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# Dissociation of $\beta$ 1 and $\beta$ 2 Adrenergic Receptor Subtypes in Retrieval and Reconsolidation of a Cocaine Conditioned Place Preference

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DISSOCIATION OF  $\beta$ 1 AND  $\beta$ 2 ADRENERGIC RECEPTOR SUBTYPES  
IN RETRIEVAL AND RECONSOLIDATION OF A  
COCAINE CONDITIONED PLACE PREFERENCE

by

Michael K. Fitzgerald

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## ABSTRACT

### DISSOCIATION OF $\beta$ 1 AND $\beta$ 2 ADRENERGIC RECEPTOR SUBTYPES IN RETRIEVAL AND RECONSOLIDATION OF A COCAINE CONDITIONED PLACE PREFERENCE

by

Michael K. Fitzgerald

The University of Wisconsin - Milwaukee, 2013  
Under the Supervision of Devin Mueller

Drug-seeking behavior is maintained by encounters with drug-associated cues, and disrupting retrieval or reconsolidation of the drug-cue associations could reduce the risk of relapse. Previous work has shown  $\beta$ -adrenergic receptor ( $\beta$ -AR) antagonists can prevent retrieval or reconsolidation of a cocaine conditioned place preference (CPP) when administered either before or after test, respectively (Otis and Mueller, 2011; Otis et al., 2013). However, the specific  $\beta$ -AR subtypes that mediate retrieval and reconsolidation of a cocaine CPP remain unknown. Here we used selective blockade of  $\beta$ 1 or  $\beta$ 2-AR subtypes to determine the effects on retrieval and reconsolidation of a cocaine CPP. During conditioning, rats were trained to associate one chamber, but not another, with cocaine. Memory retrieval was then tested by allowing the rats access to both chambers, resulting in a CPP for the previously cocaine-paired side. Pre-test injection of the  $\beta$ 1-AR antagonist betaxolol at 20 mg/kg, i.p. but not 3 mg/kg prevented expression of a cocaine-induced CPP, indicating  $\beta$ 1-AR involvement in memory retrieval. During subsequent drug-free tests, saline-treated rats continued to show a CPP, whereas

betaxolol-treated rats did not. Moreover, treatment with the higher dose of betaxolol prevented subsequent reinstatement to a priming injection of cocaine. Unlike betaxolol administration, pre-test injection of the  $\beta$ 2-AR antagonist ICI 118,551 (4 or 8 mg/kg, i.p.) had no affect on expression of a cocaine-induced CPP. On subsequent tests, however, vehicle- and 4 mg/kg ICI 118,551-treated rats continued to show a CPP whereas 8 mg/kg ICI 118,551-treated rats did not, indicative of a deficit in reconsolidation following this higher dose. Moreover, ICI 118,551 treatment prevented subsequent reinstatement to a priming injection of cocaine. Thus, we conclude that retrieval of a CPP is dependent on  $\beta$ 1-ARs whereas reconsolidation of a CPP is dependent on  $\beta$ 2-ARs. Finally, co-injection of betaxolol (20 mg/kg) and ICI 118,551 (8 mg/kg) prevented CPP expression on that and subsequent CPP tests, resulting in a long-lasting retrieval deficit that prevented reinstatement to a priming injection of cocaine. Our findings support the use of  $\beta$ -AR antagonists as adjuncts for addiction therapies by preventing retrieval and reconsolidation of drug-associated memories and providing protection against relapse.

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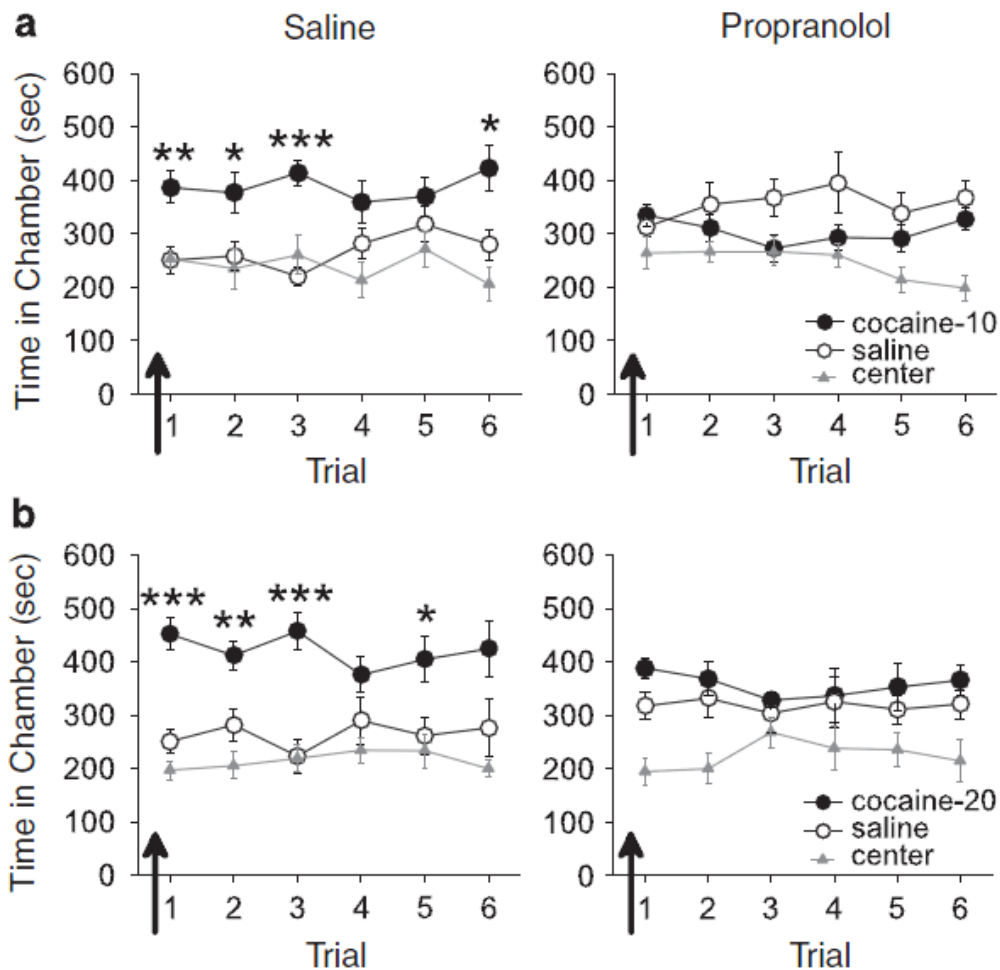


## Introduction

### Drug-associated cues

Addiction is characterized as a compulsively relapsing pattern of drug use that persists in the face of negative consequences and a desire to stop using. Despite the will to end drug use, drug-seeking behavior can be maintained by encounters with drug-associated cues. Addicts respond to cues such as the drugs themselves, drug paraphernalia, people using drugs, and environments where drugs were used (O'Brien et al., 1977; Ehrman et al., 1992; Foltin and Haney, 2000). Research indicates that cues become associated with the drug in stages, including acquisition and consolidation (Duarte et al., 2003). Following associative memory acquisition and consolidation, retrieval can be triggered by the cue resulting in behavioral expression (drug seeking) and subsequent reconsolidation of the associative memory (Sara, 2000). Preventing retrieval or reconsolidation of drug-associated memories provoked by cues could therefore limit relapse susceptibility. Previously, our lab has shown that retrieval of cocaine-associated memories is mediated by the activation of  $\beta$ -adrenergic receptors using the conditioned place preference (CPP) procedure (Otis & Mueller, 2011). The CPP procedure is a useful animal model of drug seeking because it connects the environmental stimuli with drug administration. To disrupt cocaine-associated memory retrieval, Otis and Mueller (2011) administered propranolol, a non-specific  $\beta$ -adrenergic receptor ( $\beta$ -AR) blocker which has affinity for both  $\beta_1$  and  $\beta_2$   $\beta$ -AR subtypes, immediately prior to a retrieval test (see Figure 1). However, it remains unknown if one or both  $\beta$ -AR subtypes mediate this retrieval blockade. Isolating the specific receptor subtype that prevents cue-induced memory retrieval will lead to an understanding of the mechanisms underlying cocaine-

associated memory retrieval and could provide insight into potential treatment options for cocaine addiction.



**Figure 1** Propranolol disrupts retrieval of a cocaine-associated conditioned place preference (CPP). Following conditioning with (a) 10 mg/kg or (b) 20 mg/kg doses of cocaine, propranolol but not saline injections (arrows) prevented rats from expressing a CPP for the previously cocaine-paired chamber over the previously saline-paired chamber during the first CPP trial. Propranolol- but not saline-treated rats continued to show no CPP during subsequent propranolol-free trials. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$ .

### **The conditioned place preference procedure**

The mechanisms of drug-associated memory retrieval can be investigated using the conditioned place preference (CPP) paradigm. This paradigm is an animal model of drug seeking in which rats learn to associate environmental stimuli with cocaine. During training, rats are given experimenter-delivered cocaine or saline followed by exposure to one of two chambers. Following training, rats are given full access to both the cocaine- and saline-paired chambers, along with a neutral center chamber. Rats spend more time in the previously cocaine-paired chamber during this trial, thus expressing a cocaine-induced CPP. When a CPP is expressed, investigators can be certain that the rats acquired, consolidated, and retrieved this cocaine-associated memory. Additionally, rats that express a CPP on additional test trials demonstrate that they were able to reconsolidate the cocaine-associated memory from the previous trial.

### **Memory retrieval and the noradrenergic system**

Studies have demonstrated that memory expression can be enhanced by pre-test treatment of nicotine (Faiman et al., 1992), cocaine, (Rodriguez et al., 1993), or amphetamine (Sara & Deweer, 1982). These drugs are nonspecific, but all of them enhance noradrenergic signaling. In addition, stimulation of the noradrenergic system influences memory task performance. For example, blockade of  $\alpha_2$ -adrenergic autoreceptors, which normally prevent norepinephrine from being released, leads to an increase in rat performance in maze navigation after the task had been forgotten (Sara, 1985; Sara & Devauges, 1989). Moreover, stimulation of the locus coeruleus (LC), a major nucleus of noradrenergic cell bodies (Dahlstroem & Fuxe, 1964), also enhances memory expression after forgetting. This enhancement is prevented by the noradrenergic

$\beta$ -AR antagonist propranolol (Devauges & Sara, 1991). Taken together, these studies demonstrate that memory retrieval can be enhanced by stimulation of the noradrenergic system, and that this effect is also dependent on  $\beta$ -AR signaling.

Although retrieval of cue-associated memories is enhanced by activating neurons in the LC (Sara, 2009; Sterpenich et al., 2006) to release norepinephrine (Cassens et al., 1980; Feenstra et al, 2001; Mingote et al., 2004), the necessity of norepinephrine for retrieval has only recently been explored. Seminal work by Murchison and colleagues (2004) showed that mice that lack the enzyme for the synthesis of norepinephrine (dopamine  $\beta$ -hydroxylase (DBH)) were able to acquire a contextual fear memory, but were unable to express this memory on subsequent daily trials for seven days. Similar results were found using the Morris water maze, a task requiring spatial memory. Moreover, normal rats that were administered  $\beta$ -AR antagonists showed impaired contextual fear expression while  $\beta$ -AR agonists enhanced expression. Interestingly, DBH knockout mice were able to perform as well as controls on several tasks involving distinct cues rather than contexts. From these data, the authors concluded that  $\beta$ -AR signaling is necessary for the retrieval of contextual, but not cued memory tasks.

Although Murchinson and colleagues suggest a distinct role for  $\beta$ -ARs in retrieval of contextual, but not cued memories, there is evidence that  $\beta$ -ARs are necessary for cue-associated memories as well. Rodriguez-Romaguera and colleagues (2009) showed that  $\beta$ -AR blockade with propranolol attenuated cue-induced fear expression. Additionally, in the CPP paradigm, it has been shown that propranolol blocks reinstatement of an extinguished CPP in a distinguishably different context than drug-associated cue conditioning (Mantsch et al., 2010). Human studies have shown that propranolol administration can attenuate cued recall of heroin- or emotionally-related words (Kroes,

Strange, & Dolan, 2010; Zhao et al., 2010). This evidence supports the necessity of  $\beta$ -AR activation in retrieval of both context- and cue-associated memories.

### **Different structures, different roles in drug-related memory**

Mechanisms of drug-associated memories can be investigated through pharmacological manipulation at different time points throughout CPP experiments. Otis and Mueller (2011) demonstrated that rats given an intraperitoneal (i.p.) injection of propranolol prior to an initial CPP trial were unable to *retrieve* the CPP during the first and subsequent daily CPP trials. Furthermore Otis, Dashew, and Mueller (2013) identified that infusing nadolol, another non-specific beta-AR blocker, into the prelimbic medial prefrontal cortex (PL-mPFC) prior to the rat's first CPP trial also blocked retrieval on the initial and subsequent daily trials.

While retrieval of a CPP occurs upon exposure to the apparatus, reconsolidation of the retrieved memory is a process that occurs after the rats have been removed from the apparatus. The introduction of pharmacological agents to rats after the CPP session could then disrupt the reconsolidation of the cocaine-associated memory.

Reconsolidation disruption makes it difficult to retrieve a CPP memory from long-term memory on subsequent trials. Fricks-Gleason and Marshall (2008) demonstrated that daily post-retrieval systemic injections of propranolol decreased CPP by interfering with reconsolidation of the memory for the association between the drug-paired side and the reinforcing effects of the drug rather than facilitating new extinction learning. The locus of action has since been determined. Otis et al., (2013) and Bernardi, Ryabinin, Berger, and Lattal (2009) investigated the role of amygdala  $\beta$ -ARs in reconsolidation of a cocaine CPP. After retrieval of a CPP, infusions of propranolol or nadolol into the

basolateral amygdala (BLA) immediately post-trial resulted in a retrieval deficit on subsequent daily trials. Thus reconsolidation had been disrupted by blockade of  $\beta$ -ARs in the BLA.

To show that the PL-mPFC and BLA have distinctly different roles in retrieval and reconsolidation, each structure was tested using the same pre- or post-trial timing of infusions (Otis et al., 2013). The PL-mPFC was shown to mediate retrieval of a CPP, but not reconsolidation. When propranolol or nadolol was infused into the PL-mPFC after the first CPP trial, the rats continued to express a CPP on subsequent trials. Had reconsolidation been jeopardized, the rats would not have been able to express a CPP on subsequent CPP trials as observed with post-trial BLA infusions. The BLA, on the other hand, is involved with reconsolidation and not retrieval as infusions with propranolol or nadolol prior to the first CPP trial did not disrupt expression of a CPP. However, on subsequent trials, these rats were unable to express a CPP demonstrating that reconsolidation had been blocked (Otis et al., 2013).

### **Different $\beta$ –AR subtypes, different effects on memory**

Activation of  $\beta$ -ARs in the CNS activates adenylyl cyclase to increase the levels of intracellular cAMP, which activates protein kinase A (PKA) (Mueller, Porter, & Quirk, 2008). When PKA becomes activated, it can have local effects such as increasing cell excitability, increasing NMDAr-mediated calcium current (Skeberdis et al., 2006), and facilitating AMPA receptor trafficking (Snyder et al., 2005; Hu et al., 2007). In the CNS, there are different  $\beta$ -AR sub-types. Two specifically,  $\beta$ 1-AR and  $\beta$ 2-AR, have received increasing interest. Both  $\beta$ -AR subtypes stimulate adenylyl cyclase by coupling to the guanine nucleotide protein which leads to the increase of intracellular cAMP and

activation of PKA. Interestingly, equal doses of the non-selective  $\beta$ -AR agonist, isoproterenol, stimulates  $\beta$ 2-AR adenyl cyclase approximately 50% more than  $\beta$ 1-AR even though both  $\beta$ -AR subtypes show equal affinity for isoproterenol (Green, Holt, & Liggett, 1992). Although  $\beta$ 2-AR leads to a stronger stimulation of adenyl cyclase,  $\beta$ 1-AR has been found in much higher concentration than  $\beta$ 2-AR within forebrain structures such as the cerebral cortex, caudate, hippocampus and amygdala (Rainbow, Parsons, & Wolfe, 1984).

The differences in signaling activity and subtype receptor localizations have led to many investigations on how the two subtypes are differentially involved in memory tasks. For example, Ramos et al. (2005) demonstrated that endogenous activation of the  $\beta$ 1-AR impairs working memory in both rats and monkeys. In contrast, Ramos et al. (2008) demonstrated that  $\beta$ 2-AR activation in the pre-frontal cortex (PFC) can improve spatial working memory in rats and monkeys. Consistent with this opposing effect of the two receptor subtypes is the report of no effect on working memory when  $\beta$ 1- and  $\beta$ 2-ARs were blocked with the non-selective  $\beta$ -AR antagonist propranolol. Neither microinjection of propranolol into the PFC (Li & Mei, 1994), nor systemic administration of propranolol (Arnsten & Goldman-Rakic, 1985) altered PFC function in monkeys.

The previously described studies show different effects of  $\beta$ 1- and  $\beta$ 2-ARs in memory tasks, but those tasks are not particularly arousing for the animal subjects. In the fear and drug literature, different roles of  $\beta$ 1 or  $\beta$ 2-AR activation have been reported. For example, Flexner and colleagues (1985) reported that blocking  $\beta$ 1-AR with betaxolol suppressed inhibitory avoidance retrieval testing while blocking  $\beta$ 2-AR with ICI 118,551 had no effect. Murchison and colleagues (2004) demonstrated similar effects within the context-fear paradigm, as only  $\beta$ 1-AR blockade with betaxolol (3 mg/kg) impaired

memory retrieval. Additionally, they demonstrated that blocking  $\beta$ 2-ARs with a low (1 mg/kg) or high (10 mg/kg) dose of ICI 118,551 does not block retrieval of a context fear memory. They then targeted the hippocampus with bilateral infusions of propranolol, nadolol, or betaxolol and found in all three conditions that the mice were unable to retrieve the contextual fear memory. From this evidence, it appears that contextual memory retrieval depends on activation of  $\beta$ 1-ARs. This lab has data showing hippocampal infusions of nadolol induces a persistent deficit in CPP retrieval (Otis, Fitzgerald, & Mueller, *in review*), but the role of specific  $\beta$ -AR subtypes has not yet been investigated.

Other studies using the CPP paradigm have examined the differential roles of  $\beta$ 1- or  $\beta$ 2-AR activation. Bernardi et al. (2009) discovered that rats administered ICI 118,551 after a drug-free CPP test showed an attenuated preference during a subsequent test, whereas betaxolol had no effect. Additionally, a post-test microinfusion of ICI 118,551 into the BLA also impaired subsequent expression of a CPP. Thus,  $\beta$ 2-ARs within the amygdala are responsible for the reconsolidation of a cocaine CPP.

$\beta$ -ARs are also involved in relapse, as demonstrated using the reinstatement procedure. A priming injection of cocaine reinstates a CPP after the animal has gone through extinction learning (Kelley, et. al, 2007; Mantsch, et. al, 2010; Mueller & Stewart, 2000; Otis & Mueller, 2011; Rodriguez-Arias, et. al, 2009). Our lab has shown that the retrieval deficit induced by propranolol abolishes cocaine-induced reinstatement of a CPP (Otis & Mueller, 2011). Vranjkovic, Hang, Baker, & Mantsch (2012) investigated  $\beta$ -AR subtypes in stress-induced reinstatement of an extinguished cocaine CPP. Following place conditioning and extinction, they showed that exposure to a stressor, such as forced swim, will reinstate the CPP. However, rats administered a high



dose (20 mg/kg, i.p.), but not a lower dose (10 mg/kg, i.p.), of betaxolol prior to forced swim do not show reinstatement of the CPP. Additionally, they found that both a 1 mg/kg, i.p. or a 2 mg/kg, i.p. dose of the  $\beta$ 2-AR antagonist ICI 118,551 is also capable of blocking stress-induced reinstatement of the previously extinguished CPP. Thus, both  $\beta$ 1 and  $\beta$ 2 –ARs may mediate retrieval under some conditions.

Overall, there is limited evidence suggesting that  $\beta$ 1-AR activation is necessary for memory retrieval whereas  $\beta$ 2-AR activation is necessary for reconsolidation. This dissociation, however, has not been explicitly or systematically tested. Another possibility is that  $\beta$ –ARs in general are necessary for retrieval of a CPP. Thus, this study focuses on examining the effects of different doses of specific  $\beta$ –AR subtype antagonists on retrieval and reconsolidation of a cocaine CPP.

### **Overview**

This thesis aims to determine if  $\beta$ 1 –AR or  $\beta$ 2 –AR blockers can block the initial and/or subsequent retrieval of cocaine CPP when given systemically prior to the initial CPP trial.

## Methods

### Subjects

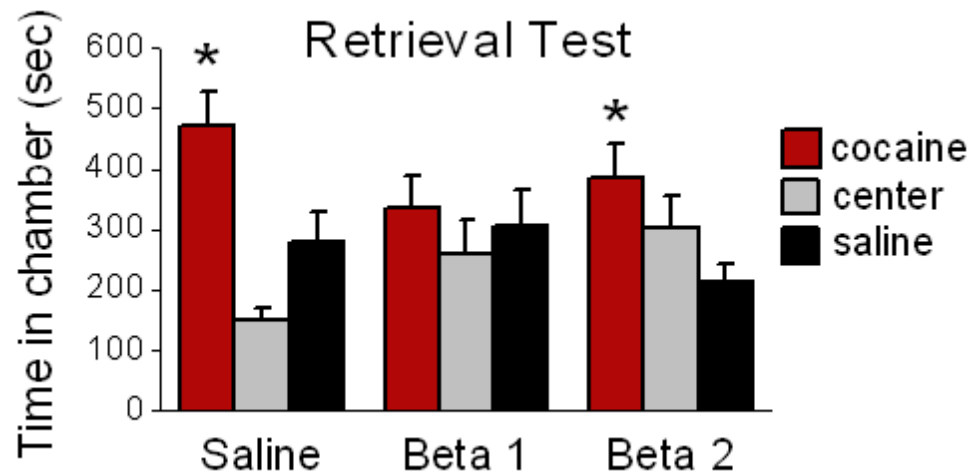
Eighty-four male Long-Evans rats weighing 250-275 grams were individually housed in clear plastic cages. Rats were maintained on a 14 hour light/10 hour dark cycle (lights on at 7am) and will have unlimited access to both water and standard laboratory rat chow (Harlan Laboratories). Rats were weighed and handled daily. All experimental protocols have been approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee in accordance with National Institutes of Health guidelines.

### Drugs and doses

For conditioning, cocaine HCl (National Institute on Drug Abuse) was dissolved in sterile 0.9% saline at a concentration of 10 mg/mL, and administered i.p. at a dose of 10 mg/kg. To investigate the optimal dose for effective disruption of cocaine CPP retrieval, we used both a high and low dose of both betaxolol and ICI 118,551. Betaxolol is a selective  $\beta_1$ -AR antagonist and ICI 118,551 is a selective  $\beta_2$ -AR antagonist. Pilot data shows that a low dose of 3 mg/kg (i.p.) of betaxolol is effective at attenuating a CPP (see Figure 2), but this dose may not be sufficient to completely disrupt retrieval on subsequent trials. Previous work by Bernardi et al. (2009) showed that betaxolol at 5 or 10 mg/kg i.p. had no effect when administered immediately after the first CPP trial (i.e., post-retrieval) on subsequent trials. However, post-retrieval administration does not influence retrieval. Since Murchison and colleagues (2004) demonstrated blockade of retrieval of contextual fear with a pre-trial injection of 3 mg/kg of betaxolol, this dose was used as the low dose for attempting to block retrieval. Vranjkovic, Hang, Baker, and

Mantsch (2012) showed that a high dose (20 mg/kg, i.p.) of betaxolol blocks stress-induced reinstatement. Thus, I also used this dose as my highest dose for attempting to block retrieval.

Since ICI 118,551 has been shown to be effective at disrupting reconsolidation of a cocaine CPP with a dose of 8 mg/kg (Bernardi et al., 2009), this will be the high dose of ICI 118,551. This effective high dose will be cut in half to 4 mg/kg for the low dose to investigate a dose response curve. Additionally, this lab has pilot data showing that betaxolol, but not ICI 118,551, is effective at blocking retrieval (see Figure 2). To determine the role of these  $\beta$ -AR subtypes in retrieval and reconsolidation, either a high or low dose of betaxolol or ICI 118,551 will be injected i.p. 20 minutes before the initial CPP trial.



**Figure 2.** Systemic administration of betaxolol 3 mg/kg, but not ICI 118,551 5 mg/kg or saline prevented rats from expressing a CPP for the previously cocaine-paired chamber during the initial CPP trial (n=8 per group). \*  $p < 0.05$

### **Place preference apparatus**

A three-chamber apparatus was used for testing and conditioning in which the two larger chambers for conditioning (13" x 9" x 11.5") are separated by a center chamber (6" x 7" x 11.5"). One of the conditioning chambers has white walls and mesh wire flooring; the other conditioning chamber has one black wall, three white walls, and a gold-grated floor. The center chamber flooring is made of aluminum. Each chamber is raised 1.5" to allow the use of removable trays for cleaning.

During conditioning, walls were placed between each chamber to isolate each rat within a specific chamber. During baseline and CPP trials the walls were removed to allow the rat access to all three chambers. Both of the larger chambers have two infrared photobeams that are separated by 3". The rat is determined to be in one of the larger chambers if the beam furthest from the door is broken or in the center chamber if the beam closest to the central chamber is broken. Throughout all phases of the experiment the room lights were turned off and a single lamp was lit to keep the room in semi-darkness.

### **Behavioral testing and injections**

Baseline preferences were determined by placing the rats into the center chamber with free access to the entire apparatus for 15 minutes. Time spent in each chamber were recorded. In previous studies using the same 3-chamber apparatus, rats spent an equivalent amount of time in the two larger conditioning chambers during baseline testing. Therefore, I used an unbiased procedure, in which rats are randomly assigned to receive cocaine in one of the two larger chambers independent of baseline preference scores.

Following baseline testing, rats were conditioned to associate one chamber, but not another, with cocaine in a counterbalanced fashion over 8 days. Injections of saline or cocaine were given immediately prior to each 20 minute conditioning session, during which the rats will be confined to their respective chamber.

Following conditioning rats were subjected to daily CPP trials during which they were placed into the center chamber and allowed free access to the entire apparatus for 15 minutes. A CPP will be determined when significantly more time is spent in the previously cocaine-paired chamber than the saline-paired chamber. To test the effects of  $\beta$ -AR subtype antagonists on retrieval, rats will be injected with one of the following 30 minutes prior to an initial CPP trial:

1. 3 mg/kg of betaxolol
2. 20 mg/kg of betaxolol
3. 4 mg/kg of ICI 118,551
4. 8 mg/kg of ICI 118,551
5. 1 ml/kg of saline (control)

Following the initial CPP trial, rats were given daily drug-free trials to determine if the betaxolol or ICI 118,551 treatment induces a persistent effect.

### **Reinstatement**

A priming injection of cocaine has been shown to reinstate a CPP after extinction (Kelley, et. al, 2007; Mantsch, et. al, 2010; Mueller & Stewart, 2000; Otis & Mueller, 2011; Rodriguez-Arias, et. al, 2009). Previous work in the lab has demonstrated that a propranolol-induced retrieval deficit persists, and importantly protects against cocaine-induced reinstatement (Otis & Mueller, 2011). Thus, I also investigated whether blockade of  $\beta$ -AR subtypes during retrieval also provides protection against cocaine-

induced reinstatement after extinction by injecting 5 mg/kg (i.p.) cocaine 15 minutes prior to a final test.

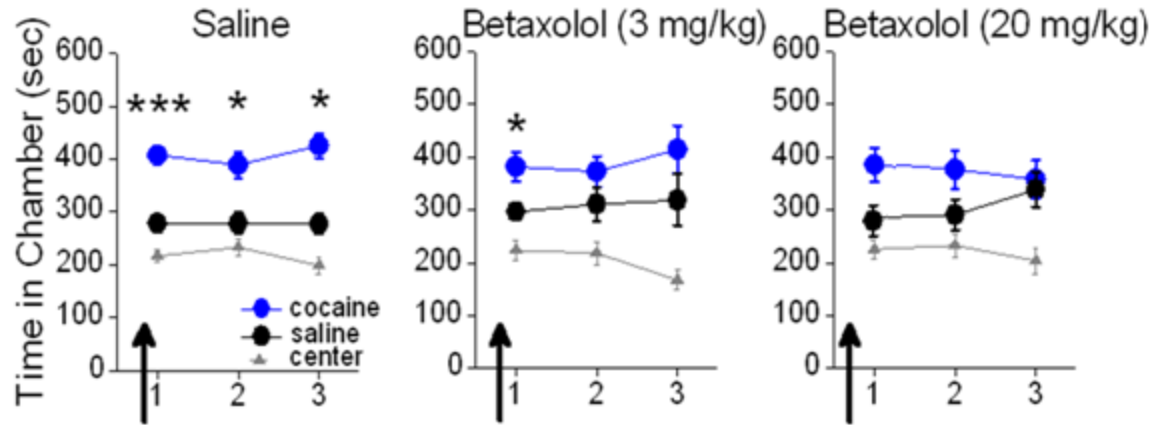
### **Data analysis and statistics**

Statistical analyses were performed using SPSS. Cocaine seeking was analyzed by comparing time spent between the cocaine, saline, and center chambers across trials and between groups using repeated measures ANOVA. If a significant effect of chamber was found, *post-hoc* Tukey's Honestly Significant Difference (HSD) tests will be used to compare the amount of time spent in the cocaine-paired and saline-paired chambers throughout the CPP trials.

## Results

### **Experiment 1A: Betaxolol attenuates retrieval of a CPP when administered prior to an initial test**

We first examined the necessity of  $\beta$ 1-adrenergic receptor activation for the retrieval of a cocaine-associated CPP. In experiment 1A, rats were pretested, conditioned with 10 mg/kg of cocaine, and subjected to daily CPP trials. Thirty minutes prior to the first CPP trial, rats were injected with either vehicle, a 3 mg/kg or 20 mg/kg dose of the  $\beta$ 1-adrenergic receptor antagonist betaxolol ( $n= 12$  per group). The saline and 3 mg/kg betaxolol group showed a CPP for the previously cocaine-paired chamber, while the 20 mg/kg betaxolol group did not (see Figure 3). Repeated measures ANOVA revealed a significant effect of chamber for the saline ( $F_{2,22}=30.20, p<0.05$ ), 3 mg/kg betaxolol ( $F_{2,22}=24.87, p<0.05$ ), and 20 mg/kg betaxolol groups ( $F_{2,22}=7.41, p<0.05$ ). *Post hoc* analyses confirmed that the saline group spent significantly more time in the previously cocaine-paired chamber than the saline-paired chamber ( $p<0.05$ ) throughout all trials. The 3 mg/kg betaxolol groups spent significantly more time in the previously cocaine-paired chamber than the saline-paired ( $p<0.05$ ) during the first trial. In contrast, the 20 mg/kg betaxolol group spent a similar amount of time in the cocaine- and saline-paired chambers ( $p>0.05$ ) throughout all trials. Thus, a single 20 mg/kg dose, but not 3 mg/kg treatment of betaxolol reduces expression of a CPP both initially and long after treatment.

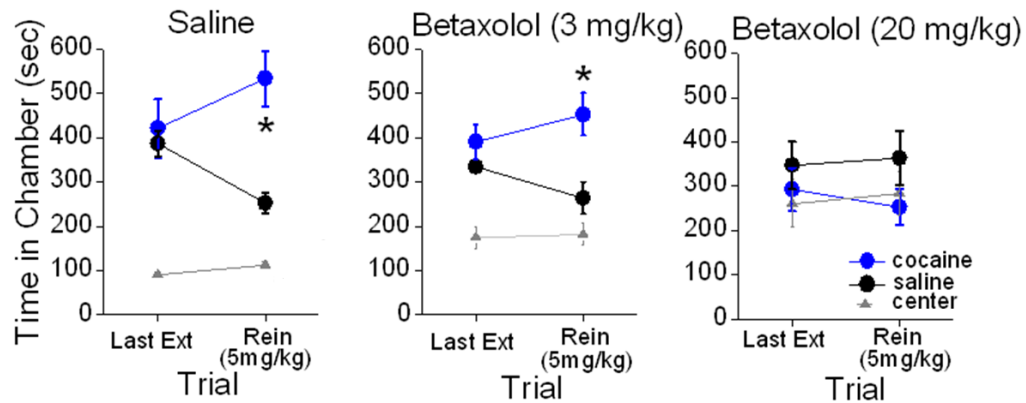


**Figure 3.** Systemic administration of betaxolol 20 mg/kg, but not 3 mg/kg or saline prevented rats from expressing a CPP for the previously cocaine-paired chamber during the initial and subsequent CPP trials (n=12 per group). \*  $p < 0.05$ .

Next we determined whether the retrieval deficit induced by betaxolol would prevent reinstatement of a CPP to a priming injection of cocaine. Rats continued to receive additional drug-free CPP trials to ensure extinction of the CPP. Analysis of the final extinction trial revealed a significant effect of chamber for the saline ( $F_{2,33}=10.73$ ,  $p < 0.05$ ) and betaxolol 3 mg/kg groups ( $F_{2,33}=10.10$ ,  $p < 0.05$ ), but not for the betaxolol 20 mg/kg group ( $F_{2,33}=.692$ ,  $p > 0.05$ ; see Figure 4). *Post hoc* analyses showed that rats spent a similar amount of time in the cocaine- and saline-paired chambers ( $p > .05$ ), and less time in the center chamber ( $p < .05$ ). Two days later, rats were injected with cocaine (5 mg/kg, i.p.) 15 min before a CPP trial to test for reinstatement of the CPP. ANOVA revealed a significant effect of chamber during the betaxolol-free cocaine-primed reinstatement trial for the saline ( $F_{2,33}=19.22$ ,  $p < 0.05$ ) and 3 mg/kg betaxolol groups ( $F_{2,33}=13.97$ ,  $p < 0.05$ ), but not for the 20 mg/kg betaxolol group ( $F_{2,33}=1.25$ ,  $p > 0.05$ ). *Post hoc* analyses showed that saline and 3 mg/kg betaxolol-treated rats spent significantly more time in the previously cocaine-paired chamber than saline-paired chamber ( $p < 0.01$ ). Thus, a high dose, but not a low dose of betaxolol, is capable of



disrupting retrieval of a CPP and preventing cocaine-induced reinstatement in the absence of further betaxolol treatment.

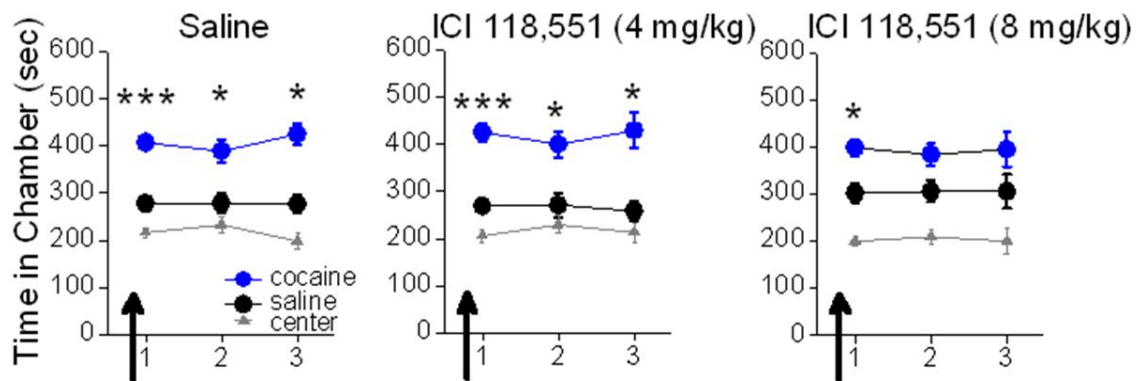


**Figure 4.** Previous administration of betaxolol 20 mg/kg, but not 3 mg/kg and saline prevented rats from expressing a CPP for the previously cocaine-paired chamber following a 5 mg/kg priming injection of cocaine. (n=12 per group). \*  $p < 0.05$ .

### Experiment 1B: ICI 118,551 attenuates reconsolidation but not retrieval of a CPP when administered prior to an initial test

We next examined the necessity of  $\beta_2$ -adrenergic receptor activation for the retrieval or reconsolidation of a cocaine-associated CPP. In experiment 1B, rats were pretested, conditioned with 10 mg/kg of cocaine, and subjected to daily CPP trials. Thirty min before the first CPP trial, they were administered either vehicle, a low 4 mg/kg, or high 8 mg/kg dose of the  $\beta_2$ -adrenergic receptor antagonist ICI 118 (n= 12 per group). The saline and 4 mg/kg ICI 118,551 group showed a CPP for the previously cocaine-paired chamber throughout all trials, while the 8 mg/kg ICI 118,551 group showed a CPP on trial 1, but not on subsequent trials (see Figure 5). Repeated measures ANOVA revealed a significant effect of chamber for the saline ( $F_{2,22}=30.20, p<0.05$ ), 4 mg/kg ICI 118,551 group ( $F_{2,22}=35.21, p<0.05$ ), and 8 mg/kg ICI 118,551 group ( $F_{2,22}=24.68, p<0.05$ ). *Post hoc* analyses confirmed that the saline group spent

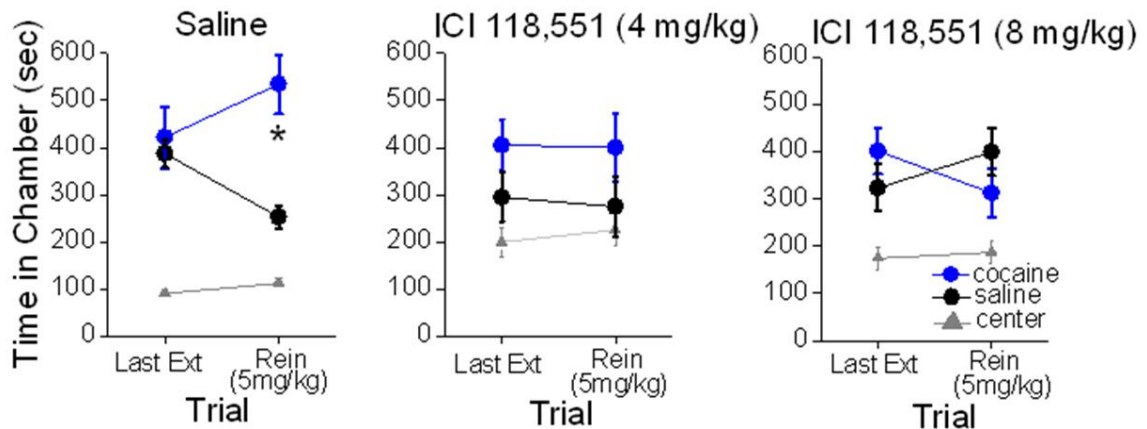
significantly more time in the previously cocaine-paired chamber than the saline-paired ( $p < 0.05$ ) throughout all trials. *Post hoc* analyses confirmed both the 4 mg/kg ICI 118,551 ( $p < 0.05$ ) and 8 mg/kg ICI 118,551 ( $p < 0.05$ ) group spent significantly more time in the previously cocaine-paired chamber than the saline chamber during the first trial. However, during the second trial ( $p < 0.05$ ) and third trial ( $p < 0.05$ ) only the 4 mg/kg ICI 118,551 group spent significantly more time in the previously cocaine-paired chamber than the saline chamber. Thus, a single 8 mg/kg but not 4 mg/kg treatment of ICI 118,551 can persistently attenuate reconsolidation of a CPP.



**Figure 5.** 4 mg/kg ICI 118,551 failed to attenuate CPP expression during the initial trial or on subsequent drug-free trials. 8 mg/kg ICI 118,551 failed to attenuate CPP expression during the initial test trial, but lead to attenuation of a CPP on subsequent trials (n = 12 per group). \*  $p < 0.05$ , \*\*\*  $p < 0.001$

Next we determined whether the reconsolidation deficit induced by ICI 118,551 would prevent reinstatement of a CPP to a priming injection of cocaine. Rats continued to receive additional drug-free CPP trials to ensure extinction of the CPP. ANOVA on the final extinction trial revealed a significant effect of chamber for the saline group ( $F_{2,33}=10.73$ ,  $p < 0.05$ ), ICI 118,551 4 mg/kg group ( $F_{2,33}=4.78$ ,  $p < 0.05$ ), and ICI 118,551 8 mg/kg group ( $F_{2,33}=7.43$ ,  $p < 0.05$ ). *Post hoc* analyses showed that during the final

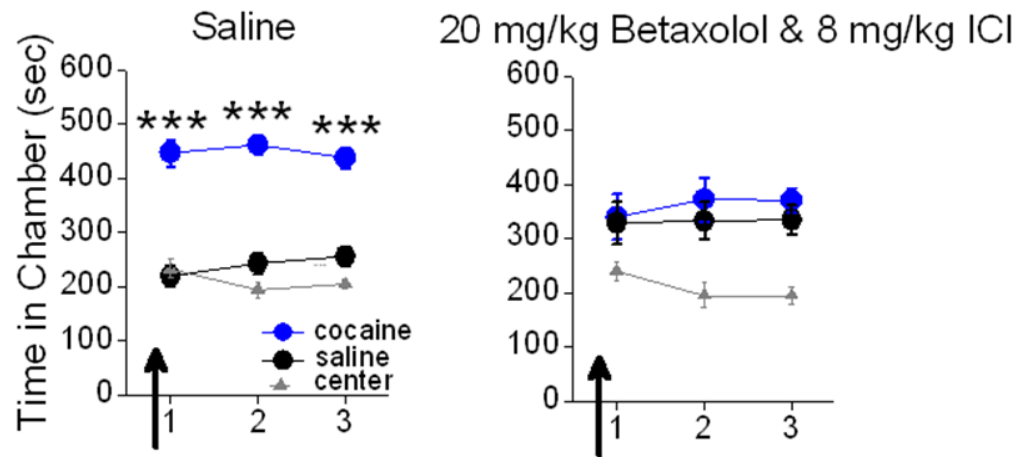
extinction trial a similar amount of time was spent in the cocaine- and saline-paired chamber ( $p>.05$ ), although a significantly greater amount of time was spent in the cocaine chamber than the center chamber for all groups ( $p<.05$ ; see Figure 6). Two days later, rats were injected with cocaine (5 mg/kg, i.p.) 15 min before a CPP trial to test for reinstatement of the CPP. ANOVA revealed a significant effect of chamber during the ICI 118,551-free cocaine-primed reinstatement trial for the saline ( $F_{2,33}=19.22, p<0.05$ ) and 8 mg/kg ICI groups ( $F_{2,33}=5.962, p<0.05$ ), but not in the 4 mg/kg ICI group ( $F_{2,33}=2.43, p>0.05$ ). *Post hoc* analyses revealed that the saline group spent more time in the cocaine- than saline-paired chamber ( $p<.05$ ), but neither ICI-treated group showed a CPP. Thus, ICI 118,551 at either a low or high dose prevents cocaine-induced reinstatement in the absence of further ICI 118,551 treatment.



**Figure 6.** Previous administration of saline failed to prevent rats from expressing a CPP whereas 4 or 8 mg/kg of ICI 118,551 prevented rats from expressing a CPP for the previously cocaine-paired chamber following a 5 mg/kg priming injection of cocaine ( $n=12$  per group). \*  $p < 0.05$ .

## **Experiment 2: Co-administration of betaxolol and ICI 118,551 persistently disrupts retrieval of CPP when administered prior to an initial test**

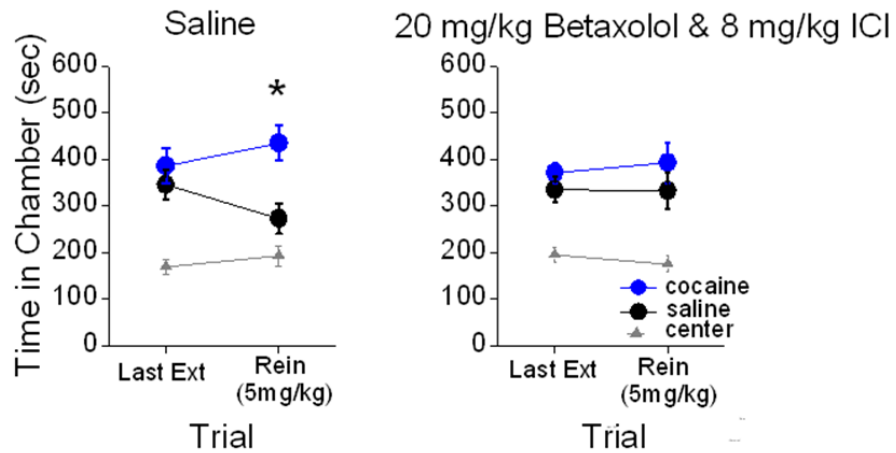
Next, we investigated the effect of co-injections of both high doses of betaxolol and ICI 118,551 on retrieval of a CPP. In experiment 2 rats were pretested, conditioned with 10 mg/kg of cocaine, and subjected to daily CPP trials. Thirty min before the first CPP trial, rats were injected with either vehicle or a 20 mg/kg dose of betaxolol combined with a 8 mg/kg dose of ICI 118,551 ( $n= 12$  per group). The saline group showed a CPP for the previously cocaine-paired chamber throughout trials whereas the co-injected group showed a retrieval deficit (see Figure 7). Repeated measures ANOVA revealed a significant effect of chamber for both the saline ( $F_{2,22}=24.87, p<0.05$ ) and the co-injected groups ( $F_{2,22}=5.42, p<0.05$ ). *Post hoc* analyses confirmed for the saline group that significantly more time was spent in the previously cocaine-paired chamber than the saline-paired chamber on all trials ( $p<0.01$ ). For the co-injected group, *post hoc* analyses showed that rats spent similar time in the cocaine- and saline-paired chambers ( $p>.05$ ), although less time was spent in the center chamber ( $p<0.05$ ). Thus, a single co-injection of 20 mg/kg betaxolol and 8 mg/kg of ICI 118,551 can persistently disrupt retrieval of a CPP throughout multiple trials, replicating previously reported results using a non-specific antagonist propranolol (Otis and Mueller, 2011).



**Figure 7.** Co-injection of  $\beta$ 1- and  $\beta$ 2-receptor antagonist injected prior to first trial induces a persistent retrieval deficit for a cocaine CPP (n = 12 per group). \*\*\* p < 0.001

Next we determined if co-injection of betaxolol and ICI 118,551 on the first CPP trial would prevent subsequent reinstatement of a CPP to a priming injection of cocaine. Rats continued to receive additional drug-free CPP trials to ensure extinction of the CPP. Analysis of the rats final extinction trial via ANOVA revealed a significant effect of chamber for the saline ( $F_{2,33}=14.69$ ,  $p<0.05$ ) and co-injected group ( $F_{2,33}=18.46$ ,  $p<0.05$ ). *Post hoc* analyses confirmed that during the final extinction trial a similar amount of time was spent in the saline- and cocaine-paired chambers ( $p>.05$ ), although rats spent less time in the center chamber ( $p<.05$ ; see Figure 8). Two days later, rats were injected with cocaine (5 mg/kg, i.p.) 15 min before a CPP trial to test for reinstatement of the CPP. ANOVA revealed a significant effect of chamber during the betaxolol-ICI 118,551-free cocaine-primed reinstatement trial for the saline ( $F_{2,33}=15.92$ ,  $p<0.05$ ) and co-injected groups ( $F_{2,33}=10.42$ ,  $p<0.05$ ). *Post hoc* analyses revealed that saline-treated rats spent significantly more time in the previously cocaine-paired than the saline-paired chamber ( $p<0.01$ ), whereas no differences were observed in the co-injected group. Thus, co-

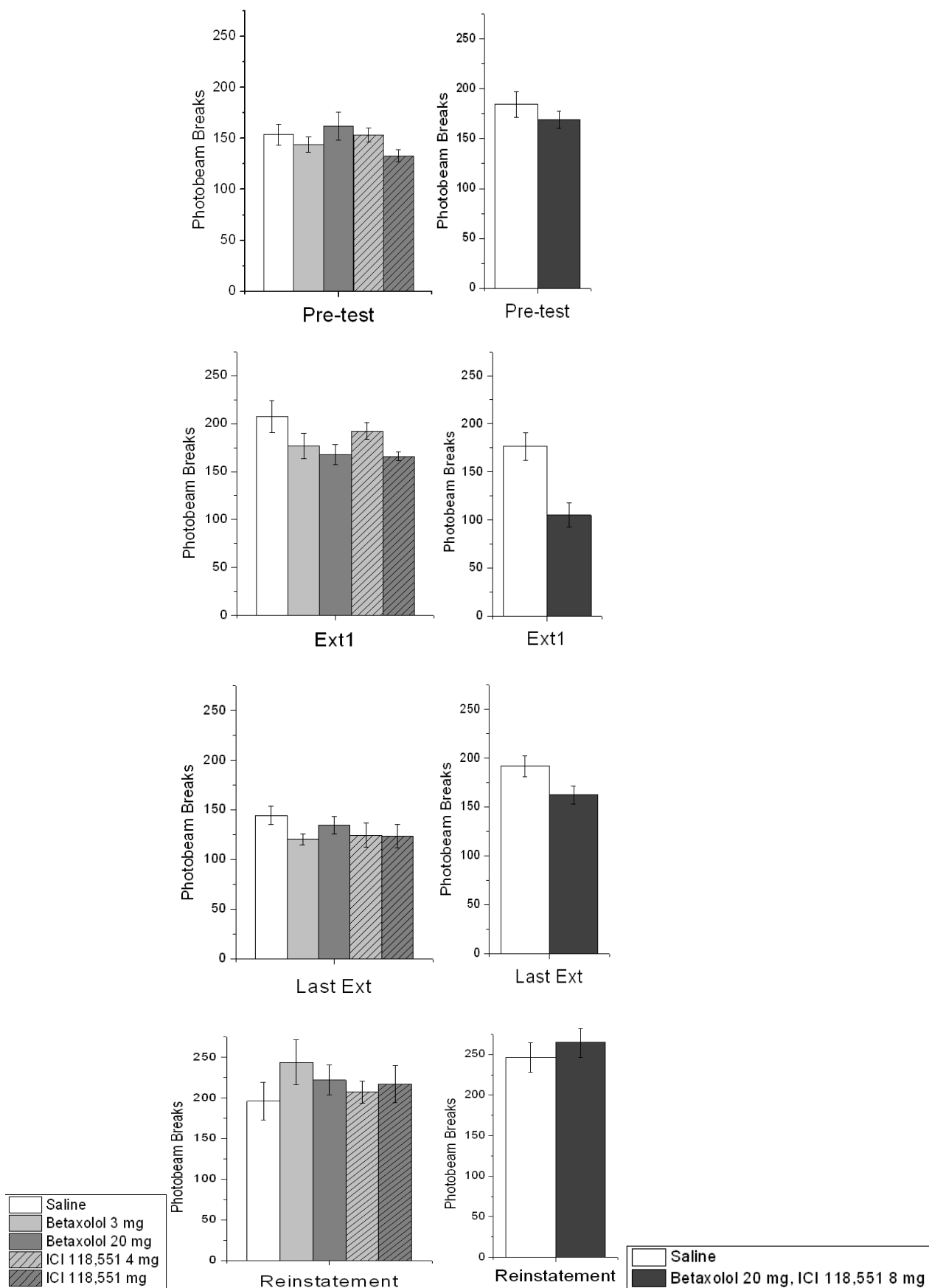
injection of betaxolol and ICI 118,551 prior to the first CPP trial prevented subsequent cocaine-induced reinstatement in the absence of further treatment.



**Figure 8.** Co-injection of  $\beta$ 1- and  $\beta$ 2-receptor antagonist injected prior to first trial prevent reinstatement of a CPP following a 5 mg/kg priming injection of cocaine \*  $p < 0.05$

### Effects of drugs on locomotion activity

To determine whether the effects of betaxolol or ICI were due to alterations in motor behavior, locomotion responses to betaxolol, ICI 118,551, and co-injections of betaxolol plus ICI 118,551 were examined (Figure 9). None of the single injections of betaxolol or ICI 118,551 had any significant effect on locomotion (ANOVA;  $F_{1,22}=9.62$ ,  $p>0.05$  for betaxolol;  $F_{1,22}=14.17$ ,  $p>0.05$  for ICI 118,551). When betaxolol and ICI 118,551 were co-injected, locomotion activity significantly decreased in comparison to controls ( $p < .05$ ). Thus, the combination of the two drugs may have altered overall motor behavior. This decrease in locomotion is similar to previous reports of 20 mg/kg propranolol to also have a decreasing effect on locomotion (Mantsch et al., 2009). However, this locomotion effect was not observed past the first trial so is not believed to have any enduring effect on the animals behavior..



**Figure 9.** Effects of locomotion activity from single injection or co-injection of betaxolol, ICI 118,551 on either pre-test, first test trial (Ext 1), last extinction trial and after receiving 5 mg/kg priming injection of cocaine. The only significant change observed was a decrease in the number of photobeam breaks for the co-injection group in comparison to their control group on Ext 1. \*  $p < 0.05$

## Discussion

### General Discussion

The present study investigated the roles of  $\beta_1$  and  $\beta_2$  adrenergic receptor subtypes in the retrieval and reconsolidation of a cocaine conditioned place preference. Consistent with our predictions, we found that  $\beta_1$ -AR blockade led to impaired retrieval whereas  $\beta_2$ -AR blockade led to impaired reconsolidation of a cocaine CPP. At the highest dose, the  $\beta_1$ -AR antagonist betaxolol induced a persistent retrieval deficit. Furthermore, rats treated with the highest dose of betaxolol did not show subsequent cocaine-induced reinstatement. In contrast, the  $\beta_2$ -AR antagonist ICI 118,551 did not affect retrieval but rather disrupted reconsolidation of a cocaine CPP. The lowest dose of ICI 118,551 had no effect, but the highest dose disrupted drug seeking on trials two and three. Additionally, ICI 118,551 treatment overall prevented subsequent reinstatement to a priming injection of cocaine, with the high dose having a greater effect than the low dose. Finally, co-injection of betaxolol and ICI 118,551 induced a persistent retrieval deficit and prevented subsequent reinstatement to a priming injection of cocaine in a similar manner as the general  $\beta$ -AR antagonist propranolol (Otis and Mueller, 2011).

Previous work has shown that structures such as the PL-mPFC (Otis, et al., 2013) or hippocampus (Otis, Fitzgerald, & Mueller, *in review*) play a role in retrieval but not reconsolidation of a CPP. In both the PL-mPFC and hippocampus  $\beta_1$ -ARs account for over 80% of the total  $\beta$ -ARs (Rainbow, Parsons, & Wolfe, 1984). Thus,  $\beta_1$ -ARs in these regions may mediate retrieval, although this remains to be determined. The present findings are consistent with previous work showing that both betaxolol and propranolol produce similar retrieval deficits in contextual fear learning (Murchinson et al., 2004; Flexner et al., 1985). Betaxolol was effective when given prior to the first trial, which



may explain why other groups did not observe any deficits following post-trial injections at various doses (Bernardi et al., 2009). We conclude that  $\beta$ 1-ARs are critical for retrieval but not reconsolidation of a cocaine CPP.

Our findings that  $\beta$ 2-ARs mediate reconsolidation of a cocaine CPP is consistent with previous findings from our lab and others (Bernardi et al., 2009). Additionally, previous work has shown that the amygdala is involved with reconsolidation but not retrieval of a CPP (Otis et al., 2013). Interestingly,  $\beta$ -AR distribution varies across different nuclei of the amygdala. The BLA in the rat brain has been reported to express 65%  $\beta$ 1-ARs, 35%  $\beta$ 2-ARs; the lateral central nucleus expresses 60%  $\beta$ 1-ARs, 40%  $\beta$ 2-ARs; and the medial central nucleus expresses roughly 53%  $\beta$ 1-ARs, 47%  $\beta$ 2-ARs (Rainbow, Parsons, Wolfe, 1984). Drug experience may alter this distribution as expression of  $\beta$ 1-AR, but not  $\beta$ 2-AR, significantly increased during withdrawal from cocaine in rats (Rudoy and Van Bockstael, 2007). In these rats, administration of 5 mg/kg,i.p. of betaxolol resulted in a significant attenuation of anxiety-like behavior which is characteristic during cocaine withdrawal (Rudoy and Van Bockstael, 2007). Since the amygdala mediates anxiety (Davis, 1992; Bremner et al., 1996; Tanaka et al., 2000; Charney, 2003) and is potently activated during withdrawal from various drugs of abuse, including cocaine (Zhou et al., 2003; Pollandt et al., 2006), it is possible that  $\beta$ 1-AR may mediate symptoms of withdrawal. Thus,  $\beta$ -ARs in the amygdala mediate reconsolidation through actions at  $\beta$ 2-ARs and may participate in withdrawal in a  $\beta$ 1-AR dependent fashion.

Although our findings implicate that retrieval of a CPP is mediated by  $\beta$ 1-ARs, one possibility for the initial attenuation of the CPP is that betaxolol may induce an aversive or appetitive state. No work has been done yet to confirm if betaxolol induces a

CPP or aversion, however in Rudoy and Van Bockstael's (2007) experiments, betaxolol did not produce anxiolytic-like effects in control animals. We do not believe betaxolol would create a preference or aversion because the retrieval deficit was observed on subsequent betaxolol-free trials. Furthermore, conditioning with the general  $\beta$ -AR antagonist propranolol does not induce a preference or aversion on a subsequent test trial (Otis and Mueller, 2011). Regarding the  $\beta_2$ -AR antagonist ICI 118,551, Bernardi et al., (2009) demonstrated that administration of ICI 118,551 following a single CS exposure had no effect on a subsequent test. This suggests that ICI 118,551 at the high dose we used was not by itself aversive. Since neither propranolol nor ICI 118,551 created a preference or aversion, we do not believe betaxolol would cause a preference or aversion effect, but a follow-up study is needed.

Another possible explanation of our results is that betaxolol or ICI 118,551 facilitated the extinction of a CPP. It is well accepted that norepinephrine facilitates, rather than impedes, memory formation through  $\beta$ -receptor activation (McGaugh, 2000). Previous work with non-selective beta blockers has demonstrated that propranolol impairs extinction learning (Mueller and Cahill, 2010) in aversive (Merlo and Izquierdo, 1967; Mueller et al., 2008; Ouyang and Thomas, 2005) and appetitive paradigms (LaLumiere et al., 2010).

### **Potential Mechanism**

Activation of  $\beta$ -ARs in the CNS activates adenylyl cyclase to increase the levels of intracellular cAMP, which activates protein kinase A (PKA) (Mueller, Porter, & Quirk, 2008). PKA in turn phosphorylates calcium activated potassium channels and blocks their activity. Accordingly, norepinephrine can increase the firing of a neuron by

removing the hyperpolarizing currents that inhibit its activity (Madison and Nicoll, 1986). Interestingly, equal doses of the non-selective  $\beta$ -AR agonist, isoproterenol, stimulates  $\beta$ 2-AR adenylyl cyclase approximately 50% more than  $\beta$ 1-AR even though both  $\beta$ -AR subtypes show equal affinity for isoproterenol (Green, Holt, & Liggett, 1992). Both  $\beta$ -AR subtypes are distributed across dendrites (Joels and Baram, 2009) and stimulate adenylyl cyclase by coupling to the guanine nucleotide protein which leads to the increase of intracellular cAMP and activation of PKA. Past work has shown that  $\beta$ -adrenergic signaling is necessary for CPP retrieval (Jasmin et al, 2006; Olson et al, 2006). Activity within the locus coeruleus, a cell body region for noradrenergic neurons, has been shown to have a graded response to stimuli, responding greatest to salient stimuli and subsequently leading to better retrieval (Sara, 2009). For example, human studies have shown that propranolol administration can attenuate cued recall of heroin- or emotionally-related words (Kroes, Strange, & Dolan, 2010; Zhao et al., 2010). Using the CPP procedure, cocaine administration is paired with a distinguishable cue of a specific conditioning chamber. Previous work has shown to block memory retrieval or reconsolidation via norepinephrine blockade at  $\beta$ -ARs within structures mediating retrieval or reconsolidation of a cocaine CPP, the  $\beta$ -AR antagonist must be active in the presence of the drug-associated cues for the specific drug associations to be disrupted (Otis et al., 2013; Bernardi et al., 2009). Thus disrupting noradrenergic signaling in the presence of the previously associated drug cues prevents drug-seeking.

### **Clinical Relevance**

Since our work and others (Vranjkovic et al., 2012) has shown that  $\beta$ 2-AR blockade is capable of blocking stress- or cocaine-induced reinstatement, ICI 118,551 could serve as a potential in or out-patient medication for both men and women that may

have to return to stressful or environmental cues during or after treatment. A recent fMRI study shows that in cocaine dependence, corticostriatal-limbic hyperactivity appears to be linked to stress cues in women and drug cues in men (Potenza et al., 2012). Although men are more likely to have a cocaine abuse or dependence disorder (Brady and Randall, 1999), women begin using cocaine at an earlier age (Chen and Kandel, 2002) and progress more rapidly from casual use to a dependence disorder (O'Brien and Anthony 2005). Differences also exist between men and women in terms of relapse to cocaine use. For example, women tend to have shorter cocaine-free periods (Kosten et al., 1996), with some reports indicating an enhanced likelihood to relapse (Hyman et al., 2008) following exposure to stressful stimuli/life events or depression, factors that have been implicated as the leading cause of relapse in people (Sinha, 2009). Taken together, those who are dealing with either stress or drug cues as a cause to use could potentially benefit from  $\beta$ 2-AR blockade.

In summary, these findings further suggest that central noradrenergic systems contribute to the retrieval and reconsolidation of drug-associated memories. Because presentation of drug-associated stimuli promotes drug seeking and relapse in addicts who have even gone through exposure therapy, using betaxolol, ICI 118,551 or a combination of the two in adjunct with exposure therapy could help limit the relapse vulnerability of addicts by disrupting stimulus-induced drug seeking even after treatment has ended.

### **Future Directions**

Future work will focus on the following aspects: the success of ICI 118,551 following both longer cocaine exposure and after multiple cocaine or stress relapses, replication of these experiments but involving female rats, microinfusions of betaxolol or

ICI 118,551 in the PL-mPFC or hippocampus, and electrophysiology using betaxolol or ICI 118,551. The current study shows the success after one priming injection, but the continuous relapse rates prevalent in people suggests an investigation is needed of the potential success of ICI 118,551 for cocaine addicts of extended use in addition to several cue or stress induced relapses. Also, the current study demonstrates the success of betaxolol or ICI 118,551 with male rats. As mentioned before there are several differences in cocaine addiction between men and women. It remains unknown if ICI 118,551 protecting effect against stress or cocaine induced reinstatement translates over from males to females.

As described before there are differences in distributions of  $\beta$ 1-AR and  $\beta$ 2-AR throughout the brain. An investigation of microinfusions of either betaxolol or ICI 118,551 into the PL-mPFC or hippocampus could open a potential discussion of specific structures in the brain that mediate retrieval rather than subtypes. At the current moment we would hypothesize that infusions of betaxolol would create similar results of nadolol (Otis et al., 2013), but it remains uncertain if ICI 118,551 could disrupt retrieval. In addition to the said microinfusion studies, electrophysiology studies could reveal what concentrations of betaxolol, ICI 118,551 or betaxolol and ICI 118,551 would be needed to replicate the effects of propranolol nullifying the increased excitability of norepinephrine on a neuron (Otis et al., 2013).

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