is ideal to replicate behavior with pharmacology but that, of course, has its own set of caveats such a brain availability and selectivity. Additionally, it is important to utilize wild type (WT) littermates to make a comparison as opposed to mice with the same background but from a different maternal breeding strategy.

"Bias" versus "unbias" CPP apparatus

Lastly, there is the consideration of the apparatus and whether the task is bias or not. "Bias" in the context of CPP is when a group of animals consistently show a preference for a particular chamber and "unbias" is when there is consistently no preference for a particular chamber (Tzschentke, 1998; Cunningham et al., 2003). However, the term "bias" is sometimes used for when a drug is paired to the non preferred side and "unbias" when drug pairing is done randomly. Thus, it is important to understand which meaning the author is trying to convey. Often, unfortunately, many

papers do not indicate what sort of conditioning apparatus they are using (Cunningham et al., 2003). The work presented here uses a modestly biased chamber (Figure 2).

With a two- or three- chamber apparatus for CPP, animals often show a preference for one side of the chamber versus the other due to an innate preference. For example animals may spend more time on the darker side of the chamber than the side that is lighter (Schramm-Sapyta et al., 2006). Additionally, it has been shown in mice that they prefer a grid floor instead of one with bars (Cunningham et al., 2003). Although efforts have been made to create non-bias chambers when measured as a group, often from one experiment to the next, the level of bias varies. Thus, it is important to report whether or not the chamber is biased with the particular experiment.

Summary

Although there are many methodological variables that must be taken into consideration when using animal models, the information obtained from the experiments provide is useful for understanding affective disorders. One such disorder, addiction, is persistent and there are models that enable experimenters to examine the disorder further. Thus, examining later stages of addiction with the use of animal models may give clues for successful treatment.

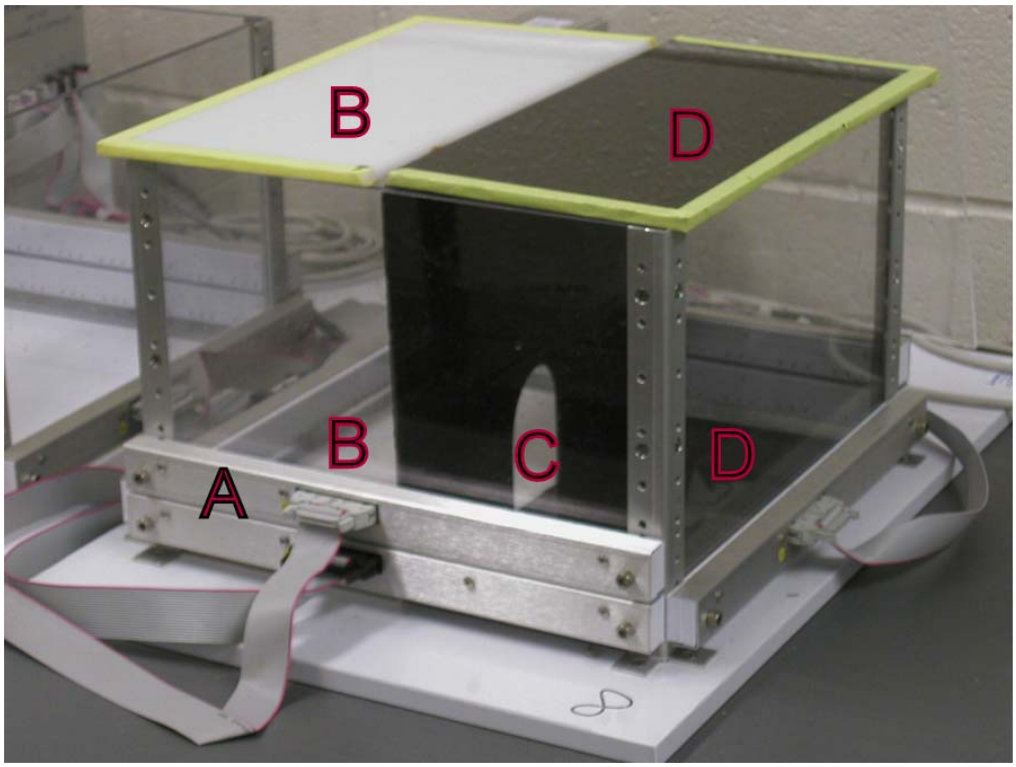


Figure 2. *The conditioned place preference apparatus*. There are infrared beams are in place all around the apparatus (A) to measure locomotor activity and the location of the mouse. The white side of the chamber (B), with sanded floor, is a distinct context from the black side of the chamber (D), which has a smooth floor. Lastly, when the door is removed, an opening is present (C) which allows the mouse to move freely between each of the chambers (B, D). Open field chamber (Med Associates; St. Albans, VT, USA), inserts made by Dr. Nicole Schramm-Sapyta.

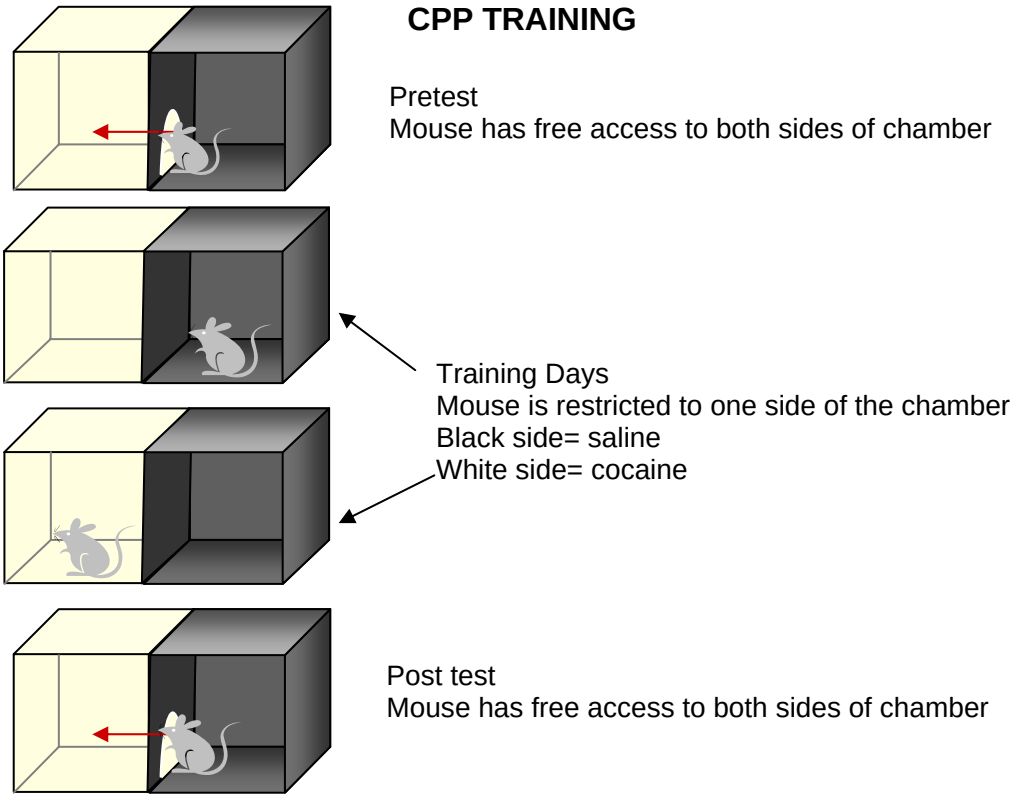


Figure 3. *Conditioned place preference training paradigm.* Animals have free access to both sides of the chamber during the pretest. During training days, animals are restricted to one side of the chamber and administered saline on one side of the chamber and cocaine (if in the cocaine group) on the other side to the chamber. Training days are separated by 24 hours. The post test occurs 24 hours following the last training session and animals have free access to both sides of the chamber.

Extinction of negative and positive valence learned behaviors

Extinction learning is when a conditioned stimulus (CS) no longer predicts a reward and thus no longer causes a conditioned response (CR). The amygdala, prefrontal cortex and hippocampus are primary areas that have been shown to be involved in extinction behavior (Myers and Davis, 2007; Quirk and Mueller, 2008).

Although it has been suggested extinction involves forgetting or "unlearning" (McClelland and Rumelhart, 1985), the widely accepted idea is extinction involves the formation of a new memory which suppresses the original memory (Rescorla and Heth, 1975; Bouton, 2002; Gale et al., 2004; Barad, 2006). The original memory of learned fear can persist months to years (Gale et al., 2004). Multiple brain systems have been implicated in fear extinction (Myers and Davis, 2007) include NE.

Recently, there has been much interest in the possibility of combining pharmaceutical agents with current extinction therapies to enhance the effect of behavioral modification following therapy (Davis et al., 2008). However, results have been mixed. For example, individuals that went for treatment for obsessive compulsive disorder and received exposure therapy treatment along with a pharmaceutical agent did not differ from the controls that did not have the addition of the pharmaceutical agent (Kushner et al., 2007). However, individuals suffering from acrophobia that were treated with a combination of exposure therapy and a pharmaceutical agent did differ benefit from treatment as evidence of being different from controls and lasting behavioral change three months later (Ressler et al., 2004). The latter data suggests that individuals suffering from other disorders can benefit from such therapy. Thus, the combination has the

potential to curb relapse of maladaptive behaviors such as addiction. However, most work in animal models thus far has focused on extinction of fear.

Animal research based on negative valence learned behavior, i.e. fear conditioning, has shown that there are three primary ways to undermine extinction behavior, i.e. to cause the original behavior to resurface: 1) Renewal- in which the context from the original learning is switched (Bouton, 1993), 2) Reinstatement- noncontingent exposure to a drug (de Wit and Stewart, 1981) or exposure to a stressor (Kreibich and Blendy, 2004; Kupferschmidt et al., 2009). that causes the behavior to resurface and 3) Spontaneous recovery- in which the extinguished behavior returns over time (Rescorla and Heth, 1975) (Figure 4).

Pharmacology of extinction

It has been found that extinction of negative valence learned behaviors can be facilitated by glucocorticoids (Yang et al., 2006), an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor potentiator (Zushida et al., 2007), d-cycloserine (DCS) (Walker et al., 2002) and other pharmaceuticals. This is of interest because of the potential of these drugs to be used in adjunct to cognitive therapy. Indeed, clinical trials for DCS (Ressler et al., 2004) and cortisol (Soravia et al., 2006) have yielded promising results as individuals benefit from the combination therapy.

Anatomy of extinction

Few studies have focused on extinction of positive valence learned behaviors such as appetitive or psychostimulant CPP extinction. The basolateral amygdala (BLA) and

ventromedial prefrontal cortex (vmPFC) have been examined in appetitive extinction studies. Lesions of the BLA prevent extinction of conditioned responding for a food reinforcer, suggesting the BLA is involved in extinction and necessary for the behavior (Burns et al., 1999). A lesion of the vmPFC impaired extinction following the actual extinction session (Maruki et al., 2003) and appetitive extinction has been reported to increase NE in the vmPFC (Mingote et al., 2004).

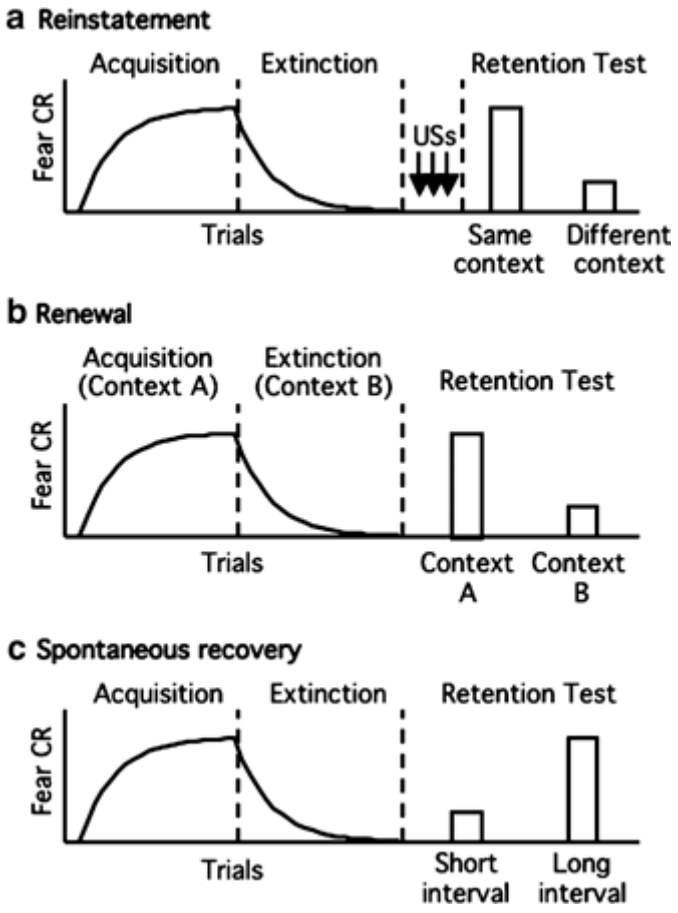


Figure 4. *Extinction involves learning new behavior.* (Myers and Davis, 2007). 4a) Extinction of a learned behavior can return upon the presentation of an unconditioned reinforcer, such as a stressor. 4b) Renewal of a behavior can occur when a behavior is acquired in one context and extinguished in another. When placed in the original context of testing, the behavior resumes. 4c) Lastly, behavior can return without being provoked, i.e. spontaneously recover. This suggests the formation of an inhibitory memory that may not be strong enough to suppress the original memory for an extended amount of time.

Extinction in humans

To date, there is little evidence that exposure treatment is effective in preventing relapse among past users of drugs (Dawe et al., 1993; Powell et al., 2005). Thus, the idea of revamping therapy to include pharmaceutical agents is appealing. Firstly, as mentioned before, it is important for animal models to model human behavior in order to gain further insight to the pathophysiology of various disorders. As with animal behavior, the amygdala is activated during extinction in humans (Knight et al., 2004). Further, the vmPFC has been shown to be involved in extinction 24 hours following the extinction sessions (Phelps et al., 2004), which is similar to what is seen in humans (Sotres-Bayon et al., 2006).

Behavioral exposure therapy is a technique that used to alter behavior by helping alter emotional behavior (Powers et al., 2009). Further, it is thought extinction is the basis of exposure therapy, which traditionally repeatedly presents fearful producing stimuli that ideally decreases the fearful response over time (Myers and Davis, 2002). It has been shown that repeated cortisol, a hormone involved in the stress response, administered prior to exposure therapy facilitated extinction of a spider phobia as well as social phobia (Soravia et al., 2006). Encouragingly, it was shown that the spider phobia remained attenuated 48 hours post exposure therapy (Soravia et al., 2006). Further, it has been shown that exposure therapy in adjunct with pharmacotherapy, DCS treatment, did positively enhance the effect of exposure therapy for anxiety disorders (Ressler et al., 2004; Kushner et al., 2007). It has been reported that DCS administered before exposure therapy is beneficial to individuals but if administered DCS during exposure therapy does not have an effect (Guastella et al., 2008). Interestingly, administration of yohimbine, an

α_2-AR antagonist, may facilitate extinction of the fear of claustrophobia (Powers et al., 2009). Conversely, yohimbine has been shown to increase the stress response in individuals suffering from PTSD (Yehuda et al., 1992; Southwick et al., 1999) and healthy individuals (Vythilingam et al., 2000).

Role of Central NE in Extinction Studies

NE has been shown to play a role in extinction of learned behaviors. A considerable amount of evidence suggests that infusion of noradrenergic agonists and antagonists into the basolateral amygdala (BLA) enhance and impair consolidation respectively, therefore affecting extinction of the learned behavior (Ferry et al., 1999). More specifically, lesions of the VNAB but not the DNAB retarded extinction of aversive conditioning (Schank et al., 2006). This suggests that regions innervated by the VNAB are important to NE mediated extinction behaviors. Additionally, an early study revealed that animals treated with yohimbine systemically were less disrupted by a CS that was previously paired with a footshock (Davidson and Lucki, 1987). Further, the involvement of noradrenergic transmission is a study by Cain and colleagues showed administration of yohimbine facilitated extinction in conditioned fear (Caine and Koob, 1994).

Recently, it has been shown that the prefrontal catecholamine system is required for a negative valence learned behavior, e.g. lithium chloride induced place aversion (Schank et al., 2006), thus NE may be involved. Also, the use of an α_2-AR agonist, clonidine, attenuated opiate induced withdrawal when injected in the BNST (Delfs et al., 2000).

Summary

Although there are limitations with the use of animal models, they provide important information about neural components that contribute to the behavior of an organism. Models investigating extinction of learned behaviors are useful because it suggests maladaptive behavior can be modified with additional learning. Lastly, NE contributes to extinction of learned behaviors and could be a system for therapeutic targets.

Norepinephrine Receptors

G-protein coupled receptors

G protein (guanyl nucleotide binding protein) coupled receptors, when activated, binds to a target protein such as a channel, changing the properties (Rens-Domiano and Hamm, 1995). They are seven membrane spanning receptors and contain three different subunits, G_α, G_β and G_γ. G_i linked proteins couple to inhibition of adenylyl cyclase in addition to activation of G-protein-coupled inwardly rectifying potassium (GIRK) channels. G_s linked proteins couple to stimulation of adenylyl cyclase. Lastly, G_q linked proteins couple to the activation of phospholipase C (DeVivo and Iyengar, 1994; Rens-Domiano and Hamm, 1995).

Adrenergic Receptors

Norepinephrine exerts its effect by signaling through adrenergic receptors (ARs). There are nine distinct adrenergic receptors that fall into G_q linked α_1 (α_{1A}, α_{1B}, α_{1D}), G_i linked α_2 (α_{2A}, α_{2B}, α_{2C}) and G_s linked β (β_1, β_2, β_3) ARs categories, which are all G-protein, seven transmembrane receptors (Bylund et al., 1994; DeVivo and Iyengar, 1994; Haapalinna et al., 1997). All of the ARs have been shown to play a role in addictive behaviors as measured by animal models with pharmacology and genetic models. It has been shown that an α_1 –AR antagonist, prazosin, attenuates psychostimulant sensitization (Drouin et al., 2002). Additionally, there is a decrease in sensitization in α_{1B}-AR knockout animals (Drouin et al., 2002). As for β ARs, it has been shown these receptors

are necessary for cocaine induced anxiety (Schank et al., 2006) and amphetamine sensitization (Colussi-Mas et al., 2005).

The focus of this work is on α_2-ARs, which are G_i linked and bind epinephrine and norepinephrine, which have effects peripherally and centrally, respectively (Saunders and Limbird, 1999). The three α_2 subtypes include α_{2A}, α_{2B} and α_{2C}. The α_{2A} -ARs are expressed in the LC, hippocampus and the brainstem (Sallinen et al., 1997). The α_{2C} -ARs are expressed in the cortex, hippocampus and brainstem. Finally the α_{2B} –AR receptor is not readily detected (Bucheler et al., 2002) but there is evidence of mRNA for the receptor in the thalamus, pyramidal cell layer of the hippocampus and the olfactory system (Weinshank et al., 1990; Scheinin et al., 1994). The distinct distribution of the ARs suggests they mediate different functions.

Radioligand and autoradiography studies delineated that approximately 90% of the α_2 –AR receptors located centrally are α_{2A}-AR subtype and 10% are the α_{2C} subtype (Rescorla and Heth, 1975; Bucheler et al., 2002). The $\alpha_{2A/C}$-ARs are considered the primary autoreceptors (Bucheler et al., 2002) for norepinephrine with the α_{2A}-AR playing the more prominent role in NE regulation. Further agonists of the α_2-ARs that produce sedation and analgesia are mediated through the α_{2A}-ARs (Lakhlani et al., 1997). Due to the lack of subtype specific ligands for the α_2 receptor, gene targeting studies have been used to elucidate the role of the various subtypes.

In addition to regulating the release of NE, the α_2-ARs also mediate the release of other transmitters and hence they are also known as heteroceptors (Bylund et al., 1994). For example, the receptors have been shown to regulate the release of 5-HT (Raiteri et al., 1990; Scheibner et al., 2001) and DA (Scheibner et al., 2001; Bucheler et al., 2002).

α_{2B} adrenergic receptor knockout animals

Although these receptors are not readily detected in the CNS (Bucheler et al., 2002), this receptor has been involved in many peripheral responses. The α_{2B}-AR is an essential component of nitric oxide analgesia (Sawamura et al., 2000). Further, with the use of a KO mouse, these receptors are implicated embryonic development (Link et al., 1996; Cussac et al., 2001) as well as in the development of placenta vascularization (Philipp et al., 2002).

α_{2C} adrenergic receptor knockout animals

These knockout mice produced a stress protective effect as measured by the forced swim test (Sallinen et al., 1999). Further, α_{2C}-AR KO mice showed enhanced startle responses, lack of prepulse inhibition, and shortened attack latency (Sallinen et al., 1998). Additionally, mice over expressing the α_{2C}-AR are impaired in spatial and non-spatial tasks (Bjorklund et al., 1998). However, fairly recently, a novel highly selective α_{2C}-AR antagonist was developed (Sallinen et al., 2007) which will further the understanding of the receptor contribution to neuropsychiatric disorders .

α_{2A} adrenergic receptor knockout animals

Genetic manipulations have been instrumental in providing information on the role of NE in extinction of learned behaviors. Mice lacking the α_{2A}-AR have increased blood pressure and heart rate. It has been suggested the neurons in the LC are more active in these mice compared to wildtype (Davies et al., 2003). Mice lacking the α_{2A}-ARs acquire amphetamine CPP (Juhila et al., 2005). Further, KO mice for the α_{2A}-ARs resist

extinction of conditioned fear only with the cue and not the context (Davies et al., 2003) suggesting receptors present in the hippocampus are not involved. Other authors have suggested that adrenergic signaling during context exposure is required for extinction and is likely to involve a brain region other than the hippocampus (Ouyang and Thomas, 2005). Hence, NE signaling is likely to be a key component in extinction of learned behaviors involving drugs of abuse and may involve regions innervated by the VNAB. Data from our laboratory reveals that α_{2A}-AR expression levels are high in the hippocampus as well as the hippocampal projection to the BNST (Shields, personal communication). It is possible that extinction of learned behaviors is dependent on signaling through the α_{2A}-ARs containing terminals terminating on BNST neurons. Although the lack of involvement of the hippocampus in acquisition of cCPP is disputed (Meyers et al., 2006), it is likely that signaling through the adrenergic receptors is involved in extinction of learned behaviors. In a recent paper, mice were created that expressed α_2-ARs on only adrenergic cells and found that many physiological effects were mediated by non adrenergic cells (Gilsbach et al., 2006).

Yohimbine

Yohimbine is an active chemical found in the tree bark of the *Pausinystalia yohimbe* tree which was originally used by West African tribes in fertility rituals. Today, this drug is often used for treating erectile dysfunction. Yohimbine is primarily known as an α_2-AR antagonist. An early human study administered yohimbine to PTSD patients and controls to measure the acoustic startle response. This response was increased in PTSD patients indicating it was exacerbating their condition (Morgan et al., 1995).

However, a very recent study showed that yohimbine administration to claustrophobic patients actually improved their condition (Powers et al., 2009).

As with human studies, there are still inconsistencies with rodent studies. It has been shown that yohimbine administration does facilitate the extinction of fear (Caine and Koob, 1994) in a context dependent manner (Moser et al., 1998). Conversely, there is also evidence that yohimbine, although does decrease freezing, retention of fear is unaltered (Erb et al., 2000; Davis et al., 2008).

The major caveat to all of these studies utilizing yohimbine is that yohimbine is a nonspecific drug and acts on receptors other than the α_2-ARs. Yohimbine has a modest 4.2 fold more selectivity for α_2-ARs than the serotonin 5-HT_{1A} receptors (Newman-Tancredi et al., 1998). A more selective antagonist, atipamezole, does not have activity at the serotonergic receptor (Juhila et al., 2005). This lends support to the possibility that behavior seen after yohimbine administration is not mediated by the α_2-ARs. Indeed, there is behavioral evidence of other receptors playing a role in behaviors measured following yohimbine administration. Administration of yohimbine and atipamezole, a more specific α_2-AR antagonist during prepulse inhibition produces distinct effects (Powell et al., 2005). Yohimbine significantly disrupted prepulse inhibition while atipamezole had only a weak effect (Powell et al., 2005).

A receptor that may mediate some of the behavioral effects elicited by yohimbine is the 5-HT_{1A} receptor. Indeed, it has been shown that yohimbine does have affinity at the 5-HT_{1A} receptor (Newman-Tancredi et al., 1998). Further, it has been shown that yohimbine generalizes to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-

HT_{1A} agonist, in drug discrimination (Winter and Rabin, 1992). Lastly, it was suggested that yohimbine disrupts prepulse inhibition via the 5-HT_{1A} receptors (Powell et al., 2005).

Orexin

Another potential target of yohimbine is the orexinergic system. Orexin was identified in the late 1990s by two individual groups (de Lecea et al., 1998; Sakurai et al., 1998) in the lateral hypothalamus (LH). There are two neuropeptides, orexin A and orexin B (also known as hypocretin 1 and hypocretin 2, respectively) were initially investigated for their role in feeding (Sakurai, 2007) due to their presence in the LH. Further, it has been shown to play a role in sleep and wakefulness (Sakurai, 2007).

More recently, it has been orexin has been shown to play a role in the reward system. Orexin neurons project to regions implicated in reward such as the VTA and NAc (Nakamura et al., 2000). Orexin A induced hyperlocomotion can be blocked by both a D1 and D2 antagonist (Nakamura et al., 2000), suggesting dopamine plays a modulatory role in this behavior. LH activation reinstates extinguished drug seeking and orexin A into the VTA reinstated drug seeking (Harris et al., 2005). Cocaine sensitization (Borgland et al., 2006) is blocked with an orexin antagonist SB 334867. Additionally, yohimbine reinstatement of ethanol and sucrose seeking is blocked by this same antagonist (Richards et al., 2008), suggesting a role for orexin in the response. The authors suggested the effect seen by SB 334867 is an orexin-1 receptor effect but SB 334867 has nanamolar affinity for both of the orexin receptors. Thus, it is likely that orexin plays a role in some of the behavior effects following yohimbine administration. Lastly, the α_2- AR agonist, clonidine, attenuated orexin A induced reinstatement of drug

seeking. However, SB 334867 had no effect on yohimbine induced reinstatement of food (Nair et al., 2008). Thus the role of orexin in reinstatement is controversial and poorly understood.

Interestingly, it has been shown that rats that underwent place preference training showed an increase in neuronal activity after conditioning as measured by Fos in the LH compared to controls (Harris et al., 2005). However, the activation of orexinergic LH neurons was only seen after cocaine, morphine and food place conditioning and not with novel object conditioning (Harris et al., 2005). This suggests that the LH neurons are activated by only certain types of reinforcers and their cues.

There are two known receptors for orexin, orexin-1 (OxR1) and orexin-2 (OxR2). The highest levels of the orexin receptors are in the LC, VTA and NAc (Trivedi et al., 1998; Sakurai, 2007). An early study showed OxR1 shows higher affinity for orexin A, while OxR2 shows equal affinity for the two ligands (Sakurai et al., 1998). Both OxR1 and OxR2 are G-protein coupled receptors; OxR1 appears to signal through G_q while OxR2 can couple to G_i/G_o as well as G_q subunits (Sakurai et al., 1998). It is thought the orexin receptors are located on cell bodies of orexin neurons for reward related behavior, Intracerebroventricular (i.c.v.) infusions of the orexin A peptide reinstated both cocaine and food seeking that had been extinguished (Boutrel et al., 2005).

There are orexin knockout animals and they are narcoleptic (Chemelli et al., 1999). No studies have looked at psychostimulant effects in these animals but it has been shown that KO mice shown attenuated morphine dependence as measured by withdrawal (Georgescu et al., 2003).

Summary

The α_{2A}-ARs are the most abundant of the α_2-AR and play a role in many behaviors. The use of the α_{2A}-AR KO mice and pharmaceuticals has the potential to shed light on the role of this receptor in a number of behaviors. Additionally, the use of yohimbine, an nonselective α_2-AR antagonist has yielded information about possible systems involved in extinction. The serotoninergic and orexinergic systems are the two that are the focus of the work. In additional to animal models, synaptic transmission can be examined to gain a better understanding how the systems interact.

Synaptic Transmission and Behavior

In addition to animal behavior models, researchers in the synaptic physiology field have investigated molecular mechanism of addiction. The CNS consists of neurons and glia that communicate through electric-chemical signals. In general a neuronal response starts with depolarization of the axon. If the sodium dependent depolarization is sufficient, calcium influx through voltage-gated calcium channels triggers the release of neurotransmitters from the presynaptic active zones. Upon release, neurotransmitters can then act on the dendritic post-synaptic receptors on the adjacent neurons.

One such type of stimulation is excitatory and mediated via glutamate, the major excitatory neurotransmitter in the adult brain, through activation of ionotropic glutamate receptors. Function of ionotropic glutamate receptors determine the strength of the synaptic signal and therefore determine whether the signal will propagate through the circuit resulting in behavior. There are many glutamate receptors both ionotropic and metabotropic and these receptors play a role in various behaviors, including rewarding behaviors (Schramm et al., 2001; Weitlauf et al., 2004; Kauer and Malenka, 2007).

A popular hypothesis suggests that long lasting changes in synaptic strength, termed synaptic plasticity, underlies learning and memory. It has been shown that environmental stimuli that initiate learning also induce long term potentiation (LTP) in vivo. An early study that utilized fear conditioning found that a drug accelerated learning of the task but did not alter the levels of conditioned fear and the authors suggested that common mechanisms may underlie fear conditioning and LTP (Rogan et al., 1997). Another group around the same time found that fear conditioning increased AMPA receptors, a type of ionotropic glutamate receptor, in the amygdala, suggesting synaptic

plasticity had taken place (McKernan and Shinnick-Gallagher, 1997). Further, a one-trial inhibitory avoidance occluded LTP produced by high frequency stimulation in the hippocampus, suggesting learning induces LTP in this brain region(Whitlock et al., 2006). Lastly, it was shown that saturation of LTP disrupted learning. Rats had one hippocampus lesioned and the other hippocampus had electrodes placed in it. This allowed for repeated cross-bundle tetanization which caused cumulative potentiation. Then the animals underwent a spatial learning task, morris water maze, and found it impaired learning compared to controls (Moser et al., 1998). This is all evidence that LTP is a form of learning and memory. However, there are exceptions to this argument. Mice that have a genetic mutation that mimics mental retardation and impaired in fear conditioning show an enhancement of LTP (Trivedi et al., 1998). Thus, although there is a plethora of evidence that LTP is important for learning and memory, there are exceptions.

As suggested in above, it is thought that events that induce learning change synaptic strength. This is true for addictive substances as well (Nestler, 2001; Hyman et al., 2006). As for one example, it has been shown that chronic injections of cocaine cause an increase in synaptic strength (Borgland et al., 2004; Wanat and Bonci, 2008).

There is an increasing amount of literature looking at both changes in animal behavior as well as synaptic changes by examining alterations in synaptic strength measured by electrophysiology and/or neuronal components. For example, it has been shown that extinction training causes an increase, or upregulation, of AMPA receptors subtypes in the NAc (Sutton et al., 2003). Further, when specific subtypes of the AMPA receptor are temporally over expressed, the extinction is facilitated (Sutton et al., 2003).

Additionally, animals that self administer cocaine exhibited reduced synaptic strength in the NAc (Schramm-Sapyta et al., 2006).

NE modulation of glutamate

It has been shown that NE inhibits the release of glutamate (Forray et al., 1999). Additionally, glutamate excitatory transmission can be modulated by NE. For example, application of an α_{2A}-AR receptor agonist increases excitatory transmission in the BNST (Egli et al., 2005). Further, it has been shown that NE application results in a depression in the BNST which is dependent on α_1-ARs (Davis et al., 2008).

Summary

The use of electrophysiology along with behavior is a powerful tool for understanding molecular mechanisms that occur as a result of learning and memory.

Hypothesis

The α_{2A} adrenergic receptor plays a critical role in extinction of cocaine conditioned place preference (CPP).

Specific Aims

Aim 1

Test the hypothesis that the α_{2A} adrenergic receptor plays a significant role in acquisition and demonstration of preference in the cocaine conditioned place preference (CPP) paradigm.

Aim 2

Test the hypothesis that extinction of cocaine CPP is regulated by the α_{2A} adrenergic receptor.

Aim 3

Test the hypothesis that extinction of lithium chloride CPA is regulated by the α_{2A} adrenergic receptor.

Aim 4

Test the hypothesis that yohimbine depresses excitatory transmission via orexinergic or serotonergic mechanisms in the dorsal BNST.

CHAPTER II

MATERIALS AND METHODS

Behavior studies

Subjects

Experiments on C57BL/6J mice were conducted using males obtained from The Jackson Laboratory (Bar Harbor, ME) aged 8-12 weeks. Male α_{2A}-AR KO mice were generated as previously described (Altman et al., 1999) and backcrossed onto a C57BL/6J genetic background for a minimum of 8 generations. KO and WT littermate controls were bred from heterozygous parents to minimize any potential genotype-related maternal abnormalities (Wellman et al., 2007). Mice were housed on a 12 hr light/dark cycle in groups of 2-5 with *ad libitum* access to food and water. Testing commenced at least 1 week after acclimation to the facilities. All procedures were approved by the Vanderbilt University Animal Care and Use Committees and in accordance with the Animal Welfare Act and the guidelines outlined in 'Using Animals in Intramural Research.' The number of mice used is reported in the figure legends.

Drug Treatment

Cocaine (20 mg/kg) was administered (i.p.) in a saline vehicle in a volume of 10 mL/kg body weight based on previous experiments in adult mice (Schramm-Sapyta et al., 2006). For cocaine CPP, yohimbine was administered (i.p.) in saline vehicle in a volume of 10 mL/kg body weight 30-35 mins prior to extinction testing at a dose of 2.5 mg/kg or

5 mg/kg, based upon extinction facilitating doses in C57BL/6J mice (Caine and Koob, 1994). For cocaine CPP and fear extinction, atipamezole was administered subcutaneously (s.c.) in saline vehicle in a volume of 10mL/kg body weight 30-35 minutes prior to extinction testing at a dose of 3 mg/kg, based upon studies in rats and mice (Seppala et al., 1994; Newman-Tancredi et al., 1998; Millan et al., 2000; Powell et al., 2005; Risbrough and Geyer, 2005).

Cocaine conditioned place preference and extinction

The apparatus was as previously described (Schramm-Sapyta et al., 2006). Med Associates (St Albans, VT, USA) open field chambers were fitted with acrylic inserts that created two distinct environments. One environment had a black smooth floor/black ceiling and the other a white sanded floor/white ceiling (cleaned with 30% EtOH) which creates a moderately biased chamber with a preference for the black smooth floor/black ceiling side of the chamber. Mice were first acclimated to handling and intraperitoneal (i.p.) injections for 5 days before testing. There was then a pre-conditioning session in which each a mouse was placed in the black compartment and allowed to explore the 2 compartments for 15 minutes (900 seconds). Any mouse spending >67% of the pre-conditioning session in any 1 compartment was excluded from the study. Mice in the saline group received saline (i.p.) on both sides of the chamber. Mice in the cocaine group received CS- conditioning sessions occurred on experimental days 1, 3 and 5 and CS+ conditioning sessions occurred on experimental days 2, 4 and 6. For CS+ sessions, mice received an i.p. injection of 20 mg/kg cocaine and were exposed to the white

compartment alone for 15 min. For CS- sessions, mice received an i.p. injection of saline and were exposed to the black compartment alone for 15 min.

Place preference was tested on day 7 (post-test). Mice received an i.p. injection of saline, were placed in the black compartment and were for allowed to freely explore both compartments for 15 minutes. Extinction of the CPP was then tested on days 8-13. Extinction sessions were the same as the preference session, with the exception that mice received injections of saline (i.p.), 5 mg/kg yohimbine hydrochloride (i.p.), or 3 mg/kg atipamezole hydrochloride (subcutaneous, s.c.) 30-35 min prior to session and then (to mimic preference testing) i.p. saline again immediately before each session. All drugs and saline were administered in a volume of 10 mL/kg body weight. Doses of yohimbine (Tocris; Ellisville, Missouri) were based on fear extinction affiliating doses in mice (Caine and Koob, 1994). Dose of atipamezole (Pfizer; New York, NY) was based on previous behavioral studies in mice (Seppala et al., 1994; Newman-Tancredi et al., 1998; Millan et al., 2000; Powell et al., 2005; Risbrough and Geyer, 2005). Two groups of C57BL/6J mice cocaine trained and saline pre-treated during extinction were run with the yohimbine and atipamezole pre-treated animals as controls. There was no difference and the groups were collapsed (Figure 5A). Additional control groups, mice that received saline during cocaine CPP training and pre-treated with yohimbine or atipamezole, were run in parallel with mice that were cocaine trained and pre-treated with drug (yohimbine or atipamezole).

The main dependent measure of behavior during preference and extinction sessions was time spent in each compartment during each session as recorded by Med Associates Activity Monitor software. As for preference, it was inferred from a

significantly greater amount of time spent in the cocaine-paired side relative to the saline-paired side. Extinction was indicated by 1) a significantly greater amount of time spent in the saline-paired side relative to the cocaine-paired side, and 2) the absence of percent difference in time spent on the cocaine-paired side during extinction days relative to during pre-conditioning. Additionally, locomotor activity (centimeters traveled) was recorded to determine whether drug administration altered activity.

Conditioned Place Aversion and extinction

The same apparatus used for place preference was used for conditioned place aversion (CPA). Mice were handled and acclimated similarly to animals used for place preference. During training, animals received an i.p. injection of lithium chloride, 3.0 and 3.5 mEq/kg , based on previous literature (File, 1986; Risinger and Cunningham, 2000) and placed on the black side of the chamber. Mice were placed on the black side of the chamber on days 1, 3, and 5. On the alternative days, mice received saline i.p. and were placed on the white side of the chamber on days 2, 4, and 6. The test day was identical to the test day for place preference. Extinction of CPA was conducted the same as extinction of CPP (discussed in detail above).

Electrophysiology

Extracellular field recordings

Male C57BL/6J mice were used between the ages of 5-10 weeks. They were taken from the animal facility in a new cage and allowed to acclimate in the lab for 1 hour. After the 1 hour acclimation, animals were anesthetized and killed by decapitation.

Brains were quickly removed and placed in ice cold sucrose rich artificial cerebral spinal fluid (ACSF in mM: 194 sucrose, 20 NaCl, 4.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10 glucose, 26 $NaHCO_3$). Slices of the brain were made in 300 micron thickness and only slices using the BNST were used. These slices were placed in an interface holding chamber (~28° C) at and allowed to recover for 1 hour in ACSF (in mM: 124 NaCl, 4.4 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1 NaH_2PO_4, 10 glucose, and 26 $NaHCO_3$). Picrotoxin (25μM), a $GABA_AR$ antagonist, was added to the bath for all recordings to block inhibitory transmission.

Electrodes were pulled with a Flaming-Brown Micropipette Puller (Sutter) and filled with ACSF. Stimulating electrodes consisted of formvar-coated nichrome wire. The BNST was stimulated in the dBNST which borders the internal capsule. Responses from stimulation included a N1 and N2. The N1 is the depolarization produced by direct activation of voltage gated channels and in large part is representative of the number of axons accumulated. The N2 is the synaptic response from the stimulation and is believed to be a product of glutamatergic transmission. Data points were collected every 20 seconds and the peak amplitude for each data point was averaged at 1 minute intervals.

Data analysis was performed with minutes 5-10 of the baseline and 50-55 or 60-65 minutes (indicated in figures) of the N2 response and compared with a paired t-test. All data are presented with the SEM.

All experiments were performed in the presence of picrotoxin (25μM) dissolved in dimethyl sulfide (DMSO). SB 334867 (5μM), WAY 100,135 (50μM) and yohimbine (50μM) were purchased from Tocris.

CHAPTER III

ROLE OF α_2-ARs IN ACQUISITIOIN AND EXTINCTION OF COCAINE INDUCED PLACE PREFERENCE

Introduction

Extinction is a form of learning that is thought to involve the formation of a new memory that suppresses behavioral responses to a learned stimulus (Bouton, 2002; Gale et al., 2004; Myers and Davis, 2007), although degradation of the original memory may also be involved (Mao et al., 2006). The modulation of extinction processes is increasingly recognized for its clinical potential to reshape maladaptive behavior. A major focus of research has been on the possibility of combining pharmaceutical agents with extinction-based behavioral therapy to enhance therapeutic outcome for anxiety disorders (e.g. phobia, posttraumatic stress disorder (Ressler et al., 2004). Extinction therapies could also be of benefit in the treatment of addiction. Although there have been studies investigating extinction of cue-induce craving responses in humans (O'Brien et al., 1992; Carter and Tiffany, 1999), there have been relatively few clinical or preclinical studies investigating pharmacological manipulations of extinction of human drug seeking and addiction-related behaviors in animal models (Sutton et al., 2003).

Norepinephrine (NE) plays a role in a variety of aspects of learning and memory (Ferry et al., 1999). Further, NE has emerged as a key regulator of various aspects of addiction-related behaviors (Baraban and Aghajanian, 1980; Redmond and Krystal, 1984; Schank et al., 2006; Schank et al., 2008). The source of central norepinephrine arises from two projections, the ventral noradrenergic bundle (VNAB) and the dorsal

noradrenergic bundle (DNAB), that both heavily innervate brain regions that are strongly implicated in extinction: the prefrontal cortex and the amygdaloid complex encompassing the bed nucleus of the stria terminalis (Moore and Bloom, 1979; Aston-Jones et al., 1999) Additionally, the infralimbic cortex (a prefrontal cortex region) is also critical for fear conditioning (Quirk et al., 2000; Wellman et al., 2007), and has been implicated in extinction behaviors. NE exerts its actions at these and other regions by signaling through adrenergic receptors (ARs) of which there are nine distinct AR receptors that fall into α_1, α_2 and β ARs categories, which are all G-protein, seven transmembrane receptors. The focus of our studies, the α_2-ARs, are widely distributed in the central nervous system (Nicholas et al., 1993; Wang et al., 1996).

Previous work has demonstrated that manipulation of the NE system affects extinction of conditioned fear behaviors (Caine and Koob, 1994; Erb et al., 2000). Cain et al. (2004) found that systemic administration of the α_2-AR antagonist yohimbine, a compound with strong anxiety-promoting properties in humans (Holmberg and Gershon, 1961; Redmond and Huang, 1979; Murburg et al., 1991) and laboratory animals (Bylund et al., 1994; Hagan et al., 1999; Davis et al., 2008), facilitated long-term extinction of conditioned fear in mice. While these studies suggest a contribution of α_2-ARs (as assayed by yohimbine) to fear extinction, little is known about their role in the extinction of reward-related memories formed by exposure to drugs of abuse.

In the present study, we investigated the role of α_2-ARs in extinction of cocaine-induced conditioned place preference (CPP) (and for comparison – fear extinction), using a combination of pharmacological and genetic strategies. Interestingly, while we replicated yohimbine-induced facilitation of extinction of fear (Caine and Koob, 1994),

we observed impairment of extinction learning of cocaine CPP after yohimbine administration. Moreover, we found that the impairment of cocaine CPP produced by yohimbine in C57BL/6J mice was not mimicked by the more specific α_2-AR antagonist atipamezole in this strain, and was actually exacerbated rather than attenuated in α_{2A}-AR knockout mice. Additionally, we found yohimbine elicited a slowly evolving decrease in glutamatergic transmission in the extended amygdala, which also not mimicked by atipamezole. Overall, our study provides converging lines of evidence suggesting that yohimbine has complex actions on extinction of reward behaviors that are likely independent of their effects on α_2-ARs.

Results

Cocaine conditioned place preference and extinction

Two groups of C57BL/6J mice underwent CPP training (Figure 5A) with cocaine (20 mg/kg) at two different times and there were no group differences in time spent on the drug paired side of the two groups (546 ±18 seconds, n=18; 520 ±20 seconds, n=12, t-test *ns*) and thus the groups were collapsed. There was a significant increase in time spent on the CS+ side from the pre-conditioning and post-test as expected (Figure. 5B, [t=7.77, df=58], p<.0001, t-test). We show the time spent on each side of the chamber for each of the extinction sessions (Figure 5D) and we compared the post-test time value with each extinction value (previously cocaine paired side (CS+)).

For extinction, a one-way RMANOVA of CS+ values was significant ($F_{6,209}$=27.08, p<.0001). *Post hoc* analysis shows that extinction sessions 1 through 6 are all significantly different from the post test value (Figure 5D). In regard to control

groups, a one-way RMANOVA did not find a difference between pre-conditioning, post-test, and day 6 extinction for a group that received only saline during CPP training and pre-treatment of saline during extinction (Table 1, p=0.8290).

Effect of yohimbine on cocaine CPP extinction

We next investigated the effects of yohimbine on cocaine CPP extinction. Significant cocaine CPP was produced in mice cocaine trained, as demonstrated by a significant increase in time spent in the cocaine-paired side during the pre-conditioning relative to the post-test (n=18, t-test, [t=7.77, df=17] p<.0001; Fig. 1C).

For extinction, a one-way RMANOVA of CS+ values was significant ($F_{6,125}$=5.59, p<.0001). *Post hoc* analysis shows that extinction sessions 2 through 6 are significantly different from the post test value (Figure 5E). Yohimbine did not affect locomotor activity during extinction sessions (session 1: 2276 ±115 cm traveled, session 6: 2508 ±162 cm traveled).

Cocaine CPP extinction in α_{2A}-AR KO mice and WT littermates

In order to investigate α_2-AR subtype contribution of yohimbine's effect on extinction of cocaine CPP, we tested α_{2A}-AR KO mice. Both KO and WT littermates acquired place preference after cocaine CPP training (KO: [t=12.05, df=21],p<.0001; WT [t=5.84, df=21], p<.0001 t-test, Figure 6A, 6D).

For extinction within the WT saline pre-treated mice, a one-way RMANOVA comparing CS+ time across sessions was significant ($F_{6,55}$=5.64, p<.001). *Post hoc* analysis shows that extinction sessions 3 through 6 are significantly different from the

post test value (Figure 6B). WT yohimbine pre-treated mice, a one-way RMANOVA comparing CS+ time was not significant (Fig. 3C, $F_{6,55}$=21.83, p=0.07). Locomotor activity was not affects in WT mice that received yohimbine during extinction (session 1: 1347 ± 129 cm traveled, session 6: 1839 ± 113 cm traveled).

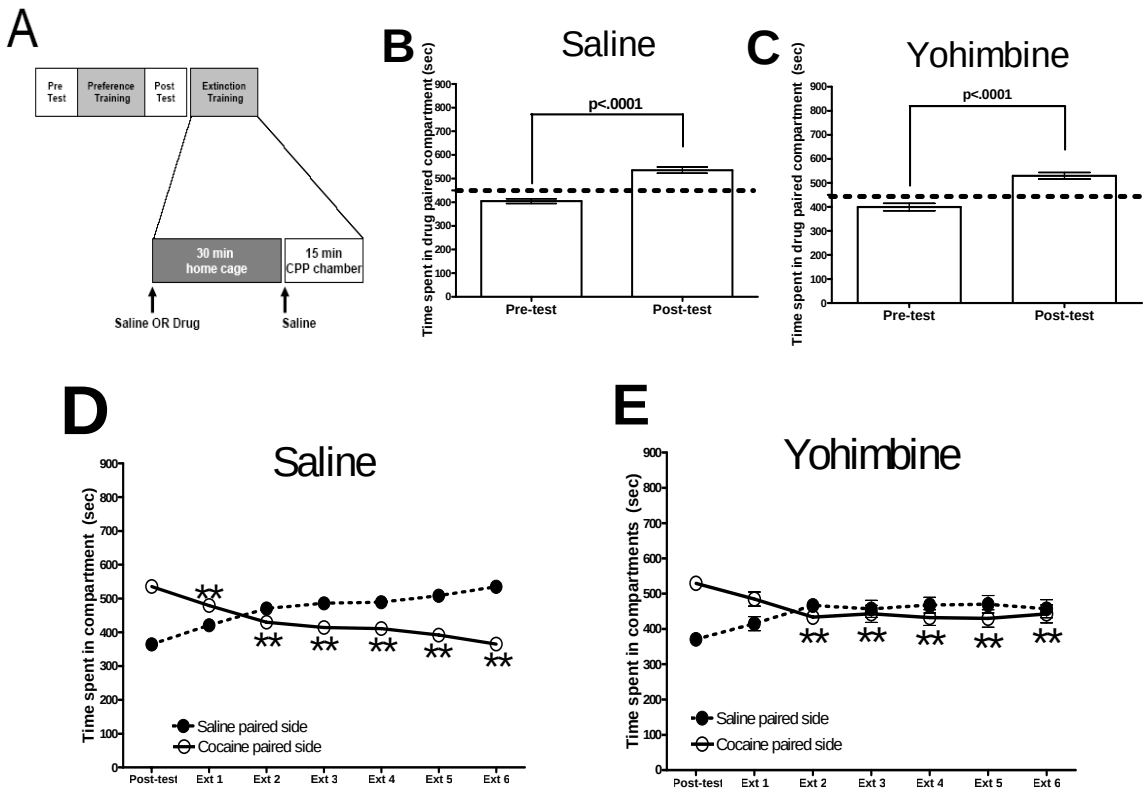


Figure 5. *C57BL/6J mice administered yohimbine 5 mg/kg (i.p.) during extinction sessions display impaired extinction* 5A) Schematic of cocaine CPP extinction. Mice are trained with 20 mg/kg cocaine during CPP training. After mice show a preference for the cocaine paired side (CS+), they are subjected to extinction training. 5B,C) C57BL/6J mice acquire place preference for the cocaine paired side (N=30, saline group; N=18, yohimbine group). 5D, E) Mice were extinguished over six days. Shown is time spent on the cocaine paired side and saline paired side during post-test and extinction. Mice were given injection of saline or yohimbine and placed in home cage. 30-35 minutes later, mice were given an injection of saline and placed in chambers. Error bars represent ± SEM. **p<.01 comparison of time spent white side of chamber in comparison to the post-test value. Dotted line in panels B,C indicates half of test period (450 seconds).

Extinction within the KO saline pre-treated mice, a one-way RMANOVA comparing CS+ time across sessions was significant ($F_{6,83}$=8.10, p<.0001). *Post hoc* analysis shows extinctions sessions 1 through 6 are significantly different from the post test value (Figure 6E). KO yohimbine pre-treated mice, a one-way RMANOVA comparing CS+ time was significant (Figure 6F, $F_{6,69}$=7.54, p<0.05). *Post hoc* analysis did not find any difference between the pretest and days 1 through 6 of extinction. Additionally, a three-way ANOVA (genotype x session x treatment) of percentage for three extinction sessions 2, 4 and 6 revealed an effect of treatment ($F_{1,76}$=4.97, p<.05) and session ($F_{2,113}$=9.38. p<.02). *Post hoc* analysis showed a difference between KO yohimbine pre-treated and KO saline pre-treated on extinction session 2, and between KO yohimbine pre-treated and both WT saline pre-treated (p<.05) and KO saline pre-treated (p<.01) on extinction session 4 (Figure 7). A three-way RMANOVA for CS+ values for the WT and KO, both treatment groups revealed an effect of genotype ($F_{1,265}$=12.00, p<.001), a genotype x treatment interaction ($F_{1.265}$=125.26, p<.0001), an effect of session ($F_{6,265}$=79.75, p<.0001), treatment x session interaction ($F_{6,265}$=17.48, p<.0001), and a genotype x treatment x session interaction ($F_{6,265}$=8.10, p<.05). *Post hoc* analysis revealed WT saline pretreated mice differed from WT yohimbine treated mice on days 3 and 6 of extinction, KO saline pretreated mice were significantly different from KO yohimbine pretreated mice on days 1 through 6 of extinction. There were no differences found between neither WT and KO saline pretreated mice nor WT and KO yohimbine pretreated mice. Regarding controls, a one-way RMANOVA did not find a difference between pre-conditioning, post-test, and day 6 extinction for both WT mice

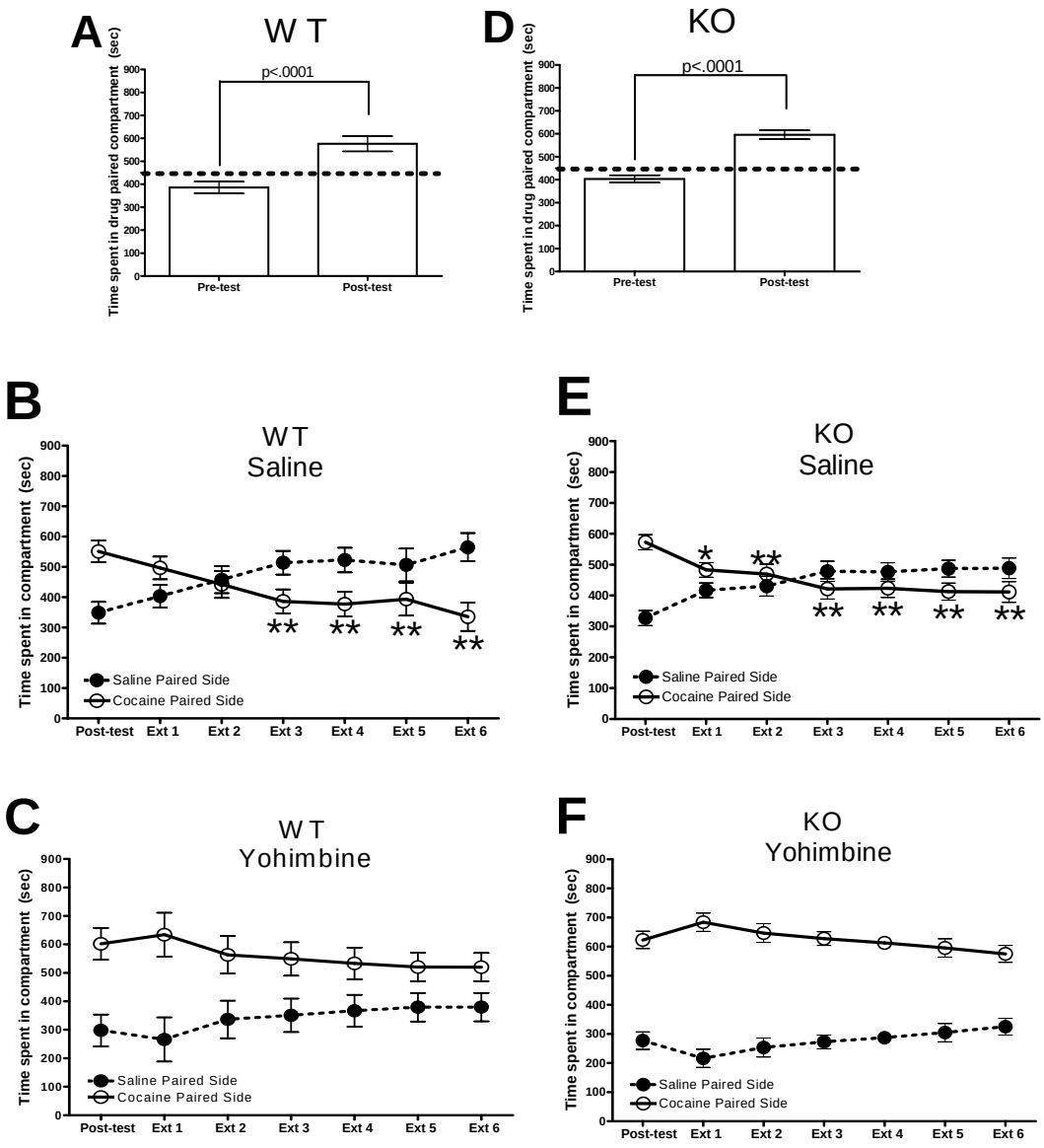
that received saline during cocaine CPP training and pretreated with either saline or yohimbine prior to extinction sessions (Table 1).

Table 1. Saline Controls:
Time spent on the white side of the chamber

	N	Treatment	Pretest	Posttest	Ext 6	p-value
C57Bl/6j	15	Sal/Sal	398.6 ± 14.8	386.8 ± 21.4	381.6 ± 27.6	0.8290
	17	Sal/Yoh	399.7 ± 8.9	429.7 ± 19.4	407.9 ± 21.5	0.3429
	6	Sal/Sal	415.3 ± 40.8	413.7 ± 45.5	359.2 ± 82.6	0.4621
	7	Sal/Ati	406.8 ± 33.4	413.9 ± 16.4	342.6 ± 30.9	0.2253
WT	4	Sal/Sal	484.0 ± 32.1	427.9 ± 40.7	501.3 ± 106.7	0.7563
	5	Sal/Yoh	399.8 ± 36.3	305.9 ± 57.6	384.5 ± 73.4	0.3879
KO	4	Sal/Sal	410.0 ± 26.5	432.8 ± 43.2	452.7 ± 49.2	0.6776
	5	Sal/Yoh	443.6 ± 32,7	518.5 ± 51.2	422.5 ± 58.1	0.2957

Additionally, one-way RMANOVA found no difference between pre-conditioning, post-test and day 6 of extinction for KO mice that received saline during cocaine CPP and pretreated with either saline or yohimbine prior to extinction sessions (Table 1).

Figure 6. *Yohimbine (5 mg/kg) administered during extinction sessions impairs extinction of cocaine CPP in α_{2A}-AR KO and WT littermates*. Mice were administered either saline or yohimbine and placed in the home cage. 30-35 minutes later, mice were given an injection of saline and placed in chambers. 3A) WT littermates obtain place preference (n=16). 3B) Shown is time spent on each side of the chamber of WT cocaine trained and saline extinguished. (N=8). 3C) Shown is time spent on each side of chamber of WT cocaine trained and yohimbine extinguished. (N=8). 3D) KO mice obtain place preference (n=22). 3E) Shown is time spent on each side of the chamber of KO mice cocaine trained and saline extinguished. (N=12). 3F) Shown is time spent on each side of chamber of KO mice cocaine trained and yohimbine extinguished. (N=10). Error bars represent ± SEM. *p<.05, **p<.01. Dotted line (panels A,D) indicates half of test period (450 seconds).


A
W T
p<.0001
Time spent in drug paired compartment (sec)
Pre-test
Post-test
D
KO
p<.0001
Time spent in drug paired compartment (sec)
Pre-test
Post-test
B
WT
Saline
Time spent in compartment (sec)
Saline Paired Side
Cocaine Paired Side
Post-test
Ext 1
Ext 2
Ext 3
Ext 4
Ext 5
Ext 6
E
KO
Saline
Time spent in compartment (sec)
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Time spent in compartment (sec)
Saline Paired Side
Cocaine Paired Side

Effect of atipamezole on cocaine CPP extinction

Mice in this experiment demonstrated significant cocaine CPP (Fig. 6A, [t=4.66, df=11], p<.0007, t-test). For extinction, a one-way RMANOVA of CS+ values was significant ($F_{6,83}$=10.96, p<.0001). *Post hoc* analysis shows that extinction sessions 1 through 6 are all significantly different from the post test value (Figure 8B).

Atipamezole did not affect locomotor activity during extinction training (session 1: 2944 ±149 cm traveled, session 6: 2957 ±193 cm traveled). For mice pre-treated with atipamezole before extinction sessions, a two-way RMANOVA was significant for treatment ($F_{5,359}$=9.95, p<.0001).

Next we analyzed differences in percent time spent on CS+ side during pre-conditioning and extinction days 2, 4, and 6 for saline, yohimbine, and atipamezole pre-treated groups. A two-way ANOVA each extinction days 2, 4 and 6 between treatment groups, which was not significant but there was trend towards an effect of session ($F_{2,179}$=2.01, p=.08). Additionally, a two-way RMANOVA of CS+ time values between the C57BL/6J saline, yohimbine and atipamezole pretreated groups revealed an significant effect of extinction sessions ($F_{6,419}$=30.25, p<.0001). *Post hoc* analysis shows on day six of extinction, there is a significant difference between saline pre-treated mice and yohimbine pre-treated mice (p<.05). As for controls, a one-way RMANOVA did not find a difference between pre-conditioning, post-test, and day 6 extinction for mice that received saline during cocaine CPP training and either saline or atipamezole during extinction (Table 1), thus the drugs (yohimbine or atipamezole) did not have an effect on its own.

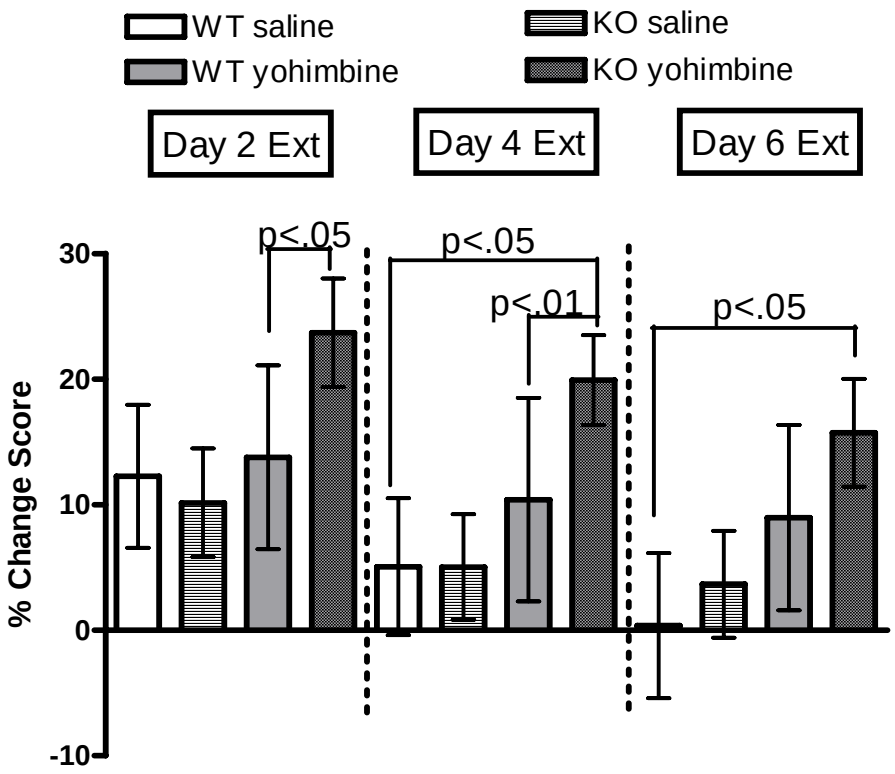


Figure 7. *Knockout mice extinguished with yohimbine exhibit impaired extinction compared to saline extinguished knockout mice.* Percent difference from pre-conditioning and extinction days for each genotype on 2, 4, and 6 for cocaine trained mice. Shown is percent time on CS+ side during pre-conditioning minus percent time on CS+ during extinction. Bars represent ± SEM. WT (N=8), KO (N=10-12).

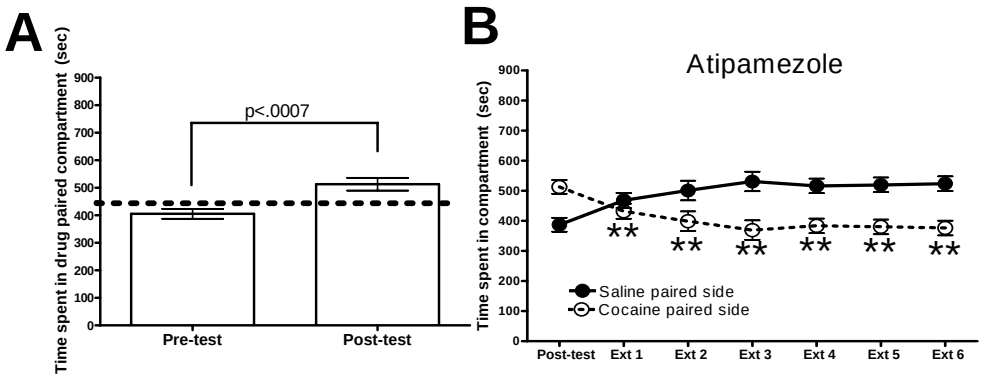


Figure 8. *C57BL/6J mice administered atipamezole extinguish place preference.* 6A) Mice acquire place preference for the cocaine paired side. 6B) Mice were given atipamezole (N=12) and placed in home cage. 30-35 minutes later, mice were given an injection of saline and placed in chambers. Error bars represent ± SEM. **p<.01 comparison of time spent on white side of chamber in comparison to post-test value.

Discussion

Here we investigated the effects of a widely used anxiogenic compound, yohimbine, on extinction of cocaine CPP. We found that yohimbine impaired extinction of cocaine CPP. Additionally we found that mice lacking the α_{2A}-AR extinguished cocaine CPP just as wild type littermates, and were hypersensitive, rather than insensitive, to the extinction impairing effects of yohimbine. We re-assessed previously reported facilitatory effects on extinction of fear by yohimbine administration (Caine and Koob, 1994) and found that yohimbine impaired fear recall without having unambiguous effects on within- or between-session fear extinction in the paradigm we employed. The specific α_2-AR antagonist, atipamezole, did not mimic yohimbine in terms of either extinction of fear conditioning or cocaine CPP. Further, we found yohimbine produced a significant depression of glutamatergic transmission in the dlBNST in brain slices prepared from both WT and α_{2A}-AR KO mice that was not mimicked by atipamezole.

Yohimbine impairs extinction of cocaine CPP

It has been reported that NE and α_2-ARs play a role in both positive and negative valence-learned behaviors (Schank et al., 2006). However, most studies exploring extinction behavior have utilized negative valence-learned behaviors. Using fear based learning, yohimbine has been shown to facilitate long-term extinction of fear in mice (Caine and Koob, 1994), although we did not observe this under current conditions possibly due to it being precluded by the strong extinction produced by our extensive massed training protocol. To investigate the effect of yohimbine on extinction of a positive valence-learned behavior, we asked whether yohimbine could facilitate

extinction of cocaine CPP, a widely used paradigm (Tzschentke, 2007). It has been reported that yohimbine causes an anxiety response when administered to humans (Murburg et al., 1991) as well as enhancing morphine place preference (Zarrindast et al., 2002). Further, it has been shown that stress enhances acquisition of morphine induced CPP (Haile et al., 2001). Thus, it may be expected that yohimbine acting as a stressor would prolong preference, i.e. impair extinction, for the CS+. We found yohimbine, indeed, impaired extinction of cocaine CPP.

To provide more insight into the mechanistic basis of yohimbine's effects on extinction of cocaine CPP after showing cocaine CPP extinction was inconsistent with the effect of yohimbine on fear extinction, we utilized mice with targeted deletion of the α_{2A}-AR gene. The α_{2A}-ARs make up 90% of the centrally located α_2-ARs (Bucheler et al., 2002). By using the α_{2A}-AR KO mice, we asked whether yohimbine was acting through the α_{2A}-ARs or other receptors. Interestingly, we found that loss of α_{2A}-AR not only failed to prevent the extinction impairing effects of cocaine CPP, but that mice lacking the α_{2A}-AR actually showed significantly exaggerated impairment of extinction by yohimbine. A noteworthy point was that, despite being on a C57BL/6J background, WT littermates of the KO mice exhibited a stronger yohimbine impairment profile than we saw in C57BL/6J mice. C57BL/6J mice and WT littermates could be subtly different because of 1) maternal effects from the heterozygote breeders as well as 2) handling issues of ordered mice (C57BL/6J from Jackson) while the WT littermates were bred in house. The difference is interesting and would be beneficial to examine in the future.

Next we tested the effects of atipamezole, a more specific α_2-AR antagonist, that does not have subtype selectivity (Newman-Tancredi et al., 1998). In contrast to

yohimbine, atipamezole failed to alter cocaine CPP extinction and showed a trend towards impairing long-term fear extinction. Taken together, these data suggest a) that yohimbine regulates cocaine CPP through an α_{2A}-AR-independent mechanism, and b) that the α_{2A}-AR is not required for extinction learning, but plays a permissive role in modulation of extinction. It should be noted that only a single dose of atipamezole was used for these experiments. However, these data are consistent with previous suggestions that anxiety behaviors emitted after yohimbine administration are independent of the α_2-ARs in mice (Schank et al., 2006) and rats (Redfern and Williams, 1995). However, the role of the α_2-ARs was largely unexplored in extinction behaviors.

Yohimbine produces depression of glutamatergic transmission in the dlBNST through an off-target action

To address the possible mechanistic basis of the behavioral effects of yohimbine and atipamezole, we utilized whole cell patch clamp techniques and recorded from dlBNST neurons. The BNST receives a dense noradrenergic input from the VNAB (Aston-Jones et al., 1999). When the VNAB is lesioned, there is an impairment of negative valence-learned extinction (Schank et al., 2006) suggesting regions innervated are essential to extinction of learned behaviors. We found atipamezole was able to reverse the depression caused by UK14,304, an α_2-AR agonist, whereas yohimbine did not reverse the depression. Furthermore, we found in α_{2A}-AR KO mouse slices, the depression caused by yohimbine persists. One interpretation is that yohimbine is acting through a non- α_2-AR mechanism in this brain region which may contribute to impairment of extinction in cocaine CPP.

Chapter IV

ACTIVE EXTINCTON OF COCAINE INDUCED PLACE PREFERENCE

Introduction

As shown in Chapter 3, yohimbine impairs extinction of cocaine CPP when the extinction sessions are conducted with the mice having access to both sides of the chamber. However, we also wanted to investigate how yohimbine effected extinction of cocaine CPP when during extinction sessions, the mouse is restricted to one side of the chamber and then having an actual test day. This type of facilitated extinction is referred to as "active" extinction in this work.

Results

To examine extinction, mice are first trained with a cocaine CPP protocol to establish a preference for the CS+ side, which is measured during the posttest. Following the posttest, mice underwent either 'active' or 'passive' extinction. During 'active' extinction, mice are restricted to either the CS+ or CS- side.

For 'active' extinction, we first trained mice with 20 mg/kg cocaine and assessed a preference for the cocaine paired side (CS+) over the saline paired side (CS-). The 2-day 'active' extinction group developed a preference ($p=.0428$, t-test, Figure 9) as well as the 6-day 'active' ext group ($p=.0404$, t-test, Figure 9). Next, we actively facilitated extinction in mice by pairing each side with saline for either two days (N=14) or six days (N=7). Unexpectedly, the mice extinguished to pretest values after the 2-day 'active' ext,

of which the mice were exposed to the CS+ and CS- side only once. The 6-day 'active' ext (3 days on either CS+ or CS-) yielded the same results as the two day 'active' ext. There is no statistical difference between the pretest (p=.8334, t-test), the posttest (p=.9964, t-test) or extinction (p=.9911, t-test) between 2-day and 6-day "active" extinction groups (Figure 9).

Discussion

Although facilitated extinction is useful to answer many behavioral questions, it would be difficult to examine the effect of drugs in facilitating extinction of cocaine CPP because of how quickly the mice extinguished their preference for the CS+ side. A similar study was performed in which they utilized two types of extinction, response and latent extinction (Gabriele and Packard, 2006). Response extinction utilized an approach response whereas latent extinction there was no approach response. The two types of extinction were able to be neuroanatomically dissociated suggesting they require different circuitry (Gabriele and Packard, 2006).

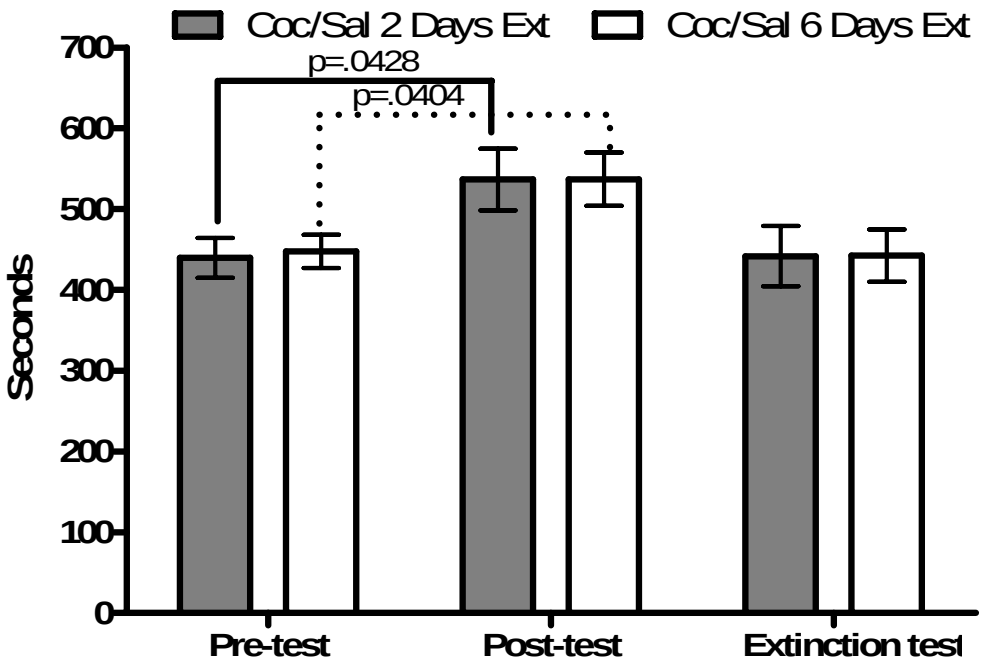


Figure 9. *Active extinction of cocaine CPP in C57BL/6J mice does not differ between 2 and 6 day groups.*. Mice underwent either 2 or 6 days of active extinction following cocaine CPP training. Both groups obtained place preference (2 day ext., N=14; 6 day ext, N=7). Error bars represent ± SEM.

CHAPTER IV

INFLUENCE OF THE OREXINERGIC AND SEROTONERGIC SYSTEMS ON YOHIMBINE INDUCED DEPRESSION OF GLUTAMATERGIC TRANSMISSION

Introduction

Yohimbine is a non-selective α_2-AR antagonist (Winter and Rabin, 1992; Newman-Tancredi et al., 1998) and recently been shown to be effective in exposure therapy (Powers et al., 2009). Thus, gaining further understanding of the mechanism of the actions of yohimbine is warranted.

We previously showed with whole cell electrophysiology that yohimbine causes a depression of glutamatergic transmission that is independent of α_2-ARs (Davis et al., 2008). However, one caveat with whole cell recordings is it is unknown whether or not application of a drug causes a decrease in the number of fibers stimulated by the stimulating electrode, thus causing a decrease in response. Thus, in order to investigate this possibility that the effect of yohimbine is synaptic or axonal, we employed extracellular field recordings. A different dose was used for this recording because of different pharmacokinetics due to different chambers. The whole cell experiment used a submerged chamber while the field experiment used an interface chamber.

Interestingly, we found yohimbine extinguishes learned fear and cocaine CPP by a mechanism independent of α_2-ARs (Davis et al., 2008) and thus must work through another system. A method to investigate the mechanism of yohimbine is to use electrophysiology. An interesting candidate that may mediate the action of yohimbine in certain behaviors is orexin. Orexin is a neuropeptide and is involved in arousal states

(Sakurai, 2007) and increases firing of the VTA (Korotkova et al., 2003). It has been shown that an orexin antagonist, SB 334867 blocks cocaine sensitization (Borgland et al., 2006). Additionally, SB 334867 blocks yohimbine induced reinstatement of ethanol and sucrose seeking behavior (Richards et al., 2008). We examined whether the depression of glutamatergic transmission after yohimbine application could be blocked by SB 334867.

Lastly, yohimbine has been shown to act through the 5-HT_{1A}. Yohimbine has only a 4.2 fold selectivity for the α_2-ARs over 5-HT_{1A} (Newman-Tancredi et al., 1998) and it acts as a partial agonist at the 5-HT_{1A} (Newman-Tancredi et al., 1998). Behaviorally, it has been shown that the effect of yohimbine on prepulse inhibition was mediated by 5-HT_{1A} (Powell et al., 2005). Further, yohimbine generalizes to a 5-HT_{1A} agonist, 8-OH-DPAT, in drug discrimination studies (Winter and Rabin, 1992). This suggests that yohimbine may mediate some of its effects via this serotonin receptor.

Results

We first investigated whether the yohimbine caused a significant decrease in the N1. We found no difference in the N1 amplitude pre- and post- application of yohimbine measured at 5-10 minutes for baseline compared to 50-55 minutes (N=6, p=0.7238) (Figure 10). Additionally, we found that a 30 minute application of 50μM yohimbine caused a significant decrease in the N2 amplitude compared to baseline (N=6, p=0.0002) (Figure 11). Thus, we were able to replicate the depression in glutamatergic transmission observed with whole cell recordings with extracellular field recordings. Further, the depression in glutamatergic transmission was not due to a decrease in the N1 after yohimbine application.

Next we attempted to examine whether or not the yohimbine induced depression of glutamatergic transmission could be blocked by a 5-HT_{1A} antagonist, WAY 100,135. We observed that WAY 100,135 (50μM) was not able to prevent the depression in glutamatergic transmission following yohimbine (50μM) application (Figure 12). However, the data cannot be interpreted because application of both WAY 100,135 and yohimbine caused a decrease in the N1 (Figure 13).

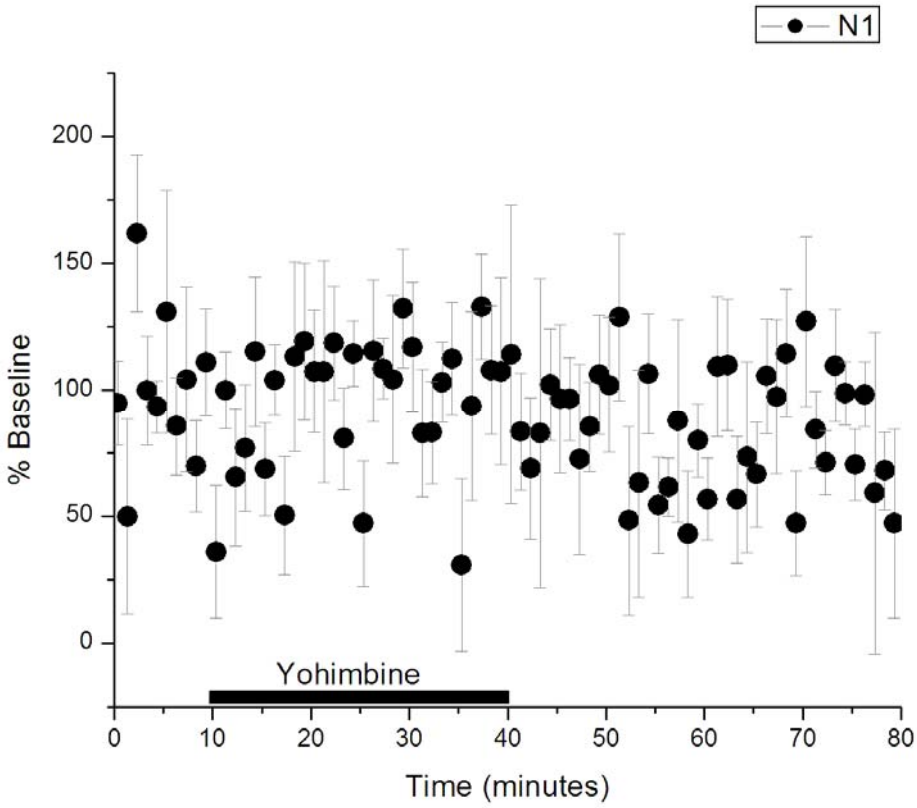


Figure 10. *Yohimbine does not alter the N1.* Bath application of yohimbine (50μM) for 30 minutes had no effect on the N1. N=6.

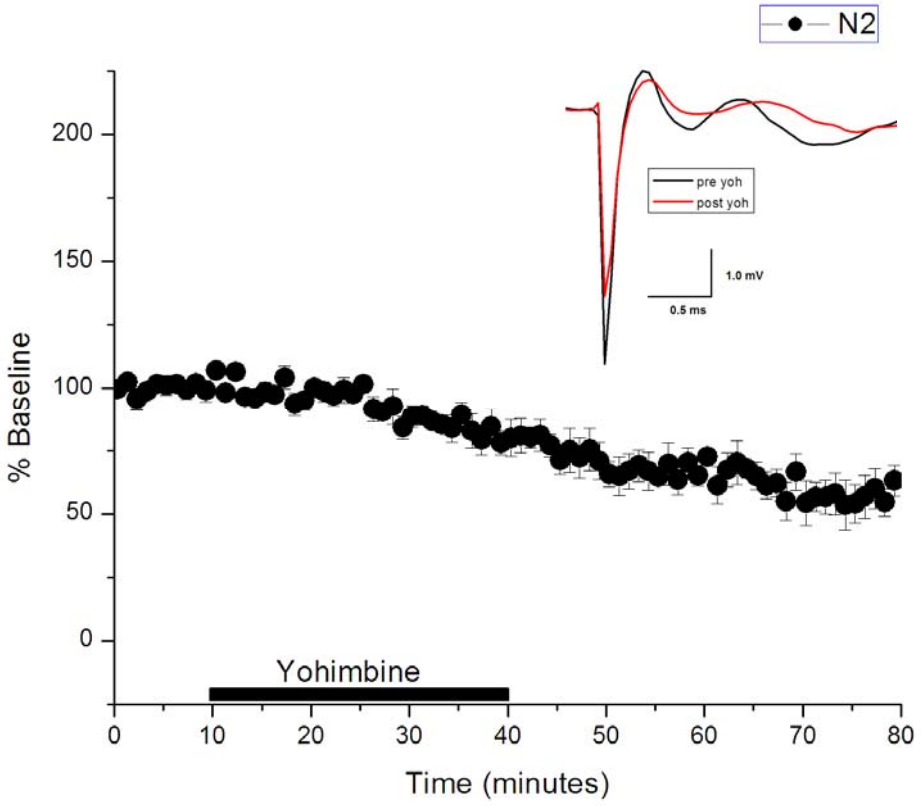


Figure 11.*Yohimbine decreases glutamatergic transmission.* Bath application of yohimbine (50µM) for 30 minutes caused a depression in the dBNST measured by extracellular field recording. N=6. Inset: Representatives trace pre- and post- yohimbine application. Each trace represents an average of 5 minutes of baseline (minutes 5-10) and 5 minutes following drug application (minutes 50-55).

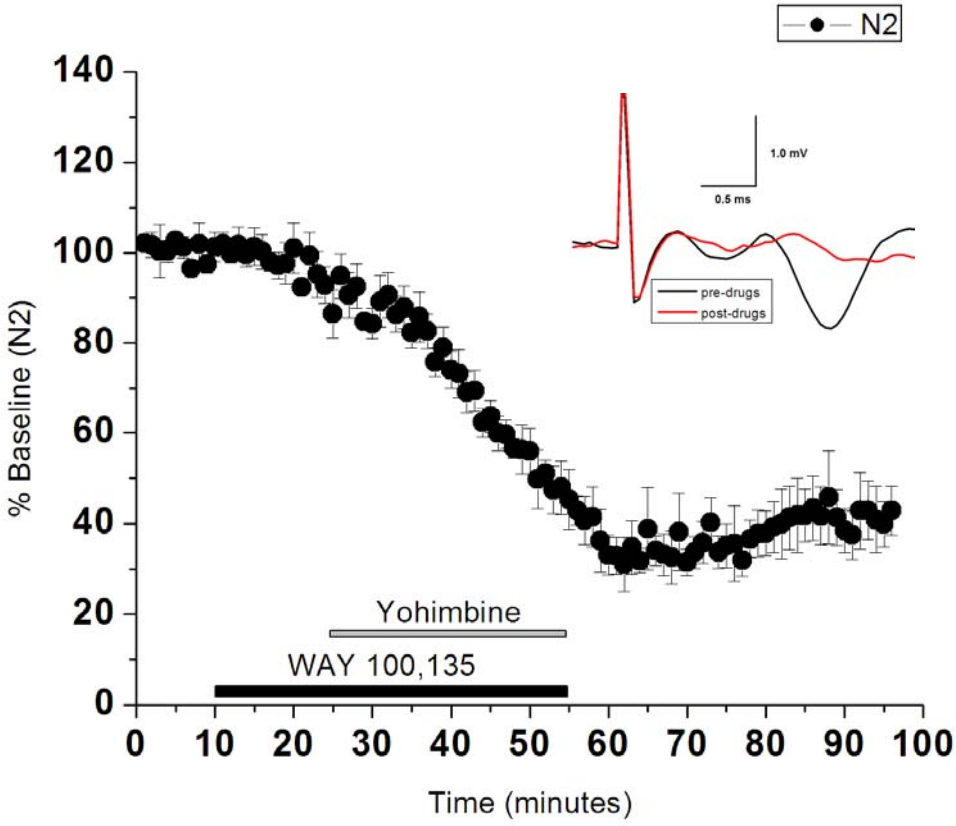


Figure 12. *Effect of WAY 100,135 on yohimbine induced depression of glutamatergic transmission.* Bath application of WAY 100,135 (50µM) did not block depression induced by yohimbine (50µM). N=4. Inset: Representatives trace pre- and post- WAY 100,135/ yohimbine application. Each trace represents an average of 5 minutes of baseline (minutes 5-10) and 5 minutes following drug application (minutes 50-55).

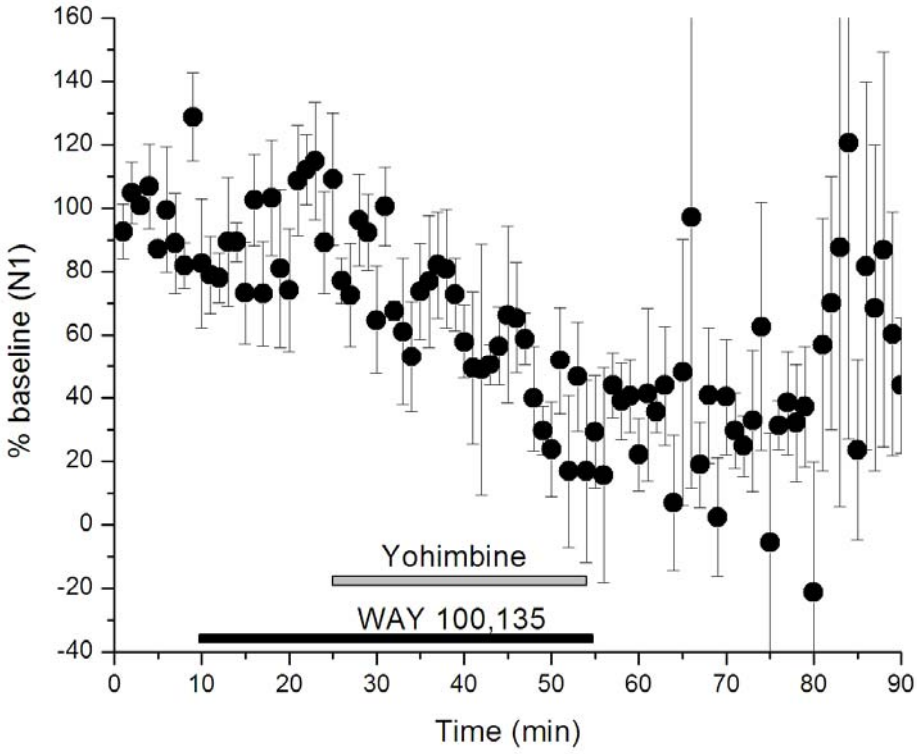


Figure 13. *Effect of co-application of WAY 100,135 and Yohimbine on N1.* Bath application of WAY 100,135 (50µM) and yohimbine (50µM) caused a decrease in the N1. N=4.

Next we examined if the yohimbine effect could be blocked by antagonism of the orexin receptors. The time used for comparison was 5-10 minutes of baseline and 60-65 minutes. First we applied SB 334867 (5μM) to observe whether it had an effect on glutamatergic transmission by itself. The 5 μM application of SB 334867 had an effect on the N2 (N=7, p=0.0087) (Figure 12) but not the N1 (N=7, p=0.5029) (Figure 13). Although the SB 334867 has a slight effect on its own by causing an approximately 10% depression, we found that a 15 minute pre-application of SB 334867 (5μM) was able to block an approximately 50% yohimbine induced depression (N=5, p=0.2802) (Figure 10). Additionally, there was no effect of the drugs on the N1 amplitude pre- and post-drug application (N=5, p=0.4847) (Figure 15). This suggests that the noradrenergic and orexinergic systems interact to cause the depression.

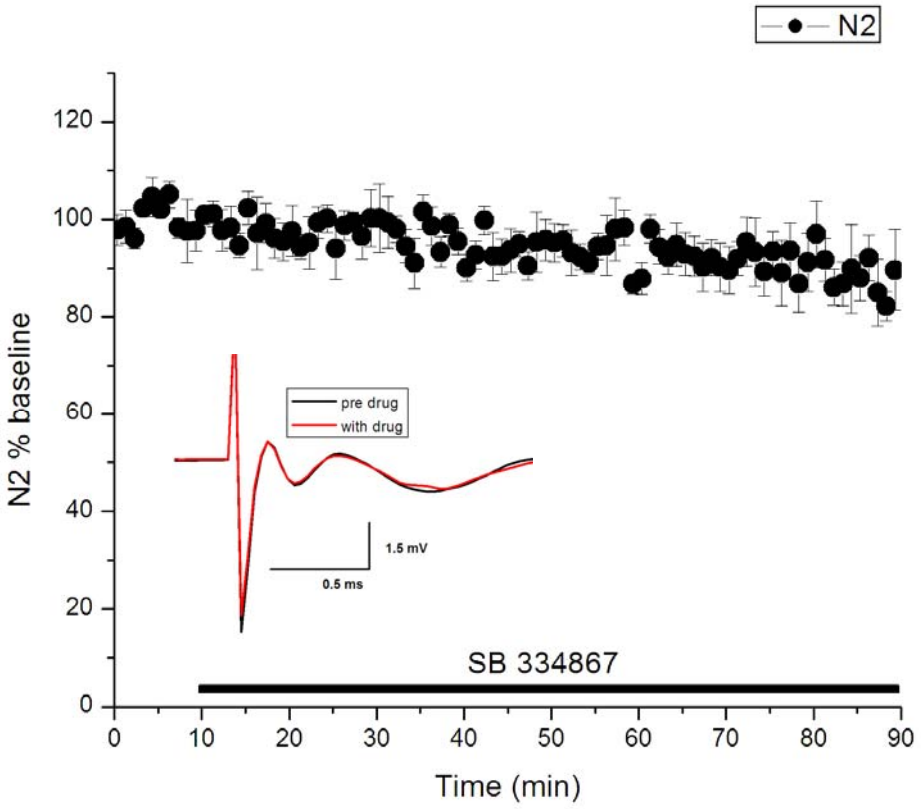


Figure 14. *SB 334867 slightly decreases glutamatergic transmission.* Bath application of SB 334867 (5µM) for 80 minutes had a modest effect on the N2. N=7. Inset: Representatives trace pre- and during SB 334867 application. Each trace represents an average of 5 minutes of baseline (minutes 5-10) and 5 minutes following drug application (minutes 60-65).

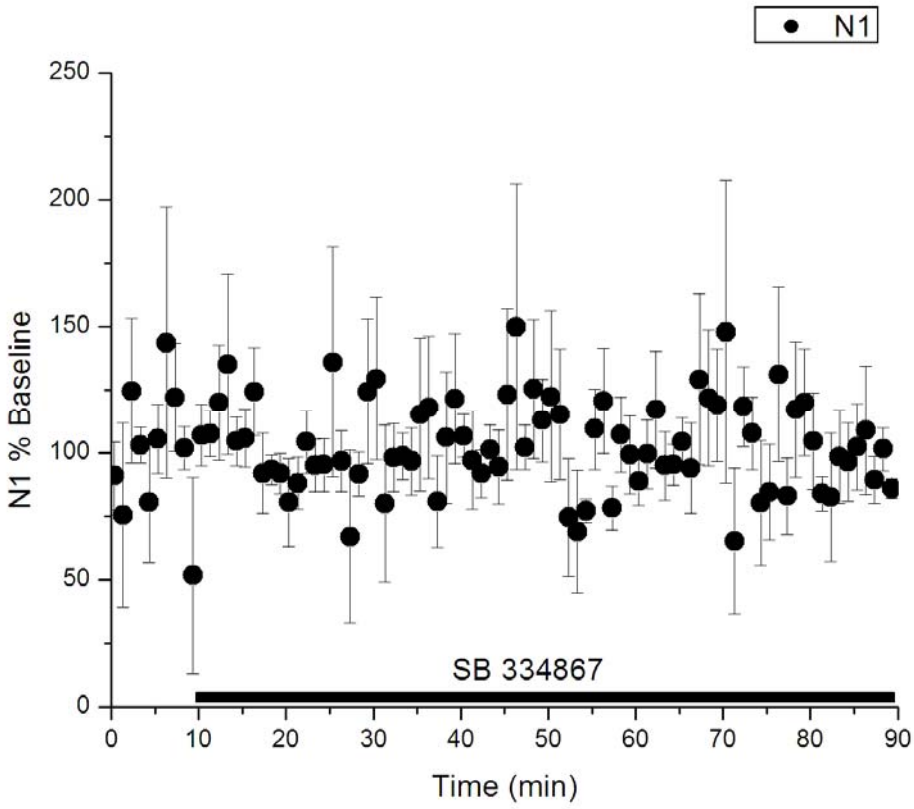


Figure 15. *SB 334867 does not alter the N1*. Bath application of SB 334867 (5μM) for 80 minutes had no effect on the N1. N=7.

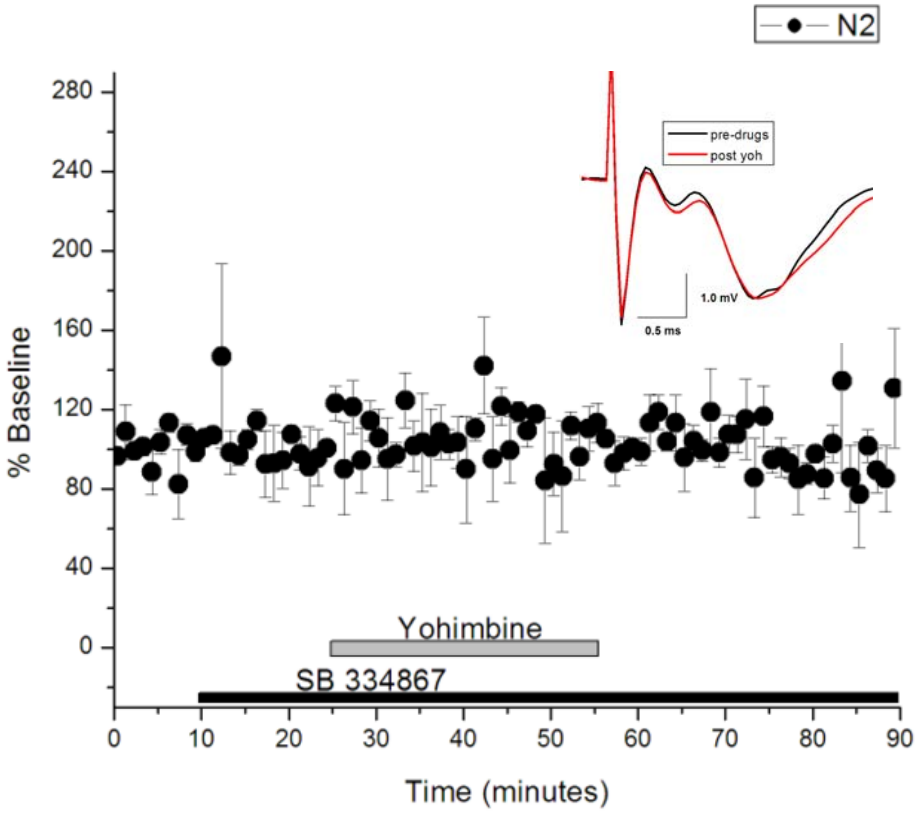


Figure 16. *SB 334867 blocks yohimbine induced depression.* Bath applied SB 33467 (5µM) was able to block yohimbine (50µM) induced depression. N=5. Inset: Representatives trace pre- yohimbine/SB 334867 and during SB 334867 application. Each trace represents an average of 5 minutes of baseline (minutes 5-10) and 5 minutes following drug application (minutes 60-65).

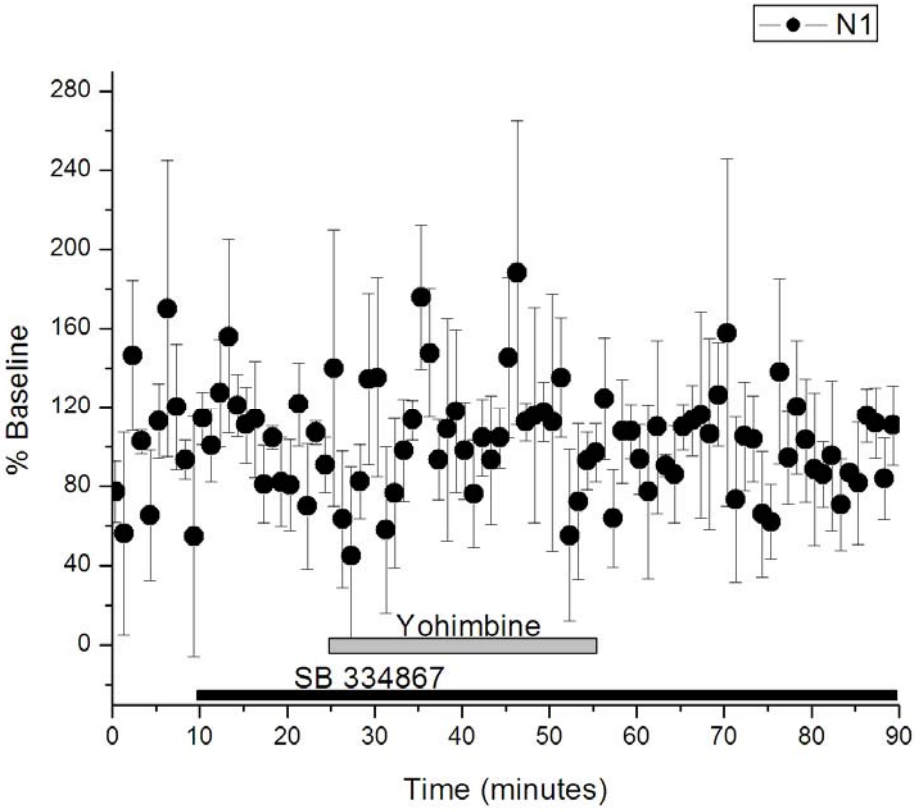


Figure 17. *The co-application of yohimbine and SB 334867 had no effect on the N1.* Bath applied SB 33467 (5µM) and yohimbine (50µM) did not cause an alteration of the N1. N=5.

Discussion

We previously reported yohimbine caused a decrease in glutamatergic transmission in the dBNST with the use of whole cell patch recordings (Davis et al., 2008). Consistent with the previous data, we were able to replicate the finding of yohimbine induced depression of glutamatergic transmission in extracellular field recordings in the dBNST (Figure 10). The finding that the N2 changed independently of the N1 suggests the depression of glutamatergic transmission is not because of an axonal effect of yohimbine.

We also found that an orexin antagonist, SB 334867 blocked the yohimbine induced depression seen with extracellular field recordings. This is of interest because it suggests a possible mechanism by which SB 334867 blocks reinstatement of ethanol and sucrose seeking by yohimbine (Richards et al., 2008). We also found that SB 334867 has a modest effect on its own. In a recent paper, it was shown that many α_2-AR effects are mediated by neurons that are not noradrenergic (Gilsbach et al., 2006). Additionally, with recent evidence that yohimbine facilitates extinction of a phobia in humans (Powers et al., 2009), the orexinergic system should be investigated in extinction of addiction behaviors in animal models.

Additionally, yohimbine is a partial agonist for the 5-HT_{1A} (Newman-Tancredi et al., 1998). We could not decipher the results of WAY 100,135 and yohimbine due to an effect of WAY 100,135 on the N1. This effect may be due to 5-HT_{1A} as a somatodendritic autoreceptor on cell bodies of serotonergic neurons (Knight et al., 2004). However, as mentioned briefly, behavioral evidence suggests that some actions of yohimbine is due to the 5-HT_{1A} (Winter and Rabin, 1992; Powell et al., 2005).

Lastly, it is possible for all of these systems to overlap. There is evidence that cells that contain orexin fibers overlap with the LC (Date et al., 1999) and orexin A increases firing of the LC (Hagan et al., 1999). Additionally, it has been suggested that orexin fibers project to the dorsal raphe (Grogan et al., 2002). Thus, it is likely all of these systems interact with produce the aforementioned behavioral effects.

CHAPTER V

EFFECT OF YOHIMBINE ON EXTINCTION OF LITHIUM CHLORIDE INDUCED PLACE AVERSION

Introduction

There are many studies examining the effect of various pharmaceutical agents on the extinction of fear (Caine and Koob, 1994; Erb et al., 2000; Bouton, 2002; Walker et al., 2002; Chhatwal et al., 2005; Myers and Davis, 2007; Zushida et al., 2007; Quirk and Mueller, 2008; Brinks et al., 2009) but there are few studies that examine the effect of the pharmaceutical agents on extinction of a positive valence learned behavior. This is relevant because of the high overlap of anxiety disorders and addiction (Coffey et al., 2002; Brady and Clary, 2003; Sareen et al., 2006).

Lithium chloride (LiCl) induce place aversion is a useful assay to explore the role of neurotransmitters, etc., in negative valence learned behaviors. Lithium chloride is a toxin that produces visceral illness symptoms in animals that are cannot vomit (Bernstein et al., 1992) and in those that can (Ossenkopp and Eckel, 1995). It has been shown that catecholamines are involved in motivational salience in both aversion and reward related stimuli (Schank et al., 2006). Interestingly, it has been shown that CPP and CPA differ in mechanism as CPP was blocked pharmacologically with a drug that inhibits nitric oxide signaling and CPA was unaffected (Grabus et al., 2006). As with most behaviors, there is evidence of strain dependence in the magnitude of aversion produced (Risinger and Cunningham, 2000).

Results

Mice that received saline during CPA training and either yohimbine or saline during extinction show a trend towards spending more time on the black side of the chamber (Figure 18 A,B). Although those that received yohimbine did not show such a clear difference but the behavior was expected due to the bias nature of the apparatus. Mice that received 3.0 mEq/kg LiCl during training and saline during extinction show a trend of avoiding the side paired with the LiCl in the posttest, but the aversion decreases by the sixth extinction day (Figure 18 C). Mice that received 3.0 mEq/Kg LiCl during training but yohimbine prior to extinction sessions also so an aversion during the posttest (Figure 18 D). Interestingly, the magnitude of the aversion increases on the first extinction day and diminishes by the sixth extinction day. Mice that received 3.5 mEq/kg LiCl during training and saline during extinction show a similar behavior of that of mice trained with 3.0 mEq/kg and extinguished with saline (Figure 18 C,E). However, mice that received 3.5 mEq/kg during training and yohimbine during extinction show a trend towards having an increased aversion during the posttest that stays elevated over all of the extinction sessions (Figure 18 F).

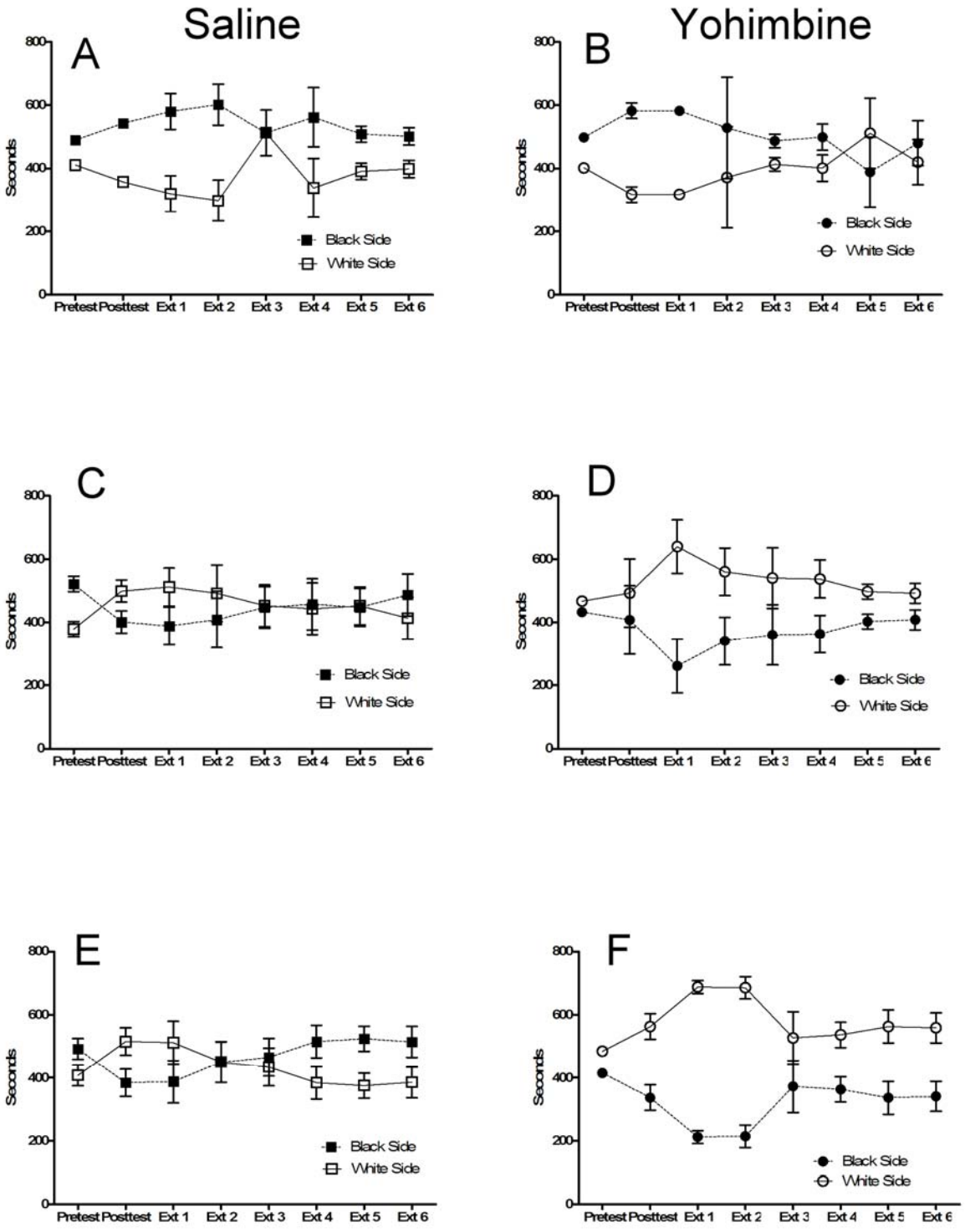
Discussion

NE has been implicated in acquisition of a negative learned valence behavior (Schank et al., 2006). Yohimbine facilitates extinction of fear (Caine and Koob, 1994) although this seems to be a within testing effect (Erb et al., 2000; Davis et al., 2008). We found yohimbine administration during place aversion extinction produced a behavioral trend towards impairing extinction of the behavior. This is interesting because it suggests

that the impairment recruits similar circuitry regardless of the valence of the learned behavior.

There is also likely a disconnect between what facilitates extinction of fear and CPA due to the nature of each test. A likely reason is fear extinction recruits a "processive" stressor circuitry whereas lithium chloride CPA utilizes a "systemic" stressor. A "processive" stressor requires higher order processing whereas a "systemic" stressor does not require the higher order processing (Ziegler et al., 1999). In support of this idea, it has been shown that corticosterone, which facilitates extinction of fear in mice (Brinks et al., 2009), does not facilitate extinction of lithium chloride induced CPA (Tenk et al., 2006). However, this effect is strain dependent (Brinks et al., 2009). Lastly, human studies found cortisol facilitates extinction of phobic fear (Soravia et al., 2006).

Figure 18. *Effect of yohimbine on extinction of conditioned place aversion in C57BL/6J mice.* A) Mice trained with saline and extinguished with saline B) Mice trained with saline and extinguished with yohimbine C) Mice trained with 3.0 mEq/kg lithium chloride and extinguished with saline D) Mice trained with 3.0mEq/kg lithium chloride and extinguished with yohimbine E) Mice trained with 3.5 mEq/kg lithium chloride and extinguished with saline, F) Mice trained with 3.5 mEq/kg lithium chloride and extinguished with yohimbine


Saline
Yohimbine
A
B
C
D
E
F
Seconds
800
600
400
200
0
Black Side
White Side
Pretest
Posttest
Ext 1
Ext 2
Ext 3
Ext 4
Ext 5
Ext 6

CHAPTER VI

GENERAL DISCUSSION

Investigation of extinction behaviors of positive and negative learned behavior is important due to the overlapping human affective disorders such as addiction and post traumatic stress disorder (PTSD). Additionally, these affective disorders may overlap in circuitry which may prove to be either useful or a downfall for therapeutic interventions.

Yohimbine impairment of extinction of cocaine CPP

We observed that yohimbine impaired extinction of cocaine CPP and this has been found to be the case in SA of cocaine (Kupferschmidt et al., 2009). This is very interesting for several reasons. It had previously been shown that yohimbine facilitated extinction of fear conditioning (Caine and Koob, 1994). However, the increase in rate of extinction is only seen within session (Erb et al., 2000; Davis et al., 2008).

This suggests that cocaine CPP and fear conditioning utilize different circuitries in extinction. However, there is an example of a drug, DCS, facilitating the extinction of fear in mice (Walker et al., 2002), cocaine conditioned place preference in rats (Botreau et al., 2006) and enhancing cognitive therapy in humans (Ressler et al., 2004). So, it is likely there is some overlap among all of the behaviors.

Fear conditioning and place preference are two very different behavioral paradigms, of course. The timing of when the behaviors are measured are also drastically different. For CPP extinction, the behavior was measured on a daily basis, i.e. a between

session collection. For fear extinction, the freezing behavior measured is measured within session. When fear extinction was examined 24 hours later, we observed no differences between the control and yohimbine treated group.

Yohimbine impairment of cocaine CPP extinction is independent of α_{2A}-ARs

We found that yohimbine exerted its effect on extinction independently of the α_{2A}-ARs with the utilization of genetic and pharmacological methods. This is particularly interesting since yohimbine is often referred to as the prototypical α_2-AR antagonist. Mice lacking the α_{2A}-ARs showed significant impairment of extinction of cocaine CPP (Davis et al., 2008), suggesting yohimbine is acting at a receptor other than the α_{2A}-ARs. This is not surprising as yohimbine only has a 4.2 fold selectivity for α_2-ARs from 5-HT_{1A} receptors (Newman-Tancredi et al., 1998). Further the effect of yohimbine on extinction was not mimicked by the more selective α_2-AR antagonist, atipamezole (Davis et al., 2008). This lack of ability of atipamezole to replicate yohimbine induced behavior has been reported with prepulse inhibition (Powell et al., 2005) and exploratory behavior (Juhila et al., 2005). Further, yohimbine induced reinstatement of ethanol and sucrose seeking was blocked by an orexin antagonist (Richards et al., 2008), suggesting the orexin receptors are an additional potential target of yohimbine.

The dramatic impairment of extinction in the α_2-AR mutant mice may be due to up regulation of other receptors since the α_{2A}-AR receptor is lacking during development and compensation may occur. However, it is unknown whether there is an upregulation of NET or other adrenergic receptors in the KO mice. Future studies should examine neurochemical and molecular (mal)adaptations in that animal.

Yohimbine induced depression of glutamatergic transmission in the dBNST

Utilizing whole cell patch electrophysiology, we found that yohimbine caused a depression that was not mimicked by atipamezole and was present in α_{2A}-AR KO mice (Davis et al., 2008). However, it was unknown whether yohimbine caused a decrease in the number of cells recruited for the response. Thus we examined the effects of yohimbine in field recordings which has the benefit of measuring the cells stimulated, referred to as the N1. We found that the N1 was unaltered after a 50μM bath application of yohimbine. The dose is different from what was used in our whole cell patch experiments because of the type of chamber used. Whole cell patch experiments occur in submerged chambers whereas our field experiments used interface chambers. Interface chambers are not as efficient in delivering drug to the brain slice. With the finding of a depression of glutamatergic transmission with field recordings, it suggests the depression in glutamatergic transmission seen with whole cell electrophysiology and extracellular field recordings is independent of alterations in the N1.

Yohimbine effect on glutamatergic transmission blocked by SB 334867

Orexin has been shown to play a role in addictive behaviors (Boutrel et al., 2005; Borgland et al., 2006; Richards et al., 2008; Sharf et al., 2008). We observed that a 15 minute pre-application of the antagonist of orexin receptors blocked depression which occurred by yohimbine. This observation suggests that the orexinergic and adrenergic system interact within the CNS. This has also been seen with behavior as yohimbine-mediated reinstatement of ethanol and sucrose seeking is blocked by an orexin antagonist (Richards et al., 2008) .

Another off target site of yohimbine action is the 5-HT_{1A} receptor. Yohimbine only has a 4.2 fold selectivity for the α_2-ARs over 5-HT_{1A} receptor and it acts as a partial agonist at 5-HT_{1A} receptors (Newman-Tancredi et al., 1998). Thus, we attempted to examine the role of the 5-HT_{1A} receptor in the yohimbine induced depression of glutamatergic transmission with the use of a 5-HT_{1A} antagonist. Thus, we were not able to decipher the serotonergic 5-HT_{1A} antagonist WAY 100,135 and yohimbine experiments. This is interesting because it suggests that even blocking the somatodendritic receptor, the decrease in glutamatergic transmission and N1 is due to another receptor. Indeed, WAY 100,135 is a drug that is also non selective (Fornal et al., 1996). Thus, it is likely another system is recruited for the depression seen following co-application of yohimbine and WAY 100,135.

Yohimbine impairs extinction of lithium chloride CPA

We next examined extinction of a negative valence behavior that utilizes a similar set up as CPP. We established lithium chloride induced place aversion using the same place conditioning apparatus (Figure 1). However, instead of pairing lithium chloride with the least preferred side as we do with cocaine, we paired with the preferred black side of the apparatus. Utilization of a negative valence learned task in a setting similar to cocaine CPP would be ideal to compare mechanism of positive and negative valence learned behaviors.

We identified when yohimbine was administered prior to the extinction session, yohimbine impaired extinction of the CPA. This is in contrast to fear extinction data. One possible reason is fear extinction recruits a “processive” stressor circuitry whereas

lithium chloride CPA utilizes a “systemic” stressor. A “processive” stressor requires higher order processing whereas a “systemic” stressor does not require the higher order processing (Ziegler et al., 1999). Another possibility is fear conditioning and extinction uses one context for behavior learning whereas CPA uses to distinct contexts creating an unavoidable and avoidable stressor environment. Yet another possibility is the timing of assessment. Fear extinction study examined the effect of yohimbine on facilitation of learning 30 minutes following yohimbine administration and then 24 hours later whereas CPP extinction is examined 30 minutes after yohimbine administration over days. Therefore, compounds that facilitate extinction of fear may not translate to other negative valence learned behaviors.

Implications

A number of behavioral studies in rats and mice have used yohimbine for a variety of assays as a prototypical α_2-AR antagonist. It has been recently used in the study of reinstatement of positive valence-behaviors (Shepard et al., 2004; Boutrel et al., 2005; Ghitza et al., 2006; Ghitza et al., 2007). Our data suggest that yohimbine may impair extinction of the learned behavior, thus the mice may still have a preference for the CS+ (side or lever) when tested. In cases where mice must reach criteria for extinction and then receive yohimbine to reinstate drug seeking, the reinstatement is likely not due to impaired extinction. A recent study examining extinction of a cocaine SA also found yohimbine impaired extinction learning (Kupferschmidt et al., 2009). Further, we are aware of only one behavioral study in rats that utilized atipamezole and made a comparison to yohimbine treated rats (Powell et al., 2005). Thus, for various behaviors, it is unknown whether yohimbine's actions in reinstating positive valence behaviors are mediated by α_2-ARs or other actions. Interestingly, yohimbine has recently been shown to reduce claustrophobia in humans (Powers et al., 2009). However, with the data of yohimbine impairing extinction of positive valence behaviors, if an individual also suffers from addiction, it may exacerbate the disease.

Orexin is a neuropeptide that was initially investigated for sleep related behaviors (Sakurai, 2007). More recently, orexin has been implicated in addiction related behaviors (DiLeone et al., 2003; Georgescu et al., 2003; Boutrel et al., 2005; Borgland et al., 2006; Richards et al., 2008; Aston-Jones et al., 2009). Our electrophysiological recordings in the dBNST suggest that orexin may be involved in yohimbine induced behavior. This

area requires more investigation as it may provide therapeutic target for addiction intervention.

Overall, our data suggest that it is likely that extinction of fear conditioning and positive valence-learned behavior, CPP, recruit different circuitries. Additionally, with a high overlap of afflictions, such as posttraumatic stress disorder (PTSD) and addiction, a drug may extinguish one behavior and exacerbate the other. Evidence of this is when individuals suffering from PTSD and addiction are shown trauma related stimuli, craving of drugs occurs (Coffey et al., 2002). However, a recent paper suggests there is overlap of the circuitries in the PFC (Peters et al., 2009).

In addition to PTSD, age may play a significant role in extinction. It has been shown that there are high levels of NE in the BNST of young animals compared to old animals (Jorm and Stamford, 1993) as well as a retardation of extinction in animals that receive drug when young (Rodd-Henricks et al., 2002). Lastly, it is important for future studies that utilize yohimbine to also use a more specific α_2-AR antagonist to help elucidate the mechanism behind the behaviors measured after yohimbine administration.

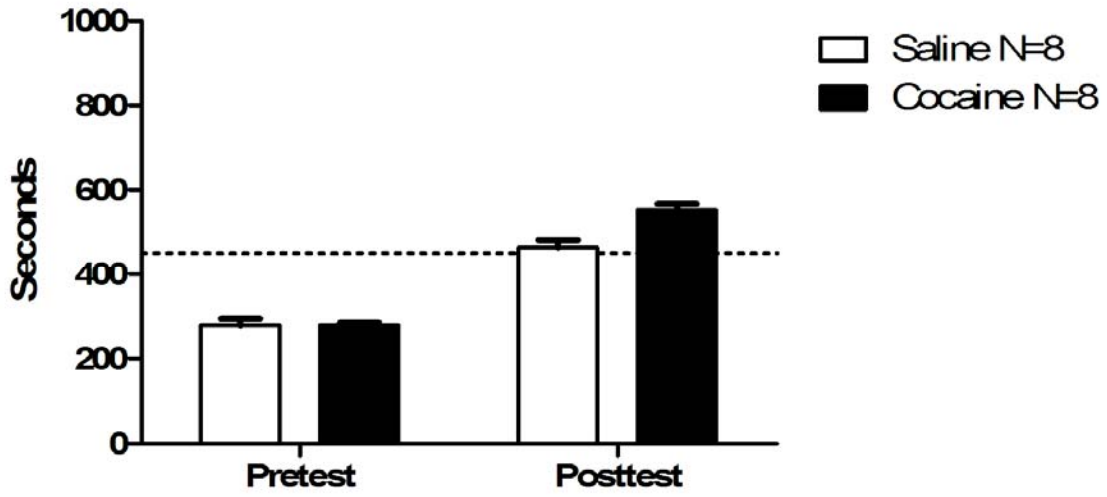
The BNST is part of the extended amygdala and is a key integrator of the limbic systems' influence on the hypothalamic pituitary axis (HPA), which is involved in the stress response. The BNST is a limbic forebrain structure that surrounds the crossing of the anterior commissure (Dong et al., 2001). The amygdala, PFC, and ventral subiculum of the hippocampus send afferents to the BNST (Cullinan et al., 1993; Canteras et al., 1995; Crane et al., 2003). Consistent with the idea of the BNST as a key regulator, stimulation in the posterior region is involved in inhibition of the HPA axis (Dunn and Berridge, 1990) whereas stimulation of the anterior region stimulate HPA activity (Dunn

and Berridge, 1990). The BNST also projects to the VTA, which is a part of the reward pathway. It sends a glutamatergic projection to the VTA, suggesting activation of the BNST causes excitation of the VTA, thus modulating dopamine release (Georges and Aston-Jones, 2002). The combined information of the role of the BNST suggests that this region could play a critical role in stress-induced relapse. Additionally, with the data that yohimbine causes a depression in the BNST (Davis et al., 2008) and yohimbine induced reinstatement blocked by orexin antagonist (Richards et al., 2008), it suggests the BNST may play a role in the impairment of extinction of cocaine CPP.

The most frequently cited reason by addicts for using drugs again after a period of being abstinent is stress (Sinha et al., 1999). Our data suggests that either 1) yohimbine may work through a mechanism independent of the α_2-AR or 2) that the α_2-AR play a specific role in reinstatement and does not play a role in extinction, suggesting two distinct circuitries. Studies that use inactivation of different brain regions could shed light on differences in neural substrates required for each behavior.

Appendix A

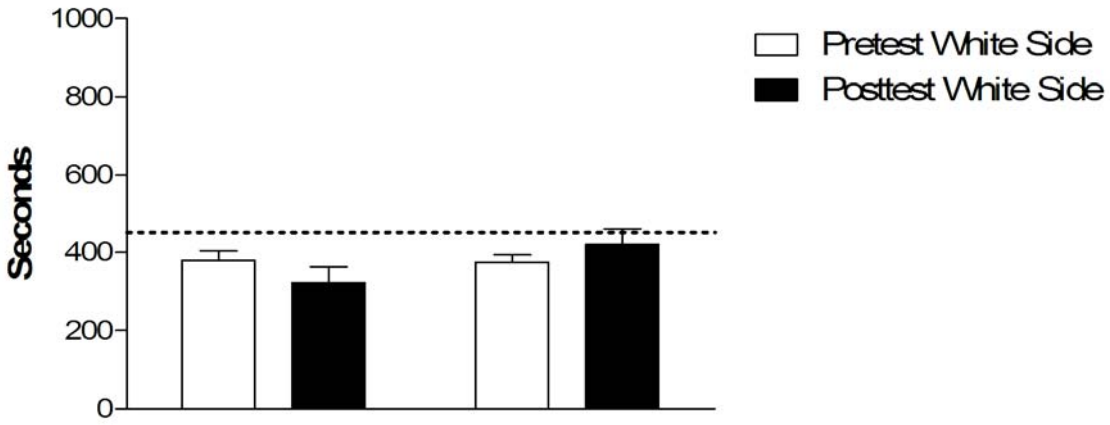
The CPP apparatus used in all of the studies is biased, in that the mice prefer the black side of the chamber. This is expected as innately mice prefer dark places as well as corners. This bias introduces anxiety as a factor which may or may not prevent place preference. We investigated whether or not we could obtain place preference with no lights on, relying only on the textural differences between the two sides of the chamber. The black side of the chamber has a smooth floor and the white side has a textured floor. It has been shown that rats will obtain place preference without lighting (Roma and Riley, 2005) but unexplored in mice. The C57BL/6J mice used in the following study were delivered at 6 weeks of age and acclimated to the facility for one week, thus at the time of CPP training, they were 7 weeks of age. The cocaine group was trained with 10 mg/kg cocaine given i.p. and placed in chamber for 15 minutes (900 seconds).
Day 1: Pretest
Day 2: Morning- Both groups received saline, black side
Day 2: Afternoon- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 3: Morning- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 3: Afternoon- Both groups received saline, black side
Day 4: Morning- Both groups received saline, black side
Day 4: Afternoon- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 5: Posttest



As seen here in a small cohort of mice, we were able to obtain modest place preference in C57Bl6/J mice. This implicates that the texture alone in our apparatus is sufficient enough to obtain place preference. Dotted line indicates half of session, 450 seconds.

Appendix B

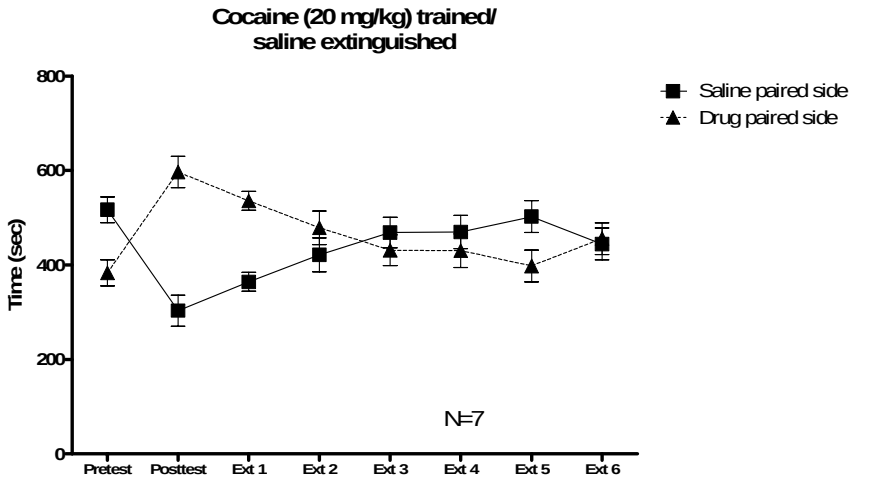
We utilized Swiss Webster mice for CPP. However, we were not able to obtain place preference in these mice with 20 mg/kg of cocaine, i.p. The pharmacokinetic profile of these mice is likely different from that of C57BL/6J mice. Also, many studies utilize doses far greater than 20 mg/kg without any negative effect reported.
Day 1: Pretest
Day 2: Morning- Both groups received saline, black side
Day 2: Afternoon- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 3: Morning- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 3: Afternoon- Both groups received saline, black side
Day 4: Morning- Both groups received saline, black side
Day 4: Afternoon- Cocaine group received cocaine and placed on the white side and saline group received saline and placed on white side
Day 5: Posttest

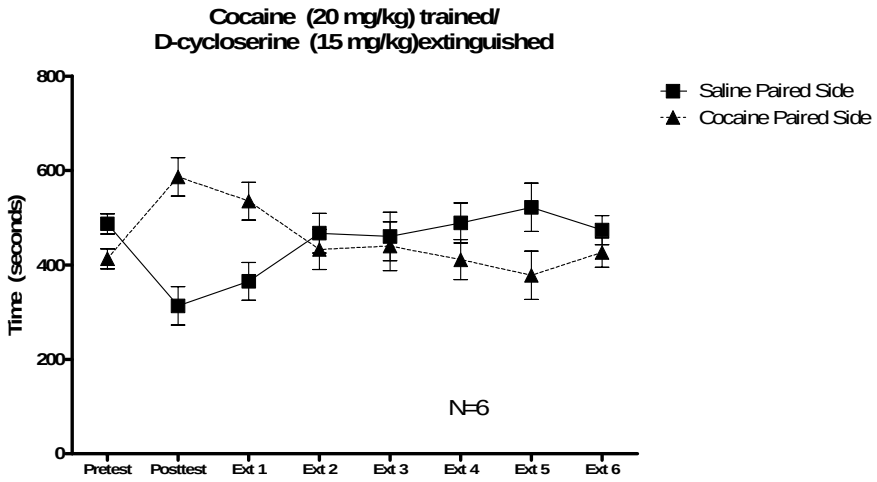


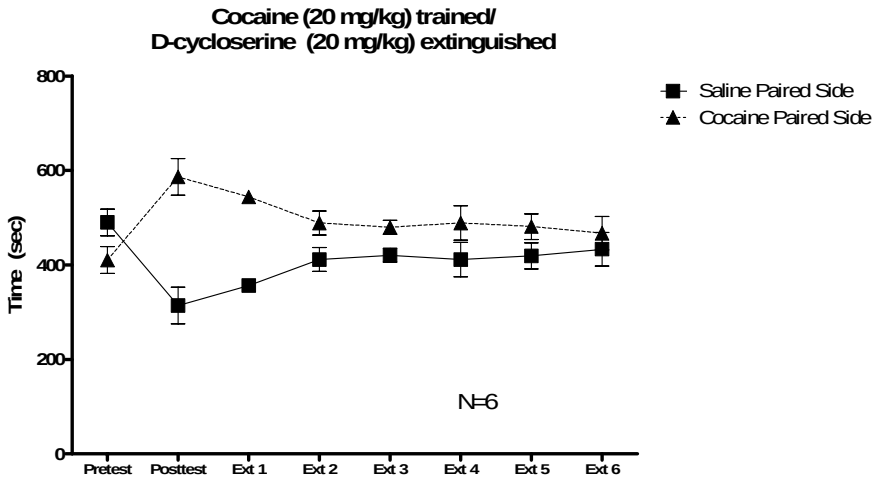
Dotted line indicates half of test session, 450 seconds.

Appendix C

We examined the effect of d-cycloserine (DCS) on extinction of cocaine CPP. It has previously been shown that facilitated extinction of fear in (Ledgerwood et al., 2005) and cCPP in rats (Botreau et al., 2006). Additionally DCS has been effective in the human affective condition of anxiety (Davis et al., 2008). Our extinction paradigm is identical to what was used in examining the effect of yohimbine on extinction (Davis et al., 2008). As seen here, we did not see the facilitation of extinction in C57BL/6J mice. N=7 for saline extinguished and N=6 for other two DCS groups.

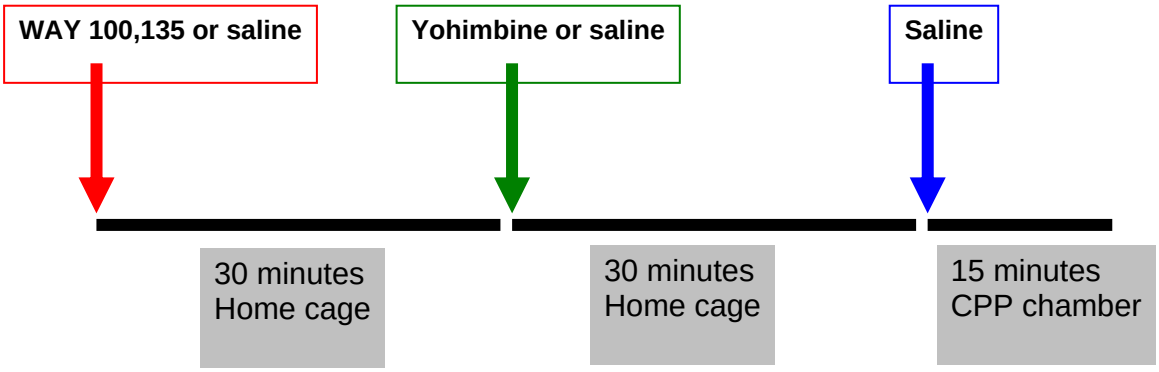

Cocaine (20 mg/kg) trained/
saline extinguished
Saline paired side
Drug paired side
Time (sec)
800
600
400
200
0
N=7
Pretest
Posttest
Ext 1
Ext 2
Ext 3
Ext 4
Ext 5
Ext 6


Cocaine (20 mg/kg) trained/
D-cycloserine (15 mg/kg)extinguished
Saline Paired Side
Cocaine Paired Side
Time (seconds)
800
600
400
200
0
N=6
Pretest
Posttest
Ext 1
Ext 2
Ext 3
Ext 4
Ext 5
Ext 6

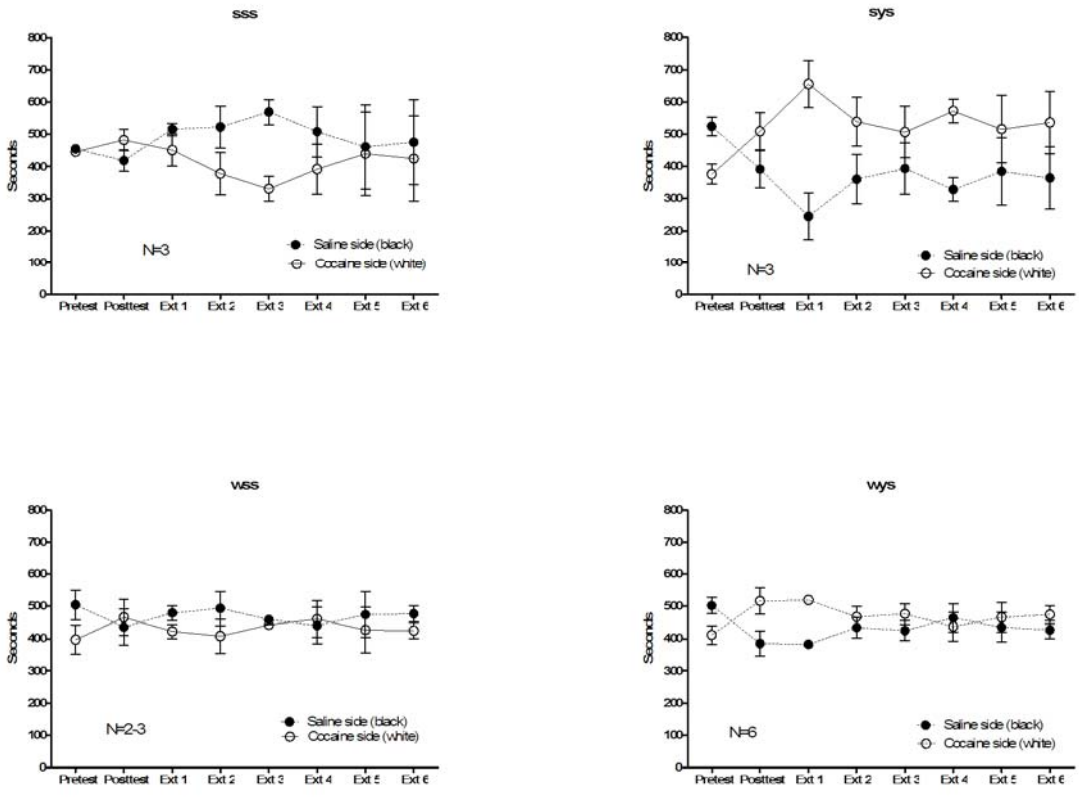

Cocaine (20 mg/kg) trained/
D-cycloserine (20 mg/kg) extinguished
Saline Paired Side
Cocaine Paired Side
Time (sec)
800
600
400
200
0
N=6
Pretest
Posttest
Ext 1
Ext 2
Ext 3
Ext 4
Ext 5
Ext 6

Appendix D

Preliminary studies were conducted to determine if WAY 100,135, a 5-HT_{1A} antagonist could block the effect of yohimbine on extinction of cocaine CPP. Administration of the drug/timing is illustrated below. All mice were trained with 20 mg/kg cocaine

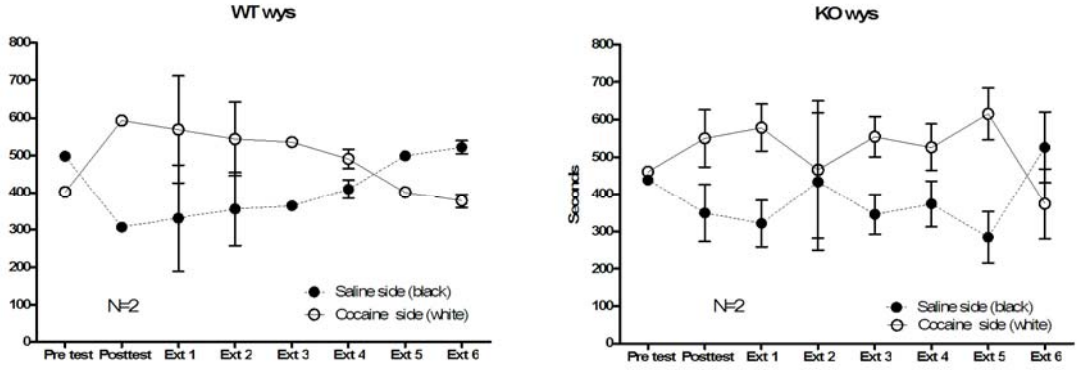


Results obtained with C56BL/6J adult male mice.



SSS= online saline injections
SYS= saline, yohimbine, saline injection (according to the schedule listed above)
WSS= WAY 100,135, saline, saline
WYS= WAY 100, 135, yohimbine, saline

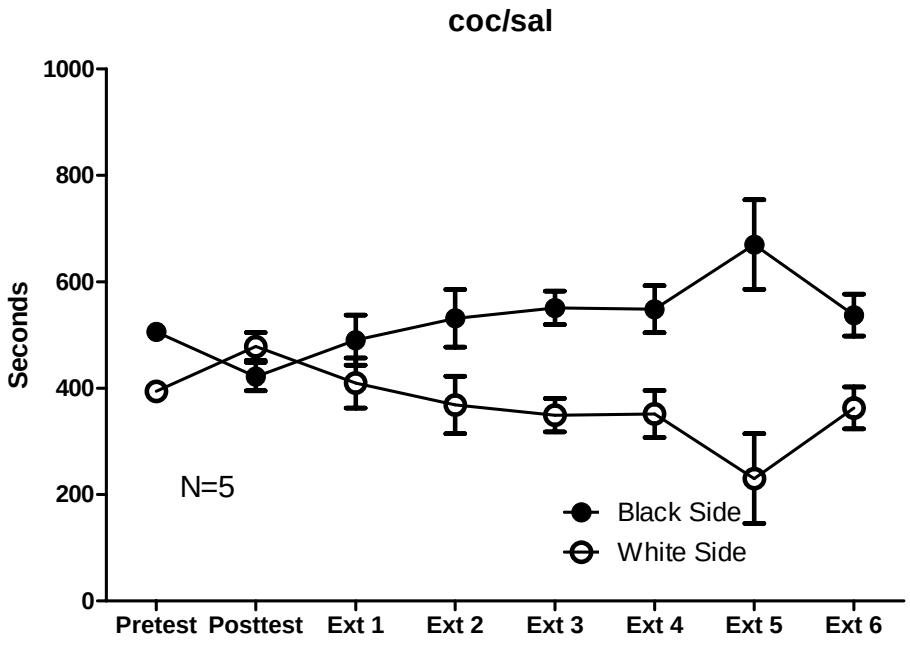
We also performed some WAY 100,135 experiments in α_{2A}-AR KO mice and WT littermates.

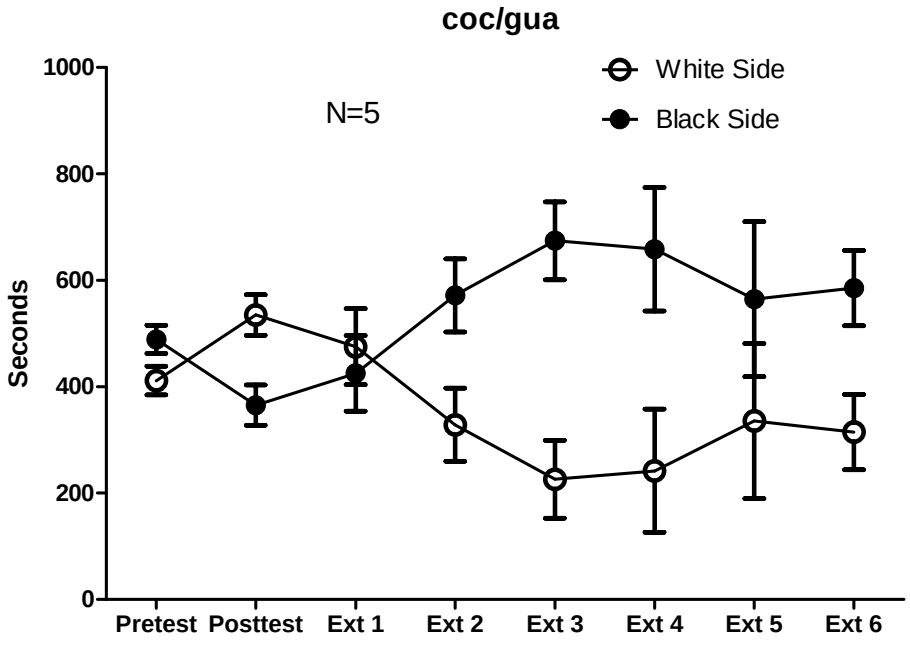


Both groups received WAY 100,135, yohimbine then a saline injection.

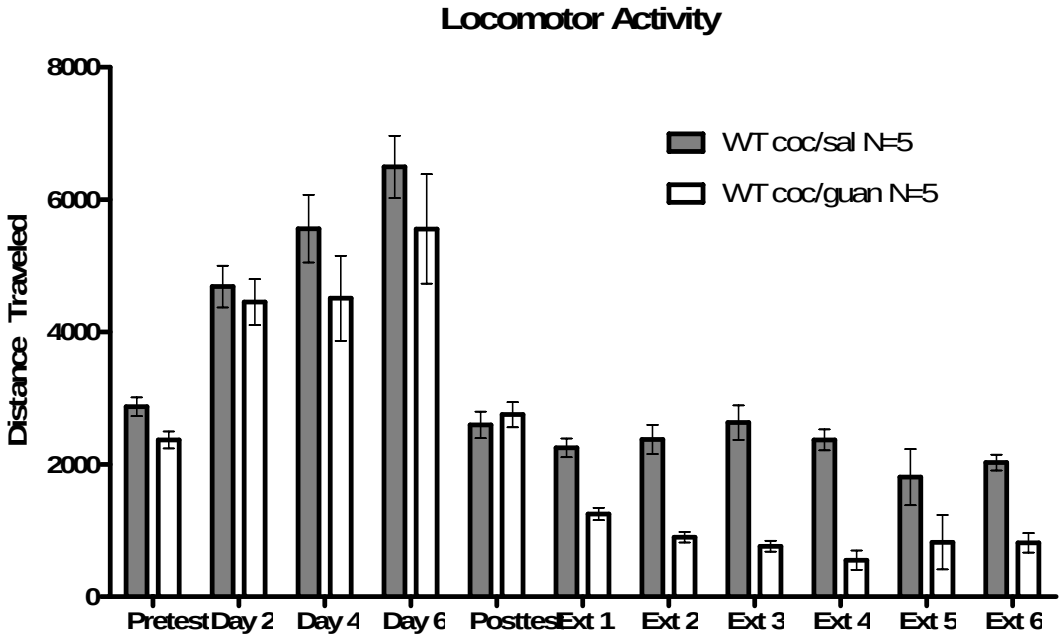
Appendix E

In order to examine the effect of the α_{2A}-AR on extinction of cocaine (20 mg/kg) CPP pharmacologically, we utilized guanfacine, an α_{2A}-AR agonist. We used 1.0 mg/kg based on a previous dose that was not reported to cause sedation (Franowicz et al., 2002).





We did not see a trend towards an alteration in extinction behavior. However, our mice did show a trend for impaired mobility, thus complicating the interpretation of these data.



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