THE ROLE OF ADRENERGIC RECEPTORS IN THE POTENTIAL RECONSOLIDATION OF A COCAINE CONDITIONED PLACE PREFERENCE

by

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS

α-AR – alpha-adrenergic receptor

α_1-AR – alpha-adrenergic receptor, subtype 1

α_2-AR – alpha-adrenergic receptor, subtype 2

ANOVA – analysis of variance

ANR – acquisition of a new response

AR – adrenergic receptor

ASO – antisense oligonucleotide

β-AR – beta-adrenergic receptor

β_1-AR – beta-adrenergic receptor, subtype 1, receptor

β_2-AR – beta-adrenergic receptor, subtype 2, receptor

BLA – basolateral amygdala (including lateral, basolateral, and accessory basal nuclei)

BNST – bed nucleus of the stria terminalis

cAMP – cyclic AMP

CeA – central amygdala

C/EBPβ – CCAAT enhancer binder protein β

CER – conditioned emotional response

CPA – conditioned place aversion

CPP – conditioned place preference

CR – conditioned response

CREB – cAMP-response element-binding protein

CS – conditioned stimulus

CTA – conditioned taste aversion

DA – dopamine

D-APV – D(-)-2-amino-5-phosphonopentanoic acid

DA-R – dopamine receptor

DA_1-R – dopamine, subtype 1, receptor

DCS – D-cycloserine

ECS – electroconvulsive shock

FOS-IR – FOS immunoreactivity

GABA – γ-aminobutyric acid

$GABA_A$-R – γ-aminobutyric acid, subtype A, receptor

$GABA_B$-R – γ-aminobutyric acid, subtype B, receptor

HCl – hydrochloride

IEG – immediate early gene

i.p. – intraperitoneal

LC – locus coeruleus

LTP – long-term potentiation

mAch-R – muscarinic acetylcholine receptor

MAPK – mitrogen-activated protein kinase

MEK – mitrogen-activated protein kinase kinase

NAcc – nucleus accumbens

NE – norepinephrine

NMDA – *N*-methyl-D-aspartate

NMDA-R – *N*-methyl-D-aspartate receptor

NTS – nucleus of the solitary tract

PBS – phosphate-buffered saline

PET – positron emission tomography

PKA – protein kinase A

PSI – protein synthesis inhibitor

PTSD – post-traumatic stress disorder

S1 – stimulus one

S2 – stimulus two

SA – self-administration

UR – unconditioned response

US – unconditioned stimulus

VTA – ventral tegmental area

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ABSTRACT

The disruption of memory retention following retrieval has been proposed to be due to the impairment of a memory reconsolidation process. Deficits in behavior attributed to reconsolidation have been demonstrated in a number of learning paradigms in animal models, including the associative learning that occurs between neutral stimuli and drugs of abuse. Because drug-associated stimuli can be a major factor in the persistence of addiction in humans, targeting potential reconsolidation mechanisms has been suggested as a potential target of pharmacotherapies aimed at dampening the powerful control of these stimuli over behavior. To that end, several studies have demonstrated impairment of reconsolidation as a means to reduce drug cue-mediated behaviors in animals using a variety of pharmacological treatments.

The focus of this dissertation was to examine the role of the noradrenergic system as a potential mediator of the reconsolidation of drug memories using the conditioned place preference (CPP) paradigm using adrenergic receptor (AR) antagonists administered systemically and site-specifically into the basolateral amygdala, a brain region previously demonstrated to mediate reconsolidation. The non-specific β_1/β_2-AR antagonist, propranolol, when systemically administered following an initial test of cocaine CPP, attenuated preference during a subsequent test. This result was then replicated with systemic adminstration of the β_2-AR antagonist ICI 118,551, but not the β_1-AR antagonist betaxolol, demonstrating a β_2-specific mechanism of the effect of propranolol. Furthermore, the α_1-adrenergic antagonist prazosin, when administered post-test, also attenuated a subsequent preference.

Because the BLA has been demonstrated to play an important role in reconsolidation processes in both drug and nondrug conditioning paradigms, the FOS response in the BLA was examined following the expression of a cocaine CPP, and indicated that the BLA is one potential locus of the response to cocaine-conditioned cues, as indicated by an increase in the FOS response compared to controls, and a potential site of reconsolidation. Subsequently, both ICI 118,551 and prazosin, administered directly into the BLA following an initial test of preference, both impaired cocaine CPP upon subsequent testing, consistent with the systemic results demonstrated earlier.

These findings are consistent with a growing literature targeting reconsolidation mechanisms in a variety of learning mechanisms using adrenergic antagonists. Furthermore, targeting potential reconsolidation mechanisms via noradrenergic blockade represents a unique way to examine cue-induced drug-mediated behaviors in animals that may provide insight into new treatments for cue-mediated drug-seeking in humans.

CHAPTER 1. General Introduction

For animals and humans to effectively function and survive in their environments, they must be able to acquire and store rapidly changing information regarding their surroundings. Memory formation is the process by which this information is stored-- the process that occurs following a learning event that confers stability to the information being learned-- so as to appropriately guide behavior upon retrieval of this new information. The nature of how information is processed and stored as memory and how long it persists has been a well-studied phenomenon. Some knowledge comes from examining disorders in humans in which some aspect of memory is impaired. For instance, retrograde or anterograde amnesia, the loss of old memories or the inability to form new memories, respectively, can be seen in a number of conditions, most strikingly in those causing hippocampal damage or general temporal lobe impairment, as temporal lobe structures have been shown to be critical in effective memory processing in humans (reviewed in Squire and Alvarez, 1995). However, memory impairments in humans are often difficult to characterize, as the nature of the damage is rarely confined to a single brain area, and because it is impossible to determine precisely what information was stored prior to damage (Nadel and Bohbot, 2001).

Therefore, much of what we know about the processes that occur during memory formation has come from animal studies. One goal of research examining the processes that underlie learning and memory formation in animals is to better understand the processes by which memories can become maladaptive in humans, as is the case in learned fears, post-traumatic stress disorder (PTSD), and drug addiction. For instance,

drug dependence is characterized by high rates of relapse, of which cues previously associated with drugs of abuse are a major contributor (O'Brien et al., 1998; Weiss, 2005). Studies in animals have suggested that the cue-induced retrieval of drug memories renders these memories labile and disruptable (Bernardi et al., 2007; Miller and Marshall, 2005b). This work has attempted to diminish the motivational properties of drug-related cues with pharmacological manipulations administered at the time of cue exposure. To that end, a quickly expanding preclinical literature has identified several promising lines of evidence as to the precise neurobiological mechanisms involved in potential post-retrieval processes, which are largely consistent with those mechanisms known to be involved in memory formation.

Memory Consolidation

Memory can last for a brief time, or can be long-lasting, a distinction typically dependent on the number, duration, and/or salience of learning events (Sweatt, 2003). Information that decays rapidly, lasting seconds to minutes is generally referred to as short-term memory, while information lasting hours to days and beyond is generally referred to as long-term memory (Stough et al., 2006). Although the precise nature of the relationship between short- and long-term information processing remains to be completely elucidated (Dudai, 2004), the term “consolidation” is in general ascribed to the process by which information is converted into a long-term memory trace (Dudai, 2004). Evidence for consolidation comes from a variety of research approaches, including, for example, neuroimaging studies (Bontempi et al., 1999), gene expression

studies (Guzowski and McGaugh, 1997), lesion studies (Anagnostaras et al., 1999), and most importantly, pharmacological approaches (Ferry et al., 1999a). Pharmacological manipulations of consolidation in animal studies have demonstrated the possibility of clinically enhancing and impairing adaptive and maladaptive memories, respectively (reviewed in McGaugh and Roozendaal, 2009). In fact, much of our understanding of memory consolidation is based on research that has examined how this process occurs using such pharmacological approaches. Memory consolidation has typically been demonstrated by the existence of gradients of retrograde amnesia, such that increasing the interval between the acquisition of information and the administration of various amnestic treatments decreases the effectiveness of such treatments in producing retention deficits, indicative of memory consolidation as a time-dependent process that occurs after learning (reviewed in Gold, 2006; McGaugh, 1966, 2000).

Cellular mechanisms of consolidation

Global treatments such as systemic protein synthesis inhibitors (PSIs) (Davis et al., 1976; Lu et al., 2007) have been demonstrated to impair consolidation, and do so in a temporally graded manner (Andry and Luttges, 1972; Kopp et al., 1966). Studies such as these provide just some of the evidence for the important role of protein synthesis in memory consolidation (reviewed in Davis and Squire, 1984), and the current, generally accepted view that newly acquired information is initially labile, but following a time-dependent consolidation process memory is resistant to interference (McGaugh, 1966; Squire and Alvarez, 1995). Although protein synthesis is critical in long-term memory

formation, it is now known that long-term memory storage involves a cascade of cellular and molecular events initiated by the original experience that lead to RNA transcription and the translation of new proteins involved in synaptic modifications thought to mediate memory formation (reviewed in Abel and Lattal, 2001; Silva, 2003). Studies of long-lasting synaptic plasticity thought to underlie memory consolidation that have examined long-term potentiation (LTP), as well as behavioral assessments of memory, have identified several cellular events mediating gene expression and subsequent protein synthesis. Ca^{2+}influx through the *N*-methyl-D-aspartate receptor (NMDA-R), increases in cyclic AMP (cAMP), activation of protein kinase A (PKA), and phosphorylation of cAMP-response element-binding protein (CREB) are now known to be important cellular mediators of gene expression and protein synthesis required for memory consolidation (reviewed in Abel and Lattal, 2001; Silva, 2003). Furthermore, this NMDA/cAMP/PKA/CREB signal transduction cascade, as well as the mitrogen-activated protein kinase (MAPK) cascade, which is also involved in plasticity-associated gene expression (Keifer et al., 2007; Kelleher et al., 2004), can be modulated by neurotransmitter systems that alter levels of cAMP through G protein-coupled receptors, thus modulating memory formation.

Consistent with this more intricate understanding of the cellular mechanisms involved in memory formation, selective post-training systemic pharmacological manipulations of NMDA-R antagonists (Ciamei et al., 2000), γ-aminobutyric acid receptor ($GABA_B$-R) agonists (Castellano et al., 1989), β–adrenergic (β-AR) antagonists (Cahill et al., 2000), dopamine receptor (DA-R) antagonists (Castellano et al., 1991), and muscarinic acetylcholine receptor (mAch-R) receptor antagonists (Roldan et al., 1997) have all been

demonstrated, mostly in aversive tasks, to impair memory consolidation. A key feature of these consolidation studies has been a post-learning isolation of this process. Post-learning manipulations have been critical for distinguishing between effects on learning, or the acquisition of information, and post-learning consolidation. Pre-conditioning manipulations may not impair consolidation but the initial acquisition of information (Abel and Lattal, 2001).

In animal studies, consolidation is typically measured by utilizing associative learning paradigms. In associative learning, animals learn an association between a neutral stimulus, such as a tone or light, and an unconditioned stimulus (US), such as a food reward or footshock, which by itself elicits an unconditioned behavioral response (UR). For example, in fear paradigms, in which footshock serves as the US, escape behavior is typically the UR (e.g., Lattal et al., 2007). After one or more pairings, the previously neutral, conditioned stimulus (CS) comes to reliably predict the US, and exposure to the CS in the absence of the US elicits a conditioned response (CR) oftentimes different from the UR elicited by the US (e.g., freezing as a CR when increased activity is the UR). This CR indicates learning of, and subsequent retrieval of a memory for, the CS-US association.

Memory Reconsolidation

As indicated above a great deal of interest has focused on how initially labile information is strengthened into the formation of a permanent, long-term memory. In addition, the processes that occur when memory is retrieved or reactivated have also

generated a lot of interest, as these reactivated memories guide behavior based on past experiences and allow the integration of new information related to changes in the current environment. Interestingly, retrograde amnesia has also been shown to occur in both humans and animals when an amnestic treatment is administered following the reactivation of a memory (Kindt et al., 2009; Misanin et al., 1968; Nader et al., 2000), indicating that changes in the retention of previously consolidated information can occur during memory retrieval.

In the first reported demonstration of an experimentally-induced post-retrieval memory impairment, Misanin et al. (1968) used a conditioned emotional response (CER) paradigm to measure the ability of electroconvulsive shock (ECS) to produce a memory deficit when administered following a CS-only re-exposure trial in rats. The authors paired a white noise (CS) with a footshock (US) to elicit a reduction in drinking (UR) during conditioning trials. Twenty-four hours later, ECS was administered via ear clips following either a brief re-exposure to the CS and subsequent CR (reduction in drinking) or no re-exposure. Twenty-four hours later, all rats were given a CS-only exposure trial. Rats that received ECS during the first CS-only trial showed an attenuated drinking CR (i.e., less impairment of drinking) compared to rats that received ECS without cue exposure the day before. The authors concluded that ECS produced amnesia for the CS-US association, as evidenced by reduced fear to the CS, suggesting that memory deficits could be induced not only following a CS-US learning session, but also following exposure to cues associated with that learning event (Misanin et al., 1968).

This phenomenon has more recently been termed “reconsolidation” in reference to the somewhat controversial interpretation that memories must undergo consolidation again

following retrieval to remain a long-term memory trace and are sensitive to disruption (Nader et al., 2000; Przybyslawski and Sara, 1997). Importantly, this theoretical explanation for deficits in retention following retrieval is inconsistent with the notion, as mentioned above, that memory, once consolidated, is permanent (McGaugh, 1966; Squire and Alvarez, 1995). As alluded to above, in studies examining reconsolidation in animals, sometime after a CS is paired with a US during learning, an amnestic treatment is administered before or after the presentation of a nonreinforced exposure to the CS. This CS-only exposure is intended as a retrieval trial designed to reactivate the memory of the US, as indicated by an appropriate behavioral response. Again, at some point later, subjects are tested for their response to the CS, and impairments in this response as compared to vehicle-treated controls are attributed to a disruption of the reestablishment of that memory via a consolidation process similar to that which occurred following initial learning. A critical control is the administration of the amnestic treatment in the absence of the CS, and/or administration of the amnestic treatment several hours post-CS exposure, outside the presumed window of effectiveness of such treatments on memory processes. This control implies that the behavior impairment caused by the amnestic treatment was due to reactivation of the initial memory as opposed to a residual, non-specific effect of the drug on behavior.

Cellular mechanisms of reconsolidation

Consistent with that seen with consolidation, impairment of reconsolidation in associative learning paradigms in animals has also been demonstrated using systemic

administration of PSIs (Judge and Quartermain, 1982; Milekic and Alberini, 2002; Pedreira and Maldonado, 2003), and the effectiveness of amnestic treatments has similarly shown a temporal dependency following cue exposure (Milekic and Alberini, 2002). Futhermore, several studies have employed systemic administration of drugs targeting specific receptor systems known to be either directly involved, or having a modulatory role, in learning and memory consolidation, and have subsequently demonstrated impairment of reconsolidation using NMDA-R antagonists (Lee et al., 2006; Suzuki et al., 2004), β-AR antagonists (Przybyslawski et al., 1999; Roullet and Sara, 1998), choline uptake inhibitors (Boccia et al., 2004; Boccia et al., 2006) and DA_1-R antagonists (Sherry et al., 2005). And although the majority of research investigating reconsolidation has focused on aversive learning paradigms, such as those referenced above, impairments have also been demonstrated in appetitive learning tasks using similar systemic pharmacological manipulations, including, for example, PSIs, NMDA-R and β-AR antagonists (Lee and Everitt, 2008; Przybyslawski et al., 1999; Stollhoff et al., 2008).

Memory processes in substance abuse

A great deal of research has focused on the molecular, cellular, and neural substrates of learned associations between discrete and contextual cues paired with drugs of abuse in animals. Exposure to stimuli associated with drugs of abuse is an important contributor to the persistence of addiction in humans, because in the absence of drug these stimuli can trigger responses in drug abusers that mimic those of the drug itself,

including physiological arousal and euphoria, that lead to craving and drug-seeking behaviors (Childress et al., 1988a, 1988b; Johnson et al., 1998; Negrete and Emil, 1992; O'Brien et al., 1992, 1998; Weiss, 2005). Current theories attribute the powerful control of these stimuli over behavior to maladaptive plasticity via a usurpation of neural mechanisms of learning and memory that normally subserve behaviors related to the acquisition of natural rewards and the cues that predict these rewards (Hyman, 2005; Kelley, 2004).

In terms of cocaine-mediated cue learning, a number of studies, mostly using CPP, have demonstrated the ability of a variety of pharmacological manipulations known to be involved in learning and memory, as indicated above, to impair drug-mediated preference. Most notably, the concurrent activation of NMDA-Rs and DA-Rs is likely one process involved in the attribution of salience to drug cues (reviewed in Kelley, 2004), as impairments of cocaine CPP have been demonstrated by antagonists of both NMDA-Rs (Cervos and Samanin, 1995; Kim et al., 1996; Kotlinska and Biala, 1999) and DA-Rs (Cervo and Samanin, 1995; Moreney and Beninger, 1986; Nazarian et al., 2004). Other pharmacologic treatments demonstrated to affect learning and memory have also been shown to impair cocaine preference, including, but not limited to, cholinergic antagonists and GABAergic drugs (reviewed in Tzschentke, 2007). However, these studies have largely employed pre-conditioning manipulations, making it difficult to parse out effects on acquisition of a drug-cue association versus the long-term consolidation of that association.

Very few studies have examined the consolidation of drug-cue associations using post-training systemic manipulations, although findings derived from such manipulations

are consistent with those described above mediating other forms of associative learning. Rats administered ECS immediately following a single cocaine-context pairing in a conditioned sensitization paradigm showed an impairment in the sensitized locomotor response to cocaine on a subsequent test, suggesting an impairment in associative learning. ECS had no effect when administered one hour after training, outside of the presumed window of the effectiveness of ECS on learning processes (Rothman and Pert, 1994). Using CPP in mice, Kuo et al. (2007) demonstrated that systemic post-conditioning trial injections of the PSI anisomycin disrupted a subsequent cocaine CPP, again suggesting impairment of consolidation. Although the data are limited, the consolidation of drug-mediated learning appears to be protein synthesis-dependent, consistent with the findings outlined above for other associative learning tasks, suggestive of similar memory processes.

Findings from studies examining reconsolidation using systemic manipulations in drug learning paradigms in animals have been fairly consistent with those outlined above, namely that reconsolidation of drug-cue associations requires protein synthesis and more specifically the activity of receptor systems known to be involved in memory consolidation. Furthermore, impairments in reconsolidation have been demonstrated using many different drug conditioning paradigms using a variety of drugs of abuse. For example, in the case of cocaine conditioning studies, systemic PSIs have been shown to impair reconsolidation in a conditioned locomotor sensitization paradigm (Bernardi et al., 2007). Reconsolidation of cocaine CPP has been disrupted by NMDA-R antagonists (Brown et al., 2008), β-AR antagonists (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008), and mAch-R antagonists (Kelley et al., 2007). β-AR antagonists have

also impaired reconsolidation in a cocaine cue-mediated acquisition of a new response (ANR) paradigm (Milton et al., 2008b). Thus, consistent with findings in other appetitive associative learning paradigms, as well as those in aversive learning paradigms, drug-mediated cue associations appear to undergo reconsolidation following retrieval that may be dependent upon receptor systems and subsequent protein synthesis involved in the initial consolidation of the memory.

Systems involved in memory consolidation and reconsolidation: convergence on the amygdala

The amygdala has been implicated as a site critical in the formation of CS-US associations. Traditionally thought of for its role in classically conditioned fear (LeDoux, 2000), it is now known that the amygdala-- specifically the basolateral amygdala (BLA), which includes the lateral, basal and accessory basal nuclei of the amygdala-- is also involved in appetitive conditioning (reviewed in McGaugh, 2004). It is now believed that the BLA is a site of covergence of information regarding conditioned and unconditioned stimuli during learning, and thus is involved in the attribution motivational significance to previously neutral stimuli (Holland and Gallagher, 1999; Hatfield et al., 1996; Parkinson et al., 2001)(Figure 1A). The BLA receives projections from olfactory cortex, and taste and visceral pathways, as well as receiving sensory information from the sensory association cortex (Knapska et al., 2007; Otterson and Ben-Ari, 1979; Turner and Herkenham, 1991). The BLA also receives DA and NE projections from the ventral tegmental area (VTA) and locus coeruleus (LC) (Asan, 1998; Fallon et al., 1978),

respectively. DA and NE, via DA_1-Rs and β-ARs, respectively, are the likely mediators of salience endowed upon neutral cues by rewarding or aversive unconditioned stimuli via modulation of NMDA-mediated transmission (reviewed in Harley, 2004). The BLA is thought to mediate learning as well as the associative and motivational influences of conditioned cues on behavioral output via its excitatory projections, especially to the hippocampus, nucleus accumbens (NAcc) and central nucleus of the amygdala (CeN)(Brog et al., 1993; Everitt et al., 1999; Kelley et al., 1982)(Figure 1A).

Because the BLA has been extensively identified as a site critical for the establishment of motivated, associative learning (See, 2005), many studies have focused on the role of the BLA in memory consolidation (for review, see Pare, 2003). For example, reiterating the importance of protein synthesis in memory consolidation, as well as the importance of the BLA in mediating these effects, Maren et al. (2003) found that infusions of the PSI anisomycin into the BLA of rats immediately following conditioning impaired freezing to both auditory and contextual stimuli associated with footshock upon subsequent CS presentation in a Pavlovian fear conditioning paradigm. PSI in the BLA has also been shown to impair consolidation in appetitive learning paradigms (Wang et al., 2005). These effects are likely mediated through NMDA-mediated transmission, as post-training administration of the NMDA receptor antagonists 2-amino-5-phosphonopentoic acid (AP5) and MK-801 into the BLA have also been found to impair consolidation (Liang et al., 1994).

Reconsolidation has also been shown to involve protein synthesis in the BLA. Nader et al. (2000) used a fear conditioning paradigm to show that rats administered intra-BLA infusions of anisomycin following re-exposure to an auditory cue previously paired with

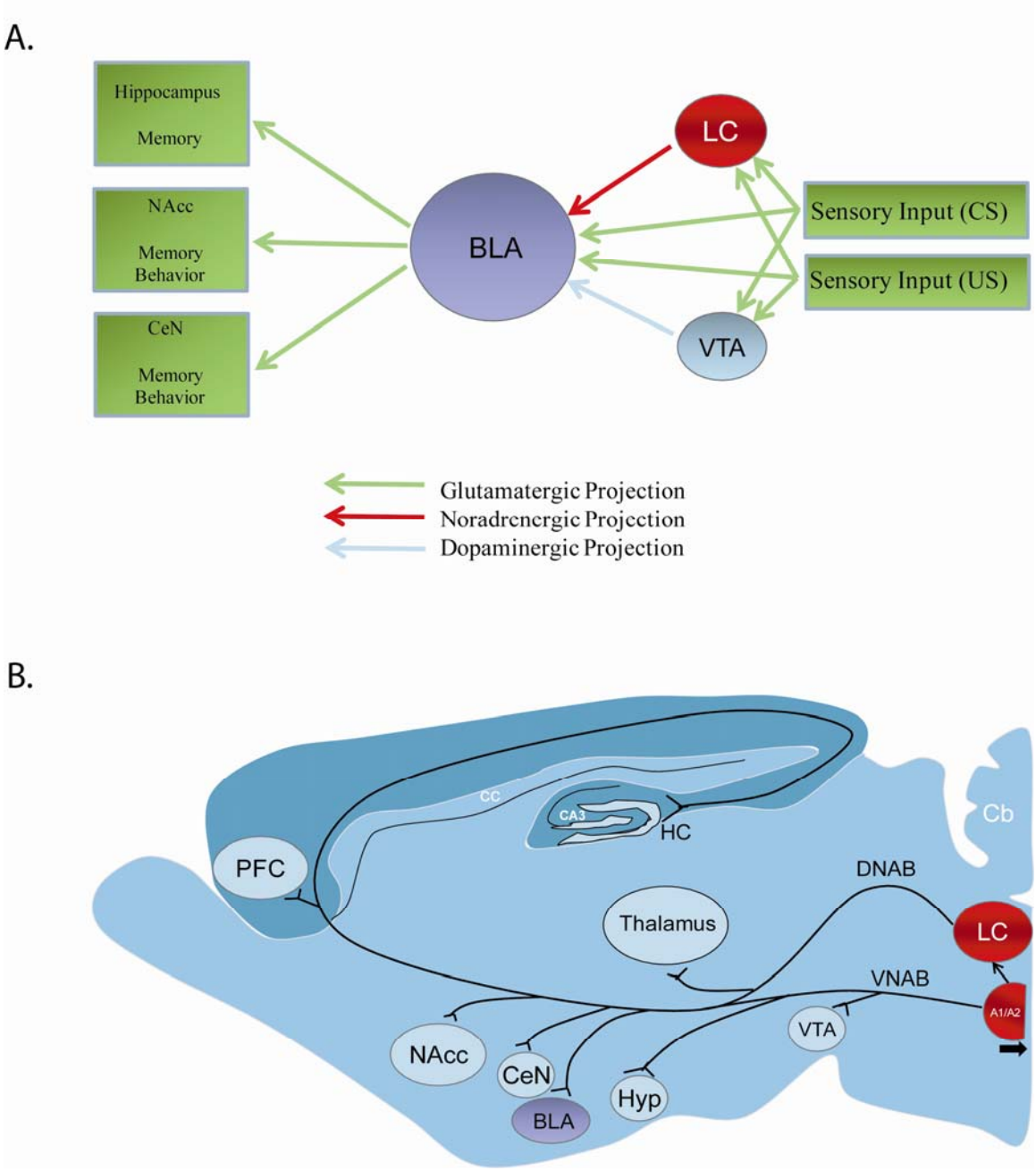

FIGURE 1. Circuitry involved in the consolidation of salient stimuli.

(A) Projections to and from the BLA involved in memory consolidation and behavior.

The BLA receives convergent sensory inputs relating information about neutral (CS) and

arousing (US) stimuli, likely responsible for associative learning. Norepinephrine and dopamine are thought to play modulatory roles in consolidation of associative learning. (B) Schematic representation of a number of norepinephrine projections arising from the locus coeruleus (LC) and the nucleus of the solitary tract (A1/A2). The BLA receives projections from both the DNAB and VNAB. Figure B modified with permission from Gang Chen and Laura Kozell. BLA, basolateral amygdala; CeN, central nucleus of the amygdala; Cb, cerebellum; cc, corpus callosum; DNAB, dorsal noradrenergic bundle; HC, hippocampus; Hyp, hypothalamus; NAcc, nucleus accumbens; PFC, prefrontal cortex; VNAB, ventral noradrenergic bundle; VTA, ventral tegmental area.

footshock showed impaired conditional freezing in response to the cue 24 hr later. Furthermore, anisomycin treatment in the absence of re-exposure to the cue left the memory and fear response intact during subsequent testing. Nader et al. concluded that reactivated fear memories return to a labile state and undergo a reconsolidation process that, like consolidation, is dependent upon the BLA. PSI in the BLA has also been shown to impair reconsolidation in appetitive learning paradigms (Wang et al., 2005). Furthermore, manipulations of cAMP-mediated signaling in the BLA have also been shown to impair reconsolidation of aversive learning tasks, using post-retrieval disruptions of PKA and CREB signaling (Koh and Bernstein, 2003; Milekic et al., 2007), consistent with the notion that similar mechanisms involved in the initial consolidation of memory are also involved in reconsolidation.

The role of norepinephrine in the BLA in memory consolidation and reconsolidation

As alluded to above, one neurotransmitter system that has been demonstrated to play an important role in memory processes in the BLA is the noradrenergic system. NE is a neuromodulator in the central nervous system that is critically involved in a variety of cognitive processes, including attention, arousal, emotion, learning, and memory consolidation (reviewed in Sara, 2009). NE is released from NE-containing neurons of the locus coeruleus (LC) and nucleus of the solitary tract (NTS)(Figure 1B). The LC is the primary source of NE in the CNS, with cell bodies located in the brainstem that send projections to a diverse number of brain structures, including, but not limited to, cortical areas, the hippocampus and the amygdala (Foote et al., 1983), with dense innervation of

the BLA (Asan, 1998). The BLA also receives projections from the NTS (Miyashita and Williams, 2003). In rats, electrophysiological recordings have shown that LC neurons are phasically activated by sensory stimuli (Aston-Jones et al., 1991), and emotionally arousing stimuli increase NE in the BLA, as measured by in vivo microdialysis (McIntyre et al., 2002). NE exerts its effects though activation of three families of ARs, β, α_1 and α_2 (Bylund et al., 1994), although the discussion here will be limited to the role of β- and α_1-ARs, based on their demonstrated role in memory consolidation, as outlined below.

NE is thought to play a role in facilitating plasticity related to learning and memory (Berridge, 2008) primarily via β-ARs, which are G-protein coupled receptors that modulate the cAMP signaling cascade via direct coupling to adenylate cyclase (AC) (Daly et al., 1981). In rat BLA in vitro, the β-AR agonist isoproterenol has been demonstrated to potentiate NMDA-R-mediated synaptic transmission (Ferry et al., 1997) and facilitate LTP in the lateral amygdala, an effect blocked by the β-AR antagonist timolol (Huang et al., 2000). In rodent studies, post-training injections of NE or β-AR agonists into the BLA have been shown to enhance memory in inhibitory avoidance (Ferry and McGaugh, 1999; Introini-Collison et al., 1991), object recognition (Roozendaal et al., 2008), fear conditioning (LaLumiere et al., 2003) and spatial learning tasks (Hatfield and McGaugh, 1999). Furthermore, intra-BLA β-AR antagonists impair consolidation (Hatfield and McGaugh, 1999; Miranda et al., 2003; Qu et al., 2008). The majority of work examining the role of the β-AR in memory consolidation via receptor antagonism has utilized non-specific β_1/β_2-AR antagonists, such as propranolol, and thus it is not clear whether the effects of β-AR antagonists administered into the BLA are selectively mediated by one or both subtypes of the receptor. In fact, only a few studies

have directly compared the roles the specific β_1 and β_2 subtypes in memory processes in general (e.g., Flexner et al., 1985; Qu et al., 2008).

α_1-ARs are G-protein coupled receptors that indirectly modulate cAMP by influencing β-AR activity (Ferry et al., 1999b; Leblanc and Ciaranello, 1984). The α_1-AR has also been demonstrated to play a role in memory consolidation. Selective activation of the α_1-AR in the BLA has been shown to enhance memory for an inhibitory avoidance task in rodents, while blockade with intra-BLA administration of the α_1-AR antagonist, prazosin, impaired long-term retention (Ferry et al., 1999b).

Based on the evidence, as presented above, that reconsolidation may involve similar receptor systems as those involved in memory consolidation, it is logical to hypothesize that ARs in the BLA may be ideally suited to play a role in post-retrieval memory mechanisms, consistent with their role in consolidation processes. However, only one study published to date has demonstrated impairment of reconsolidation via manipulation of the adrenergic system in the BLA, using the β-AR antagonist, propranolol. Debiec and LeDoux (2004) demonstrated that post-retrieval administration of propranolol into the BLA impaired an inhibitory avoidance memory in rats. However, as alluded to above, several studies have shown that systemic β-AR antagonism with propranolol appears to disrupt reconsolidation mechanisms following cued memory reactivation. These include both appetitive (Diergaarde et al., 2006; Milton et al., 2008b; Przybyslawski et al., 1999) and aversive (Abrari et al., 2008; Debiec and Ledoux, 2004; Przybyslawski et al., 1999) learning paradigms. Thus it appears clear that, in animal studies, the β-AR is likely involved in post-retrieval memory processes attributed to a reconsolidation phase, though the role of the BLA in these effects needs to be examined further.

No studies published to date have demonstrated a role for α_1-AR in reconsolidation, despite the fact that α_1-AR have been demonstrated to play a role in memory consolidation (as outlined above).

The role of the basolateral amygdala in drug conditioning

As already mentioned, stimuli associated with drugs of abuse are important contributors to the persistence of addiction in humans (Childress et al., 1988a, 1988b; Johnson et al., 1998; Negrete and Emil, 1992; O'Brien et al., 1992, 1998; Weiss, 2005). Due to its role in associative learning and control of goal-directed behaviors mediated by conditioned cues, the BLA has been demonstrated to be an important site mediating the conditioned effects of stimuli associated with drugs of abuse. In the case of cocaine, functional brain imaging studies in human addicts have shown that exposure to drug-paired stimuli increases activation in the amygdala as measured by positron emission tomography (PET) (Childress et al., 1999; Grant et al., 1996), and electrophysiological recording in rats have shown that BLA neurons that fire in response to cocaine reinforcement in the presence of an audiovisual cue also responded to the cue in the absence of reinforcement (Cammarota et al., 2008). Furthermore, the BLA has been demonstrated to be critical in the ability of cocaine-paired stimuli to elicit cocaine-seeking behaviors in animal models of addiction in acquisition and expression studies (Brown and Fibiger, 1993; Fuchs et al., 2002; McLaughlin and See, 2003; Meil and See, 1997). For example, excitotoxic lesions of the BLA disrupt the acquisition of cocaine CPP (Fuchs et al., 2002), and lesions of the BLA impair the ability of cocaine-associated

cues to reinstate extinguished responses (Meil and See, 1997), while having no effect on the ability of cocaine itself to induce reinstatement. The latter study is consistent with the role of the BLA in mediating the conditioned reinforcing properties of reward-related cues and not the reinforcing properties of the reward itself (Balleine et al., 2003).

As outlined above in regards to systemic drug work, however, only a few studies have attempted to impair consolidation using post-training BLA manipulations. Blocking consolidation of cocaine-cue associations in a self-administration model in rats with post-training tetrodotoxin (Fuchs et al., 2006) or the NMDA-R antagonist AP-5 (Feltenstein and See, 2007) has been shown to impair the cue-induced reinstatement of lever pressing following extinction trials. These data, though limited, do suggest an important role of the BLA in consolidation of cocaine-cue memories, and suggest that systems known to be involved in learning and memory, such as the NMDA-R, play an important role.

Consistent with the role of the BLA in the consolidation of cocaine-cue memories, recent evidence suggests that the reconsolidation of memories acquired through the associative learning of stimuli with drugs of abuse may also be dependent upon activity in the BLA (Lee et al., 2005; Lee et al., 2009; Milton et al., 2008a). Intra-BLA administration of the NMDA-R antagonist D(-)-2-amino-5-phosphonopentanoic acid (D-APV), prior to a reactivation session in a cocaine cue-mediated ANR procedure, impaired the ability of a conditioned reinforcement to facilitate the acquisition of a new response, which the authors attributed to a disruption of reconsolidation (Milton et al., 2008a), though alternate explanations are possible. Interestingly, although most studies of post-retrieval mechanisms examine the ability of pharmacological manipulations to impair reconsolidation, Lee et al. (2009) demonstrated that intra-BLA D-cycloserine (DCS)

administered prior to re-exposure to a cocaine-associated cue increased subsequent cocaine self-administration maintained by that cue, attributed by the authors to an enhancement of reconsolidation. Again, the BLA appears to play a critical role in post-retrieval memory mechanisms, and manipulation in the BLA is a promising approach to further characterize the potential reconsolidation of cocaine-induced memories. Theoretically, because reconsolidation mechanisms may enable continued cue reactivity for prolonged periods of time, diminishing the motivational properties of drug cues with pharmacotherapies that disrupt reconsolidation may prevent relapse.

As already mentioned, in addition to NMDA receptors, other receptor systems are likely important in modulating the post-retrieval reconsolidation of cocaine-cue associations. As outlined earlier, the noradrenergic system is a likely candidate. However, the role of adrenergic mechanisms in the BLA via manipulation of β-AR and/or α_1-AR in the BLA specifically in the reconsolidation of cocaine conditioning has yet to be studied. And although DA has a well-established role in the attribution of salience to cocaine-associated stimuli (Berglind et al., 2006; reviewed in Robinson and Berridge, 1993, 1998), NE may be more important in terms of post-retrieval reconsolidation following cue exposure in the absence of reinforcement. DA neuron firing decreases during the omission of a predicted reward (Hollerman and Schultz, 1998), while NE neurons increase firing when a reward is omitted (Sara et al., 1994). These data suggest that NE may be more involved in processes occurring during nonreinforced cue exposure (van der Meulen et al., 2007), which is, in most circumstances, the prerequisite for reconsolidation, as well as extinction, to occur.

FOS immunohistochemistry in the basolateral amygdala

c-fos is one of many inducible immediate early genes (IEGs) upregulated as a consequence of synaptic activation (reviewed in Guzowski, 2002). FOS protein is the product of c-fos and is widely used as a marker for neuronal activation (Herrera and Robertson, 1996). However, due to its role as a regulatory transcription factor, FOS mediates genomic responses to extracellular stimuli (Bing et al., 1992), and thus is likely involved in the long-lasting synaptic changes associated with the consolidation of long-term memory (reviewed in Davis and Squire, 1984; Guzowski, 2002) and has subsequently been proposed to play a role in retrieval-induced reconsolidation in the amygdala (Hall et al., 2001). Using a fear conditioning study, Hall et al. (2001) reported an increase in FOS immunoreactivity (FOS-IR) in the basal nucleus of the amygdala in rats following re-exposure to a discrete cue that had previously been paired with footshock, but no such increases in unpaired or no re-exposure animals, and no such increases in other brain areas examined. The authors concluded that the NMDA/cAMP/PKA/CREB signaling cascade following retrieval, including FOS translation, are likely involved in reconsolidation of the memory, comparable to the role of this signaling cascade in the initial consolidation of memory. Following a similar rationale with respect to cocaine-mediated associative learning, several studies have shown increases in FOS-IR in the BLA following re-exposure to cues previously paired with cocaine (e.g., Brown et al., 1992; Miller and Marshall, 2004) that may be a critical mediator of molecular events underlying reconsolidation of drug-mediated memories. Furthermore, the intra-BLA infusion of antisense oligodeoxynucleotides (ASOs) directed

against another IEG, zif268, prior to reactivation of a cocaine-stimulus memory acquired through associative learning in rats impaired reconsolidation in an ANR paradigm (Lee et al., 2005). Thus, IEG expression and the subsequent translation of their corresponding protein may play a critical role in post-retrieval memory mechanisms by mediating the expression of genes critically involved memory maintenance and/or reconsolidation. Thus, manipulation of the expression of IEGs, including c-fos, at the time of retrieval may be a promising approach to characterize the mechanisms underlying the reconsolidation of cocaine-induced memories.

Assessment of memory reconsolidation using conditioned place preference

The CPP paradigm is an animal model of Pavlovian conditioning that is commonly used to study drug-seeking behaviors (Bardo and Bevins, 2000; Cunningham et al., 2006; Tzschentke, 2007), as drugs with abuse liability in humans reliably produce CPPs in animals (Bardo and Bevins, 2000; Cunningham et al., 2006; Tzschentke, 2007). As stimuli associated with drugs of abuse are a critical component of drug-seeking behavior in humans (Childress et al., 1988a, 1988b; Johnson et al., 1998; Negrete and Emil, 1992; O'Brien et al., 1992, 1998; Weiss, 2005), CPP allows the conditioned reinforcing properties of these cues to be examined in animals. In cocaine CPP, for example, during conditioning, cocaine is paired with a distinct environment, such as one or more visual, olfactory and/or tactile cues, while vehicle is paired with a different environment. Following the completion of conditioning, animals are given a drug-free preference test, during which they have equal access to both cocaine- and vehicle-paired environments

(Cunningham et al., 2006). Animals are determined to have expressed a CPP when they spend significantly more time in the drug-associated environment relative to the vehicle-paired environment. Because of the associative learning that occurs through conditioning and the drug-free testing, CPP is dependent upon memory processes, including acquisition, consolidation, and retrieval (Schroeder and Packard, 2003). Because of the importance of cue-induced memory retrieval in studies of reconsolidation, the CPP paradigm seems ideally suited for the examination of post-retrieval impairment attributed to reconsolidation mechanisms. In fact, several studies have shown demonstrated impairments in reconsolidation of drug-mediated CPP using a variety of pharmacological manipulations (Bernardi et al., 2006; Brown et al., 2007; Fricks-Gleason and Marshall, 2008; Itzhak and Anderson, 2007; Kelley et al., 2007; Miller and Marshall, 2005b; Robinson and Franklin, 2007a, 2007b; Sadler et al., 2007; Wang et al., 2008; Zhai et al., 2008).

Dissertation Goals

Learning and memory processes are necessary for the associations that occur between neutral stimuli and drugs of abuse in humans, and the retrieval of memories produced by drug-associated stimuli can facilitate drug use or precipitate relapse following abstinence (O'Brien et al., 1992; See, 2005; Weiss, 2005). Thus, identifying ways in which to dampen the impact of drug stimuli-mediated memories represents one potential treatment for drug dependence, and pharmacologically targeting reconsolidation mechanisms, as well as extinction mechanisms, has been proposed as a possible means by which to

achieve this goal (Taylor et al., 2009). Because the noradrenergic system has been shown to play an important role in animal models of associative learning (outlined above), the focus of the studies reported here was to examine the potential involvement of this system in the reconsolidation of cocaine memories using the CPP paradigm. Furthermore, if impairment of reconsolidation with noradrenergic blockade can be demonstrated preclinically, this could result in the use of noradrenergic antagonists currently prescribed for other uses being potentially therapeutic when administered in the presence of drug-associated cues in humans.

Based on the demonstrated role of the non-specific β_1/β_2-AR antagonist, propranolol, in impairing reconsolidation in other learning paradigms (outlined above), the first study examined the effect of propranolol when systemically administered following an initial test of cocaine CPP, or in the absence of that initial test, on place preference during a subsequent test (Chapter 2).

Based on the results of Chapter 2, in which post-test propranolol did impair a subsequent preference, follow-up studies examined the subtype specificity of the propranolol-mediated effect. Pharmacotherapies targeting reconsolidation would benefit from a clearer understanding of the specific receptor subtypes that mediate propranolol's behavioral effects, which is important because more specific medications may be equally efficacious with less adverse effects. Alternatively, more specific medications may be more efficacious and thus lower doses could be used for treatment purposes. Furthermore, studies of the role of NE in memory consolidation have identified the β_2-AR as the likely mediator of NE-related changes in memory retention (e.g., Ferry and McGaugh, 1999; but see Qu et al., 2008). To date, no studies have examined

reconsolidation-like impairments using subtype-specific β-AR antagonists. Thus, the effects of post-test administration of the β_1- and β_2-AR antagonists betaxolol and ICI 118,551, respectively, on a subsequent cocaine CPP, were examined (Chapter 3). Furthermore, the α_1-AR prazosin, when administered post-test, was also examined (Chapter 3).

To identify a potential role for the BLA in mediating the systemic impairments of reconsolidation produced by adrenergic antagonists (Chapters 2 and 3), the FOS response in the BLA was then examined following the expression of a cocaine CPP under a number of different conditions (Chapter 3). In the first analysis, FOS expression was compared in groups of rats that received exposure to different configurations of the floor cues used in the CPP paradigm. In other words, FOS expression was compared in groups that received, during testing, exposure to either the choice floor, consisting of both floors previously paired with cocaine or saline, the drug-paired floor, or the saline-paired floor. This study was partly undertaken to elucidate inconsistent reports of conditioned cued FOS response in the BLA following cocaine conditioning (Franklin and Druhan, 2000; Miller and Marshall, 2005a).

In the second FOS analysis, these same groups were tested following pretreatment with the β_2- or α_1-AR antagonist ICI 118,551 or prazosin, respectively, to determine the effect of pretreatment with these drugs on CPP and FOS expression, as these drugs were demonstrated to impair preference during a second test when administered immediately following an initial test of cocaine CPP (Chapter 3).

Site-specific injections represent an important step in determining the locus of a drug's effects in the brain. The final study examined the effect of ICI 118,551 or

prazosin administered directly into the BLA following an initial test of cocaine CPP on a subsequent test of preference (Chapter 3).

To summarize, the work presented here examined the role of α_1- and β-AR antagonism on the potential reconsolidation of a cocaine CPP, and the role of the BLA in the effects of AR blockade, via systemic and site-specific administration of AR antagonists and FOS immunohistochemistry. The implications of the findings reported here are presented in Chapter 4.

CHAPTER 2: Post-retrieval propranolol disrupts a cocaine conditioned place preference

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Abstract

The current study examined whether a post-retrieval drug memory could be disrupted by the β-AR antagonist propranolol, administered following reactivation in a cocaine-mediated CPP paradigm. Following cocaine conditioning, rats were given a test of CPP, followed immediately by intraperitoneal administration of propranolol or saline. Rats that received propranolol following the preference test showed no preference for the cocaine-paired floor during a subsequent test, while vehicle-treated rats continued to express a preference for the cocaine-paired floor. These deficits in behavior were specific to retrieval of the cocaine-mediated memory, suggesting that post-retrieval propranolol induced an impairment of drug-seeking behavior that is consistent with disruption of a reconsolidation phase following retrieval.

Introduction

Newly acquired memories are initially labile, but are stabilized through a consolidation process (McGaugh, 1966), following which memories are thought to be resistant to interference (Squire and Alvarez, 1995). However, some evidence suggests that when a memory is retrieved or reactivated it undergoes a "reconsolidation" phase (Sara, 2000), during which it is again vulnerable to disruption (Judge and Quartermain, 1982; Misanin et al., 1968). And although the reconsolidation hypothesis remains speculative, many laboratories have reported behavioral deficits following post-retrieval memory manipulations (e.g., Nader et al., 2000; Pedreira et al., 2002).

Several studies of memory in fear conditioning and appetitive learning paradigms have suggested a role of the β-AR in the reconsolidation of aversive and appetitive learning following cued retrieval (Debiec and Ledoux, 2004; Diergaarde et al., 2006; Przybyslawski et al., 1999). Only recently, however, have studies begun to examine the memory processes that occur following retrieval of memories associated with drugs of abuse. Through associative learning, neutral stimuli repeatedly paired with an abused drug acquire motivational properties similar to those of the drug itself (Weiss, 2005) and subsequently produce craving and arousal in humans that provoke continued drug use (O'Brien et al., 1992; See, 2005). Thus, studying the memory mechanisms involved following exposure to stimuli associated through Pavlovian conditioning with drugs of abuse in animals may reveal novel treatments for drug-seeking behaviors induced by drug-associated cues and provide insight into human addiction and relapse. Examination of the β-AR in drug conditioning is important because β-AR antagonists, such as

propranolol, have shown promise in the treatment of recurrent, maladaptive memories in humans (Vaiva et al., 2003). And though recent evidence does indeed suggest that, following reactivation, drug memories may undergo a post-retrieval reconsolidation phase (Lee et al., 2005; Miller and Marshall, 2005b), the specific role of the β-AR in these memories has yet to be characterized.

The purpose of the current experiment was to determine whether reactivated memories in a cocaine-mediated CPP paradigm could be impaired by post-retrieval administration of the β-AR antagonist propranolol. It was hypothesized that if drug memories do indeed undergo a reconsolidation phase following retrieval, propranolol would attenuate cocaine-seeking behavior in this paradigm.

Materials and Methods

Subjects

Forty-eight male Sprague Dawley rats (Harlan, Indianapolis, Indiana) weighing 250-350 gm served as subjects. Subjects were housed two per cage in a temperature-controlled (21 °C) environment maintained on a 12-hr light-dark cycle (lights on at 7 a.m.). Food and water were available *ad libitum*. All experiments were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and the Institutional Animal Care and Use Committee of the Portland VA Medical Center. All behavioral testing was conducted during the light phase between 0800 h and 1700 h.

Drugs

Cocaine Hydrochloride (HCl) was obtained from Sigma (St. Louis, MO) and dissolved in physiological saline (0.9% NaCl) for intraperitoneal (IP) injection (1 ml/kg) and administered at a dose of 20 mg/kg. (±)-Propranolol HCl (Sigma) was dissolved in physiological saline for IP injection (1 ml/kg) and administered at a dose of 10 mg/kg. Vehicle (saline) injections were identical in volume (1 ml/kg) to those of individual drugs.

Apparatus

CPP was assessed using an unbiased design (Cunningham et al., 2006) in two automated one-compartment place conditioning chambers (modified from San Diego Instruments, San Diego, CA). Each chamber consists of a clear acrylic test cage (70 cm x 23 cm x 38.5 cm) with removable floors composed of interchangeable halves (left/right) of two distinct floor types. A grid floor consists of 2.3-mm stainless steel rods mounted 13 mm apart in an acrylic frame. A hole floor consists of perforated black acrylic with 13-mm round holes on 19-mm staggered centers (modified from Bormann and Cunningham, 1997). Pilot experiments have demonstrated that rats show approximately equal preference for the two floor types. Position in the chamber (left/right side) and general activity are assessed by computer software that records and analyzes beam interruptions from 16 infrared photocell emitter/detector pairs (8 evenly-spaced pairs per left/right side) located along the long axis of the chamber, 1.5 cm above the chamber floor. Place conditioning chambers are housed in sound-attenuated, black acrylic

enclosures (Flair Plastics, Portland, OR) designed to eliminate noise from the external environment, and have no illumination (i.e., experiments are run in the dark). Inside each chamber, a fan provides ventilation and a low level of masking noise.

Behavioral Procedures

The use of place conditioning to examine the effect of post-retrieval propranolol on cue-induced drug-seeking behaviors involved the following phases performed on consecutive days: habituation, conditioning and testing. Prior to the first phase, rats were randomly assigned to one of four groups: propranolol [PRO; n = 12 (n = 6/6 per GRID+/GRID- subgroup as described below)], vehicle [VEH; n = 12 (n = 7/5 per GRID+/GRID- subgroup)], propranolol no re-exposure [PRO-NR; n = 12 (n = 5/7 per GRID+/GRID- subgroup)], and vehicle no re-exposure [VEH-NR; n = 12 (n = 6/6 per GRID+/GRID- subgroup)]. Rats in the PRO and VEH groups received propranolol or vehicle, respectively, immediately following a drug-free test for place preference (Test 1) and tested again for a CPP 24 hr later (Test 2). Rats in the PRO-NR and VEH-NR groups did not receive Test 1, but received propranolol or vehicle in their home cages, followed 24 hr later by a test for CPP during Test 2.

<u>Habituation (1 session).</u> During habituation, rats were injected with saline (IP, 1 ml/kg) and placed in the apparatus without floors for 25 min to reduce the stress associated with injections and exposure to the apparatus.

Conditioning (8 sessions). Rats in each of the four groups outlined above were assigned to one of two conditioning subgroups (cocaine on grid floor = GRID+; cocaine on hole floor = GRID- and exposed to an Pavlovian discrimination conditioning procedure (Cunningham et al., 2006). Thus, on alternate days over eight conditioning sessions (four cocaine sessions and four saline sessions), rats in the GRID+ subgroup received cocaine (20 mg/kg IP) immediately prior to 25-min conditioning trials on the grid floor and saline (1ml/kg IP) immediately prior to 25-min trials on the hole floor. Alternatively, rats in the GRID- subgroup received cocaine (20 mg/kg IP) immediately prior to 25-min conditioning trials on the hole floor and saline (1ml/kg IP) immediately prior to 25-min trials on the grid floor. The order of treatment exposure was counterbalanced within each GRID+ and GRID- subgroup, such that half of the rats in each subgroup received conditioning to cocaine during the first conditioning trial and half of the rats received saline during the first trial. During conditioning trials, left and right floor types were identical and rats had access to both sides of the apparatus (Cunningham et al., 2006).

Testing (1 or 2 sessions). During Test 1, rats in the PRO and VEH groups received a saline injection (1 ml/kg IP) immediately prior to placement into the apparatus with half grid floor and half hole floor for a 15-min preference test designed to serve as a retrieval trial intended to reactivate the memory of the cocaine-cue association acquired during the conditioning phase. For the half grid floor and half hole floor combination, the position of the floors (left vs. right) was counterbalanced within each GRID+ and GRID- subgroups, and preference was determined by comparing the amount of time spent on the GRID floor between the GRID+ and GRID- conditioning subgroups (Cunningham et al.,

2006). Immediately following this test session, rats received either 10 mg/kg propranolol or vehicle and were returned to their home cages. Rats in the PRO-NR and VEH-NR groups received similar drug treatments without re-exposure to the apparatus. Thus, these rats were administered a saline injection 15 min prior to administration of either propranolol or vehicle in their home cages. Twenty-four hours later, rats in each of the four groups received a saline injection (1 ml/kg IP) immediately prior to placement into the apparatus with half grid floor and half hole floor for a 15-min drug-free preference test (Test 2) to assess the effect of post-retrieval propranolol on a cocaine-induced CPP. Again, preference was determined by comparing the amount of time spent on the GRID floor between the GRID+ and GRID- conditioning subgroups.

Data analysis

Statistical analyses were conducted with SPSS software (Chicago, IL). Place preference was analyzed using two-way ANOVAs [Drug Treatment X Conditioning Subgroup (GRID+/GRID-)]. Student's t-test was used to examine *a priori* comparisons between GRID+ and GRID- subgroups (with a Bonferroni correction for multiple comparisons). Activity data during Test 2 were analyzed using a one-way ANOVA (Drug Treatment). Significance was set at $p < .05$.

Results

Both propranolol- and vehicle-treated rats showed a significant place preference during Test 1. Rats that received propranolol following Test 1 showed an attenuation of

CPP during Test 2 compared to saline-treated rats. These differences were not seen in the propranolol- and saline-treated no re-exposure controls. Figure 2A shows the mean (+SEM) time spent on the GRID floor during Test 1 for groups PRO and VEH. The difference between time spent on GRID floor between the GRID+ and GRID- subgroups indicates the magnitude of the place preference. A two-way ANOVA (drug treatment X conditioning subgroup) revealed a significant main effect of conditioning subgroup [$F(1,20) = 47.5$, $p < .001$], suggesting reliable preference for the cocaine-paired floor, but no interaction or main effect of drug treatment ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that the PRO [$t(10) = 5.0$, $p < .001$] and VEH [$t(10) = 4.7$, $p < .001$] (Bonferroni-corrected $\alpha/2 = .025$) groups, prior to their respective treatments, showed significant cocaine-induced CPP.

Figure 2B shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups PRO and VEH. A two-way ANOVA again revealed a significant main effect of floor [$F(1,20) = 6.5$, $p < .05$], suggesting reliable preference for the cocaine-paired floor, but no interaction [$F(1,20) = 2.5$, $p = .13$] or main effect of drug treatment ($F < 1$). However, Student's t-test comparing time spent on GRID floor for the GRID+ and GRID- subgroups revealed that while the VEH group [$t(10) = 2.9$, $p < .025$] (Bonferroni-corrected $\alpha/2 = .025$) continued to show a significant cocaine-induced CPP during Test 2, the PRO group failed to show a preference for the cocaine-paired floor during this second test of preference ($p = .51$). These results suggest that propranolol attenuated a CPP for cocaine when administered following Test 1 re-exposure and supports the notion that drug memories may be susceptible to disruption following retrieval.

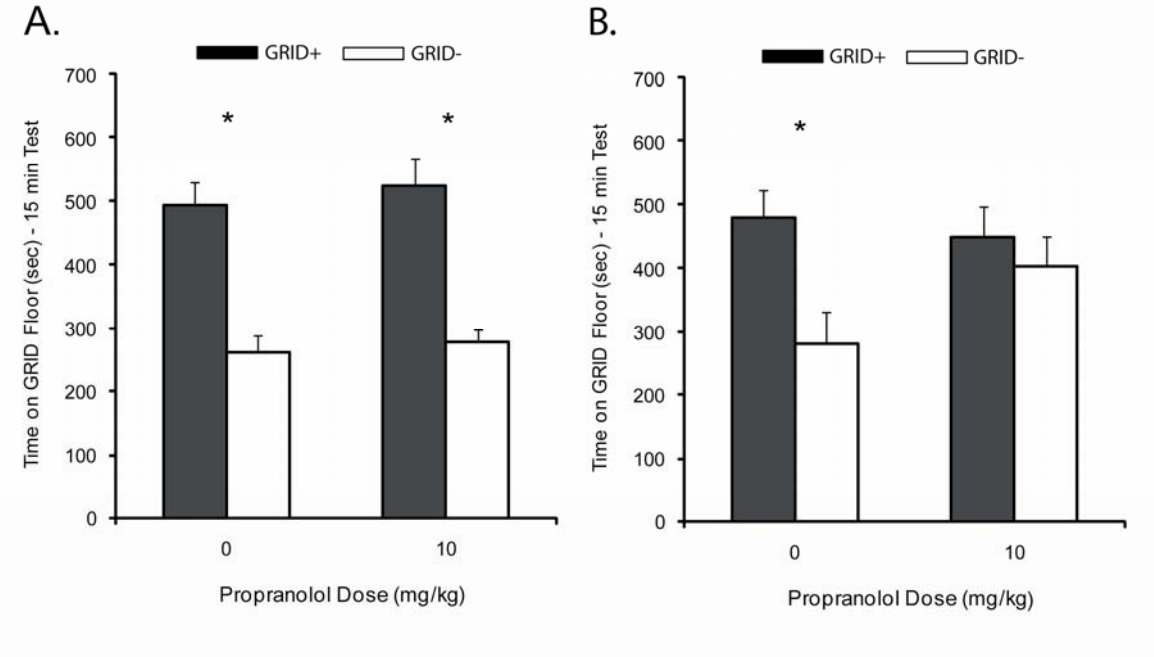

FIGURE 2. Post-retrieval administration of propranolol attenuated a subsequent cocaine CPP.

(A) Cocaine induced a CPP for the cocaine-paired floor during Test 1. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Both groups showed a significant preference for the cocaine-paired floor. (B) Post-retrieval propranolol attenuated a cocaine CPP. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Rats treated with propranolol following Test 1 showed no preference for the cocaine-paired floor during Test 2, while vehicle-treated rats continued to express a significant preference for the cocaine-paired floor. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$).

The PRO-NR and VEH-NR groups showed no difference in CPP during Test 2. Figure 3 shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups PRO-NR and VEH-NR. A two-way ANOVA revealed a significant main effect of conditioning subgroup [$F(1,20) = 12.8$, $p < .005$], suggesting reliable preference for the cocaine-paired floor, but no interaction or main effect of drug treatment ($Fs < 1$). Student's t-test revealed that the VEH-NR group [$t(10) = 2.7$, $p < .025$] (Bonferroni-corrected $\alpha/2 = .025$) showed significant cocaine-induced CPP, and although it appears in Figure 3 that rats in the PRO-NR group also showed a preference for the cocaine-paired floor, the p-value [$t(10) = 2.3$, $p = .04$] did not reach our normal criterion for Bonferroni-correction ($\alpha/2 = .025$). These results suggest that the effect seen in the PRO and VEH groups was specific to re-exposure to the testing environment.

Activity levels among the groups, as measured by one-way ANOVA, did not significantly differ between either the PRO and VEH groups ($F < 1$) or the PRO-NR and VEH-NR groups [$F(1,22) = 1.7$, $p = .21$] during Test 2, suggesting that the attenuation of CPP in the PRO group was not due to a residual effect on activity caused by propranolol. Mean (±SEM) activity counts during Test 2 were 1489.4 ± 50.2, 1511.7 ± 65.0, 1576.3 ± 50.2, and 1668.3 ± 49.6 for groups PRO, VEH, PRO-NR, and VEH-NR, respectively.

Discussion

The current study examined the effect of the β-AR antagonist propranolol on post-retrieval cocaine memories in the CPP paradigm. We found that propranolol disrupted a

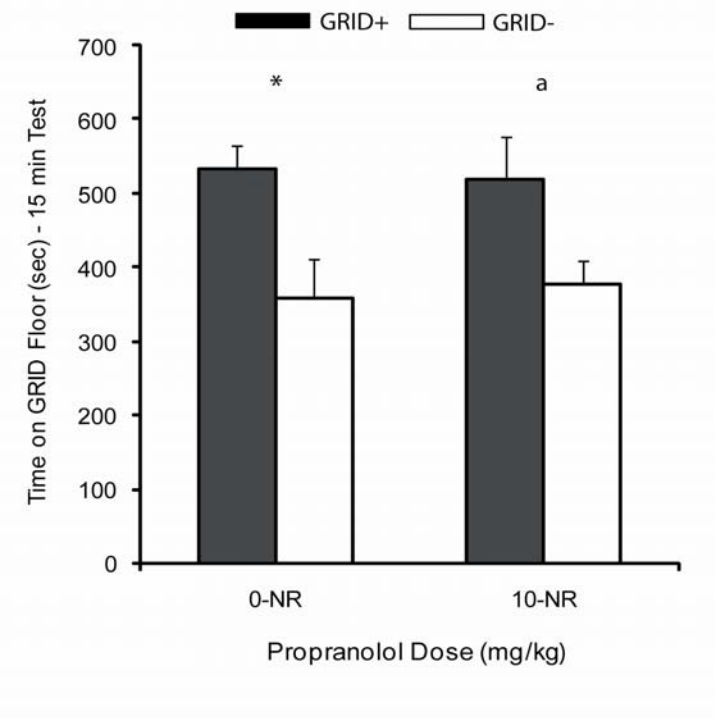

FIGURE 3. No effect of propranolol on the expression of a cocaine CPP when administered in the absence of re-exposure.

Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Groups that received propranolol or vehicle in the absence of Test 1 showed a significant or trend toward significant preference for the cocaine-paired floor during Test 2. NR = no re-exposure. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$); $^{a}p = 0.04$.

cocaine-induced CPP when administered following memory reactivation. Furthermore, propranolol administered in the absence of memory reactivation failed to disrupt a subsequent CPP. These results are consistent with recent studies demonstrating post-retrieval impairment of drug-mediated behaviors (Lee et al., 2005; Miller and Marshall, 2005b), as well as studies demonstrating that β-AR antagonists disrupt memory retrieval-mediated reconsolidation (Debiec and Ledoux, 2004; Diergaarde et al., 2006; Przybyslawski et al., 1999).

The rewarding properties of most drugs of abuse stem from their ability to increase of dopamine neurotransmission in the nucleus accumbens (Di Chiara, 1995). However, stimuli paired with drugs of abuse have been found to increase the motivational properties of drugs, and a great deal of evidence implicates the amygdala in the ability of these stimuli to elicit drug-seeking behaviors (See, 2005) via a limbic-striatal connection (Everitt et al., 1999). And although the role of β-ARs in the amygdala in mediating cue-induced drug memories has yet to be systematically characterized, noradrenergic activation in response to increased levels of norepinephrine in this brain region has been shown to be critically involved in modulating memories acquired through other associative learning paradigms (for review, see McGaugh et al., 2002). Thus it is conceivable that if inhibition of a post-retrieval reconsolidation phase mediates the deficits in drug-seeking behavior seen here as well as in other studies (Lee et al., 2005; Miller and Marshall, 2005b), β-ARs in the amygdala likely play a crucial role. Although the dose-dependency of the effect of propranolol seen here remains to be determined, the present study represents an important first step in characterizing the role of β-ARs in mediating post-retrieval drug-seeking behaviors. Because propranolol was administered

systemically, it is impossible to ascertain the brain structures involved in this effect. Thus, future studies employing site-specific β-AR manipulation will determine the site of action of these effects. Similarly, the involvement of specific β-AR subtypes in post-retrieval memory mechanisms, as well as the potential role of α-ARs in these mechanisms, needs to be evaluated.

Several researchers have suggested that behavioral impairments attributed to deficits in reconsolidation or re-storage of the original memory may be transient, and thus do not reflect permanent memory impairment (for review, see Lattal and Abel, 2004). Lattal and Abel reported that behavioral impairments caused by protein synthesis inhibition following context re-exposure were impaired 24 hr later but not 21 days later, suggesting deficits in memory retrieval that reverse with time. In contrast, previous work examining the reconsolidation of cocaine memories using the CPP paradigm has shown that impairment of drug-seeking behaviors following post-retrieval MEK inhibition was still evident 14 days following the initial preference test (Miller and Marshall, 2005b). The persistence of propranolol-induced attenuation of CPP found here, however, remains to be examined. Another interpretation of the results reported here is that propranolol facilitated extinction of the association between cocaine's putative rewarding effect and the cocaine-paired floor that serves to elicit a CPP. Thus it is conceivable that in the current study, propranolol-treated no re-exposure rats-- in contrast to those that received propranolol following an initial preference test-- showed no impairment of drug-seeking behavior because extinction mechanisms would not have been engaged in the absence of drug-free re-exposure to the testing environment (Lattal et al., 2006). However, a recent study examining extinction following contextual fear conditioning in rats found that

propranolol infused into the amygdala immediately following an initial test of conditioned freezing blocked a bicuculline-induced enhancement of extinction during subsequent tests (Berlau and McGaugh, 2006). In that same study, norepinephrine administered into the amygdala post-retrieval dose-dependently enhanced extinction, presumably via adrenergic activation. Whether drug-induced reward learning is regulated by similar processes as those involved in fear learning, however, still needs to be explored.

Drug addiction in humans is characterized by high rates of relapse, and environmental stimuli that are associated with drugs of abuse are thought to be critical to the persistence of addiction in humans and major contributors in relapse to drug-seeking following periods of abstinence (Childress et al., 1988a; O'Brien et al., 1992; Weiss, 2005). Thus, we propose that the results reported here represent an alternative technique with which to examine cue-induced drug-seeking behaviors associated with abused substances in animals, and thus may provide insight into developing novel treatments with which to treat addiction and relapse in humans. Retrieval-induced reconsolidation mechanisms may enable continued cue reactivity for prolonged periods of time. Therefore, diminishing the incentive-motivational properties of drug cues with pharmacotherapies that disrupt potential reconsolidation mechanisms may prevent relapse.

The results presented here suggest an important role for β-ARs in post-retrieval memory mechanisms associated with drugs of abuse. Whether these post-retrieval mechanisms are indicative of permanent or transient memory impairment still needs to be elucidated. In either case, however, β-AR antagonists may represent a novel target for

pharmacotherapy of cue-induced craving and drug-seeking behaviors that precipitate relapse.

CHAPTER 3: Post-retrieval disruption of a cocaine conditioned place preference by systemic and intra-basolateral amygdala β_2 and α_1-adrenergic antagonists

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Abstract

Previous work has demonstrated post-retrieval impairment in associative learning paradigms, including those mediated by drugs of abuse, using β-AR antagonism. To date, little is known about the specific β-AR subtypes involved in these effects. The current study examined the subtype-specificity of the post-retrieval effect of β-AR antagonism in the cocaine CPP paradigm, as well as the effect of α_1-adrenergic antagonism in this paradigm. In separate studies, we found that following cocaine conditioning, rats administered the β_2 antagonist ICI 118,551 (8 mg/kg I.P.) or the α_1-antagonist prazosin (1 mg/kg I.P.) following a drug-free test for CPP showed attenuated preference on a subsequent test 24 hr later, while the β_1 antagonist betaxolol (5 or 10 mg/kg I.P.) and a lower dose of prazosin (0.3 mg/kg I.P.) had no effect. ICI 118,551 and prazosin also attenuated FOS expression induced by the CPP test in the BLA. Furthermore, bilateral intra-BLA microinfusion of ICI 118,551 (6 nmol/side) or prazosin (0.5 nmol/side) also impaired a subsequent preference when administered post-test. Systemic or intra-BLA ICI 118,551 or prazosin administered to rats in their home cages, in the absence of a preference test, had no effect on CPP 24 hr later. These results demonstrate a role for adrenergic mechanisms within the BLA in post-retrieval memory processes. They also suggest that previous demonstrations of post-retrieval impairments caused by the nonspecific β-adrenergic antagonist, propranolol, may be mediated by the β_2, and not the β_1, subtype of β-AR.

Introduction

Substantial evidence indicates that new information acquired after a learning event is initially plastic, during which time memory retention can be disrupted, but is strengthened by a time-dependent consolidation process (McGaugh, 2000). Recent interest has focused on the issue of retrieval-induced plasticity, a process by which changes in the retention of previously acquired information are possible. The notion of reconsolidation, one theoretical mechanism by which such changes may occur, suggests that when a memory is retrieved it once again enters a labile state (Nader, 2003; Sara, 2000) and is vulnerable to disruption (Judge and Quartermain, 1982; Misanin et al., 1968). The existence of a distinct memory reconsolidation phase has generated a great deal of interest in the possibility that, from a clinical perspective, the dramatic impact of recurring, maladaptive memories in humans could be lessened through treatments aimed at interfering with reconsolidation. Thus, pharmacotherapies targeting reconsolidation mechanisms might represent a novel treatment strategy for conditions such as learned fears, post-traumatic stress disorder (PTSD) and drug addiction. To that end, a quickly expanding preclinical literature has identified several promising lines of evidence as to the precise neurobiological mechanisms involved in potential reconsolidation processes. And although the theoretical mechanisms underlying retrieval-induced plasticity remain unclear, the behavioral effects seen have been robust across many different learning paradigms using many different neurobiological manipulations (reviewed in Diergaarde et al., 2008; Tronson and Taylor, 2007).

Many studies have implicated the noradrenergic system, via β-AR blockade, in the memory processes that follow cued reminder trials. These include fear- (Abrari et al., 2008; Debiec and Ledoux, 2004; Przybyslawski et al., 1999), natural reward- (Diergaarde et al., 2006; Milton et al., 2008b), and drug reward-mediated (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008; Milton et al., 2008b; Robinson and Franklin, 2007a) associative learning paradigms, all using the non-specific β-AR antagonist, propranolol. In humans, drug-associated stimuli can facilitate continued drug use or precipitate relapse to drug-seeking following periods of abstinence (O'Brien et al., 1992; See, 2005; Weiss, 2005). Using an animal model of cocaine-conditioned behaviors, Bernardi et al. (2006) demonstrated that systemic post-retrieval administration of propranolol impaired a subsequent cocaine CPP. Furthermore, other studies have also demonstrated reconsolidation-like impairments in drug learning paradigms using systemic propranolol (Milton et al., 2008b; Robinson and Franklin, 2007a). Thus, it appears likely that the β-AR may play an important role in processes occurring following drug memory retrieval.

However, most of what is known about the noradrenergic system in the memory processes that follow cued reminder trials comes from studies that use non-specific β-AR antagonists, such as propranolol. As a consequence, several issues regarding ARs and post-retrieval memory processes remain unresolved. First, because propranolol has affinity for both β_1- and β_2-AR subtypes, it is unclear which subtype mediates these effects. To date, no studies have examined reconsolidation-like impairments using subtype-specific β-AR antagonists, which is important because more specific medications may be equally efficacious with less adverse effects. Second, no studies to date have examined α-ARs regarding a potential role in reconsolidation-like effects. α-ARs--

specifically α_1-ARs-- have a demonstrated role in memory consolidation (Ferry et al., 1999a, 1999b) and may also mediate post-retrieval processes. Third, although the BLA has had a demonstrated role in reconsolidation in numerous studies, the behavioral conditions during retrieval of drug-associated memories leading to gene expression within the BLA have not clearly been defined. Specifically, in the CPP paradigm used here, it is unclear whether exposure to a cocaine cue alone will induce gene expression or whether a preference for the drug-associated environment needs to be expressed for BLA involvement (Franklin and Druhan, 2000; Miller and Marshall, 2005a).

Here, we first examined the effects of systemic post-test β_1-, β_2-, and α_1-AR antagonism on cocaine CPP. We then focused on the BLA due to its involvement in reconsolidation-like effects in drug learning paradigms (e.g., Lee et al., 2005), measuring FOS immunoreactivity (FOS-IR) and employing microinfusions of AR antagonists to examine the BLA as a potential site of AR-mediated impairments.

Materials and Methods

Subjects

Sprague Dawley rats (Harlan, Indianapolis, Indiana) weighing 300-350 gm (375-425 gm for intracranial studies) at the beginning of experiments served as subjects. Subjects were housed two per cage in a temperature-controlled (21 °C) environment maintained on a 12-hr light-dark cycle (lights on at 6 a.m.). Food and water were available ad libitum. All experiments were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and the Institutional Animal Care

and Use Committee of the Portland VA Medical Center. All behavioral testing was conducted during the light phase between 0700 h and 1600 h.

Drugs

Cocaine HCl was obtained from Sigma (St. Louis, MO) and dissolved in physiological saline (0.9% NaCl) for intraperitoneal (IP) injection (1 ml/kg), and administered at a dose of 20 mg/kg. For systemic (IP) administration studies, betaxolol HCl (Sigma) was at doses of 5 and 10 mg/kg (1 ml/kg in physiological saline), ICI 118,551 HCl (Sigma) was administered at a dose of 8 mg/kg (2 ml/kg in sterile water), and prazosin HCl (Sigma) was administered at doses of 0.3 and 1 mg/kg (2 ml/kg in sterile water). For intra-BLA administration studies, ICI 118,551 and prazosin were dissolved in sterile saline and administered at doses of 6 nmol/side and 0.5 nmol/side, respectively. Intra-BLA infusions were administered at a volume of 0.5 µl delivered over a 2-min period, and microinjectors were left in place for 1 min following infusions. Vehicle injections were administered at volumes equal to those described for individual drugs.

Apparatus

CPP was assessed using an unbiased design (Cunningham et al., 2006) in four automated one-compartment place conditioning chambers (modified from San Diego Instruments, San Diego, CA). Each chamber consists of a clear acrylic test cage (70 cm x 23 cm x 38.5 cm) with removable floors composed of interchangeable halves (left/right) of two distinct floor types. A GRID floor consists of 2.3-mm stainless steel rods mounted 13 mm apart in an acrylic frame. A HOLE floor consists of perforated black

acrylic with 13-mm round holes on 19-mm staggered centers (modified from Bormann and Cunningham, 1997). Pilot experiments have demonstrated that rats show approximately equal preference for the two floor types. Position in the chamber (left/right side) and general activity are assessed by computer software (San Diego Instruments, San Diego, CA) that records and analyzes beam interruptions from 16 infrared photocell emitter/detector pairs (8 evenly-spaced pairs per left/right side) located along the long axis of the chamber, 1.5 cm above the chamber floor. Place conditioning chambers are housed in sound-attenuated, black acrylic enclosures (Flair Plastics, Portland, OR) designed to eliminate noise from the external environment, and have no illumination (i.e., experiments are run in the dark). Inside each chamber, a fan provides ventilation and a low level of masking noise.

Behavioral Procedures (Experiments 1, 3, and 4)

Place conditioning involved the following phases performed on consecutive days: habituation, conditioning and testing.

Habituation (1 session). During habituation, rats were injected with saline (IP, 1 ml/kg) and placed in the apparatus without floors for 25 min to reduce the stress associated with injections and exposure to the apparatus.

Conditioning (8 sessions). Rats in each drug treatment group per experiment (outlined below) were randomly assigned to one of two conditioning subgroups (cocaine on GRID floor = GRID+; cocaine on HOLE floor = GRID-) and exposed to a Pavlovian

discrimination conditioning procedure (Cunningham et al., 2006). Thus, on alternate days over eight conditioning sessions (four cocaine sessions and four saline sessions), rats in the GRID+ subgroup received cocaine (20 mg/kg IP) immediately prior to 25-min conditioning trials on the GRID floor and saline (1ml/kg IP) immediately prior to 25-min trials on the HOLE floor. Alternatively, rats in the GRID- subgroup received cocaine (20 mg/kg IP) immediately prior to 25-min conditioning trials on the HOLE floor and saline (1ml/kg IP) immediately prior to 25-min trials on the GRID floor. The order of treatment exposure was counterbalanced within each GRID+ and GRID- subgroup, such that half of the rats in each subgroup received conditioning to cocaine during the first conditioning trial and half of the rats received saline during the first trial. During conditioning trials, left and right floor types were identical and rats had access to both sides of the apparatus (Cunningham et al., 2006).

Testing (1 or 2 sessions). During Test 1, rats received a saline injection (1 ml/kg IP) immediately prior to placement into the apparatus with half GRID floor and half HOLE floor for a 15-min preference test designed to serve as a retrieval trial intended to reactivate the memory of the cocaine-cue association acquired during the conditioning phase. For the half GRID floor and half HOLE floor combination, the position of the floors (left vs. right) was counterbalanced within each GRID+ and GRID- subgroup, and magnitude of the place preference was determined by comparing the amount of time spent on the GRID floor between the GRID+ and GRID- conditioning subgroups (Cunningham et al., 2006). In Experiments 1 and 4, immediately following this test session, rats received drug treatment injections as outlined below and were returned to

their home cages. Rats in no re-exposure groups (Experiments 1C, 1E and 4B) received similar drug treatments without re-exposure to the apparatus. Thus, these rats were administered drug treatments in their home cages. Twenty-four hours later, all rats received a saline injection (1 ml/kg IP) immediately prior to placement into the apparatus with half GRID floor and half HOLE floor for a 25-min drug-free preference test (Test 2) to assess the effect of post-retrieval drug treatments on a cocaine-induced CPP. Twenty-five-min tests were administered for Test 2 to match conditioning trial duration, but data for Test 2 was analyzed only for the first 15 min for direct comparison to Test 1 retrieval trials. Again, preference was determined by comparing the amount of time spent on the GRID floor between the GRID+ and GRID- conditioning subgroups. Activity during Test 2, as measured by the number of photobeam interruptions, was measured to determine if drug injections following Test 1 had effects on locomotion that might affect a subsequent preference.

In Experiments 3A and 3B, animals were given a 15-min preference test and euthanized for FOS immunohistochemistry. Furthermore, in Experiment 3B, animals were pretreated with vehicle, prazosin, or ICI 118,551 30 min prior to this 15-min preference test as indicated below.

Immunohistochemistry Procedures (Experiment 3)

Tissue Preparation. Ninety minutes after the end of the 15-min preference test in Experiment 3, rats were euthanized with sodium pentobarbital and transcardially perfused with phosphate-buffered saline (PBS), followed by 2% paraformaldehyde in PBS. Brains were then post-fixed overnight in the same 2% paraformaldehyde solution, followed by

cryoprotection in a 20%, then 30%, sucrose (in PBS) solution until saturated. Forty-five μm coronal slices were cut on a CM 3050S cryostat (Leica Microsystems, Inc., Deerfield, IL, USA) and collected into PBS containing 0.1% NaN_3.

Immunohistochemistry. Staining for FOS was conducted using free-floating slices of the BLA. Endogenous peroxidase activity was inhibited with 0.3% peroxide in PBS, and a 4.5% goat serum (Vector Laboratory, Inc., Burlingame, CA, USA) in a 0.3% Triton-X 100/PBS solution was used for blocking. Slices were incubated overnight in a 1:2500 dilution of a rabbit polyclonal antibody against FOS (sc-52; Santa Cruz Biotech, Santa Cruz, CA, USA) in PBS/Triton-X with 0.1% bovine serum albumin (BSA). Slices were then incubated in a 0.3% Triton-X 100/PBS solution containing a goat anti-rabbit secondary antibody (Vector Laboratories Inc., Burlingame, CA, USA), followed by detection of the immunoreaction using the Vectastain ABC kit (Vector Laboratory Inc., Burlingame, CA) in a 0.3% Triton-X 100/PBS solution, and enzymatic development using the Metal Enhanced DAB kit (Thermo Scientific, Rockford, IL). Slices were subsequently mounted on gelatinized slides, dehydrated, and coverslipped.

FOS Quantification. The number of FOS–containing cells was counted manually using a Leica DM4000 microscope (Bartels and Stout, Inc., Bellevue, WA, USA). The number of FOS-positive neurons from the left and right BLA (including the basal, lateral, and accessory basal nuclei; see Figure 10D) per section was totaled, and multiple sections per rat were averaged to produce a single data point for statistical analysis. For experiment 3A, 10-12 slices across the BLA [approximate bregma positions: -1.80 to -3.30 mm; 2-6

slices/bregma range: -1.80 to -2.30 (A), -2.30 to -2.80 (B), -2.80-3.30 (C)] were assessed. For experiment 3B, 4-8 slices per bregma range C were assessed.

Intracranial implantation, microinjection procedures, and histology (Experiment 4)

Intracranial implantation and microinjection procedures. For intracranial implantation, rats were mounted onto a stereotaxic device (Stoelting, Wood Dale, IL) under deep isoflurane-induced anesthesia (4% for induction and 1.5–2.5% maintenance) administered via inhalation. Rats were surgically implanted with bilateral 22-gauge guide cannulae (Plastics One, Roanoke, VA) 4 or 5.5 mm above the BLA at the following coordinates: A/P –2.9, M/L ±5.0, D/V -4.5 or A/P –2.9, M/L ±4.8, D/V -3.5 (Paxinos and Watson, 1998), with respect (in mm) to Bregma, the midsagittal sinus and the surface of the level skull, respectively. The guide cannulae were secured to the skull with screws and dental acrylic. Cannulae were kept patent with 28-gauge dummy cannula (Plastics One). Bilateral intra-BLA microinjections were administered using a multi-syringe microinfusion pump (KD Scientific, Holliston, MA) and Hamilton syringes (25 μl) with 28-gauge microinjectors (Plastics One) connected via pre-loaded PE 20 tubing. When inserted, microinjectors extended 4 or 5.5 mm beyond the chronically-implanted guide shaft to reach the BLA.

Histology. After the behavioral testing was completed, rats were euthanized and injected with coomassie blue dye into their cannulae for placement verification. Brains were removed and flash frozen in methyl butane chilled on dry ice/isopropyl alcohol, then stored in a -80°C freezer. Brains were later sectioned on a cryostat, and stained using

cresyl violet and coverslipped, and placement in the BLA was verified. Only rats in which bilateral placement of the dye were correctly located within the BLA (including the basal, lateral, and accessory basal nuclei) were included in the statistical analyses of behavioral measures. Of 103 rats cannulated, 29 were removed from the study due to post-surgical illness or death, cannula removal, or incorrect placement.

Experimental Procedures

Experiment 1A – Post-retrieval betaxolol administration. This experiment examined the effect of post-test administration of the selective β_1-adrenergic antagonist, betaxolol, on a subsequent test of cocaine CPP. Prior to behavioral procedures, rats were randomly assigned to one of three groups: vehicle (BET-0); n = 24; 12 per GRID+/GRID- conditioning subgroups), 5 mg/kg betaxolol (BET-5; n = 24; 12 per GRID+/GRID- conditioning subgroups), and 10 mg/kg betaxolol (BET-10; n = 24; 12 per GRID+/GRID- conditioning subgroups). All rats were subjected to the habituation, conditioning, and testing procedures described above. Rats in the each group received the appropriate dose of betaxolol or vehicle immediately following a drug-free test for place preference (Test 1) and tested again for a CPP 24 hr later (Test 2).

Experiment 1B – Post-retrieval ICI 118,551 administration. This experiment examined the effect of post-test administration of the selective β_2-adrenergic antagonist, ICI 118,551, on a subsequent test of cocaine CPP. Rats were randomly assigned to one of two groups: vehicle (ICI-0; n = 24; 12 per GRID+/GRID- conditioning subgroups) and 8 mg/kg ICI 118,551 (ICI-8; n = 24; 12 per GRID+/GRID- conditioning subgroups). Rats

were subjected to the habituation, conditioning, and testing procedures described above, and received ICI 118,551 or vehicle immediately following a drug-free test for place preference (Test 1) and tested again for a CPP 24 hr later (Test 2).

Experiment 1C – ICI 118,551 administration in the absence of retrieval. This experiment was a follow-up to Experiment 1B. Rats were assigned to one of two groups: vehicle-no re-exposure (ICI-0-NR; n = 24; 12 per GRID+/GRID- conditioning subgroups) and 8 mg/kg ICI 118,551-no re-exposure (ICI-8-NR; n = 24; 12 per GRID+/GRID- conditioning subgroups). Rats were subjected to the habituation and conditioning procedures described above. However, rats in the ICI-0-NR and ICI-8-NR groups did not receive Test 1, but received ICI 118,551 or vehicle in their home cages, followed 24 hr later by a test for CPP during Test 2. This omission of Test 1 was intended to determine whether the effect of ICI 118,551 in Experiment 1B was specific to re-exposure to the cocaine environment.

Experiment 1D – Post-retrieval prazosin administration. This experiment examined the effect of post-test administration of the α_1-adrenergic antagonist, prazosin, on a subsequent test of cocaine CPP. Rats were randomly assigned to one of three groups: vehicle (PRAZ-0; n = 32; 16 per GRID+/GRID- conditioning subgroups), 0.3 mg/kg prazosin (PRAZ-.3; n = 32; 16 per GRID+/GRID- conditioning subgroups), and 1 mg/kg prazosin (PRAZ-1; n = 32; 16 per GRID+/GRID- conditioning subgroups). All rats were subjected to the habituation, conditioning, and testing procedures described above. Rats in the each group received the appropriate dose of prazosin or vehicle immediately

following a drug-free test for place preference (Test 1) and tested again for a CPP 24 hr later (Test 2).

Experiment 1E – Prazosin administration in the absence of retrieval. In a follow-up to Experiment 1D, rats were again assigned to one of two groups: vehicle-no re-exposure (PRAZ-0-NR; n = 24; 12 per GRID+/GRID- conditioning subgroups) and 1 mg/kg prazosin-no re-exposure (PRAZ-1-NR; n = 24; 12 per GRID+/GRID- conditioning subgroups). Rats were subjected to the habituation and conditioning procedures described above. However, rats in the PRAZ-0-NR and PRAZ-1-NR groups did not receive Test 1, but received prazosin or vehicle in their home cages, followed 24 hr later by a test for CPP during Test 2. This omission of Test 1 was intended to determine whether the effect of 1 mg/kg prazosin in Experiment 1D was specific to re-exposure to the cocaine environment.

Experiment 2 – Test of the aversive effects of prazosin and ICI 118,551. It is possible that prazosin and ICI 118,551 have aversive properties that might result in a conditioned aversion that could mask preference upon subsequent testing (Bormann and Cunningham, 1997). This experiment was conducted to determine if ICI 118,551 and prazosin affected preference during Test 2 in Experiments 1B and 1D by the conditioning of an aversion to the cocaine-paired cue due to the temporal proximity of post-test injections with exposure to that cue, on which rats spend the majority of their time during preference testing. Such an effect would weaken preference by altering the value of the cocaine-paired cue. This experiment, unlike those above, was conducted over four consecutive days, involving one

habituation session (as described above), two conditioning sessions (one GRID, one HOLE) and one test (as described above). In this case, conditioning trials consisted of a saline pre-injection and a 15 min CS exposure that was followed by prazosin, ICI 118,551 or vehicle administration after exposure to one floor cue, and vehicle administration after the other. This experiment was intended to emulate the post-15 min test drug injections used in Experiments 1B and 1D to determine if these injections were aversive. Thus, rats were randomly assigned to one of three groups: vehicle (VEH; n = 16; 8 per GRID-/GRID- conditioning subgroups), 1 mg/kg prazosin (PRAZ-1; n = 16; 8 per GRID+/GRID- conditioning subgroups), and 8 mg/kg ICI 118,551 (ICI-8; n = 16; 8 per GRID+/GRID- conditioning subgroups). Counterbalancing was as described above. Animals were tested for an aversion 24 hr after the last of the two conditioning trials.

Experiment 3A: FOS expression in the BLA following test for cocaine CPP. The purpose of this experiment was to determine how different floor configurations during testing would affect the FOS response in the BLA. As exposure to the choice, drug-paired, and saline-paired floors should all retrieve a specific memory, we examined the FOS response as a potential indicator of any differences, which might suggest a role for FOS in the BLA in post-retrieval memory processes. Rats were randomly assigned to one of three groups: choice floor (CS+/CS-; n = 8; 4 per GRID+/GRID- conditioning subgroups), drug-paired floor (CS+; n = 8; 4 per GRID+/GRID- conditioning subgroups), and saline-paired floor (CS-; n = 8; 4 per GRID+/GRID- conditioning subgroups). Rats were subjected to the habituation and conditioning procedures described above, and given a 15-min drug-free exposure to either the CS+/CS+ floor configuration (as in a typical

CPP test), the CS+ floor, or the CS- floor. Following testing, animals were processed for immunohistochemistry as described above.

Experiment 3B: Systemic effects of prazosin and ICI 118,551 on a test for cocaine CPP and FOS expression in the BLA. This study sought to determine the role of the BLA as a possible neural substrate for the post-retrieval impairment seen in Experiments 1B and 1D. Table 1 outlines the groups used in this experiment and the number of animals per group. The FOS response in the BLA was again examined following exposure to different configurations of the cues present during conditioning, but animals were pretreated 30 min prior to testing with systemic vehicle, 1 mg/kg prazosin, or 8 mg/kg ICI 118,551, drug doses that were effective in producing impairment of CPP. It was hypothesized that pretreatment with prazosin or ICI 118,551 might impair the FOS response in the BLA induced by the expression of CPP without altering the behavior

PreTx / Floor	VEH	PRAZ-1	ICI-8
CS+/CS- (choice)	n = 9 (8)	n = 8 (8)	n = 8 (7)
CS+	n = 8 (8)	n = 8 (7)	n = 8 (7)
CS-	n = 8 (7)	n = 8 (8)	n = 8 (8)
CS-/CS- (choice)	n = 9 (7)		
NP	n = 6 (6)		

TABLE 1. The number of animals in each group for Experiment 3B.

Across the top row are the three drug pre-treatments, and down the far-left column are the different test floor configurations. In parentheses are the numbers of animals included in FOS expression analysis for each group.

itself, based on preliminary data suggesting that propranolol had no effect on the expression of a cocaine CPP (see Appendix), thus confirming a post-retrieval effect of these drugs. Rats were randomly assigned to one of nine groups, based on floor condition (identical to Experiment 3A) and drug treatment (VEH, PRAZ-1, and ICI-8). These groups of rats were subjected to the habituation and conditioning procedures described above, and following drug pretreatment, given a 15-min exposure to the appropriate floor configuration during testing. Two further groups were included here. A no procedures (NP) group consisted of rats that were not subjected to any of the conditioning or testing procedures of CPP, included to determine changes in the FOS response in the BLA based solely on experimental procedures. The second group (CS-/CS-) received training and testing identical to the VEH CS+/CS- group, but received no cocaine during conditioning, instead receiving saline prior to conditioning trials with both floors. This group was included to control for the possibility that increases in the FOS response in the BLA were mediated by the novelty of the choice floor configuration. Following testing, animals were processed for immunohistochemistry as described above.

Experiment 4A: Post-retrieval intra-BLA prazosin and ICI 118,551 administration. This experiment examined the effect of post-test intra-BLA administration of the α_1-adrenergic antagonist, prazosin, and the β_2-adrenergic antagonist, ICI 118,551, on a subsequent test of cocaine CPP. Rats were randomly assigned to one of three groups: vehicle (VEH; n = 16; 9/7 per GRID+/GRID- conditioning subgroups), 0.5 nmol/side prazosin (PRAZ; n = 17; 8/9 per GRID+/GRID- conditioning subgroups), and 6 nmol/side ICI 118,551 (ICI; n = 16; 8/8 per GRID+/GRID- conditioning subgroups). All

rats were subjected to the habituation, conditioning, and testing procedures described above. Rats in the each group received intra-BLA microinfusions of vehicle, prazosin, or ICI 118,551 immediately following a drug-free test for place preference (Test 1) and tested again for a CPP 24 hr later (Test 2).

Experiment 4B: Post-retrieval intra-BLA prazosin and ICI 118,551 administration in the absence of retrieval. In a follow-up to Experiment 4A, rats were randomly assigned to one of three groups: vehicle no re-exposure (VEH-NR; n = 9; 4/5 per GRID+/GRID- conditioning subgroups), 0.5 nmol/side prazosin (PRAZ-NR; n = 9; 5/4 per GRID+/GRID- conditioning subgroups), and 6 nmol/side ICI 118,551 (ICI-NR; n = 7; 3/4 per GRID+/GRID- conditioning subgroups). Rats were subjected to the habituation and conditioning procedures described above. However, rats in the VEH-NR, PRAZ-NR and ICI-NR groups did not receive Test 1, but received drug infisions in their home cages, followed 24 hr later by a test for CPP during Test 2. This omission of Test 1 was intended to determine whether the effect seen in Experiment 4A was specific to re-exposure to the cocaine environment.

Data Analysis

Statistical analyses were conducted with SPSS software (Chicago, IL). Place preference was analyzed using two-way ANOVAs [Drug Treatment Dose X Conditioning Subgroup (GRID+/GRID-)]. Student's t-test was used to examine *a priori* comparisons between GRID+ and GRID- subgroups (with a Bonferroni correction for

multiple comparisons). Activity data during Test 2 were analyzed using a one-way ANOVA (Drug Treatment).

For FOS analysis, one-way or two-way ANOVAs were used to analyze the number of FOS-positive cells per treatment group, as specified. Student's t-test was used for post-hoc analysis where indicated. Significance was set at $p < .05$.

Results

Experiment 1A - Post-retrieval Betaxolol administration failed to alter a subsequent CPP. Rats in each group showed a CPP during Test 1, prior to betaxolol or vehicle administration. Figure 4A shows the mean (+SEM) time spent on the GRID floor during Test 1 for groups BET-0, BET-5, and BET-10. A two-way ANOVA (Betaxolol Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,66) = 90.6$, $p < .001$], indicating reliable preference across drug treatments for the cocaine-paired floor, but no significant interaction or main effect of betaxolol ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that groups BET-0 [$t(22) = 6.5$, $p < .001$], BET-5 [$t(22) = 6.7$, $p < .001$], and BET-10 [$t(22) = 3.9$, $p < .001$] (Bonferroni-corrected $\alpha/3 = 0.017$), prior to their respective treatments, all showed significant cocaine-induced CPP.

Rats that received betaxolol following Test 1 continued to show a CPP during Test 2. Figure 4B shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups BET-0, BET-5, and BET-10. A two-way ANOVA (Betaxolol Dose X

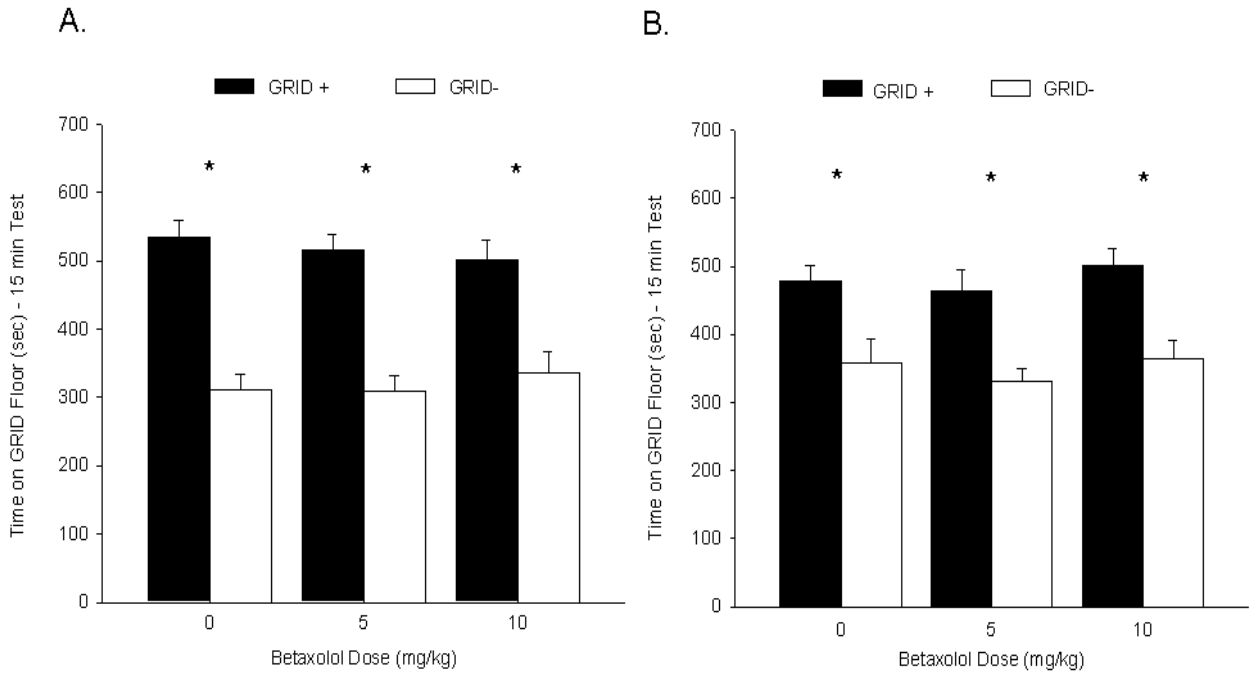

FIGURE 4. Post-retrieval administration of betaxolol had no effect on a subsequent cocaine CPP.

(A) Cocaine induced a CPP for the cocaine-paired floor during Test 1. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. All groups showed a significant preference for the cocaine-paired floor. (B) Post-retrieval betaxolol following Test 1 did not affect a cocaine CPP during Test 2. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the first 15-min of a 25-min drug-free test. Rats treated with betaxolol following Test 1 continued to show a significant preference for the cocaine-paired floor during Test 2. *$p < 0.017$ (Bonferroni-corrected $\alpha/3$).

Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,66) = 35.7$, $p < .001$], again indicating reliable preference for the cocaine-paired floor, but no significant interaction or main effect of betaxolol ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment revealed that groups BET-0 [$t(22) = 3.0$, $p < .01$], BET-5 [$t(22) = 3.7$, $p < .005$], and BET-10 [$t(22) = 3.7$, $p < .005$] (Bonferroni-corrected $\alpha/3 = 0.017$) again all showed significant cocaine-induced CPP during Test 2. Thus, the β_1-adrenergic antagonist betaxolol failed to attenuate a CPP for cocaine when administered following Test 1.

A one-way ANOVA [$F(2,69) = 2.0$, $p = .14$] revealed that activity during Test 2 did not differ significantly between the three groups BET-0 (1341±49), BET-5 (1477±43), and BET-10 (1391±54).

Experiment 1B: Post-retrieval ICI 118,551 administration attenuated a subséquent CPP. Rats in each group showed a CPP during Test 1, prior to ICI 118,551 or vehicle administration. Figure 5A shows the mean (+SEM) time spent on the GRID floor during Test 1 for groups ICI-0 and ICI-8. A two-way ANOVA (ICI 118,551 Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,44) = 46.3$, $p < .001$], indicating reliable preference across drug treatments for the cocaine-paired floor, but no significant interaction or main effect of ICI 118,551 ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that groups ICI-0 [$t(22) = 4.3$, $p < .001$]

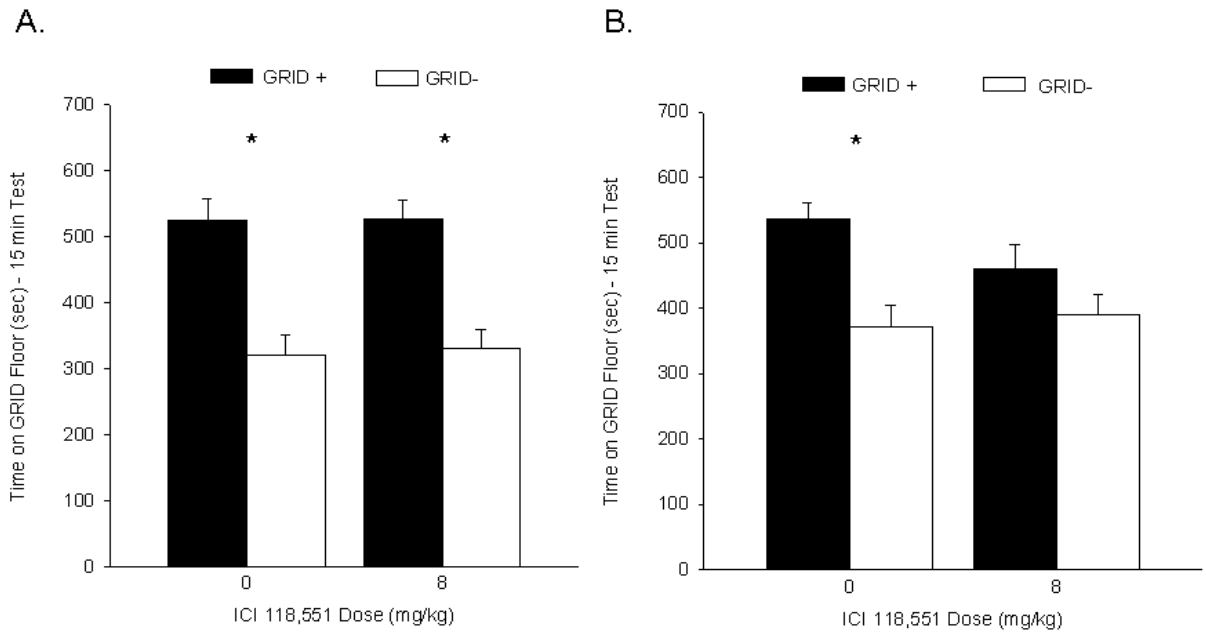

FIGURE 5. Post-retrieval administration of ICI 118,551 attenuated a subsequent cocaine CPP.

(A) Cocaine induced a CPP for the cocaine-paired floor during Test 1. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Both groups showed a significant preference for the cocaine-paired floor. (B) Post-retrieval ICI 118,551 following Test 1 attenuated a cocaine CPP during Test 2. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the first 15-min of a 25-min drug-free test. Rats treated with ICI 118,551 following Test 1 showed no significant preference for the cocaine-paired floor during Test 2, while vehicle-treated rats continued to show a significant CPP. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$).

and ICI-8 [$t(22) = 5.4$, $p < .001$] (Bonferroni-corrected $\alpha/2 = 0.025$) both showed significant cocaine-induced CPP prior to their respective treatments.

Rats that received vehicle following Test 1 continued to show a CPP during Test 2, while ICI 118,551-treated rats failed to show a significant place preference during Test 2. Figure 5B shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups ICI-0 and ICI-8. A two-way ANOVA (ICI 118,551 Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,44) = 13.7$, $p < .001$], indicating preference for the cocaine-paired floor, but no significant interaction [$F(1,44) = 2.2$, $p = .14$] or main effect of ICI 118,551 ($F < 1$). However, Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment revealed that while group ICI-0 [$t(22) = 3.9$, $p < .001$] (Bonferroni-corrected $\alpha/2 = 0.025$) continued to show a significant place preference for the cocaine-paired floor, group ICI-8 [$t(22) = 1.5$, $p > .15$] (Bonferroni-corrected $\alpha/2 = 0.025$) no longer showed a significant preference during Test 2 . These results suggest that the β_2-adrenergic antagonist ICI 118,551 attenuated a CPP for cocaine when administered following Test 1.

Activity during Test 2 did not differ between the two groups ICI-0 (1391±53) and IC1-8 (1368±57), as revealed by a one-way ANOVA ($F < 1$). Thus, the difference in preference between the two groups was not due to a residual or conditioned locomotor effect of the drug.

Experiment 1C: ICI 118,551 administration had no effect on CPP in the absence of retrieval. Rats receiving ICI 118,551 or vehicle in their home cages, in the absence of

Test 1, showed no difference in CPP during Test 2. Figure 6 shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups ICI-0-NR and ICI-8-NR. A two-way ANOVA (ICI Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,44) = 156.2$, $p < .001$], suggesting reliable preference for the cocaine-paired floor, but no significant interaction or main effect of ICI 118,551 ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups confirmed that both the ICI-0-NR [$t(22) = 9.1$, $p < .001$] and ICI-8-NR [$t(22) = 8.5$, $p < .001$] (Bonferroni-corrected $\alpha/2 = 0.025$) groups showed significant cocaine-induced CPP, suggesting that the effect seen in the ICI-8 group in Experiment 2 was specific to exposure to the testing environment.

A one-way ANOVA revealed no difference between the ICI-0-NR (1587±43) and ICI-8-NR (1604±45) groups ($F < 1$), suggesting no residual locomotor effect of the drug.

Experiment 1D: Post-retrieval prazosin administration attenuated a subsequent CPP.

Rats in each drug treatment group showed a CPP during Test 1, prior to prazosin or vehicle administration. Figure 7A shows the mean (+SEM) time spent on the GRID floor during Test 1 for all groups. A two-way ANOVA (Prazosin Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,90) = 73.8$, $p < .001$], indicating reliable preference across drug treatments for the cocaine-paired floor, but no interaction [$F(2,90) = 2.4$, $p = .10$] or main effect of prazosin ($F < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that groups PRAZ-0 [$t(30) = 6.8$, $p < .001$], PRAZ-.3

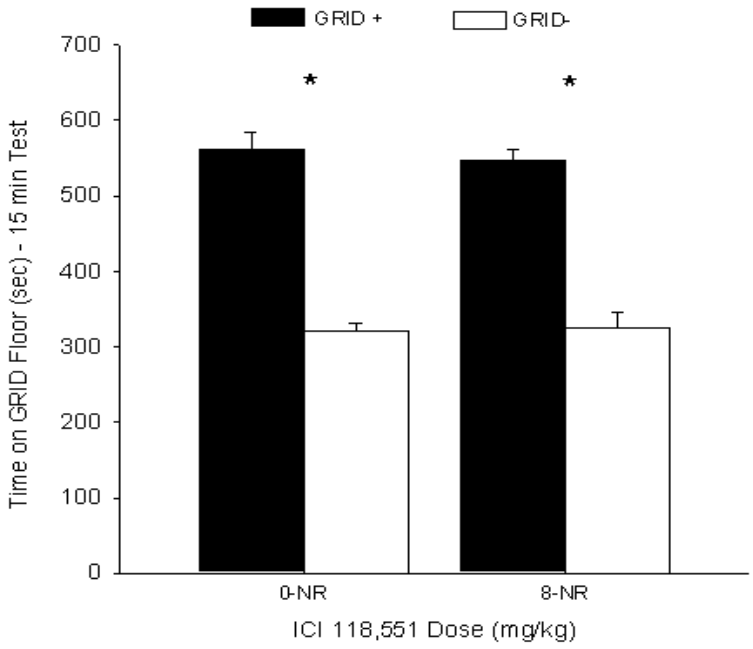

FIGURE 6. No effect of ICI 118,551 on a cocaine CPP when administered in the absence of re-exposure.

Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the first 15-min of the 25-min drug-free test. Groups that received ICI 118,551 or vehicle in the absence of Test 1 (NR) both showed a significant cocaine CPP during Test 2. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$). NR = No Re-exposure.

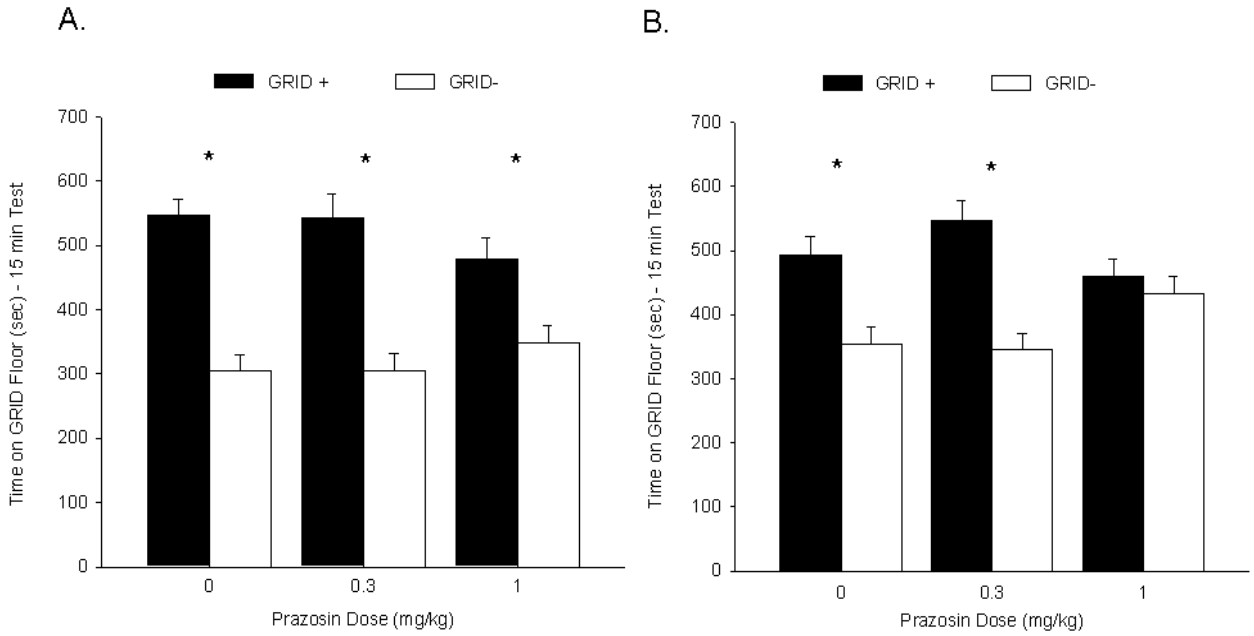

FIGURE 7. Post-retrieval administration of the highest dose of prazosin attenuated a subsequent cocaine CPP.

Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Prior to prazosin administration, all groups showed a significant preference for the cocaine-paired floor. (B) Post-retrieval prazosin attenuated a cocaine CPP. Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the first 15-min of the 25-min drug-free test. Rats treated with 1 mg/kg prazosin following Test 1 showed no preference for the cocaine-paired floor during Test 2, while rats administered vehicle or 0.3 mg/kg prazosin continued to express a significant preference for the cocaine-paired floor. *$p < 0.017$ (Bonferroni-corrected $\alpha/3$).

[t(30) = 5.2, p < .001] and PRAZ-1 [t(30) = 3.2, p < .005] (Bonferroni-corrected α/3 = 0.017) all showed significant cocaine-induced CPP prior to their respective treatments.

Rats that received vehicle and 0.3 mg/kg prazosin following Test 1 continued to show a CPP during Test 2, while 1 mg/kg prazosin-treated rats failed to show a significant place preference during Test 2. Figure 7B shows the mean (+SEM) time spent on the GRID floor during Test 2 for all groups. A two-way ANOVA (Prazosin Dose X Conditioning Subgroup) revealed a significant interaction [F(2,90) = 5.2, p < .01] and a significant main effect of conditioning subgroup [F(1,90) = 30.5, p < .001], but no main effect of prazosin dose (F < 1). Follow-up two-way ANOVAs for each pair of prazosin doses revealed significant interactions between the PRAZ-0 and PRAZ-1 groups [F(1,60) = 4.2, p < .05] and the PRAZ-.3 and PRAZ-1 groups [F(1,60) = 10.5, p < .005] and significant main effects of conditioning subgroup [Fs(1,60) > 9.3, ps < .005] but not prazosin dose (Fs < 1), indicating a difference in preference based on dose. There was no significant interaction between the PRAZ-.0 and PRAZ-.3 groups [F(1,60) = 1.3, p = .26] or main effect of prazosin dose (F < 1), but a significant effect of conditioning subgroup [F(1,60) = 10.5, p < .005], indicating reliable preference. Student's t-tests comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each prazosin dose confirmed that groups PRAZ-0 [t(30) = 3.5, p < .005] and PRAZ-.3 [t(30) = 5.3, p < .001] continued to show a preference during Test 2, the PRAZ-1 group [t(30) = 3.2, p = .47] no longer showed a preference for the cocaine-paired floor (Bonferroni-corrected α/3 = 0.017). These results suggest that a 1 mg/kg dose of the α_1-adrenergic antagonist prazosin attenuated a CPP for cocaine when administered following Test 1.

Activity during Test 2 did not differ between the three groups PRAZ-0 (1427±41), PRAZ-.3 (1362±51), and PRAZ-1 (1448±47), as revealed by a one-way ANOVA ($F < 1$), suggesting that the difference in preference in the PRAZ-0 group was not due to a residual or conditioned locomotor effect of the drug.

Experiment 1E: Prazosin administration had no effect on CPP in the absence of retrieval. Groups that received vehicle or 1 mg/kg prazosin in the absence of Test 1 showed no difference in CPP during Test 2. Figure 8 shows the mean (+SEM) time spent on the GRID floor during Test 2 for groups PRAZ-0-NR and PRAZ-1-NR. A two-way ANOVA (Prazosin Dose X Conditioning Subgroup) revealed a significant main effect of conditioning subgroup [$F(1,46) = 156.2$, $p < .001$], but no interaction or main effect of Prazosin ($Fs < 1$), suggesting reliable preference for the cocaine-paired floor. Student's t-test confirmed that both the PRAZ-0-NR [$t(22) = 8.4$, $p < .001$] and PRAZ-1-NR [$t(22) = 9.5$, $p < .001$] (Bonferroni-corrected $\alpha/2 = 0.025$) groups showed significant cocaine-induced CPP, suggesting that the effect seen in the PRAZ-1 group in Experiment 1 was specific to exposure to the testing environment.

A one-way ANOVA revealed no difference between the PRAZ-0-NR (1591±55) and PRAZ-1-NR (1601±56) groups ($F < 1$), suggesting no residual locomotor effect of the drug.

Experiment 2 – Prazosin and ICI 118,551 had no conditioned aversive effect. Prazosin and ICI 118,551, when administered following CS exposure, failed to alter the neutral preference for the distinct floor cues. Figure 9 shows the mean (+SEM) time spent on the

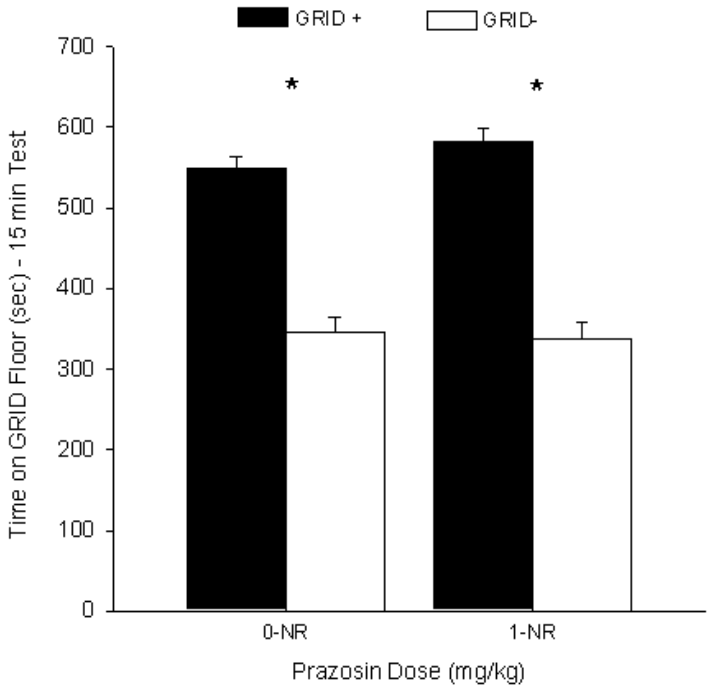

FIGURE 8. No effect of prazosin on a cocaine CPP when administered in the absence of re-exposure.

Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the first 15-min of the 25-min drug-free test. Groups that received vehicle or 1 mg/kg prazosin in the absence of Test 1 (NR) both showed a significant cocaine CPP during Test 2. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$). NR = No Re-exposure.

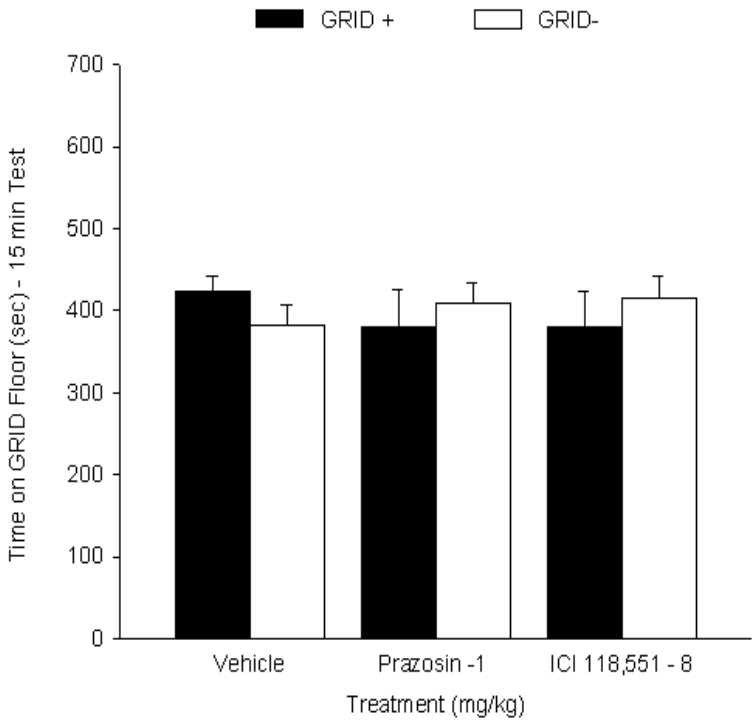

FIGURE 9. No effect of prazosin or ICI 118,551 when administered after one of two distinct CS exposures.

Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the first 15-min of the 25-min test. Groups that received vehicle, 1 mg/kg prazosin, or 8 mg/kg ICI 118,551 after exposure to one of the floor cues (and vehicle after the other) showed no change in neutral preference for the two cues.

GRID floor during a preference test for groups VEH, PRAZ-1 and BET-8. Separate two-way ANOVAs (Drug Treatment X Conditioning Subgroup) between the PRAZ-1 or BET-8 groups and the VEH group revealed no significant interactions [$Fs(1,28) < 1.7$, $ps > .20$] or main effects ($Fs < 1$). Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that the VEH [$t(14) = 1.4$, $p = .19$], PRAZ [$t(14) = -0.5$, $p = .60$], and ICI [$t(14) = -0.7$, $p = .51$](Bonferroni-corrected $\alpha/3 = 0.017$) groups all failed to show a significant preference for either floor cue. Therefore, at the doses used here, these drugs are not by themselves aversive, suggesting a conditioned aversion to the preferred, cocaine-paired cue following the initial tests of preference is not a likely explanation for the Test 2 impairments in CPP seen in Experiments 1B and 1D.

Experiment 3A: FOS expression in the BLA following test for cocaine CPP. FOS-IR in the BLA in response to differential exposure to floor cues during testing was higher in the choice condition than when these cues are presented separately. Behaviorally, rats in the choice floor condition of FOS expression analysis showed cocaine CPP (Figure 10A), as confirmed by Student's t-test [$t(6) = 3.3$, $p < .05$].

Across the BLA, FOS-IR was higher in response to exposure to the choice CS+/CS- cues than that of either of the cues presented separately (Figure 10B), an effect that was prominent in a more posterior region of the BLA (Figure 10C). Figure 10D shows representative FOS-IR in the BLA for each of the three groups. For FOS analysis across the BLA (bregma -1.80 to -3.30)(Figure 10B) and between ranges [-1.80 to -2.30 (A),

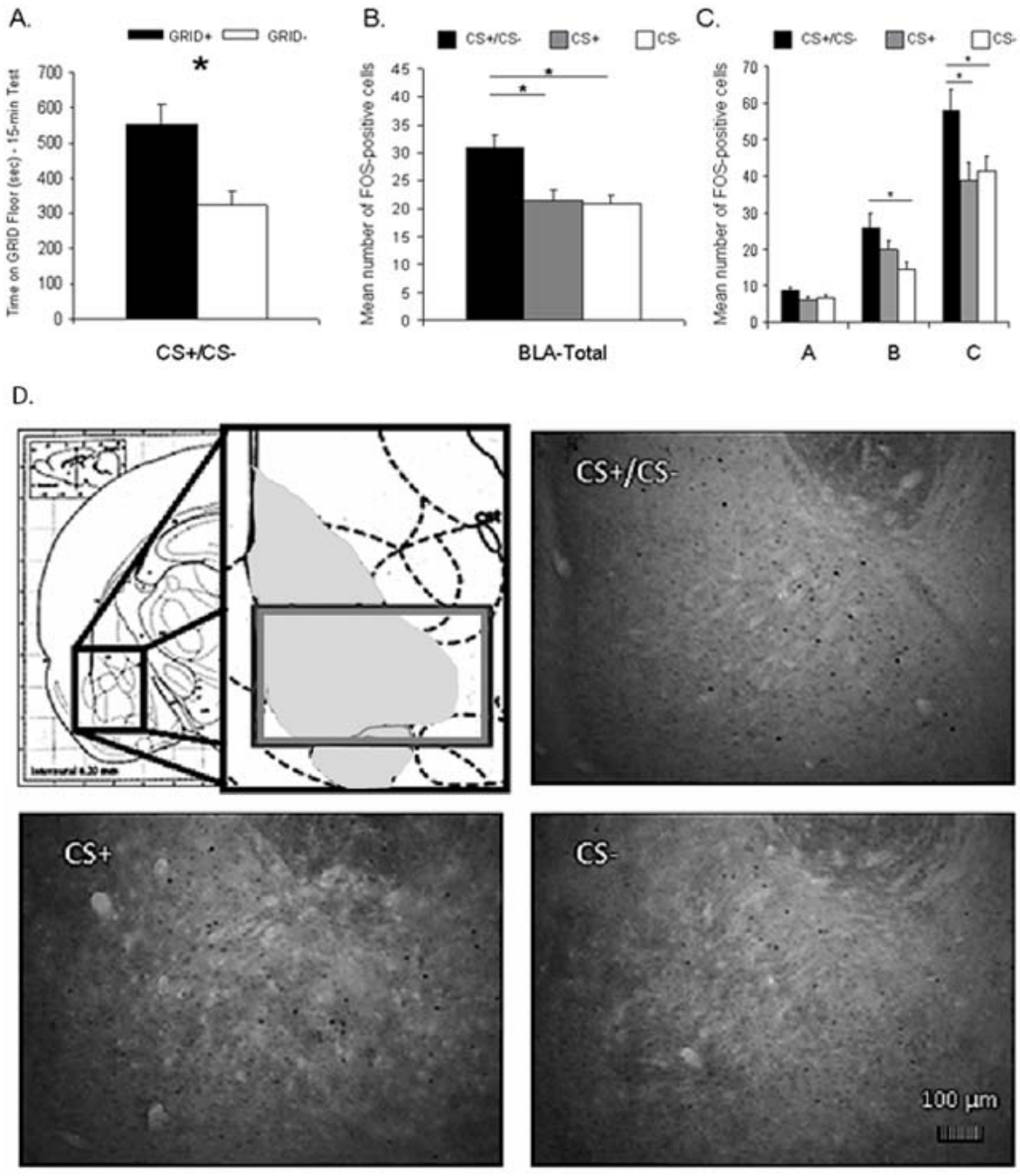

FIGURE 10. FOS-IR in the BLA after exposure to the CS+/CS- condition (choice floor) was higher than after exposure to either the CS+ or CS- conditions.

(A) Animals in the CS+/CS- condition showed a CPP. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. (B) Across the BLA (-1.80 to -3.30), FOS-IR was significantly higher in the CS+/CS- condition than in the CS+ or CS- conditions. Data represent mean number of FOS-positive cells. (C) Across distinct bregma levels of the BLA, a similar pattern emerged in the most posterior region. Data represent mean number of FOS-positive cells. There were no differences in FOS-IR between the three floor conditions in bregma level A (-1.80 to -2.30). In bregma level B (-2.30 to -2.80), there was significantly higher FOS-IR in the CS+/CS- condition as compared to the CS- condition, whereas the CS+ was not significantly different than either of the other two floor conditions. In bregma level C (-2.80 to -3.30), FOS-IR was significantly higher in the CS+/CS- condition than in both the CS+ or CS- conditions, which showed similar levels of FOS response. (D) Representative FOS-IR from each of the three floor configurations. Shaded in light grey in the upper left panel is the entire BLA as analyzed for immunohistochemistry. The dark grey box on the right side of the upper left panel indicates the area represented by the representative FOS-IR panels. *$p < 0.05$.

-2.30 to -2.80 (B), and -2.80 to -3.30 (C)] (Figure 10C), a repeated measures ANOVA (Bregma Range X Test Floor), with a Greenhouse-Geisser correction for a violation of sphericity, revealed a significant interaction [$F(2.7,28.5) = 4.4$, $p < .05$], as well as significant main effects of test floor [$F(2,21) = 4.5$, $p < .05$] and bregma range [$F(1.4,28.5) = 211.9$, $p < .001$]. Follow-up ANOVAs between ranges A and B, B and C, and A and C all revealed significant interactions [$Fs(2,21) > 4.2$, $ps < .05$] and main effects of both test floor [$Fs(1,21) > 4.0$, $ps < .05$] and bregma range [$Fs(2,21) > 108.0$, $ps < .05$]. One way ANOVAs within each bregma range revealed a significant effect of floor in ranges B and C [$Fs(2,21) > 4.5$, $ps < .05$], but not A ([$F(2,21) = 1.7$, $p = .20$]. A Student's t-test examining range B revealed difference between the CS+/CS- and CS- groups [$t(14) > 2.8$, $p < .05$], a trend towards a difference in the CS+ and CS- groups [$t(14) = 1.9$, $p = .08$], and no difference between the CS+/CS- and CS+ groups [$t(14) = 1.5$, $p = .17$]. A Student's t-test examining range C revealed differences between the CS+/CS- group and each of the other two groups (CS+ and CS-) [$ts(14) > 2.4$, $ps < .05$], but no difference between the CS+ and CS- group [$t(14) = .43$, $p = .67$]. These results imply that FOS-IR is higher in response to exposure to the choice CS+/CS- cues than that of either of the cues presented separately. Because of the higher FOS response in range C, and the differences seen between floor conditions here, we decided to focus on this bregma range for Experiment 3B.

<u>Experiment 3B: Systemic effects of prazosin and ICI 118,551 on a test for cocaine CPP and FOS expression in the BLA.</u> Rats in the choice floor conditions showed preference for the cocaine-paired floor that was not affected by prazosin or ICI 118,551

pretreatment. Figure 11A shows the mean (+SEM) time spent on the GRID floor during the test for all groups. A two-way ANOVA (Drug Pretreatment X Conditioning Subgroup) between the PRAZ-1 and VEH groups revealed significant effects of conditioning subgroup [$F(1,13) = 12.1$, $p < .005$], but no significant interaction or main effect of drug pretreatment ($Fs < 1$). Between the ICI-8 and VEH groups, there was also a significant effect of conditioning subgroup [$F(1,13) = 18.5$, $p < .005$], but no significant interaction or main effect of drug pretreatment ($Fs < 1$). Although it is clear that rats in groups VEH [$t(7) = 2.4$, $p = .05$], PRAZ-1 [$t(6) = 2.7$, $p < .05$], and ICI-8 [$t(6) = 5.4$, $p < .005$] showed a preference for the cocaine-paired floor, due to the small number of animals used for FOS analyses, all p-values did not reach our normal criterion for Bonferroni-correction ($\alpha/3 = 0.017$).

FOS-IR was examined in BLA bregma range C (-2.80 to -3.30)(Figure 11B). Animals with poor representation in bregma range C due to poor staining or tissue destruction were removed from analysis. Table 1 shows the number of animals per group used for FOS analyses in parentheses. To determine replication of the FOS-IR here to that seen in Experiment 3A, an ANOVA was used to examine the effect of Test Floor [$F(2,18) = 39.2$, $p < .001$]. Similar to the results in Experiment 3A, Student's t-test revealed that groups CS+ and CS- did not differ in FOS-IR [$t(13) = 1.4$, $p = .18$], while expression in the CS+/CS- group was significantly higher than that of the CS- group [$t(13) = 2.7$, $p < .05$], and trended toward significantly higher than that of the CS+ group [$t(14) = 1.8$, $p = .10$]. A one-way ANOVA comparing FOS-IR in the VEH CS+/CS-, CS-/CS- and NP groups revealed a significant group effect [$F(2,18) = 39.2$, $p < .001$]. Student's t-test between the CS-/CS- and NP groups [$t(11) = 8.2$, $p < .001$] confirmed the procedural

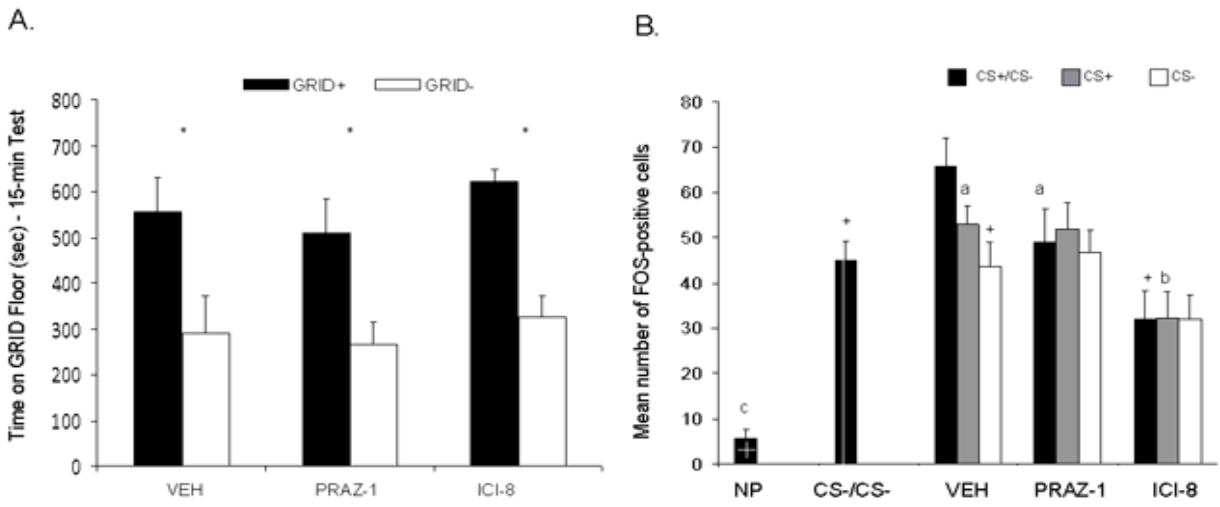

FIGURE 11. Pretreatment with ICI 118,551 attenuated the FOS response in bregma level C of the BLA, while pretreatment with prazosin had only a small effect.

(A) Animals in the CS+/CS- conditions showed a CPP that was unaffected by pretreatment. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Animals pretreated with vehicle, prazosin or ICI-118,551 all showed place preference (but see Results). (B) In bregma range C of the BLA (-2.80 to -3.30), FOS-IR was significantly attenuated in both the CS+/CS- and CS+ conditions in animals pretreated with 8 mg/kg ICI 118,551. FOS-IR was largely unaffected by prazosin pretreatment, though there was a nonsignificant decrease in the CS+/CS- condition. Furthermore, FOS-IR in the vehicle-treated CS+/CS- was significantly higher than in animals that did not receive cocaine during conditioning but were tested in the presence of the choice floor (CS-/CS-). Data represent mean number of FOS-positive cells in each condition. *$p < 0.05$; $^{+}p < 0.05$ (compared to VEH CS+/CS-); $^{a}p = .10$ (compared to VEH CS+/CS-); $^{b}p < 0.05$ (compared to VEH CS+); ^{c}p

< 0.05 (compared to CS-/CS-). VEH = Vehicle; PRAZ-1 = 1 mg/kg prazosin; ICI-8 = 8 mg/kg ICI 118, 551; NP = No procedures.

effects of the CPP paradigm on FOS-IR in the BLA, as rats that received no CPP training showed very little FOS-IR in the BLA. Furthermore, the CS+/CS- showed significantly higher FOS-IR than the CS-/CS- group [$t(13) = 2.8$, $p < .05$], suggesting that the novelty of the choice floor condition is not likely mediating the increase in the FOS response in the choice floor condition as seen in Experiment 3A.

Between the VEH and PRAZ groups, a two-way ANOVA (Drug Pretreatment X Test Floor) revealed no significant interaction [$F(2,40) = 1.7$, $p = .19$] or main effect of drug [$F(1,40) = 1.2$, $p = .29$] or floor [$F(2,40) = 2.4$, $p < .11$]. Thus, prazosin pretreatment had no effect on FOS-IR in the three floor conditions as a whole, though it appears in Figure 11B that there may be a trend toward attenuation of the response in the PRAZ CS+/CS- group, verified by t-test [$t(14) = 1.8$, $p = .10$]. Between the VEH and ICI groups, a two-way ANOVA revealed a significant main effect of drug [$F(1,39) = 24.3$, $p < .001$], but no significant interaction [$F(2,39) = 2.1$, $p = .14$] or main effect of floor [$F(2,39) = 2.1$, $p = .14$]. Student's t-test revealed that between the VEH and ICI groups, FOS-IR was significantly different in the CS+/CS- [$t(13) = 3.9$, $p < .005$] and CS+ [$t(13) = 3.0$, $p < .01$] conditions, but not the CS- [$t(13) = 1.5$, $p = .16$] condition. Thus, ICI 118,551 pretreatment attenuated the FOS response in both groups associated with the cocaine cue.

<u>Experiment 4A: Post-retrieval intra-BLA prazosin and ICI 118,551 administration attenuated a subsequent CPP.</u> Rats in each drug treatment group (VEH, PRAZ, and ICI) showed a CPP during Test 1, prior to drug treatment. Figure 12A shows the mean (+SEM) time spent on the GRID floor during Test 1 for all groups. Separate two-way ANOVAs (Post-test Treatment X Conditioning Subgroup) were conducted between the

PRAZ or ICI groups and the VEH group. Between the PRAZ and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,29) = 83.1$, $p < .001$], indicating reliable preference across drug treatments for the cocaine-paired floor, but no interaction [$F(1,29) = 2.4$, $p = .13$] or main effect of prazosin [$F(1,29) = 3.9$, $p = .06$]. Between the ICI and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,28) = 44.8$, $p < .001$], again indicating reliable preference across drug treatments for the cocaine-paired floor, but no interaction ($F < 1$) or main effect of ICI 118,551 [$F(1,28) = 1.2$, $p = .28$]. Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that the VEH [$t(14) = 4.9$, $p < .001$], PRAZ [$t(15) = 8.3$, $p < .001$] and ICI [$t(14) = 4.6$, $p < .001$] (Bonferroni-corrected $\alpha/3 = 0.017$) groups all showed a significant preference during Test 1, prior to their respective treatments.

Rats that received vehicle following Test 1 continued to show a CPP during Test 2, while prazosin- and ICI 118,551-treated rats failed to show a significant place preference during Test 2. Figure 12B shows the mean (+SEM) time spent on the GRID floor during Test 2 for all groups. Separate two-way ANOVAs (Post-test Treatment X Conditioning Subgroup) were conducted between the PRAZ or ICI groups and the VEH group. Between the PRAZ and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,29) = 9.8$, $p < .005$], indicating reliable preference across drug treatments for the cocaine-paired floor, but no interaction or main effect of prazosin ($Fs < 1$). Between the ICI and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,28) = 7.3$, $p < .05$], again indicating reliable preference across drug treatments for the cocaine-paired floor, but no

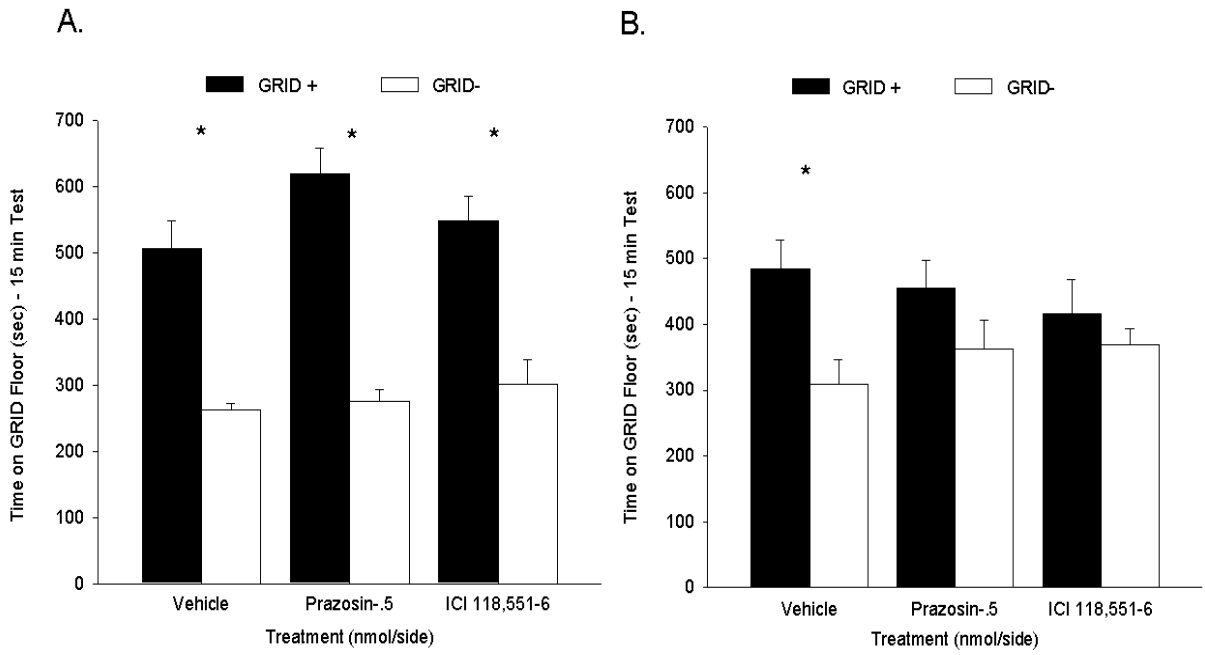

FIGURE 12. Post-retrieval intra-BLA administration of prazosin and ICI 118,551 attenuated a subsequent cocaine CPP.

(A) Cocaine induced a CPP for the cocaine-paired floor during Test 1. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. All groups showed a significant preference for the cocaine-paired floor. (B) Post-retrieval intra-BLA prazosin and ICI 118,551 following Test 1 attenuated a cocaine CPP during Test 2. Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the first 15-min of a 25-min drug-free test. Rats treated with prazosin or ICI 118,551 following Test 1 showed no significant preference for the cocaine-paired floor during Test 2, while vehicle-treated rats continued to show a significant CPP. *$p < 0.017$ (Bonferroni-corrected $\alpha/3$).

interaction [$F(1,28) = 2.5$, $p = .13$] or main effect of ICI 118,551 ($F < 1$). However, Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment revealed that while the VEH group [$t(14) = 2.9$, $p < .017$] continued to show a preference during Test 2, groups PRAZ [$t(15) = 1.5$, $p = .15$] and ICI [$t(14) = 0.8$, $p = .42$] no longer showed a significant preference for the cocaine-paired floor (Bonferroni-corrected $\alpha/3 = 0.017$). Thus, intra-BLA microinfusion of either prazosin or ICI 118,551 immediately after Test 1 attenuated a preference during Test 2. These findings are consistent with systemic findings reported earlier (Figures 5 and 7).

A one-way ANOVA revealed that the VEH (1424±73), PRAZ (1315±73), and ICI (1545±82) groups did not differ in locomotor activity [$F(2,46) = 2.2$, $p = .13$], again suggesting that conditioned or residual effects of the drugs on locomotion do not explain the impairments in CPP seen during Test 2.

Figure 14 shows the general placement range of injections into the BLA and a representative bilateral BLA cannulation.

Experiment 4B: Post-retrieval intra-BLA prazosin and ICI 118,551 administration had no effect on CPP in the absence of retrieval. The VEH, PRAZ, and ICI groups showed no difference in CPP in the absence of Test 1. Figure 13 shows the mean (+SEM) time spent on the GRID floor during Test 2 for the three groups. Separate two-way ANOVAs (Post-test Treatment X Conditioning Subgroup) were conducted between the PRAZ or ICI groups and the VEH group. Between the PRAZ and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,14) = 43.7$, $p < .001$], indicating reliable preference across drug treatments for the cocaine-paired floor,

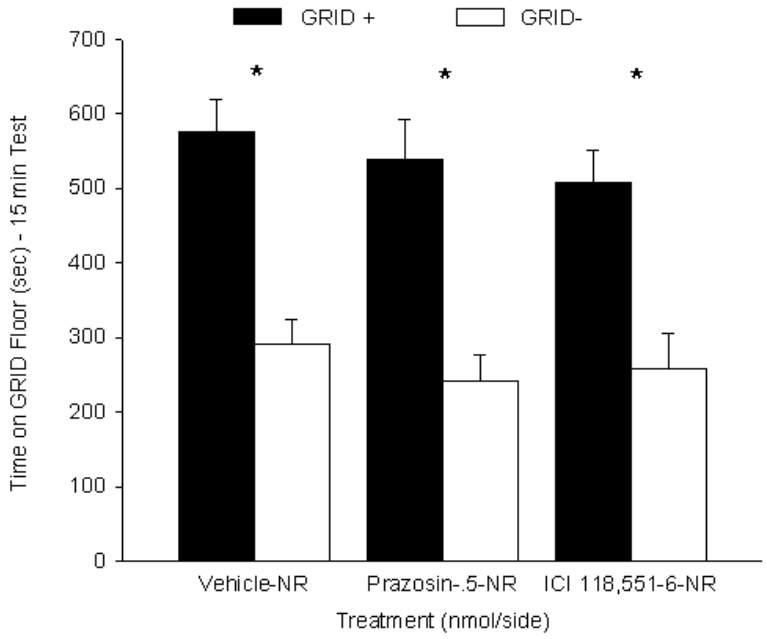

FIGURE 13. No effect of intra-BLA administration of prazosin or ICI 118,551 on a cocaine CPP when administered in the absence of re-exposure.

Data represent mean (+SEM) time spent on GRID floor for the GRID+ and GRID- subgroups during the first 15-min of the 25-min drug-free test. Groups that received vehicle, prazosin, or ICI 118,551 in the absence of Test 1 (NR) all showed a significant cocaine CPP during Test 2. *$p < 0.017$ (Bonferroni-corrected $\alpha/3$). NR = No Re-exposure.

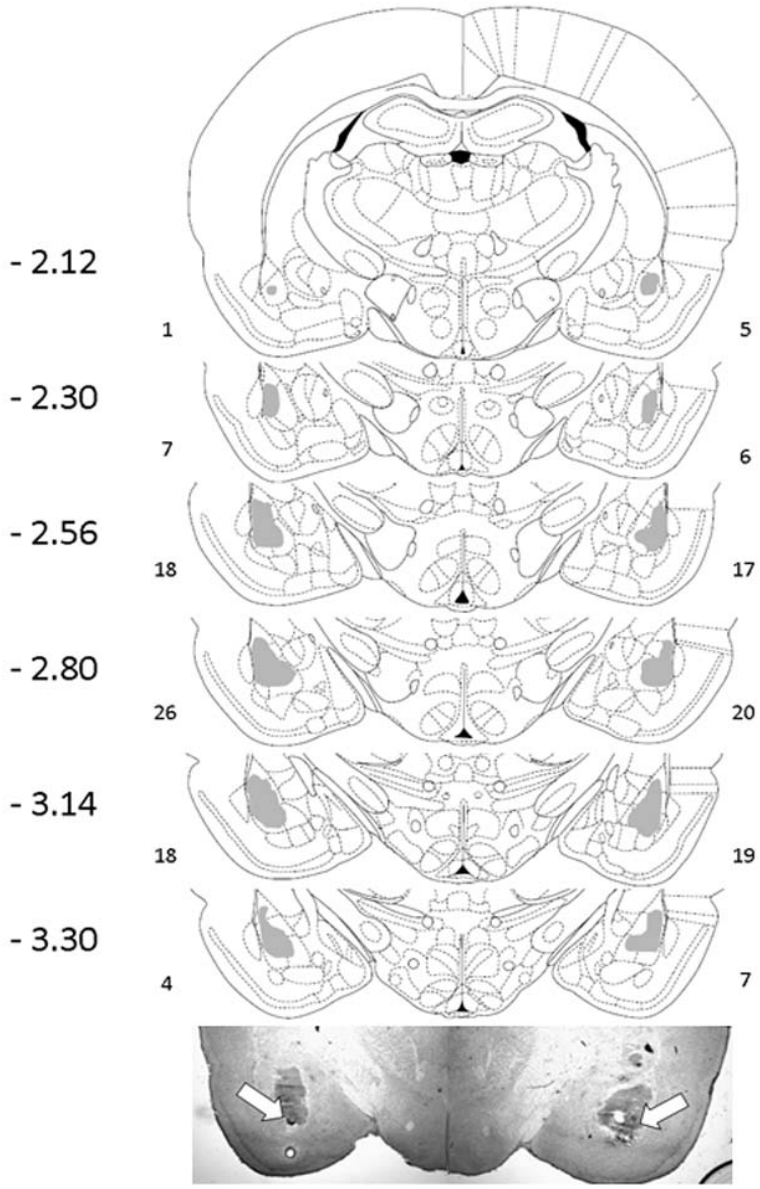

FIGURE 14. Areas in grey represent the cannula placements for intra-BLA studies. All cannulae were located within the grey areas. The far left represents A/P coordinate with respect to bregma. Smaller numbers to the right and left of figure represent number of cannula placements within that bregma level. The bottom figure is a representative slice from an intra-BLA bilaterally-cannulated animal. White arrows indicate placement of dye injection.

but no interaction or main effect of prazosin (Fs < 1). Between the ICI and VEH groups, a two-way ANOVA revealed a significant main effect of Conditioning Subgroup [$F(1,12) = 38.9$, $p < .001$], again indicating reliable preference across drug treatments for the cocaine-paired floor, but no interaction ($F < 1$) or main effect of ICI 118,551 [$F(1,12) = 1.4$, $p = .26$]. Student's t-test comparing time spent on the GRID floor for the GRID+ and GRID- subgroups within each drug treatment confirmed that the VEH [$t(5) = 3.7$, $p < .017$], PRAZ [$t(7) = 4.3$, $p < .005$] and ICI [$t(7) = 5.2$, $p < .005$] (Bonferroni-corrected $\alpha/3 = 0.017$) groups showed significant cocaine-induced CPP, suggesting that the effects seen in Experiment 4A were specific to exposure to the testing environment.

A one-way ANOVA revealed that the VEH (1459±92), PRAZ (1391±114), and ICI (1326±168) groups did not differ in locomotor activity ($F < 1$).

Figure 14 shows the general placement range of injections into the BLA and a representative bilateral BLA cannulation.

Discussion

These experiments are the first to demonstrate a role for α_1- and β_2-AR antagonists in retrieval-induced plasticity in cocaine CPP. Behavioral effects were observed with both systemic and intra-BLA administration, demonstrating the importance of the noradrenergic system in the BLA in post-retrieval memory mechanisms. Immunohistochemistry confirmed this and demonstrated that in CPP, stimulus configuration was an important mediator of cue-induced activation in the BLA.

We first examined the effects of post-retrieval administration of the β_1- and β_2-AR antagonists, betaxolol and ICI 118,551. We found that ICI 118,551 attenuated a subsequent CPP, while betaxolol had no effect at either dose tested. Furthermore, ICI 118,551 administered to control rats in the absence of memory reactivation failed to affect a cocaine CPP. These results are consistent with previous work demonstrating post-retrieval impairment of a cocaine CPP using the nonspecific β-AR antagonist propranolol (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008), and suggest that the effect of propranolol demonstrated in these previous studies may be mediated by the β_2-AR. The results presented here are also consistent with a number of other studies demonstrating impairment of possible reconsolidation processes via systemic β-AR blockade in drug-mediated learning paradigms, including morphine CPP (Robinson and Franklin, 2007a) and cocaine self-aministration (Milton et al., 2008b), and several other non-drug learning tasks (Abrari et al., 2008; Diergaarde et al., 2006; Przybyslawski et al., 1999; Roullet and Sara, 1998). Propranolol administered following retrieval of a fear memory in humans has recently been reported to completely abolish the expression of fear (Kindt et al., 2009), and thus our results extend findings in both human and animal studies by suggesting that the disruption of maladaptive memories can be achieved via more targeted AR antagonism.

The α_1-AR antagonist, prazosin, at the highest dose tested, also attenuated a cocaine-induced CPP when administered following memory retrieval. Furthermore, prazosin administered to rats in the absence of re-exposure to the drug environment failed to affect a subsequent preference. To date, no studies have demonstrated post-retrieval

impairments using prazosin, though it has been demonstrated to play an important role in emotional memory processing (Ferry et al., 1999b).

One explanation for an attenuation of preference is an aversive conditioning effect of the post-test drug treatment. If ICI 118,551 or prazosin were aversive, the temporal proximity of the drug with the preferred, drug-paired floor during the initial test via immediate post-test administration might decrease preference on a subsequent test (Bormann and Cunningham, 1997). We demonstrated here that administration of ICI 118,551 or prazosin following a single CS exposure, identical to the parameters in our post-retrieval studies, had no effect on a subsequent test. This suggests that ICI 118,551 and prazosin, at the doses we used, were not by themselves aversive. This does not preclude the possibility, however, that interactions of these drugs with the conditioned properties of cocaine present during testing may have different effects, and further studies are needed to elucidate these potential interactions.

Our FOS-IR studies were undertaken to distinguish between the FOS response in the BLA during a choice test versus the separate cues individually, and to determine the effect of ICI 118,551 and prazosin on conditioned changes in FOS-IR to confirm the BLA as a mediator of post-retrieval effects. Previous studies have shown inconsistent results of conditioned cued FOS response in the BLA following cocaine conditioning. Franklin and Druhan (2000) found no conditioned increase in the FOS response in the BLA in rats exposed to a context in which they had previously received multiple cocaine pairings. However, Miller and Marshall (2005a) saw an increase in the FOS response in the BLA following a test for cocaine CPP. We found that FOS-IR in the BLA was higher after exposure to the CS+/CS- condition (choice) during testing than after exposure to

either the CS+ or CS- floors. This effect was not due to the novel configuration of the chamber in the CS+/CS- group, because a control group that received conditioning trials without cocaine and testing on the choice floor had a significantly lower FOS response in the BLA compared to cocaine-conditioned animals.

One possible explanation for the increased FOS response of the choice floor is an effect of behavioral contrast (Bevins, 2005). Upon exposure to either the drug- or saline-paired floor, FOS-IR in the BLA is similar. However, upon exposure to the choice floor, the differential value of the cues is unmasked and the FOS response is increased in the BLA. Another possible explanation is that FOS-IR in the BLA upon expression of CPP reflects the learning of a CS+ approach response (Gremel and Cunningham, 2008). As the choice floor can conceivably be thought of as a distinct, new environment, it is likely that this new experience requires processing of a variety of new information, including recognizing a new floor configuration, discriminating between the value of the two floors, and making a response based on this information. Thus it is possible that adrenergic antagonists disrupted memory formation based on this new information, resulting in an attenuated preference response upon subsequent testing, an idea consistent more with impaired consolidation rather than reconsolidation (Tronel et al., 2005).

With respect to the drug pretreatments, ICI 118,551 attenuated the increased FOS response in the choice floor condition, as well as in the CS+ floor condition, but had no effect on the behavioral expression or retrieval of CPP. This suggests a role for the BLA, and possibly FOS activation, in mediating post-retrieval processes via β_2-ARs. Prazosin, which also failed to affect preference, had only a minor effect on the FOS response, and only in the choice floor condition, suggesting a limited role for α_1-ARs in the BLA in

post-retrieval processes. As noted above, these attenuations of the FOS response, because they were more apparent in the choice floor conditions, could be related to an impairment of consolidation of information learned during the CPP test. Regardless, our FOS results indicate that the BLA was undoubtedly involved in the conditioned effects of cocaine cues in our experiments. And though not definitive, the FOS results were suggestive of a post-retrieval effect of noradrenergic antagonism in the BLA. Given some of the differences in gene expression that occur after induction or retrieval of cue-associated learning (Lee et al., 2004), it will be important to further characterize the role of FOS in memory processes related to drug learning.

Based on the large body of evidence indicating the importance of the BLA in mediating impairments of reconsolidation effects (Debiec and Ledoux, 2004; Milekic et al., 2007; Nader et al., 2000), as well as the suggestive data provided by our FOS studies, we examined the role of the BLA in mediating the post-retrieval impairments seen here with systemic adrenergic antagonists. Only one study to date has demonstrated AR blockade in the BLA as a useful tool for post-retrieval impairment. Debiec & LeDoux (2004) demonstrated that propranolol administered into the BLA following memory reactivation impaired an inhibitory avoidance memory in rats. Here, we have shown that noradrenergic blockade in the BLA translates to studies of drug conditioning. Our studies indicate, at least in the cocaine CPP paradigm examined here, that the post-retrieval effect of β-AR antagonism on subsequent cue responding can be achieved via intra-BLA β_2-AR antagonism with ICI 118,551, consistent with our systemic findings. We further demonstrated that the α_1-AR antagonism with prazosin impaired a subsequent

preference when administered into the BLA following memory retrieval, again consistent with our systemic findings.

A number of other findings have also suggested that the BLA mediates impairments of reconsolidation in drug learning paradigms (e.g., Lee et al., 2005; Lee and Everitt, 2008; Wang et al., 2008). For example, the NMDA receptor antagonist AP-5 administered into the BLA of rats disrupted new learning mediated by a cocaine-associated CS when administered prior to a CS-only retrieval trial (Lee and Everitt, 2008). In a CPP paradigm, Wang et al. (2008) found that corticosterone, as well the glucocorticoid agonist RU28362, both impaired a subsequent morphine CPP in rats when microinjected into the BLA following an initial test. Glucocorticoids have been shown to interact with the adrenergic system in the BLA to modulate memory formation (Roozendaal et al., 2002). Interestingly, post-retrieval administration of the protein synthesis inhibitor anisomycin into the BLA had no effect on a subsequent morphine CPP in rats (Yim et al., 2006). However, anisomycin administered into the BLA in rats has been shown to produce immediate and dramatic increases in norepinephrine there, as measured by microdialysis (Canal et al., 2007), which would have an effect opposite to that of adrenergic antagonism, and may be one reason post-retrieval anisomycin failed to impair morphine CPP.

In summary, we found that α_1- and β_2-AR antagonists can attenuate a subsequent cocaine CPP when administered after an initial test. This effect was seen both systemically and intra-BLA, confirming the BLA as one critical mediator of post-retrieval impairments. Because environmental stimuli that are associated with drugs of abuse are critical to the persistence of addiction in humans (Childress et al., 1988b;

Weiss, 2005), targeting post-retrieval mechanisms via adrenergic blockade in the BLA represents a unique way to examine cue-induced drug-mediated behaviors in animals.

CHAPTER 4. General Discussion

The ability to disrupt memories presumed to be previously consolidated following retrieval has been proposed to be due to the impairment of a memory reconsolidation process. The majority of reconsolidation experiments have focused on aversive learning paradigms (e.g., Misanin et al., 1968; Nader et al., 2000), suggesting disruption of reconsolidation as a potential treatment for conditioned fears and PTSD (Nader et al., 2000). More recently, impairments in reconsolidation have been demonstrated in appetitive learning paradigms, including those examining the associative learning that occurs between neutral stimuli and drugs of abuse. Because drug-associated stimuli can be a major factor in the persistence of addiction in humans (Childress et al., 1988b; Weiss, 2005), targeting potential reconsolidation mechanisms has been suggested as a potential target of pharmacotherapies aimed at dampening the powerful control of these stimuli over behavior (Taylor et al., 2009). To that end, several studies have demonstrated impairment of reconsolidation as a means to reduce drug cue-mediated behaviors in animals using a variety of pharmacological treatments (e.g., Bernardi et al., 2007; Lee et al., 2005; Miller and Marshall, 2005b).

The focus of the studies reported here was to examine the role of the noradrenergic system as a potential mediator of the reconsolidation of drug memories using the CPP paradigm. It was first demonstrated that the non-specific β_1/β_2-AR antagonist, propranolol, when systemically administered following an initial test of cocaine CPP, attenuated preference during a subsequent test (Chapter 2). This study demonstrated that

post-retrieval impairment could be induced in a drug conditioning paradigm via β-AR antagonism, consistent with what had been found in other studies reporting propranolol-induced reconsolidation in non-drug learning paradigms (Abrari et al., 2008; Diergaarde et al., 2006; Przybyslawski et al., 1999). Furthermore, this result has since been demonstrated using a morphine CPP paradigm (2007a). Robinson & Franklin (2007a) also demonstrated that the effect of propranolol was centrally, and not peripherally, mediated, as nadolol, a β-AR antagonist that poorly penetrates the blood-brain barrier, had no effect.

It was next demonstrated that the effect of propranolol was mediated by the β_2-, and not the β_1-, AR subtype, as the β_2-AR antagonist ICI 118,551 replicated the post-retrieval impairment of CPP by propranolol (Chapter 3). In contrast, the β_1-AR betaxolol had no effect. To date, no other studies have examined the subtype specificity of propranolol's effect in a reconsolidation paradigm. Furthermore, it was also demonstrated that the α_1-AR prazosin, when administered post-test, also attenuated a subsequent preference (Chapter 3). To date, no studies have demonstrated post-retrieval impairment with prazosin, though a recent study demonstrated a lack of impairment of post-retrieval prazosin on a subsequent nicotine CPP (Forget et al., 2009). However, procedural differences could account for this lack of effect. Nonetheless, the results reported here demonstrate that β_2- and α_1-ARs both induce post-retrieval impairments in the cocaine CPP paradigm.

Because the BLA has been demonstrated to play an important role in reconsolidation processes in both drug (Lee et al., 2005; Milton et al., 2008a) and nondrug (Debiec and Ledoux, 2004; Milekic et al., 2007) conditioning paradigms, FOS expression in the BLA

was examined following the expression of a cocaine CPP (Chapter 3). One of the major findings here was that FOS expression in response to preference testing was higher in the BLA in cocaine-conditioned animals than in animals that received no cocaine during conditioning, confirming the BLA as one potential locus of the response to cocaine-conditioned cues, consistent with a previous report demonstrating an increase in FOS activation here (Miller and Marshall, 2005a). Another major finding was that pretreatment with ICI 118,551, which behaviorally failed to impair the expression of a conditioned place preference in the FOS studies when administered prior to testing, completely attenuated the conditioned increase in FOS activation, suggesting perhaps post-retrieval involvement of the FOS response, consistent with other studies suggesting disruptions of IEG expression as a mediator of reconsolidation impairments (Lee et al., 2005). Prazosin, which also had a post-retrieval behavioral effect, modestly (but nonsignificantly) attenuated FOS expression in response to preference of a CPP, while also having no effect on the behavioral expression of CPP. However, the strongest conclusion that can be made of these and other FOS results in Chapter 2 is that the BLA is activated during a test for cocaine CPP. What this activation and its attenuation by adrenergic antagonists represent in terms of memory processes remains speculative.

The last study demonstrated that both ICI 118,551 and prazosin, administered directly into the BLA following an initial test of preference, both impaired cocaine CPP upon subsequent testing (Chapter 3), consistent with the systemic results presented in Chapters 2 and 3. Though neither of these drugs have, to date, been examined as tools for post-retrieval impairments, these findings are consistent with the ability of post-training infusions of propranolol (Miranda et al., 2003) and prazosin (Ferry et al., 1999b) into the

BLA to impair memory formation and the ability of NE or the β_2-AR agonist clenbuterol into the BLA to enhance memory formation (Ferry and McGaugh, 1999). Furthermore, both propranolol and prazosin have been demonstrated to block the enhancements in memory produced by clenbuterol, with propranolol acting at β-ARs and prazosin influencing β-AR activity (Ferry et al., 1999a). More importantly, the results presented here are consistent with findings that post-retrieval administration of propranolol into the BLA impaired purported reconsolidation of an inhibitory avoidance memory in rats (Debiec and Ledoux, 2004). Thus, NE release into the BLA in response to salient unconditioned and conditioned stimuli during training and retrieval, respectively, likely serves to enhance or maintain memory for those events, and this effect of NE can be blocked with certain adrenergic antagonists.

Alternative explanations to a reconsolidation interpretation

The notion that memories thought to be consolidated can in fact be disrupted upon reactivation has obviously been met with some scrutiny, despite the fact that deficits following retrieval have been demonstrated in a number of different tasks with a variety of manipulations (reviewed in Diergaarde et al., 2008; Tronson and Taylor, 2007). This scrutiny, rightly so, has stemmed from a number of studies that have failed to demonstrate impairment of reconsolidation following retrieval (Cammarota et al., 2004; Hernandez and Kelley, 2004), or found that these deficits are transient (Lattal and Abel, 2004; Prado-Alcala et al., 2006) or can be reversed with reminder treatments (Eisenberg and Dudai, 2004; Fischer et al., 2004). These findings suggest that behavioral impairment

attributed to disruptions in reconsolidation may have alternate explanations. Some of these alternate explanations are discussed below, specifically with regard to the present findings.

Conditioning effects of post-retrieval drugs

One alternative to a reconsolidation interpretation is a conditioning effect of the post-test drug treatment, which could result in a new association or alter the value of the current CS. In terms of CPP, these possibilities could be manifested in a new memory associated with the CS+, as this is the predominant cue with which rats are in contact during expression of a preference, or a change in the value of the conditioned reinforcing properties of that cue. A new CS-US association, based on what would presumably be aversive properties based on the decrease in preference seen in the experiments presented here, is not likely. Previous studies have demonstrated that the dose of propranolol used here (10 mg/kg) failed to induce a place preference on its own (Milton et al., 2008b), alter motor activity (Harris et al., 1996), induce a conditioned taste aversion (Freeman et al., 2008), or devalue food reward incentive (Przybyslawski et al., 1999). Here, ICI 118,551 and prazosin failed to alter the neutral preference for the two distinct floor CSs when administered following CS+ exposure (Chapter 3). Thus, these three drug treatments are not by themselves aversive.

A more plausible scenario is that an interaction between these drugs and the putative conditioned reinforcing properties of the CS+ through immediate post-test administration is in fact aversive, or otherwise diminishes the conditioned reinforcing value of the CS+.

Limited data exists as to whether adrenergic antagonists affect cocaine reinforcement. However, systemic prazosin has been found to attenuate cocaine-induced reinstatement following extinction in a self-administration (SA) paradigm in rats (Zhang and Kosten, 2005), but neither prazosin or propranolol systemically administered prior to cocaine priming had any effect on reinstatement in squirrel monkeys (Platt et al., 2007). Furthermore, ICI 118,551, when administered in combination with betaxolol into either the bed nucleus of the stria terminalis (BNST) or central amygdala (CeA), had no effect on cocaine-primed reinstatement in rats (Leri et al., 2002). Thus, in terms of the CPP studies reported in Chapters 2 and 3, it is unclear if post-retrieval adrenergic antagonism might have decreased the conditioned reinforcing value of the CS+, based on the available evidence.

In addition to its well-studied rewarding/reinforcing effects, cocaine has also been shown to have acute anxiogenic properties that occur when the rewarding effects subside, an effect demonstrated in both human (Williamson et al., 1997) and rodent (Hayase et al., 2005; Paine et al., 2002; Schank et al., 2008) studies. This acute effect of cocaine has been demonstrated to be mediated, in part, by increased noradrenergic activity, as the anxiogenic effects of cocaine, as measured in mice in the elevated plus maze, was shown to be blocked by propranolol, though not by prazosin or the α_2-adrenergic antagonist yohimbine (Schank et al., 2008). The conditioned anxiogenic effect of cocaine has been demonstrated using the CPP paradigm. While animals express a preference for an environment associated with the immediate rewarding effects of cocaine (e.g., Bernardi et al., 2006; Ettenberg and Bernardi, 2007), a conditioned place aversion (CPA) has been shown to develop when conditioning trials with cocaine are delayed relative to

administration of the drug, indicating an avoidance of the environment associated with cocaine (Ettenberg and Bernardi, 2007; Ettenberg et al., 1999). The adrenergic antagonists used in the present study, through a mediation of anxiety, may thus alter the conditioned reinforcing value of the cocaine-conditioned cue.

A study using the conditioned taste aversion (CTA) paradigm illustrates the potential effects of adrenergic antagonists on cocaine-conditioned behaviors. In the CTA paradigm, an animal learns to avoid solution paired with the administration of an aversive drug. Cocaine has been demonstrated to be aversive in CTA (Hunt and Amit, 1987), and Freeman et al. (2008) examined the effect of propranolol and prazosin on a cocaine CTA. Forty minutes after access to a saccharin solution, rats were pretreated with systemic propranolol (10 mg/kg) or prazosin (0.3 mg/kg), doses that did not by themselves induce a CTA, followed 20 min later by an injection of systemic cocaine (10, 18 or 32 mg /kg). Upon later testing of saccharin solution drinking, both prazosin and propranolol failed to attenuate cocaine CTA. In fact, prazosin enhanced cocaine CTA at the two lower doses of cocaine, while propranolol enhanced cocaine CTA at the lowest dose, suggesting that a decrease in noradrenergic transmission increased the aversive properties of cocaine. Although in the CTA study prazosin and propranolol were administered essentially in combination with cocaine, which is procedurally different from the studies outlined in Chapters 2 and 3, it indicates that these drugs may increase the aversive qualities of cocaine. Thus, it is reasonable to speculate that post-retrieval administration of these drugs following a test for CPP conditionally increased the aversive properties of the cocaine representation mediated by the CS+. Assuming that the expression of CPP can be, based on the parameters by which it is measured, a balance between the conditioned

rewarding and aversive properties of a drug, such that a preference results from a greater attribution of positive versus negative value to the cocaine-paired cue, it is possible that the adrenergic antagonists employed in Chapters 2 and 3 conditionally tipped the balance the other way. In other words, these drugs may have decreased the conditioned reinforcing value of the cocaine cue by increasing the aversive quality of the cue, thus attenuating preference upon subsequent testing. However, this idea is not entirely consistent with a general anxiolytic effect of adrenergic antagonists, both systemically (Manion et al., 2007; Rodriguez-Romaguera et al., 2009) and into the amygdala (Graeff et al., 1993), and is not consistent with the CPP results reported here (Chapter 2), in which both prazosin and ICI 118,551 failed to alter the expression of a CPP when administered prior to testing. Pretreatment might also be expected to cause an increase in the conditioned aversive properties of the CS+ due to the interoceptive effects of either of the two adrenergic antagonists. Admittedly, however, this potential conditioned aversion may have been more likely to be seen upon subsequent testing, which was not done in the study referred to above.

Another alternative for the findings reported here, in contrast to that presented above, is that the attenuation of preference during Test 2 in Chapters 2 and 3 is due to a conditioned decrease in anxiety mediated by the cocaine cue. Though not consistently demonstrated, cocaine withdrawal in humans can be characterized by depression and anxiety, (Rudoy and Van Bockstaele, 2007), which may perpetuate drug-seeking behavior (Sarnyai et al., 1995), and in rats withdrawal-induced anxiety is manifested within 2 days of discontinued administration of cocaine (Harris and Aston-Jones, 1993; Sarnyai et al., 1995). Moreover, the noradrenergic system is dysregulated in both

humans (McDougle et al., 1994) and rats (Harris and Williams, 1992) during acute withdrawal and propranolol has been shown to eliminate cocaine withdrawal-induced anxiety in rats (Harris and Aston-Jones, 1993). Withdrawal can also be conditioned to cues associated with drug-induced anxiety. Thus, if rats were associating not only the positive, but also the negative properties of cocaine with the CS+ during conditioning, a conditioned withdrawal during testing might provoke approach toward the CS+ in an effort to obtain the drug to alleviate these negative symptoms (Koob et al., 1997). Decreased anxiety through AR antagonism paired temporally with the cocaine cue might attenuate anxiety-mediated approach on a subsequent test. However, recent evidence using the elevated plus maze following repeated daily injections of cocaine suggests that acute cocaine withdrawal is mediated by the β_1 subtype of the β-AR (Rudoy and Van Bockstaele, 2007), contrary to the findings here (Chapter 3) in which post-retrieval betaxolol, at an identical dose used in that study to attenuate withdrawal, had no effect on a subsequent preference. Thus it seems likely that the effects seen using β-adrenergic antagonists in Chapters 2 and 3 are due to processes mechanistically different from those mediating cocaine-induced anxiety. To date, prazosin has not been studied with regard to the anxiogenic effects of cocaine, though it has been demonstrated to attenuate ethanol withdrawal (Walker et al., 2008). Importantly, though, it is unlikely that anxiogenic effects are, in general, mediating CPPs. For example, the 5-HT_{1A} partial agonist and anxiolytic buspirone had no effect on the expression of a cocaine CPP when conditioning trials were immediately preceded by cocaine administration. In contrast, when conditioning trials were delayed following cocaine administration, which normally results in a CPA, buspirone blocked the conditioned anxiety and subsequent CPA, and rats

instead demonstrated preference for the cocaine-paired environment (Ettenberg and Bernardi, 2007).

Enhanced extinction

The relationship between mechanisms mediating a potential reconsolidation phase and those mediating extinction have complicated interpretations of post-retrieval impairment because both reconsolidation and extinction have been demonstrated to be induced under similar circumstances. In other words, both mechanisms can be initiated by nonreinforced presentation of the cue(s) present during learning. In general, proponents of reconsolidation now believe that there is a balance between reconsolidation and extinction that is in large part based on the duration of the nonreinforced trial, such that whichever process is dominant will determine the behavioral result (Tronson and Taylor, 2007). More specifically, this dominance theory suggests that which memory is affected depends on the amount of extinction that occurs during the retrieval trial (Eisenberg et al., 2003). Retrieval trials of short duration have been poroposed to result in reconsolidation and a strengthening or maintenance of the existing memory, while longer duration retrieval trials are thought to result in extinction. The switch between these two processes likely depends upon the predictive ability of the CS. In the early stages of nonreinforced CS exposure, as the CS continues to reliably predict the US, reconsolidation processes are thought to be dominant, and amnestic treatments administered during this stage would impair reconsolidation. With longer retrieval trials, the CS loses its ability to reliably predict the US, and a new CS-no US extinction

memory results, and amnestic treatments administered during this stage would impair extinction. For example, Pedreira and Maldonado (2003) found that the PSI cyclohexamide impaired a contextual fear memory in crabs when administered prior to a short, 5-min context re-exposure, but impaired extinction of that memory when administered prior to a longer, 60-min re-exposure duration, suggesting that reconsolidation processes were dominant following the shorter duration context re-exposure while extinction processes were dominant following the longer duration re-exposure. Interestingly, although the current set of studies did not determine the effects of post-retrieval adrenergic antagonists with different exposure durations or determine the supposed temporal boundaries between reconsolidation and extinction, extinction of CPP anecdotally appeared to occur in vehicle-treated control animals in some cases (Chapter 3), as suggested by visual decreases in magnitude of CPP from Tests 1 to Tests 2 (e.g., Figures 5 and 10), raising the possibility that in these animals, potential reconsolidation mechanisms neither strengthened nor fully maintained the CS-US memory, as is typically touted (Tronson et al., 2006). Although it is possible that a shorter duration retrieval trial may have resulted in less extinction, it is likely that if reconsolidation processes do exist, they likely occur in parallel with extinction processes. Furthermore, there is ample evidence demonstrating extinction with brief retrieval trials (e.g., Lattal, 2007). Therefore, the relationship between these two mechanisms cannot simply be attributed to the duration of nonreinforced trials, and thus needs to be further elucidated.

More important to the discussion of extinction in terms of impairments attributed to reconsolidation is the fact that the behavioral manifestations of impairments in reconsolidation are identical to those resulting from extinction (i.e., a decrease in

responding to the CS+), and thus it has been suggested that impairments in reconsolidation represent a facilitation of extinction (Fischer et al., 2004). Following contextual fear conditioning, Fischer et al. (2004) administered the PSI anisomycin into the hippocampus of mice following the first of several brief, daily nonreinforced trials and found reduced fear expression over the course of these trials in anisomycin-treated animals as compared to vehicle controls. The authors concluded, largely based on the ability of a reminder shock to reinstate freezing behavior, that anisomycin had facilitated extinction. They further proposed a protein synthesis-dependent mechanism that prevents extinction when the CS does not reliably predict the absence of the US, and thus anisomycin essentially inhibited this mechanism, resulting in rapid extinction (Fischer et al., 2004).

Although very few studies of reconsolidation attribute impairments to facilitated extinction, it remains a possibility and worth discussion with respect to the findings outlined here. Importantly, any decrease in the conditioned reinforcing properties of the cocaine-paired cue, as indicated above, would be expected to facilitate extinction. Furthermore, the drugs used in the current studies may themselves modulate extinction. To date, it appears that no studies have examined the effects of prazosin and ICI 118,551 on extinction memories, and very few have looked at extinction using propranolol. The majority of studies that have, however, indicate that propranolol impairs extinction (Berlau and McGaugh, 2006; Cain et al., 2004; Mueller et al., 2008) or has no effect (Rodriguez-Romaguera et al., 2009), indicating that in some circumstances, but not others, propranolol disrupts the formation of extinction memories. Berlaugh and McGaugh (2006) found that intra-BLA administration of NE enhanced extinction in a

contextual fear conditioning study, suggesting an important role for noradrenergic transmission in the formation of extinction memories. Furthermore, propranolol blocked the extinction-enhancing effect of the GABA antagonist bicuculline, suggesting that propranolol indeed impairs extinction in the BLA. Alternatively, a study using cocaine CPP found that propranolol administered systemically to rats following a single test of cocaine CPP had no effect on either a subsequent test [in contrast to the findings reported here (Chapter 2)] or, following further nonreinforced tests, on cocaine-primed reinstatement (Fricks-Gleason and Marshall, 2008). However, they further reported that propranolol administered after each of a number of subsequent tests resulted in a decrease in preference across trials as compared to controls, suggesting a propranolol-induced facilitated extinction. Because no reinstatement of preference was seen during a cocaine-primed reinstatement test, the authors interpreted this finding as being inconsistent with propranolol-induced facilitated extinction, but rather an impairment of reconsolidation (Fricks-Gleason and Marshall, 2008). However, is important to recognize that the absence of recovery is consistent with both impairments in reconsolidation and enhancements of extinction (Lattal and Stafford, 2008). Because repeated testing resulted in lower levels of preference over several trials in propranolol-treated rats as compared to vehicle-treated rats, reinstatement would be weaker in the propranolol-treated rats, and differences in preference would be expected to occur. Thus, the enhanced extinction explanation is not entirely unlikely with respect to the current findings, but not consistent with findings demonstrating, especially in the BLA, that adrenergic antagonists impair the formation of new memories (Ferry et al., 1999b; Miranda et al., 2003).

New Learning

One difference between the retrieval sessions in the CPP paradigm and other behavioral measures of reconsolidation is that during the initial test of preference a new response, previously not expressed, is required to demonstrate the conditioned reinforcing value of the cocaine-paired cue. Thus, in the current set of experiments, it is possible that post-treatment adrenergic antagonism impaired the consolidation of an approach response mediated by the conditioned reinforcer. Futhermore, the choice floor during a preference test is also a new environment. Alberini (2005) proposed that memory reactivation likely represents a process by with new information is integrated with old memories, and that retrieval-induced reconsolidation episodes, though acknowledged as a distinct process, may not be involved in this updating process. In an elegant demonstration of this, Tronel et al. (2005) first showed in an inhibitory avoidance paradigm that a light cue (S1) present during avoidance conditioning (with a shock US) in one context could be second-order conditioned to elicit fear in a second context (S2) via a nonreinforced reactivation trial in S2, such that in the absence of S1, S2 elicited the same fear response (as measured by the latency to enter the dark side of the chamber). Next, in a separate experiment, the authors demonstrated that reactivation of the original fear memory via exposure to S1 in S2, followed by systemic administration of the PSI anisomycin, resulted in an almost complete suppression of the fear response in both the original context and S2 upon subsequent testing. In other words, both the original S1-US and S1-S2 associations underwent a protein synthesis phase. Furthermore, the authors replicated their own

previous results showing that the transcription factor CCAAT enhancer binder protein β (C/EBPβ) administered into the hippocampus impaired consolidation, but not reconsolidation, of inhibitory avoidance, while C/EBPβ administered into the BLA impaired reconsolidation, but not consolidation, of inhibitory avoidance (Taubenfeld et al., 2001). The authors then demonstrated that C/EBPβ administered into the BLA following reactivation as described above impaired retention of the original S1-US memory (as measured by fear latencies in the original and S2 contexts), but had no effect on the S1-S2 memory, while C/EBPβ administered into the hippocampus impaired retention of the S1-S2 memory, but not the original S1-US memory. In other words, the impairment of a memory that incorporates new information into an existing, reactivated memory is likely mediated by disruption of consolidation, and not reconsolidation (Tronel et al., 2005).

In terms of the current set of experiments, the fact that a test for preference comprises a new floor configuration, and thus likely requires the integration of new information (i.e., both floors simultaneously) with previously established memories (i.e., one floor represents putative reinforcing effects while the other does not), it is possible that the initial test for CPP represents a new learning experience in which, not entirely unlike the study outlined above, second-order conditioning could occur between the CS+ and/or the CS- and the new choice environment, resulting in a new association that is disrupted by adrenergic blockade. Alternatively, as this new experience requires recognizing a new floor configuration, retrieving and discriminating between the value of the two floors, and making a response based on this information, it is possible that learning and subsequent consolidation in this elaborate scheme is compromised by post-retrieval adrenergic

antagonism. These scenarios might also explain the FOS expression results reported in Chapter 2, in which exposure to the choice floor increased FOS expression over either of the floors independently, which could be indicative of a new learning experience. However, the findings by Tronel et al. (2005) outlined above do not preclude the possibility of a distinct reconsolidation phase, but suggest, in terms of the current set of experiments, that this phase would likely be more appropriately examined with exposure solely to the discrete CS+ floor in the CPP paradigm. Nonetheless, the new learning hypothesis represents an intriguing possibility for demonstrations of post-retrieval impairments of CPP as well as fear paradigms, in which retrieval sessions are usually carried out in a context different than that in which conditioning occurred.

Future Directions

Several future studies merit examination with respect to the current findings. For instance, repeated testing following the initial demonstration of preference would have indicated the persistence of the effects reported here. More importantly, as the clinical efficacy of a treatment for dependence lies primarily in its ability to decrease the possibility of relapse, a cocaine-primed reinstatement session following impairment of CPP would indicate the magnitude of the impairment. Robinson & Franklin (2007a) demonstrated that post-test propranolol-induced disruption of a morphine place preference did in fact persist for at least 7 days, but this effect was reversed by a morphine-primed retest. Higher or repeated doses of post-test treatments might attenuate this drug-primed reinstatement, as indicated in a study alluded to earlier in which no

reinstatement of preference was seen when propranolol was administered following repeated testing in a cocaine CPP paradigm (Fricks-Gleason and Marshall, 2008). Another way to examine the persistence of effects is with longer retention intervals with respect to the initial retrieval trial and treatment administration (2004). Miller & Marshall (2005b) found no recovery of preference following a 14-day retention interval following impairment of reconsolidation using MEK inhibitors in a cocaine CPP paradigm, suggesting lasting impairment. Admittedly the authors did not attempt to reinstate preference with a priming dose of cocaine. Thus, a longer retention interval following intra-BLA administration of ICI 118,551 or prazosin, followed by a cocaine-primed reinstatement of preference test, would further indicate the efficacy of these potential treatments on drug-induced behaviors measured by CPP. Yet another potential follow-up to the studies reported here would be to recondition adrenergic-impaired animals to the floor cue on which they previously got vehicle to determine if they reacquire CPP slower than control animals that never received an adrenergic antagonist. This is similar to a procedure used by Milton et al. (2008b), who showed that propranolol administered prior to a CS-only retrieval trial following self-administration of cocaine in the presence of that cue inhibited the ability of that cue to mediate learning of a new response to acquire cocaine. In addition, it has been argued by that same group that CPP is not a relevant procedure by which to model cocaine cue-mediated behavior in animals because of the limited number of cocaine exposures typically used in this paradigm (Milton et al., 2008b). Thus, examining the potential post-retrieval effects of β_2- and α_1-ARs following overtraining of cocaine CPP (i.e., a larger number of conditioning sessions) would further elucidate whether the effects reported here are due to weak

conditioning. Stronger memories have been reported to be less amenable to impairments of reconsolidation (Suzuki et al., 2004).

Another area that deserves examination is a comparison between the effects of the treatment drugs here on consolidation versus those achieved by post-retrieval administration. It would be interesting to discover whether post-training adrenergic blockade results in greater impairments in retention than post-retrieval AR blockade. Few studies have examined differences in post-training and post-retrieval impairments in drug conditioning paradigms (see Robinson and Franklin, 2007b; Yu et al., 2009) and to date, none using adrenergic antagonists. In general, however, impairments in consolidation have been demonstrated to be stronger than post-retrieval impairments (Judge and Quartermain, 1982; Stafford and Lattal, 2009; Yu et al., 2009). The difficulty in comparing consolidation and reconsolidation in cocaine CPP, however, is the difficulty in obtaining preference with a single cocaine exposure, as the effects of noradrenergic blockade reported here were obtained with a single administration.

One last area of interest concerning the current experiments is determining whether post-retrieval manipulation could actually strengthen an existing memory. If a distinct memory reconsolidation phase actually exists, impairments in reconsolidation cannot be the only way to demonstrate its existence. Enhancements of memory must also be demonstrated. Some studies have claimed to enhance memory reconsolidation (Lee et al., 2009; Tronson et al., 2006). In terms of drug conditioning studies, as mentioned earlier, Lee et al. (Lee et al., 2009) reported an enhancement of reconsolidation in their ANR procedure using the NMDA agonist DCS. Though not a direct assessment of an enhancement of reconsolidation, Bernardi et al. (2007) showed in a cocaine conditioned

sensitization paradigm that post-retrieval administration of anisomycin impaired the conditioned locomotor response to cocaine upon subsequent testing. Interestingly, vehicle-treated controls that received the same re-exposure showed a greater conditioned locomotor response than animals tested without the re-exposure, suggesting that the brief re-exposure enhanced the memory for the context. In terms of the current set of experiments, post-retrieval enhancements of preference could be examined using intra-BLA administration of NE, the β_2-AR agonist clenbuterol, or the α_1-AR agonist phenylephrine, all of which have been shown to enhance the consolidation of memory when administered into the BLA (Ferry and McGaugh, 1999; Ferry et al., 1999b), though not in drug conditioning studies. Altenatively, enhancement could be examined with post-retrieval administration of an antagonist of the α_2-AR, a presynaptic autoreceptor of which blockade would be expected to enhance NE release.

Summary and Conclusions

In summary, the work presented here demonstrated that in a cocaine CPP paradigm, systemic post-test administration of the nonspecific β-AR antagonist, propranolol, attenuated a cocaine CPP upon subsequent testing. This was the first study to demonstrate a post-retrieval effect of propranolol in a drug learning paradigm, confirming a role for β-AR following retrieval of drug memories. This effect of propranolol has since been demonstrated in a morphine CPP paradigm (Robinson and Franklin, 2007a), as well as a drug-mediated ANR paradigm (Milton et al., 2008b). This effect of propranolol on cocaine CPP was replicated here using the β_2-AR antagonist ICI 118,551,

but not the β_1-AR antagonist betaxolol. This is the first demonstration of a subtype-specific effect of β-ARs in any paradigm examining post-retrieval impairments. The α_1-AR antagonist, prazosin, administered post-test also attenuated a subsequent cocaine CPP, again the first demonstration of a role for α_1-ARs in mediating post-retrieval memory processes in any paradigm. Furthermore, ICI 118,551 and prazosin had an identical effect when administered post-test directly into the BLA, implicating the BLA as a likely mediator of post-retrieval impairments induced by AR antagonism. To date, this is the first demonstration of intra-BLA AR antagonism impairing reconsolidation in a drug conditioning paradigm, but are consistent with findings showing that β_2-ARs and α_1-ARs in the BLA are important modulators of initial consolidation in fear learning paradigms, suggesting similar mechanisms in the BLA mediating consolidation and reconsolidation. The results presented here are also consistent with other studies purporting impairments in reconsolidation in the CPP and other drug learning paradigms using a variety of manipulations administered systemically or site-specifically (Bernardi et al., 2006, 2007; Brown et al., 2007; Fricks-Gleason and Marshall, 2008; Itzhak and Anderson, 2007; Kelley et al., 2007; Miller and Marshall, 2005b; Milton et al., 2008a; Milton et al., 2008b; Robinson and Franklin, 2007a, 2007b; Sadler et al., 2007; Wang et al., 2008; Zhai et al., 2008).

The work presented here indicates that the noradrenergic system is a likely mediator of the salience of drug-associated cues, and that blockade of this system in combination with exposure to these cues may dampen their ability to drive drug-seeking behaviors. Because environmental stimuli that are associated with drugs of abuse are critical to the persistence of addiction in humans (Childress et al., 1988b; Weiss, 2005), targeting

potential reconsolidation mechanisms via noradrenergic blockade represents a unique way to examine cue-induced drug-mediated behaviors in animal that may provide insight into new treatments for cue-mediated drug-seeking in humans. Importantly, several alternative explanations have been posited as to mechanisms by which adrenergic antagonism following retrieval might diminish cocaine CPP that are inconsistent with the notion that memory retrieval induces a reconsolidation phase that serves to maintain or strengthen existing memories. Thus, the precise mechanism by which the reported results resulted in a decrease in the ability of the cocaine-associated to induce "drug-seeking" behavior need to be further elucidated.

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APPENDIX

The purpose of the following preliminary study was to determine the effect of propranolol on the expression or retrieval of a cocaine CPP. Subjects were twenty-four Sprague Dawley rats, housed as described in Chapter 2. Cocaine HCL and (±)-Propranolol HCl were dissolved and administered as described in Chapter 2. Cocaine place conditioning was conducted as described in Chapters 2 and 3. Rats received a single 25-min test of CPP, preceded 30 min by an injection of vehicle or propranolol (10 mg/kg), and data were analyzed and for the first 15 min of the test as described in Chapters in Chapters 2 and 3, using a two-way ANOVA [Drug Treatment X Conditioning Subgroup (GRID+/GRID-)] and Student's t-test to examine *a priori* comparisons between GRID+ and GRID- subgroups (with a Bonferroni correction for multiple comparisons). Activity data during were analyzed using a one-way ANOVA (Drug Treatment). Significance was set at $p < .05$. Figure 15 shows the mean (+SEM) time spent on the GRID floor during the test for CPP. Rats in the vehicle [n = 12 (n = 6/6 per GRID+/GRID- subgroup] and propranolol [n = 12 (n = 6/6 per GRID+/GRID- subgroup] groups showed reliable preference for the cocaine-paired floor, as indicated by a significant main effect of conditioning subgroup [$F(1,20) = 16.9$, $p < .005$], and no interaction or main effect of drug treatment ($Fs < 1$). Student's t-test revealed that the propranolol group [$t(10) = 4.8$, $p < .001$] (Bonferroni-corrected $\alpha/2 = .025$) showed significant cocaine-induced CPP, and although it appears in Figure 15 that rats in the vehicle group also showed a preference for the cocaine-paired floor, the p-value [$t(10) = 1.8$, $p = .10$] did not reach our normal criterion for Bonferroni-correction ($\alpha/2 = .025$).

Activity levels (±SEM) did not differ significantly between the vehicle (1444.3 ± 75.8) and propranolol (1671.8 ± 95.7) groups, though there was a trend toward higher activity in the propranolol-treated group [$F(1,22) = 3.5$, $p = .08$]. This preliminary data suggests that propranolol did not reduce the expression of a cocaine CPP, but an increased number of animals is needed to better draw this conclusion.

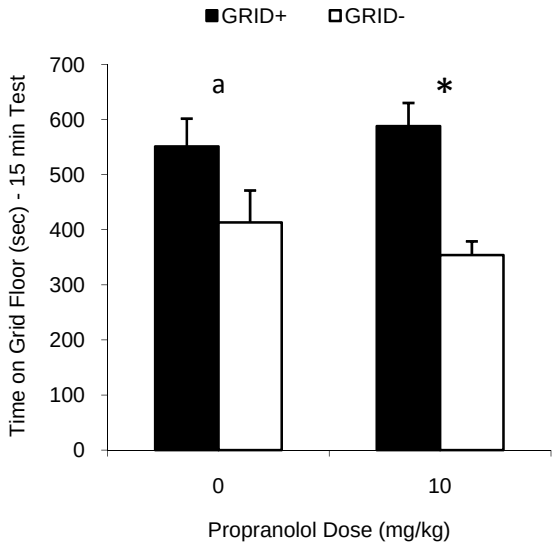

FIGURE 15. The effect of propranolol on the expression of a cocaine CPP.
Data represent mean (+SEM) time spent on the GRID floor for the GRID+ and GRID- subgroups during the 15-min drug-free test. Groups that received propranolol or vehicle prior to testing showed a significant or trend toward significant preference for the cocaine-paired floor during Test 2. NR = no re-exposure. *$p < 0.025$ (Bonferroni-corrected $\alpha/2$); $^{a}p = 0.10$.