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Memory Retrieval Is Maintained By Intrinsic and Synaptic Plasticity in Prelimbic Cortex

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MEMORY RETRIEVAL IS MAINTAINED BY INTRINSIC AND SYNAPTIC PLASTICITY IN PRELIMBIC CORTEX

by

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ABSTRACT

MEMORY RETRIEVAL IS MAINTAINED BY INTRINSIC AND SYNAPTIC PLASTICITY IN PRELIMBIC CORTEX

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Under the Supervision of Devin Mueller

Abnormally strong memories underlie common disorders including addiction and post-traumatic stress disorder (PTSD). Memory disruption would therefore be beneficial for treatment of these disorders. Evidence reveals that cocaine conditioned place preference (CPP) memories are susceptible to long-lasting disruption during memory retrieval. For example, inhibition of β-adrenergic receptor (β-AR) activity within the prelimbic medial prefrontal cortex (PL-mPFC) prevents cocaine CPP memory retrieval, and this retrieval impairment is both long-lasting and prevents subsequent reinstatement of the CPP. Despite this, whether PL-mPFC β-AR activity is a fundamental mechanism required to maintain retrieval of other memories is unclear. Furthermore, how PL-mPFC β-AR activity maintains memory retrieval is unknown. Thus, here I use a combination of behavioral and electrophysiological techniques to 1) evaluate how PL-mPFC β-AR

activity regulates retrieval of memories related to a natural reward and to an aversive stimulus and 2) to determine the mechanism of memory retrieval deficits.

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Introduction

Potentially rewarding or threatening stimuli are particularly relevant to an organism. Presentation of these stimuli augments arousal and attention, and ensuing improvement of sensory perception, learning, and memory allows fast and accurate responses to a potential reward or threat (Sara and Bouret, 2012). Although this increases the likelihood of survival within an environment, stimulus-induced enhancement of learning and memory can also become problematic.

Pathological forms of memory drive inappropriate behaviors that are destructive to normal health and behavior. Disruption of these memories would therefore be beneficial for treatment of memory-related psychiatric diseases. Here, I use drug addiction and post-traumatic stress disorder (PTSD) as model diseases to describe how pathological forms of memory can lead to disordered behaviors. Moreover, research regarding memory disruption, particularly by β-adrenergic receptor (β-AR) blockade during memory retrieval, is described. The mechanisms by which β-AR blockade disrupts memory retrieval are not known. Thus, a series of experiments were performed using a combination of behavioral and electrophysiological techniques to 1) determine the mechanism of memory retrieval impairments and 2) evaluate whether β-AR activity during memory retrieval is a fundamental mechanism required for the maintenance of memories.

Natural and pathological appetitive learning

Survival and reproduction depend on obtaining rewards such as food, shelter, and sex. To obtain these rewards efficiently an organism must learn based on previous

experiences. For example, environmental stimuli become associated with a reward as positively reinforced behaviors are completed (Pavlov, 1927). Following this learning, presentation of reward-related cues can motivate behaviors which have previously been reinforced by that reward (Hyman, 2005). Unfortunately, stimulus-reward learning can become too strong, leading to pathological forms of appetitive memory.

Drug abuse induces the formation of strong associations between environmental stimuli and drug effects. Presentation of drug-associated cues can subsequently provoke abnormal autonomic responses and subjective reports of craving among addicts (Childress et al., 1986a, b; Ehrman et al., 1992), and these cravings drive compulsive drug seeking (Herman, 1974; O'Brien et al., 1991). Unfortunately, compulsive drug seeking can persist despite negative consequences (e.g., withdrawal, illness, anxiety, and intoxication) and this behavior can supplant healthy, positively reinforcing behaviors. However, disruption of drug-associated memories would prevent cue-induced drug seeking, limiting relapse susceptibility.

Natural and pathological fear learning

Survival also depends on learning to avoid and escape danger. Stimuli that are coupled with a perceived threat, such as the howl of a predator, become associated with the danger. Following this learning, presentation of the threat-associated stimuli can drive the behaviors that promote avoidance of the threatening stimulus. However, fear learning can also become problematic.

Traumatic experiences, such as combat or a violent assault, can lead to PTSD. This disorder develops gradually following a trauma, and initial symptoms include

cognitively re-experiencing the event even with attempts to avoid such thoughts (Grillon et al., 1996). This leads to a pathological form of memory, in which cues associated with the event are particularly capable of reminding the patient of the trauma. Thus, presentation of trauma-related cues (e.g., a noise) can induce an exaggerated fear response, which may include startle, perspiration, shortness of breath, and panic. However, disruption of traumatic memories would be beneficial for treatment of PTSD (Pitman et al., 2002; Brunet et al., 2008).

Memory acquisition, consolidation, retrieval, and reconsolidation

Appetitive and aversive learning require overlapping neural mechanisms (Peters et al., 2009). Research reveals that formation and expression of these memories occurs in several stages, including acquisition, consolidation, retrieval, and reconsolidation (Abel and Lattal, 2001; Nader and Hardt, 2009). First, memory acquisition is the earliest stage of learning, during which an association exists as a short-term memory. Second, memory consolidation is the process by which a short-term memory becomes long-term. Third, memory retrieval is the reactivation of a consolidated memory, allowing memory recall and/or behavioral expression of the memory. Therefore, consolidated memories are not functional if memory retrieval is disrupted. Last, memory reconsolidation is the process by which a retrieved memory becomes labile and is again consolidated into long-term storage. Reconsolidation likely reflects "memory updating", and recent research reveals that many types of memories are susceptible to disruption during this process (Nader and Hardt, 2009). Thus, memory retrieval and reconsolidation are of particular importance to disorders associated with pathological forms of memory, as disruption of these processes

could prevent cue-induced behaviors. To understand the mechanisms required for retrieval and reconsolidation, neuroscientists use rodent memory models. Next, I focus on three rodent models that are used to study memory.

Conditioned place preference

The conditioned place preference (CPP) paradigm is commonly used to investigate the neural mechanisms of drug-associated memories. In this paradigm, rats are trained to associate one chamber, but not another, with a drug of abuse. Following training, all rats are given full access to both chambers while in a drug-free state. During these CPP trials more time is spent within the drug-paired chamber than within the saline-paired chamber. Thus, a CPP for the previously drug-paired chamber is expressed. When a CPP is expressed investigators can be certain that animals acquired, consolidated, and retrieved the drug-associated memory. Moreover, the mechanisms underlying these processes can be investigated through pharmacological manipulation at different time points throughout CPP experiments. For example, the mechanisms underlying drug-associated memory retrieval can be examined via pharmacological manipulation before a drug-free CPP trial. The effects of such manipulation can be determined by assessing CPP expression during the CPP trial. In contrast, memory reconsolidation can be studied by manipulating the rodents immediately after a retrieval trial. The effects of that manipulation can then be assessed during another CPP trial the following day. Thus, the CPP procedure allows for the investigation of drug-associated memory retrieval and reconsolidation.

Self-administration

The self-administration paradigm is seldom used to study drug-associated memories, possibly due to confusion regarding the proper methods required to investigate memory within this model. In the self-administration paradigm, rats learn to press a lever for an intravenous infusion of a drug. Moreover, drug infusions are paired with cues that become associated with drug availability. Drug self-administration training takes a series of days to weeks, such that the behavior becomes well-rehearsed and probably habitual. Consistent with this idea, even limited cocaine self-administration can cause insensitivity to devaluation of cocaine, indicating that self-administration becomes a behavior that is not goal-oriented (Zapata et al., 2010). Moreover, this habitual cocaine seeking is blocked by inactivation of the dorsal striatum, a structure that is critical for habit learning (Zapata et al., 2010). In contrast, goal-oriented cocaine seeking is not blocked by dorsal striatum inactivation (Zapata et al., 2010). Thus, drug self-administration becomes a habitual behavior, and likely depends on neural circuits responsible for habit learning, but not memory retrieval.

Consistent with the idea that self-administration becomes a habitual behavior; data suggest that self-administration becomes independent of brain structures that are critical for memory retrieval, particularly the prelimbic medial prefrontal cortex (PL-mPFC). PL-mPFC is critical for memory retrieval (Corcoran and Quirk, 2007; Peters et al., 2009; Otis et al., 2013), and PL-mPFC activation is necessary for acquisition of an operant response before an action becomes habitual (Killcross and Coutureau, 2003; Ostlund and Balleine, 2005). Thus, drug self-administration may become independent of PL-mPFC during training. Indeed, inactivation of the PL-mPFC prevents the acquisition

of self-administration, but has no effect directly after self-administration training (Di Ciano et al., 2007). Thus, drug self-administration becomes 1) habitual and dependent on neural circuits responsible for habit learning and 2) independent of goals and independent of neural circuits responsible for memory retrieval. Importantly, extinction learning is likely to reverse this distinction, such that drug self-administration becomes dependent on memory retrieval.

Following drug self-administration training, rats undergo extinction during which lever presses do not lead to drug infusions. To study memory retrieval, extinction must occur in the absence of drug-associated cues or within an alternative context. This allows for extinction of the habitual behavior (lever pressing), but not extinction of the drug-cue or drug-context memories. Following successful extinction training, rats no longer press the lever for the drug. However, drug seeking can be reinstated upon re-presentation of the drug-associated cue or context. As drug seeking at this time is no longer habitual, cue or context-induced reinstatement is likely to require memory retrieval. In support of this, many studies reveal that cue-induced and context-induced reinstatement of drug self-administration is PL-mPFC dependent (Capriles et al., 2003; McLaughlin and See, 2003; Fuchs et al., 2005; Ball and Slane, 2012). Thus, cue-induced reinstatement and context-induced reinstatement allow for investigation memory retrieval within the self-administration paradigm.

Fear conditioning

Fear conditioning is a commonly used model of aversive learning and memory. In this paradigm, rats are trained to associate a conditional stimulus (CS), such as a context or an auditory tone, with an aversive unconditional stimulus (UCS), such as a foot shock. Along with context fear conditioning, two distinct types of cued fear conditioning are commonly used. First, delay fear conditioning involves the presentation of a CS that terminates with the UCS. Second, trace fear conditioning involves the presentation of the CS and UCS, but these stimuli are separated in time by a silent 'trace interval'. Thus, trace fear learning is slightly more complex and requires more neural processing, such as PL-mPFC activation during the trace interval (Runyan et al., 2004; Gilmartin and McEchron, 2005; Gilmartin and Helmstetter, 2010; Gilmartin et al., 2013). Following context, delay, or trace fear conditioning rats are tested via presentation of the CS (context or discrete cue) in the absence of the UCS during fear memory retrieval tests. During this test, presentation of the CS induces a conditional response (CR), such as a rise in heart rate, blood pressure, and/or freezing of movement. If rats express a CR following presentation of the CS, investigators can conclude that the animals acquired, consolidated, and retrieved the fear-associated memory. Similar to place conditioning, the mechanisms underlying fear memory retrieval can be studied via manipulations before a CS test, whereas the mechanisms underlying reconsolidation can be studied via manipulations after a CS test.

Memory retrieval and noradrenergic signaling

Memory retrieval can be operationally defined as the neural process by which a consolidated memory is reactivated. Retrieval allows for conscious recollection and behavioral expression of memory. It is important to note that investigations of memory retrieval in rodents actually use behavioral expression to make inferences regarding memory retrieval. Although impairments in memory retrieval would prevent behavioral expression of the memory, behavioral expression can also be disrupted by influencing nonspecific factors such as motor activity, motivation, and attention. Thus, researchers should be careful with interpretations when investigating memory retrieval. Moreover, when making conclusions regarding memory retrieval it is critical to run experiments that control for nonspecific effects of the manipulations on behavioral expression of memory.

The neural mechanisms underlying memory retrieval are not well understood. Early studies revealed that behavioral expression of memories can be enhanced by cocaine (Rodriguez et al., 1993), amphetamine (Sara and Deweer, 1982), and nicotine (Faiman et al., 1992). These drugs are nonspecific, although each is capable of enhancing noradrenergic signaling. More specific agonists of noradrenergic signaling have also been given during a retrieval test in the memory forgetfulness task. In this paradigm, rats learn and over time forget a path which will guide them to the end of a maze. Enhancement of noradrenergic signaling, via inhibition of α_2-adrenergic autoreceptors which inhibit norepinephrine release, enhances behavioral performance in the maze after forgetting (Sara and Devauges, 1989). Similarly, stimulation of the locus coeruleus (LC), a major nucleus of noradrenergic cell bodies (Dahlstroem and Fuxe, 1964), also enhances memory expression in this task (Sara and Devauges, 1988). Finally,

the enhancement of memory expression by LC stimulation is prevented by the β-adrenergic receptor (β-AR) antagonist propranolol (Devauges and Sara, 1991). Taken together, these studies indicate that memory retrieval may be enhanced by stimulation of noradrenergic signaling, and that this effect is dependent on β-AR activation.

Stimulation of noradrenergic signaling enhances memory expression, although it was unclear whether noradrenergic signaling is necessary for memory retrieval. Murchison and colleagues (2004) addressed this issue by using mice which lack dopamine β-hydroxylase (DBH), a necessary enzyme for norepinephrine and epinephrine synthesis from dopamine. DBH knockout mice expressed fear during the learning phase of a contextual fear task but were unable to express this memory 1 to 4 days later. Similar results were also found in the Morris water maze, another spatial memory paradigm. Interestingly, memory expression for DBH knockout mice was intact in a variety of discrete cue-induced memory tasks. Furthermore, wild-type mice treated with β-AR antagonists expressed less fear during a contexual retrieval test, whereas mice treated with β-AR agonists expressed more fear during a contextual retrieval test (Murchison et al., 2004). Interestingly, these effects were not present in a variety of discrete cued tasks, allowing the researchers to control for nonspecific effects of the manipulations on behavioral expression of the memory. Thus, these data demonstrated that retrieval of contextual memories is dependent on β-AR signaling.

Although the evidence described indicates that β-AR signaling mediates retrieval of contextual, but not discrete cued memories, other evidence is at odds with this distinction. For example, systemic injections of propranolol, a β-AR antagonist, reduces cue-induced fear expression in rats (Rodriguez-Romaguera et al., 2009). Moreover,

propranolol reduces the firing rate of prelimbic medial prefrontal cortex (PL-mPFC) neurons *in vivo* (Rodriguez-Romaguera et al., 2009), a structure that is necessary for expression of fear memories (Corcoran and Quirk, 2007). Thus, whether β-AR activation is required for both context and discrete cue-induced memory retrieval remains unclear.

Persistent retrieval impairments in humans and rodents

Despite evidence supporting β-AR involvement in retrieval, only a few studies have examined whether this activity is required to successfully maintain future memory retrieval. Recent data reveals that propranolol disrupts recall of visual memories and emotional words in humans (Kroes et al, 2010; Kroes et al, 2012 *SfN Abstracts*) and heroin-related words in human heroin addicts (Zhao et al., 2010). Moreover, the effects of β-AR blockade on memory recall in humans are long lasting (Kroes et al., 2010), and may prevent memory reinstatement (Kroes et al, 2012 *SfN Abstracts*). Although these findings are somewhat surprising given current models of memory, recent data support the conclusion that memory retrieval can be persistently impaired by β-AR blockade.

We recently investigated the effects of β-AR blockade on drug-associated memory retrieval in rodents. Using a cocaine-induced CPP paradigm, we found that systemic injections (Otis and Mueller, 2011), PL-mPFC microinfusions (Otis et al., 2013), or dorsal hippocampus (dHipp) microinfusions (Otis et al., 2014a) of β-AR antagonists prevents CPP memory expression. Similar to human studies, the effects of β-AR blockade on CPP expression persisted during subsequent days in the absence of further propranolol treatment. Moreover, these memory impairments prevented subsequent cocaine-induced reinstatement of a CPP (Otis and Mueller, 2011; Otis et al.,

2014a). Below, I thoroughly describe these data and associated control studies which led to the conclusion that CPP memory retrieval can be persistently impaired by β-AR blockade during retrieval.

Otis and Mueller, 2011

We first investigated the necessity of β-AR activation for cocaine-associated CPP memory retrieval. Rats were conditioned to associate one chamber with cocaine (10 mg/kg or 20 mg/kg) and another with saline before daily CPP tests. Moreover, rats were given systemic injections of saline or the β-AR antagonist propranolol before the first CPP test only. Rats treated with saline expressed a CPP for the previously cocaine-associated chamber during the first CPP test and during all subsequent tests overall, whereas rats treated with propranolol did not (see Figure 1A and Figure 1B). We next replicated these findings, but rats were injected with saline or propranolol before the second CPP test. All rats expressed a CPP during the first injection-free test. Moreover, rats treated with saline expressed a CPP for the previously cocaine-associated chamber during the second CPP test and during subsequent injection-free trials overall. In contrast, rats treated with propranolol did not express a CPP during the second test or during subsequent propranolol-free tests (see Figure 2). We again replicated these findings but injected rats with saline or propranolol before the second, third, and fourth CPP tests. All rats expressed a CPP during the first injection-free test. Moreover, rats treated with saline expressed a CPP during all subsequent CPP tests overall, including an injection-free CPP test 2 weeks later. However, propranolol-treated rats did not express a CPP during these tests or during the injection-free test 2 weeks later (see Figure 3). Thus, systemic administration of propranolol before the first or second CPP test prevented the expression of a cocaine-induced CPP memory, and this effect persisted for a minimum of 2 weeks.

Multiple unreinforced CPP tests lead to extinction learning, resulting in no CPP expression during subsequent tests. Following extinction, reinstatement of a CPP can be induced via administration of cocaine (Mueller and Stewart, 2000). Thus, we determined whether the persistent CPP expression deficit prevented cocaine-induced reinstatement of the CPP. Following conditioning, rats were treated with saline or propranolol before the second, third, and fourth CPP tests. Behaviorally, both extinction learning and propranolol treatment abolish CPP expression. Thus, rats treated with saline received an extra 8 CPP extinction tests of longer duration (30 minutes) to ensure extinction of the CPP. During the final CPP test, neither saline- nor propranolol-treated rats expressed a CPP. The following day, rats previously treated with saline expressed cocaine-induced reinstatement of the CPP, whereas rats previously treated with propranolol did not (see Figure 4). Thus, previous treatment with propranolol provided long-lasting protection against cocaine-induced reinstatement of the CPP.

Propranolol has effects on both the central and peripheral nervous systems (Street et al., 1979), and therefore the effects of propranolol on CPP expression could be due to β-AR blockade in either system. To investigate this, we administered sotalol, a peripheral β-AR antagonist that does not cross the blood-brain barrier (Dahlof, 1981), before the second, third, and fourth CPP tests. Rats treated with saline and sotalol expressed a CPP during the first CPP test and during subsequent tests overall (see Figure 5). Thus, sotalol did not prevent CPP memory expression, indicating that the effects of propranolol are due to β-AR blockade in the central nervous system.

Propranolol has effects that are not specific to β-AR blockade when administered at high doses, including protein kinase C inhibition and serotonergic receptor blockade

(Alexander and Wood, 1987; Sozzani et al., 1992). Thus, we examined the effect of a low dose of (-)-propranolol (1 mg/kg), the more active enantiomer which is more selective for β-ARs. Following conditioning, rats were given systemic injections of saline or (-)-propranolol before the first CPP test. Rats treated with saline expressed a CPP for the previously cocaine-associated chamber during the first CPP test and during all subsequent tests overall, whereas rats treated with (-)-propranolol did not (see Figure 6A). Thus, a low dose of (-)-propranolol induced a persistent disruption of CPP expression, supporting the conclusion that the effects of propranolol on CPP expression are specific to β-AR blockade.

Propranolol and other β-AR antagonists are capable of preventing CPP memory reconsolidation (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008; Bernardi et al., 2009). Thus, the persistent effects of propranolol on CPP expression could be attributable to reconsolidation blockade. To investigate this, following conditioning rats were given systemic injections of saline or propranolol immediately before, instead of 20 minutes before the first CPP test. In this case, propranolol is unlikely to have effects until after the CPP trial, during memory reconsolidation and after a CPP has already been expressed. Rats treated with propranolol expressed a CPP for the previously cocaine-associated chamber during the first CPP test and during subsequent CPP trials overall (see Figure 6B). Thus, propranolol administration immediately before a CPP test did not prevent subsequent CPP expression. Taken together, these data reveal that propranolol persistently impairs CPP expression without having effects on memory reconsolidation.

We also examined whether propranolol induced an affective state capable of altering CPP expression, or if propranolol altered locomotor activity. Rats were

conditioned to associate one chamber with propranolol and another with saline. Following conditioning, rats were given a CPP test during which no CPP or aversion was expressed for the previously propranolol-paired chamber (see Figure 6C). Thus, propranolol itself does not induce an affective state capable of influencing CPP expression. Finally, we determined the effects of propranolol on locomotor activity by measuring the number of photobeam breaks during a CPP trial. Rats treated with saline and propranolol had equivalent photobeam breaks during the CPP trial, indicating that propranolol did not influence locomotor activity (see Figure 6D). Taken together, the effects of propranolol are not attributable to reconsolidation blockade or other nonspecific effects on behavioral expression of the memory. Thus, β-AR blockade persistently impairs CPP memory retrieval, and this provides protection against subsequent cocaine-induced reinstatement.

Figures: Otis and Mueller, 2011

Figure 1

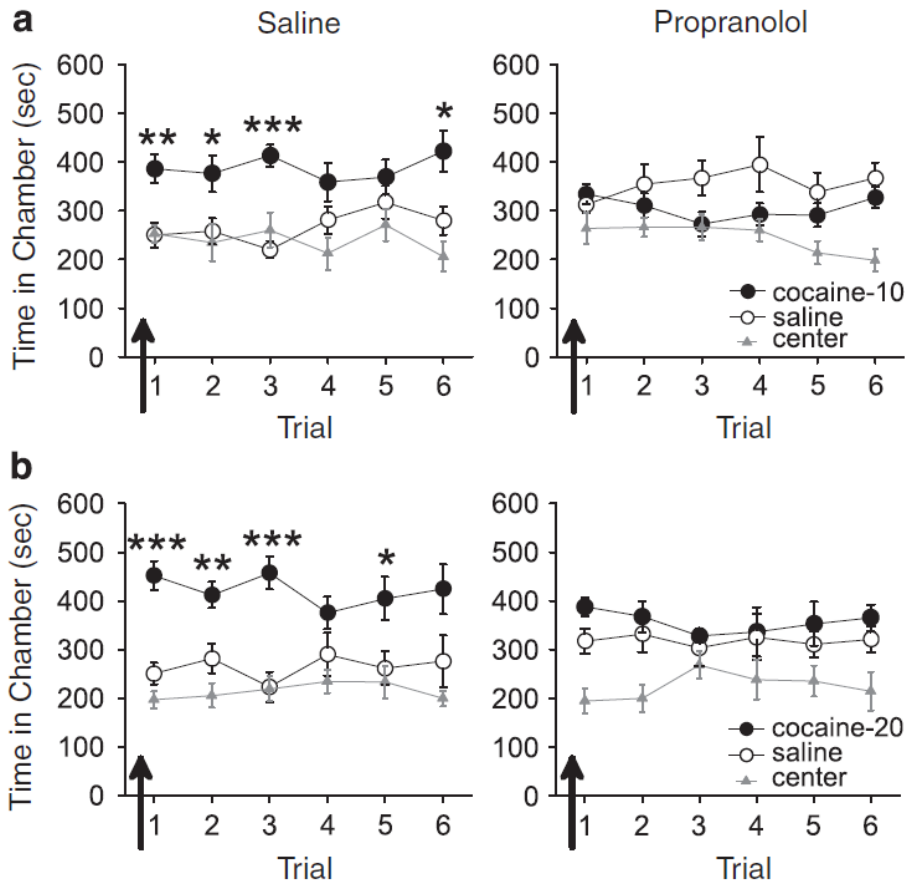

Figure 1. β-AR blockade persistently impairs cocaine CPP memory expression. Following conditioning with (A) 10 mg/kg or (B) 20 mg/kg of cocaine, systemic injections of propranolol, but not saline, before the first CPP trial prevented rats from expressing a cocaine CPP. Rats previously treated with propranolol, but not saline, continued to express no cocaine CPP during subsequent propranolol-free trials (Otis and Mueller, 2011). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 2

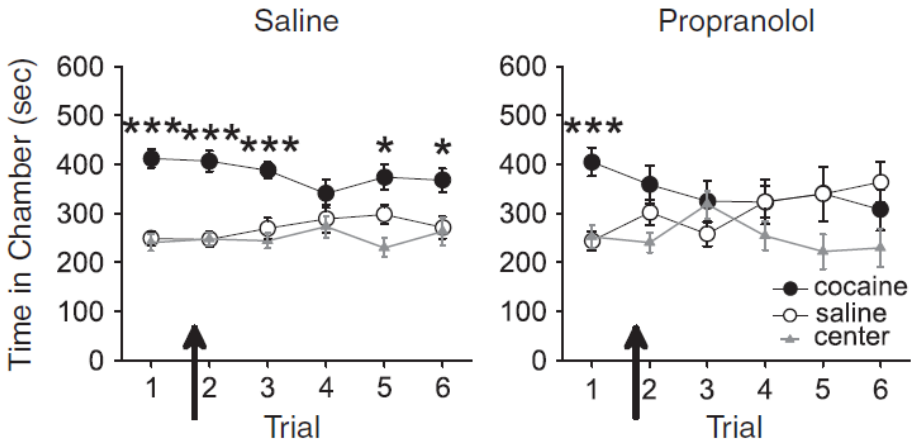

Figure 2. β-AR blockade persistently impairs cocaine CPP memory expression when administered before the second CPP trial. Following conditioning, systemic injections of propranolol, but not saline, before the second CPP trial persistently impair CPP expression (Otis and Mueller, 2011). ***$p < 0.001$ and *$p < 0.05$.

Figure 3

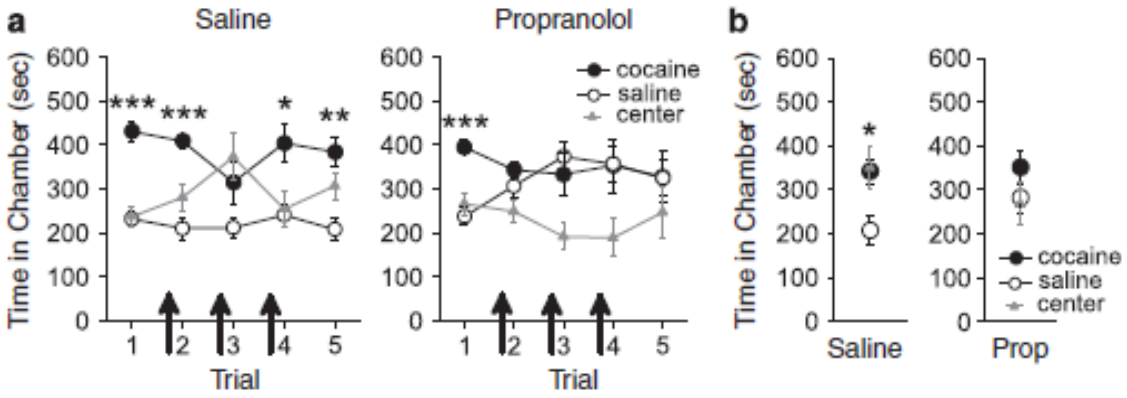

Figure 3. Effects of β-AR blockade on CPP expression are long-lasting. (A) Systemic injections of propranolol, but not saline, before the second, third, and fourth CPP trials persistently impair CPP expression. (B) Rats previously treated with propranolol, but not saline, continued to express no CPP following a 14-day break from testing (Otis and Mueller, 2011). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 4

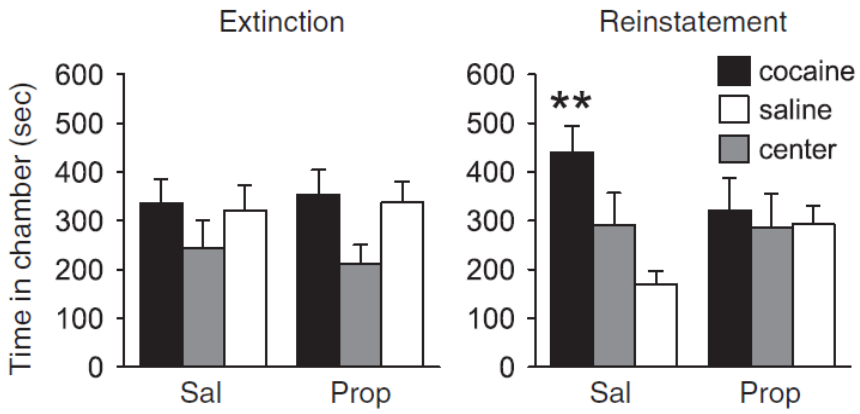

Figure 4. Effects of β-AR blockade on CPP expression prevent subsequent cocaine-induced reinstatement. Rats treated with saline or propranolol expressed no CPP during the final CPP extinction trial. Rats previously treated with saline, but not propranolol, expressed cocaine-induced reinstatement of the CPP (Otis and Mueller, 2011). Sal, saline; Prop, propranolol; $^{**}p < 0.01$.

Figure 5

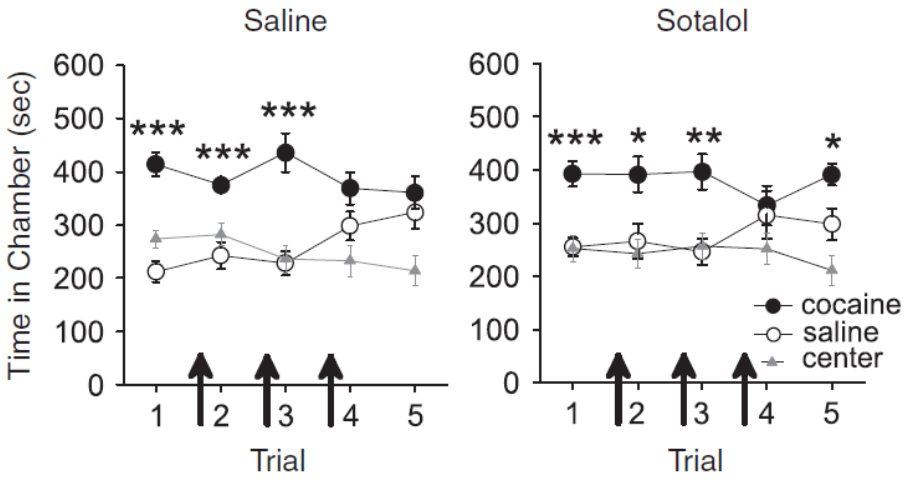

Figure 5. Peripheral β-AR blockade has no effect on CPP expression. Rats treated with sotalol did not prevent CPP expression across trials (Otis and Mueller, 2011). ***$p <$ 0.001, **$p < 0.01$, and *$p < 0.05$.

Figure 6

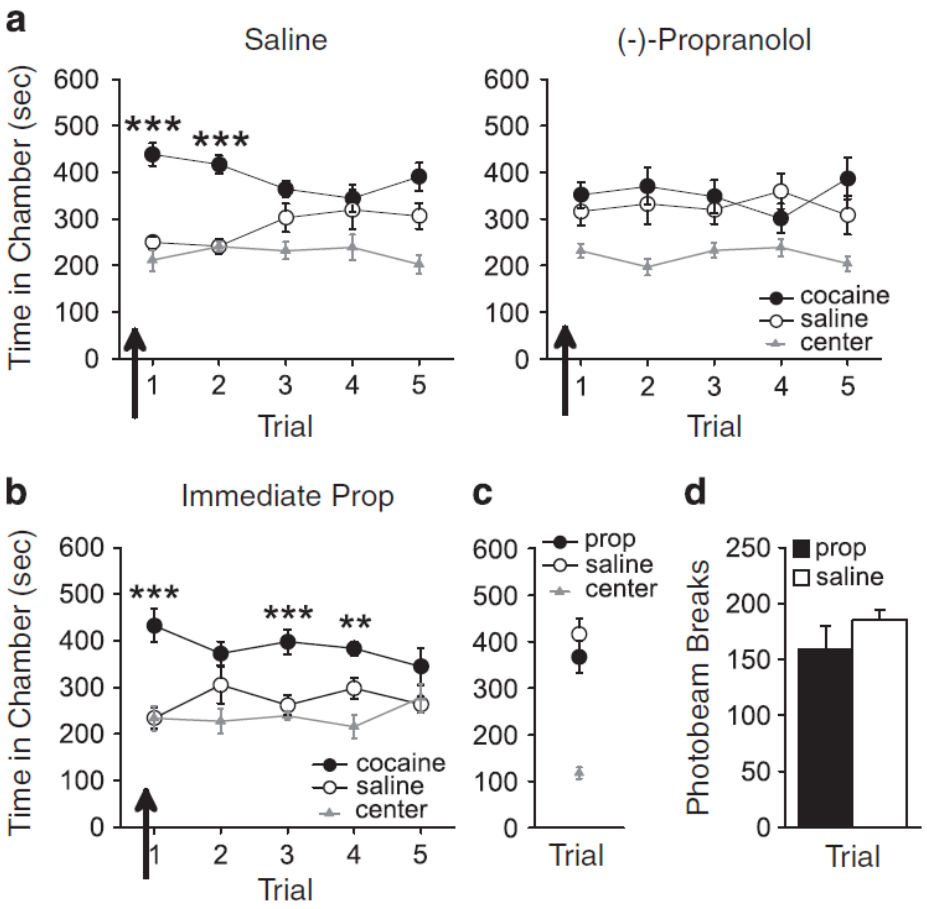

Figure 6. Effects of systemic propranolol injections on CPP expression are not due to nonspecific effects. (A) Systemic injections of (-)-propranolol, but not saline, induced a persistent deficit in CPP expression. (B) Systemic injections of propranolol immediately before a CPP trial did not completely prevent subsequent CPP expression. (C) Rats spent an equivalent amount of time within previously propranolol-paired and saline-paired chambers. (D) Systemic injections of propranolol did not affect locomotor activity, as measured by photobeam breaks during a CPP trial (Otis and Mueller, 2011). ***$p <$ 0.001 and **$p < 0.01$.

Neurobiological mechanisms of memory retrieval

The above results indicate that retrieval is dependent on β-AR activation in the central nervous system. Our next goal was to determine the locus at which β-AR activation is required for retrieval. We focused on three structures that are known to be important for the expression of learned behaviors: PL-mPFC, basolateral amygdala (BLA), and dHipp.

PL-mPFC involvement in memory retrieval

The PL-mPFC is necessary for the expression of learned fear. Unit recordings in rats reveal that PL-mPFC pyramidal neurons become active during presentation of a CS that was previously paired with an aversive US (Burgos-Robles et al., 2009; Sotres-Bayon et al., 2012). This activity likely drives fear expression, as electrical stimulation of PL-mPFC augments cue-induced fear (Vidal-Gonzalez et al., 2006). In contrast, inactivation of PL-mPFC prevents cue and context-induced fear expression, whereas PL-mPFC inactivation does not prevent innate fear of a predator or fear of open spaces (Corcoran and Quirk, 2007). Thus, PL-mPFC activity is involved in expression of learned, but not innate fear. This dissociation allows for the conclusion that PL-mPFC activation is necessary for retrieval, not only behavioral expression, of fear memories.

PL-mPFC activity may also been critical for drug-associated memory retrieval. Exposure to a previously cocaine-paired cues leads to robust PL-mPFC immediate early gene expression (Miller and Marshall, 2004, 2005; Zavala et al., 2008) indicating that presentation of a cocaine-associated context may activate PL-mPFC. Furthermore, drug-induced CPP expression is blocked by PL-mPFC lesions (Isaac et al., 1989; Tzschentke

and Schmidt, 1999) or PL-mPFC DNA-methyltransferase inhibition (Han et al., 2010). Thus, expression of a drug-associated CPP memory depends on PL-mPFC activity.

PL-mPFC also regulates cue-induced drug seeking within the drug self-administration paradigm. Pharmacological inactivation of the PL-mPFC prevents cue and context-induced reinstatement of self-administration (McLaughlin and See, 2003; Fuchs et al., 2005; Hiranita et al., 2006; Ball and Slane, 2012). Taken together, PL-mPFC is important for behavioral expression of fear and drug-associated memories.

BLA involvement in memory retrieval

The PL-mPFC has reciprocal connections with the basolateral amygdala (BLA) (Ishikawa and Nakamura, 2003; Gabbott et al., 2005), and BLA acts as a sensory interface for expression of learned fear (LeDoux, 2000). Sensory inputs converge within BLA (LeDoux et al., 1990b; LeDoux et al., 1990a; Romanski et al., 1993) , and BLA neurons spike upon presentation of a shock-associated CS (CS^{+}), but not upon presentation of a neutral CS (Quirk et al., 1995; Quirk et al., 1997). Furthermore, disruption of BLA activity prevents cue-induced fear expression (Kim et al., 1993; Maren et al., 1996a; Lee et al., 2001). Taken together, sensory inputs to the BLA allow cue-induced fear expression. BLA also receives input from the hippocampus (Canteras and Swanson, 1992), and this pathway may provide contextual information for fear expression (LeDoux, 2000). In support of this, context-induced fear expression is dependent on BLA and dHipp activity (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Helmstetter and Bellgowan, 1994; Maren et al., 1996b; Maren et al., 1997). Thus,

BLA receives input regarding sensory and contextual information for the expression of learned fear.

The BLA is also important for the expression of drug-associated memories. Exposure to a previously cocaine-paired environment leads to robust BLA immediate early gene expression (Miller and Marshall, 2004, 2005). Moreover, human studies reveal that drug-associated cue exposure increases amygdalar metabolic activity, and this activity correlates with reported drug cravings (Grant et al., 1996). BLA lesions or inactivation prevent drug-induced CPP expression (Hiroi and White, 1991; Brown and Fibiger, 1993; McDonald et al., 2010) and sucrose-induced CPP expression (Everitt et al., 1991). Inactivation of the BLA protein kinase C (PKC) and protein synthesis also transiently impairs CPP expression (Lai et al., 2008). In contrast, inhibition of BLA PKA, which is downstream of β-ARs (Pedarzani and Storm, 1993; Raman et al., 1996) has no effect on cocaine CPP expression (Lai et al., 2008). Taken together, although the BLA is critical for drug-induced CPP expression, BLA β-AR dependent signaling is unlikely to mediate drug-induced CPP expression.

Evidence reveals that BLA activity is critical for retrieval of drug-associated self-administration memories. Exposure to cues associated with cocaine self-administration induces expression of BLA immediate early genes (Neisewander et al., 2000). Moreover, BLA lesions or pharmacological inactivation prevent cue-induced reinstatement of drug seeking (Meil and See, 1997; Grimm and See, 2000; Fuchs and See, 2002; Kantak et al., 2002; Yun and Fields, 2003; Fuchs et al., 2005). In contrast, BLA inactivation has no effect on cocaine-induced reinstatement of cocaine seeking (Grimm and See, 2000). These data reveal that BLA activation is necessary for expression of cue-induced cocaine

seeking, and not simply expression of cocaine seeking. Thus, the conclusion can be made that BLA activity is critical for retrieval of drug-associated memories in the self-administration paradigm.

dHipp involvement in memory retrieval

The dorsal hippocampus (dHipp) has reciprocal connections with PL-mPFC through its ventral subregion (vHipp) and through the nucleus reuniens of the thalamus (Hoover and Vertes, 2007; Vertes et al., 2007). Moreover, the dHipp regulates expression of contextual fear memories. Lesions or pharmacological inactivation of the dHipp prevents context-induced fear (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren and Fanselow, 1997; Holt and Maren, 1999; Corcoran and Maren, 2001). In contrast, dHipp lesions or inactivation do not impair discrete cue-induced fear (Kim and Fanselow, 1992; Corcoran and Maren, 2001). Consistent with this, pharmacological or genetic disruption of dHipp β-AR signaling abolishes context, but not discrete cue-induced fear expression (Murchison et al., 2004). These data reveal that dHipp β-AR signaling is necessary for context-induced fear, not simply fear expression. Thus, the conclusion can be made that the dHipp β-AR activation mediates contextual fear memory retrieval.

The dHipp is also important for expression of contextual drug-associated memories. Immediate early genes are expressed within the dHipp following exposure to a previously drug-paired environment (Zhou and Zhu, 2006; Hearing et al., 2010). Moreover, lesions or pharmacological inactivation of dHipp prevents drug-induced CPP

expression (Meyers et al., 2003; Meyers et al., 2006; Zarrindast et al., 2006). Thus, dHipp activity is critical for expression of drug-associated CPP memories.

Evidence reveals that dHipp activity is critical for retrieval of drug-associated self-administration memories. Specifically, dHipp inactivation prevents context-induced reinstatement of cocaine self-administration (Fuchs et al., 2005). In contrast, dHipp inactivation has no effect on discrete cue-induced reinstatement or cocaine-induced reinstatement of cocaine self-administration. These data reveal that dHipp activation is necessary for context-induced cocaine seeking, and not simply expression of cocaine seeking. Thus, the conclusion can be made that dHipp activation is required for retrieval of contextual drug-associated memories within the self-administration paradigm.

Retrieval versus reconsolidation impairments: distinct or identical mechanisms?

The results described above reveal that PL-mPFC, BLA, and dHipp are involved in the expression of fear and drug-associated memories. As described above, we found that disruption of central β-AR activation induces persistent impairments in retrieval of a cocaine-induced CPP memory (Otis and Mueller, 2011). Thus, given the role of these structures in memory retrieval, we hypothesized that β-AR activity within PL-mPFC, BLA, or dHipp may be critical for maintaining cocaine-induced CPP memory retrieval.

When considering the involvement of a structure in the maintenance of memory retrieval, it is critical to also consider memory reconsolidation. Specifically, any manipulations that induce persistent CPP disruption can be explained by 1) a persistent memory retrieval disruption, 2) a transient impairment in behavioral expression of the CPP along with blockade of memory reconsolidation, or 3) persistent impairment in CPP

expression due to effects that are unrelated to memory retrieval or reconsolidation (e.g., long-lasting motor impairments). Moreover, whether the maintenance of retrieval requires distinct or identical mechanisms as stabilization of memory for reconsolidation had not been examined. Thus, I next discuss the possible involvement of PL-mPFC, BLA, and dHipp in memory reconsolidation.

Neurobiological mechanisms of memory reconsolidation

PL-mPFC involvement in memory reconsolidation

Above I describe data supporting PL-mPFC involvement in expression of fear and drug-associated memories. However, very few investigations have examined the role of PL-mPFC in memory reconsolidation. PL-mPFC protein synthesis inhibition has been shown to impair the consolidation, but not reconsolidation, of a trace fear memory (Blum et al., 2006). In contrast, PL-mPFC inactivation or α_1-AR blockade impairs fear memory reconsolidation (Do Monte et al., 2013; Stern et al., 2013). For drug-associated memories, one study revealed that PL-mPFC inactivation or protein synthesis inhibition following context re-exposure had no effect on subsequent context-induced cocaine self-administration (Ramirez et al., 2009). Taken together, although some studies support PL-mPFC involvement in memory reconsolidation, this area of research has been mostly ignored.

BLA involvement in memory reconsolidation

In contrast to PL-mPFC, many studies have revealed a critical role of the BLA for the stabilization of memory during reconsolidation. This was first demonstrated with fear

conditioning, as BLA microinfusions of the protein synthesis inhibitor anisomycin immediately following CS exposure impairs fear expression during a subsequent CS test (Nader et al., 2000). Moreover, BLA anisomycin infusions also prevented reinstatement and spontaneous recovery of the fear memory (Duvarci and Nader, 2004), suggesting possible memory ablation. Similarly, BLA β-AR blockade prevents the reconsolidation, but not consolidation, of an auditory fear memory (Debiec and Ledoux, 2004). Thus, BLA β-AR signaling, including downstream PKA activation, may be critical for reconsolidation. Indeed, BLA PKA inhibition prevents fear memory reconsolidation, whereas BLA PKA activation enhances fear memory reconsolidation (Tronson et al., 2006). Amygdalar β-AR signaling may also be critical for reconsolidation of human memories. Oral administration of the β-AR antagonist propranolol prevents reconsolidation of fear memories in humans (Kindt et al., 2009; Schwabe et al., 2013). Furthermore, experiments using fMRI reveal that disruption of reconsolidation in humans prevents the BLA from responding to presentation of the CS (Agren et al., 2012). Taken together, BLA β-AR signaling is critical for the reconsolidation of fear memories.

Evidence also reveals BLA involvement in reconsolidation of drug-associated memories. Initial experiments demonstrated that ablation of BLA *zif268* (an immediate early gene involved in synaptic plasticity) during cue presentation impairs subsequent cue-induced cocaine self-administration (Lee et al., 2005; Lee et al., 2006). Moreover, disruption of amygdalar NMDAr (N-methyl D-aspartate receptor) activation also disrupts reconsolidation of cue-induced cocaine self-administration (Milton et al., 2008). Pharmacological or genetic disruption of BLA activity also impairs drug-associated CPP memory reconsolidation (Li et al., 2010; Theberge et al., 2010; Wu et al., 2011). This

includes β-AR signaling, as inactivation of BLA β-ARs or downstream PKA prevents cocaine-induced CPP memory reconsolidation (Bernardi et al., 2009; Arguello et al., 2013). Taken together, BLA activity, including β-AR signaling, is critical for reconsolidation of drug-associated memories.

dHipp involvement in memory reconsolidation

The dHipp is critical for reconsolidation of contextual memories. In a contextual fear conditioning paradigm, dHipp protein synthesis inhibition following presentation of a fear-associated context disrupts subsequent context-induced fear (Debiec and Ledoux, 2004; Lee et al., 2004). In the self-administration paradigm, dHipp inactivation but not protein synthesis inhibition following context exposure disrupts subsequent context-induced reinstatement (Ramirez et al., 2009). Moreover, unilateral dHipp inactivation along with contralateral BLA protein synthesis inhibition following context exposure disrupts subsequent context-induced drug self-administration (Wells et al., 2011). In contrast, either manipulation alone has no effect. These data indicate that dHipp activity following presentation of contextual cues may be required for protein synthesis-dependent reconsolidation processes in the BLA. In the CPP paradigm, research reveals that disruption of dHipp protein synthesis or PKA following exposure to a morphine-paired context disrupts subsequent morphine-induced CPP expression (Milekic et al., 2006; Taubenfeld et al., 2010). As PKA and protein synthesis are downstream of β-AR signaling, these data indicate that reconsolidation of drug-associated CPP memories may require dHipp β-AR signaling.

Localizing the effects of β-AR blockade on cocaine CPP memory retrieval

The data above describe a critical role for PL-mPFC, BLA, and dHipp in retrieval and/or reconsolidation of fear and drug-associated memories. We previously revealed that β-AR blockade induces persistent cocaine CPP memory retrieval impairments. Thus, we next targeted PL-mPFC, BLA, and dHipp with β-AR antagonists before and after a CPP retrieval test to determine the effects of these manipulations on drug-associated memory retrieval and reconsolidation.

Otis, Dashew, and Mueller, 2013

We first determined the necessity of β-AR activation within the PL-mPFC for retrieval of a cocaine-induced CPP by administering β-AR antagonists before a CPP retrieval test. Following conditioning, rats were given PL-mPFC microinfusions of saline or propranolol before the second CPP test. Microinfusions of propranolol, but not saline, prevented CPP expression during the second test and during a subsequent microinfusion-free test (see Figure 7B). We next replicated these findings, but rats were given PL-mPFC microinfusions of saline or the more selective β-AR antagonist nadolol before the first CPP test. Rats treated with saline expressed a CPP for the previously cocaine-associated chamber during all CPP tests, whereas rats treated with nadolol did not (see Figure 7C). Moreover, PL-mPFC microinfusions of propranolol or nadolol did not affect locomotor activity, as measured by photobeam breaks during a CPP test (see Figure 7D and Figure 7E). Thus, PL-mPFC microinfusions of β-AR antagonists induced a persistent impairment in expression of a cocaine-induced CPP without affecting locomotor activity.

We next determined the necessity of β-AR activation within the BLA for retrieval of a cocaine-induced CPP. Following conditioning, rats were given BLA microinfusions of saline or propranolol before the second CPP test. Microinfusions of propranolol had no effect on CPP during the second CPP test. However, propranolol-treated rats expressed no CPP during the subsequent microinfusion-free test (see Figure 8B). We next replicated these findings, but rats were given BLA microinfusions of saline or nadolol before the first CPP test. Rats treated with saline expressed a CPP for the previously cocaine-associated chamber during all CPP tests, whereas rats treated with

nadolol only expressed a CPP during the first CPP test (see Figure 8C). BLA microinfusions of propranolol or nadolol did not affect locomotor activity, as measured by photobeam breaks during a CPP test (see Figure 8D and Figure 8E). Thus, BLA microinfusions of β-AR antagonists did not prevent initial expression of the CPP. However, BLA β-AR blockade did prevent CPP expression during subsequent microinfusion-free tests, indicating that BLA β-AR activation may be necessary for CPP memory reconsolidation.

We next determined if CPP expression deficits induced by PL-mPFC and BLA β-AR blockade were due to disruption of memory reconsolidation. First, following conditioning, rats were given PL-mPFC microinfusions of saline or nadolol immediately after the first CPP test. Rats treated with either saline or nadolol expressed a CPP across all tests, indicating that PL-mPFC nadolol microinfusions had no effect on CPP expression (see Figure 9A). Second, following conditioning rats were given BLA microinfusions of saline or nadolol immediately after the first CPP test. Rats treated with saline expressed a CPP for the previously cocaine-associated chamber during all CPP tests, whereas rats treated with nadolol only expressed a CPP during the first CPP test (see Figure 9B). Thus, β-AR blockade in BLA, but not PL-mPFC, prevented the reconsolidation of a cocaine CPP memory. These results are consistent with data revealing that microinfusions of a β_2-AR antagonist after a cocaine CPP test also attenuate subsequent CPP expression (Bernardi et al., 2009).

We next confirmed that the observed findings were not due to nonspecific effects of PL-mPFC and BLA microinfusions on memory expression. We already demonstrated that PL-mPFC or BLA propranolol and nadolol microinfusions do not affect locomotor activity. Next, we evaluated the effects of PL-mPFC or BLA β-AR blockade in the absence of a CPP test. Following conditioning, rats were given PL-mPFC or BLA microinfusions of nadolol in the absence of testing, following by a single microinfusion-free CPP test the following day. Rats that received microinfusions of saline or nadolol expressed an equivalent CPP during this test (see Figure 10), indicating that the effects of PL-mPFC and BLA β-AR blockade on CPP expression require memory reactivation (i.e., the CPP test). Finally, we determined whether PL-mPFC or BLA β-AR blockade induces an affective state capable of altering CPP expression. Rats received PL-mPFC or BLA microinfusions of nadolol in one chamber, and saline in another. Following nadolol conditioning, rats were exposed to all chambers for a nadolol-induced CPP test. Rats did not express a CPP or aversion for the previously nadolol-paired chamber (see Figure 11), indicating that nadolol did not induce an affective state capable of influencing CPP expression.

Our findings demonstrate that PL-mPFC β-AR activation during, but not after, a CPP test is critical for subsequent CPP expression. Moreover, these effects were not due to reconsolidation blockade or nonspecific effects on behavioral expression of the CPP. These data lead to the conclusion that PL-mPFC β-AR activation maintains cocaine-associated CPP memory retrieval. Thus, we provide the first evidence that the maintenance of retrieval requires neural mechanisms that are completely distinct from those required for stabilization of memory during reconsolidation. We have now further

examined the dissociation between retrieval deficits and reconsolidation blockade by evaluating the role of dHipp β-AR activation in retrieval and reconsolidation of a cocaine-induced CPP memory (Otis et al., 2014a).

Figures: Otis, Dashew, and Mueller, 2013

Figure 7

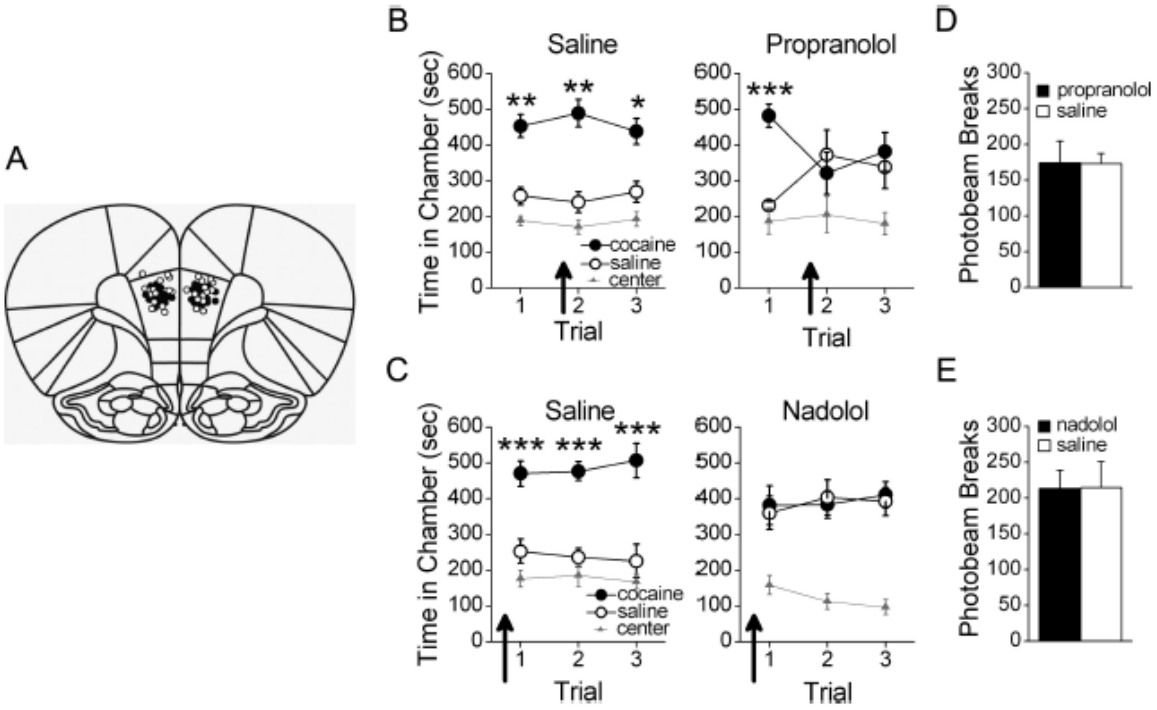

Figure 7. PL-mPFC β-AR blockade persistently impairs cocaine CPP memory retrieval. (A) Coronal drawings (bregma, +3.72) showing injector tip placements of PL-mPFC microinfusions. (B) PL-mPFC microinfusions of propranolol, but not saline, before the second CPP trial persistently impair CPP expression. (C) PL-mPFC microinfusions of nadolol, but not saline, before the first CPP trial induced a persistent deficit in CPP expression. PL-mPFC microinfusions of (D) propranolol or (E) nadolol did not affect locomotor activity, as measured by photobeam breaks during a CPP trial (Otis et al., 2013). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 8

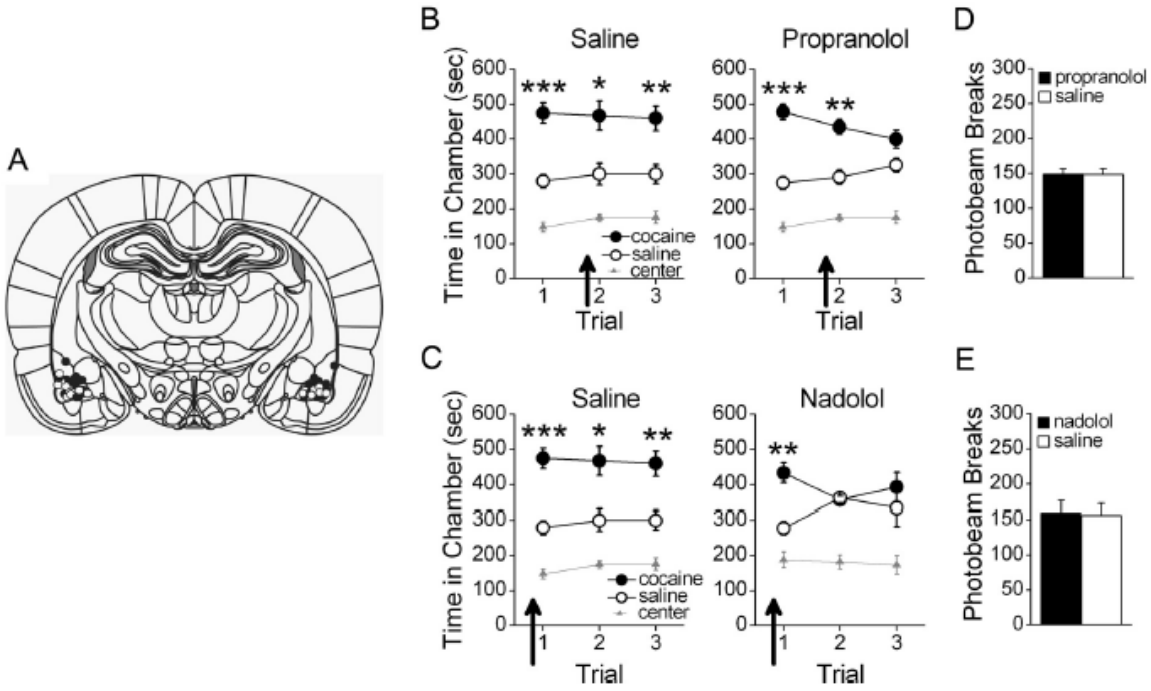

Figure 8. BLA β-AR blockade does not block initial CPP memory retrieval. (A) Coronal drawings (bregma, -2.76) showing injector tip placements of BLA microinfusions. BLA microinfusions of (B) propranolol before the second CPP trial or (C) nadolol before the first CPP trial did not affect initial CPP expression, but prevented CPP expression during subsequent trials. BLA microinfusions of (D) propranolol or (E) nadolol did not affect locomotor activity, as measured by photobeam breaks during a CPP trial (Otis et al., 2013). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 9

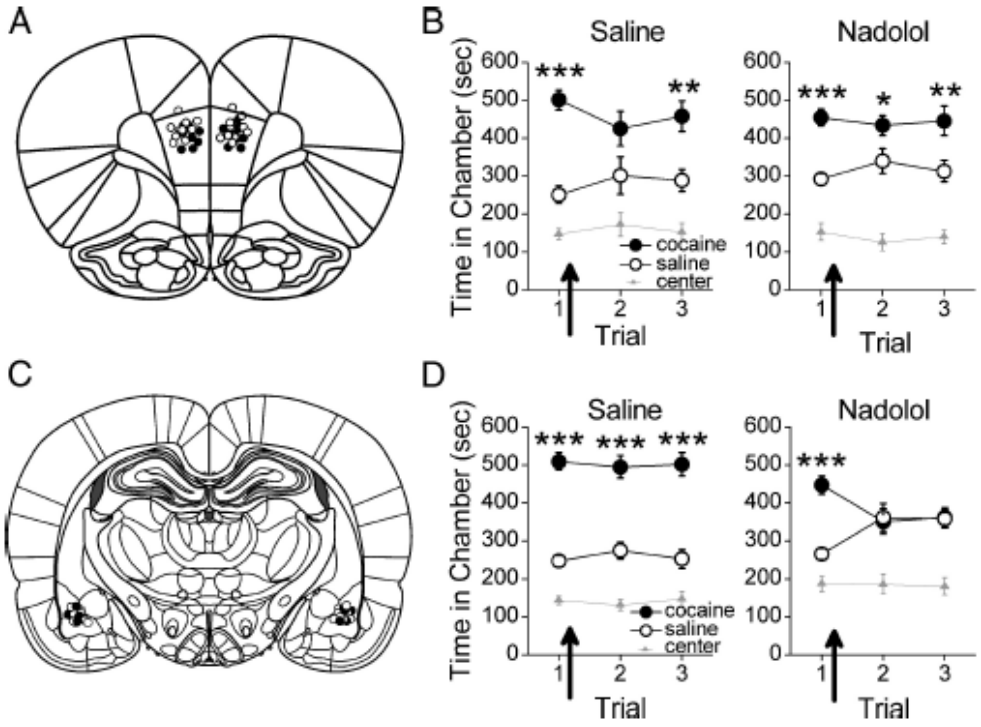

Figure 9. BLA but not PL-mPFC β-AR blockade impairs reconsolidation of a cocaine CPP memory. (A) Coronal drawings (bregma, +3.72) showing injector tip placements of PL-mPFC microinfusions. (B) PL-mPFC microinfusions of nadolol after a CPP trial did not prevent CPP expression during subsequent trials. (C) Coronal drawings (bregma, -2.76) showing injector tip placements of BLA microinfusions. (D) BLA microinfusions of nadolol, but not saline, after a CPP trial prevent CPP expression during subsequent trials (Otis et al., 2013). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 10

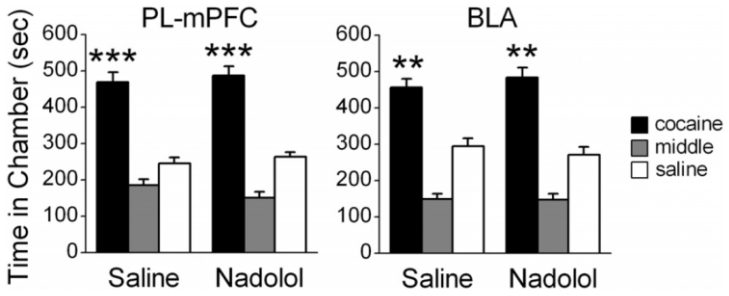

Figure 10. PL-mPFC and BLA β-AR blockade in the absence of retrieval does not prevent CPP expression. PL-mPFC (left) or BLA (right) microinfusions of nadolol do not prevent CPP expression during a CPP trial 24 hours later (Otis et al., 2013). ***$p < 0.001$ and **$p < 0.01$.

Figure 11

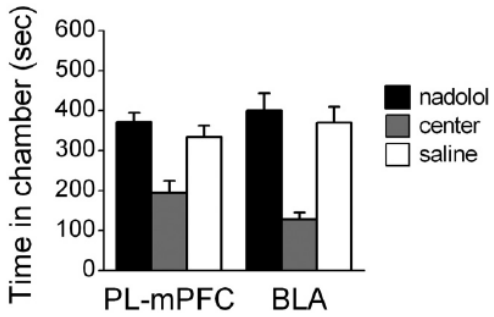

Figure 11. PL-mPFC and BLA β-AR blockade does not induce a CPP or aversion. Following conditioning with PL-mPFC or BLA microinfusions nadolol or saline, rats spent an equivalent amount of time within previously nadolol- and saline-paired chambers (Otis et al., 2013).

Otis, Fitzgerald, and Mueller, 2014a

We next determined the necessity of β-AR activation within the dHipp for retrieval of a cocaine-induced CPP by administering nadolol before a CPP retrieval test. Following conditioning, rats were given dHipp microinfusions of saline, a low dose of nadolol (1μg/μl) or a higher dose of nadolol (2μg/μl) before the first CPP test. Rats treated with saline or the low dose of nadolol expressed a CPP for the previously cocaine-associated chamber during all CPP tests, whereas rats treated with the high dose of nadolol did not (see Figure 12B). To determine if this effect required the CPP test, or simply required exposure to the previously cocaine-paired chamber, we replicated the experiment but only exposed the rats to the previously cocaine-paired chamber following dHipp microinfusions. Rats were then given daily CPP tests, during which previously saline-treated rats expressed a CPP, whereas previously nadolol-treated rats did not (see Figure 12C). Thus, dHipp microinfusions of nadolol induced a persistent impairment in expression of a cocaine-induced CPP when administered before a CPP test or when administered before exposure to the previously cocaine-paired chamber.

We next determined the effects of dHipp β-AR blockade on subsequent cocaine-induced reinstatement of the CPP. Rats that were previously treated with dHipp nadolol or saline before the first CPP test (as described above; see Figure 12) were given a week break from testing. Two final CPP tests were then given, during which no groups expressed a CPP. The following day, rats previously treated with saline or the low dose of nadolol expressed cocaine-induced (5 mg/kg) reinstatement of the CPP, whereas rats previously treated with the high dose of nadolol did not (see Figure 13A). We next replicated these findings, but administered a higher dose of cocaine during the cocaine-

induced reinstatement test (10 mg/kg). Consistent with the previous finding, rats previously treated with saline expressed cocaine-induced reinstatement of the CPP, whereas rats previously treated with the high dose of nadolol did not (see Figure 13B). Thus, dHipp nadolol-induced CPP expression deficits provided long-lasting protection against cocaine-induced reinstatement of the CPP.

We also investigated whether dHipp β-AR blockade-induced CPP expression and reinstatement deficits are due to disruption of memory reconsolidation. Following conditioning, rats were given dHipp microinfusions of saline or nadolol immediately after the first CPP test. Rats treated with saline expressed a CPP during the first, second, and third test whereas rats treated with nadolol expressed a CPP during the first, third, and fourth test (see Figure 14B). Thus, although nadolol may have impaired CPP expression during the second CPP test, this effect was transient. These findings indicate that CPP reconsolidation is not completely disrupted by dHipp β-AR blockade. We next determined the effects of post-test dHipp β-AR blockade on subsequent cocaine-induced reinstatement of the CPP. Following a week break from testing, rats were given a final CPP trial, during which neither saline nor nadolol-treated rats expressed a CPP. The following day, rats previously treated with saline or nadolol expressed cocaine-induced reinstatement of the CPP (see Figure 14C). Thus, post-test nadolol microinfusions did not induce long-lasting CPP expression deficits and did not provide protection against cocaine-induced reinstatement of the CPP.

We next confirmed that the observed findings were not due to nonspecific effects of dHipp microinfusions on memory expression. We first evaluated the effects of dHipp β-AR blockade in the absence of a CPP test. Following conditioning, rats were given

dHipp microinfusions of nadolol in the absence of testing, following by a single microinfusion-free CPP test the following day. Rats that received microinfusions of saline or nadolol expressed an equivalent CPP during this test (see Figure 15A). Next, we determined whether dHipp β-AR blockade induces an affective state capable of influencing CPP expression. Rats received dHipp microinfusions of nadolol in one chamber, and saline in another. Following nadolol conditioning, rats were exposed to all chambers for a nadolol-induced CPP test. Rats did not express a CPP or aversion for the previously nadolol-paired chamber (see Figure 15B), indicating that nadolol did not induce an affective state capable of influencing CPP expression. Finally, dHipp microinfusions of nadolol did not affect locomotor activity, as measured by photobeam breaks during a CPP test (see Figure 15C).

Our findings demonstrate that dHipp β-AR activation during, but not after, a CPP test is critical for subsequent CPP expression. Moreover, these effects were not due to reconsolidation blockade or nonspecific effects on behavioral expression of the CPP. Thus, the conclusion can be made that dHipp β-AR activation is necessary for maintaining cocaine-induced CPP memory retrieval.

Figures: Otis, Fitzgerald, and Mueller, 2014

Figure 12

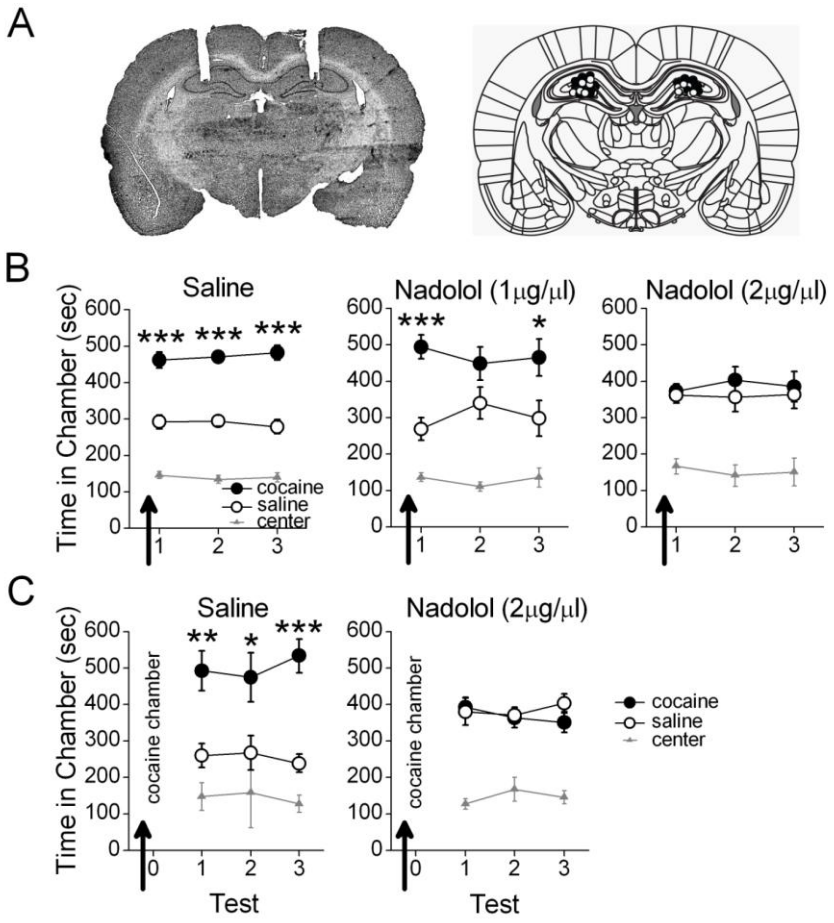

Figure 12. dHipp β-AR blockade persistently impairs cocaine CPP memory retrieval. (A) Coronal drawings (bregma, -3.24 mm) showing injector tip placements of dHipp microinfusions. (B) dHipp microinfusions of a high dose of nadolol, but not a low dose or saline, before the first CPP trial persistently impair CPP expression. (C) dHipp microinfusions of nadolol, but not saline, before exposure to the previously cocaine-paired chamber only prevented CPP expression during subsequent CPP trials (Otis et al., 2014a). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 13

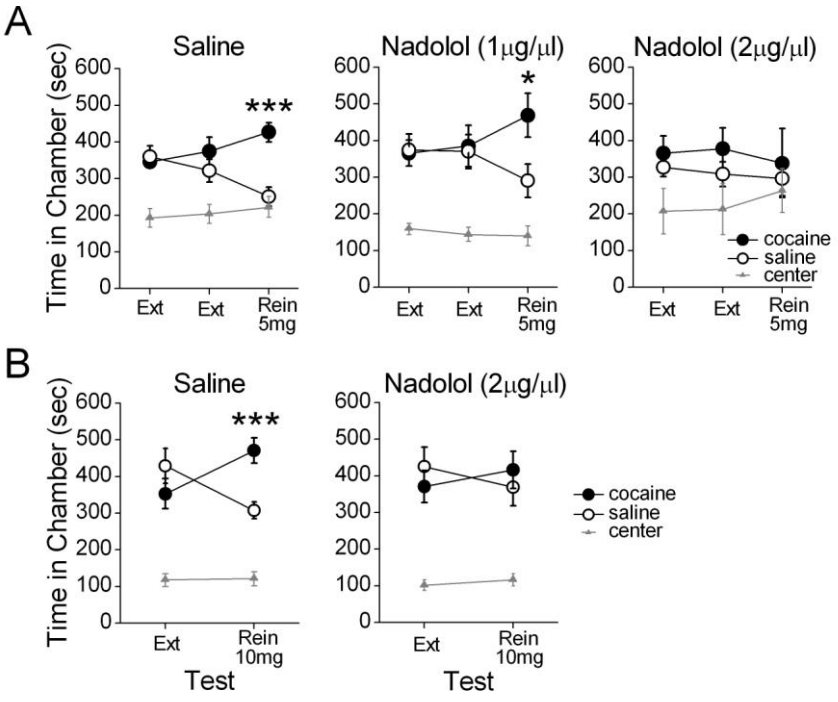

Figure 13. Retrieval impairments induced by dHipp β-AR blockade prevent cocaine-induced reinstatement. Rats treated with saline or nadolol before an initial CPP trial expressed no CPP during the final CPP extinction trials. Following injections of a (A) low or (B) high dose of cocaine, rats previously treated with dHipp microinfusions of saline or the low dose of nadolol, but not the high dose of nadolol, expressed cocaine-induced reinstatement of the CPP (Otis et al., 2014a). ***$p < 0.001$ and *$p < 0.05$.

Figure 14

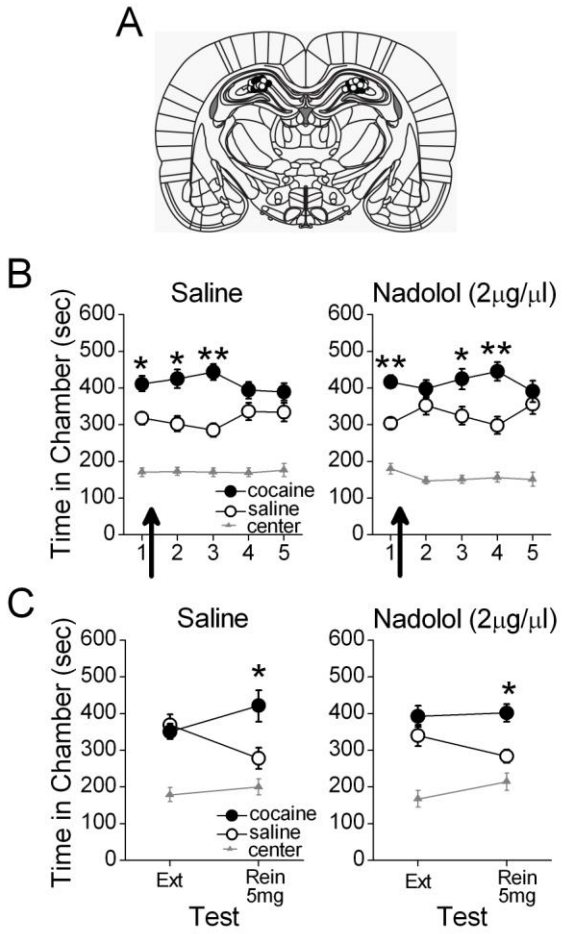

Figure 14. dHipp β-AR blockade does not abolish CPP memory reconsolidation. (A) Coronal drawings (bregma, -3.24 mm) showing injector tip placements of dHipp microinfusions. (B) dHipp microinfusions of a nadolol did not prevent rats from expressing a CPP during subsequent trials. (C) Rats treated with saline or nadolol expressed no CPP during the final CPP extinction trial. Rats previously treated with dHipp microinfusions of saline or nadolol after the first CPP trial expressed cocaine-induced reinstatement of the CPP (Otis et al., 2014a). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Figure 15

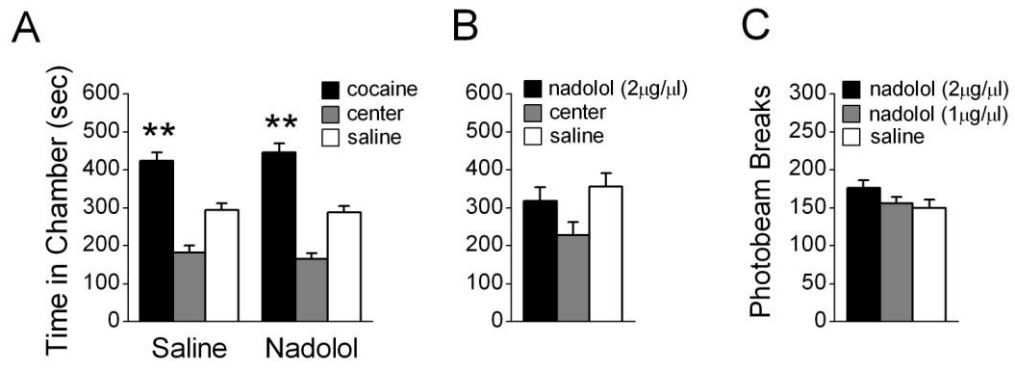

Figure 15. Effects of dHipp nadolol microinfusions on CPP expression are not due to nonspecific effects. (A) dHipp microinfusions of nadolol did not prevent rats from expressing a CPP 24 hours later. (B) Following conditioning with dHipp microinfusions of nadolol or saline, rats spent an equivalent amount of time within previously nadolol- and saline-paired chambers. (C) dHipp microinfusions of nadolol not affect locomotor activity, as measured by photobeam breaks during a CPP trial (Otis et al., 2014a). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Proposed model of drug-associated memory retrieval

The experiments described throughout this dissertation reveal some basic neural mechanisms required for memory retrieval. Next, I use these and other studies to illustrate a likely system that is required for (and maintains) drug-associated memory retrieval (see Figure 16).

PL-mPFC is necessary for retrieval of learned fear (Corcoran and Quirk, 2007) and drug-associated memories (Otis et al., 2013). Relapse to drug seeking involves perturbations in particular PL-mPFC outputs which synapse onto nucleus accumbens core (NAcc) medium spiny neurons (Kalivas et al., 2005). NAcc glutamatergic receptor activity is critical for reinstatement of drug seeking (Fuchs et al., 2004; LaLumiere and Kalivas, 2008), and NAcc extracellular glutamate levels increase during reinstatement (Baker et al., 2003; McFarland et al., 2003). Moreover, PL-mPFC microinfusions of brain-derived neurotrophic factor (BDNF) or GABA receptor agonists prevent reinstatement and prevent the rise in NAcc extracellular glutamate (McFarland et al., 2003; Berglind et al., 2009). Thus, PL-mPFC likely provides glutamatergic input to the NAcc, which drives behavioral expression of drug seeking.

The PL-mPFC has reciprocal projections with the BLA (Ishikawa and Nakamura, 2003; Gabbott et al., 2005), a structure that is also critical for drug-associated memory retrieval (Grimm and See, 2000). Moreover, evidence reveals that the BLA may activate PL-mPFC for retrieval. First, disconnection of these structures via contralateral inactivation prevents cue-induced reinstatement of cocaine seeking (Mashhoon et al., 2010). Second, PL-mPFC projecting BLA neurons express immediate early genes following exposure to a previously cocaine-associated environment (Miller and Marshall,

2005). In contrast, although some PL-mPFC neurons also express more immediate early genes upon context exposure, those neurons do not project back to the BLA (Miller and Marshall, 2005). Taken together, BLA neurons drive PL-mPFC activation for drug-associated memory retrieval.

The dHipp provides input to the BLA and has indirect projections to the PL-mPFC through its ventral subregion and through the nucleus reuniens of the thalamus (Hoover and Vertes, 2007; Vertes et al., 2007). Considering the role of the dHipp in contextual fear and drug-associated memory retrieval (Corcoran and Maren, 2001; Fuchs et al., 2005), dHipp likely provides contextual information to BLA and PL-mPFC for memory retrieval.

Finally, nuclei of noradrenergic neurons, such as the locus coeruleus (LC) and nucleus tractus solitarius (NTS) reside within the brainstem. Our data suggest that noradrenergic neurons secrete norepinephrine within PL-mPFC and dHipp to support drug-associated memory retrieval. In support of this, presentation of salient cues leads to norepinephrine release (Cassens et al., 1980). Moreover, artificial enhancement of NE release via direct stimulation of LC neurons enhances behavioral expression of memory (Sara and Devauges, 1988), an effect that is dependent on β-AR activation (Devauges and Sara, 1991). NE release is known to activate PL-mPFC and dHipp β-ARs (Pedarzani and Storm, 1993; Otis et al., 2013), and we found that this activation is critical for cocaine-induced CPP memory retrieval (Otis et al., 2013; Otis et al., 2014a). Thus, brainstem noradrenergic neurons become active following presentation of salient cues, and this activity allows memory retrieval. Interestingly, although most memory studies have focused on LC neurons, some data hint that NTS neurons may be critical for drug-

associated memory retrieval. Specifically, data reveal that genetic deletion of norepinephrine, via knockout of norepinephrine-synthesizing enzyme dopamine β-hydroxylase, prevents drug-induced CPP expression (Jasmin et al., 2006; Olson et al., 2006). Moreover, restoration of norepinephrine within the NTS rescues these impairments (Olson et al., 2006). Thus, norepinephrine released from the NTS may be critical for drug-associated memory retrieval. However, further investigations must be completed to confirm this hypothesis.

Figure 16

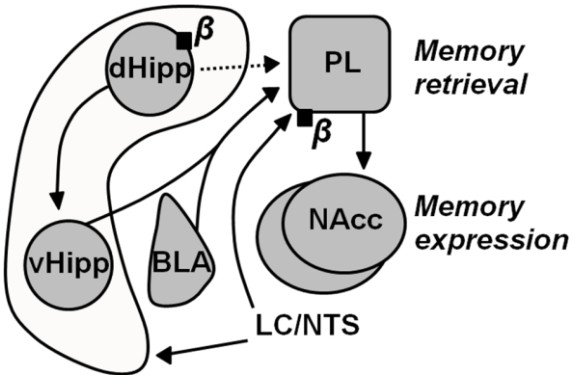

Figure 16. Proposed model of drug-associated memory retrieval. Brainstem nuclei (LC/NTS) release norepinephrine into forebrain structures (dHipp, PL-mPFC). These structures, including the BLA, interact to promote cocaine-associated memory expression through NAcc-projecting PL-mPFC neurons. LC, locus coeruleus; NTS, nucleus tractus solitarius; vHipp, ventral hippocampus; dHipp, dorsal hippocampus; PL, prelimbic medial prefrontal cortex; NAcc, nucleus accumbens core.

Prelimbic β-AR activation enhances synaptic plasticity and intrinsic excitability

The data described demonstrate that PL-mPFC input, including noradrenergic input for β-AR activation, is required for memory retrieval. However, the mechanism by which β-AR activation maintains retrieval is unknown. Next, I describe research regarding the effects of β-AR activation on synaptic and intrinsic neuronal activity. Moreover, I use this research to describe a possible mechanism by which β-AR activity maintains retrieval.

β-AR activation enhances synaptic plasticity

Synaptic strength is modified by the strength and timing of presynaptic inputs (Bliss and Lomo, 1973; Levy and Steward, 1979; Malenka and Nicoll, 1999). This synaptic plasticity is a likely mechanism of learning, as learning is associated with synaptic plasticity within brain regions that are required for that learning (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Whitlock et al., 2006). Moreover, mechanisms that are required for synaptic plasticity, including glutamate receptor activity, are also important for learning (Morris et al., 1986; Artola and Singer, 1987; Kleinschmidt et al., 1987; Miserendino et al., 1990; Tsien et al., 1996; Malenka and Nicoll, 1999; Tang et al., 1999). Thus, synaptic plasticity is a likely mechanism of learning and memory.

Synaptic plasticity is regulated by β-AR activation, although the exact role of β-AR activity in synaptic plasticity varies depending on the brain region (O'Dell et al., 2010). Studies reveal that NE or β-AR agonists enhance electrically-evoked long-term potentiation (LTP) at hippocampal mossy fiber synapses (Hopkins and Johnston, 1984).

Moreover, NE and β-AR agonists enhance LTP at these synapses via both presynaptic and postsynaptic mechanisms (Hopkins and Johnston, 1988; Huang and Kandel, 1996). β-AR agonists also promote spike-timing dependent plasticity (STDP) at mPFC pyramidal neuron synapses (Zaitsev and Anwyl, 2012), although the exact mechanism by which β-AR activation facilitates mPFC plasticity is unclear. Recently, studies revealed that mPFC β-AR activation increases evoked excitatory postsynaptic currents (eEPSCs) by increasing presynaptic neurotransmitter release and by enhancing NMDAr channel conductance (Huang and Hsu, 2006; Zaitsev and Anwyl, 2012). Taken together, NE-induced β-AR activation may facilitate mPFC synaptic plasticity by 1) increasing presynaptic glutamate release and 2) by enhancing postsynaptic NMDAr channel conductance.

β-AR activation enhances intrinsic excitability

Intrinsic excitability can be defined as the electrical properties of a neuronal membrane that are independent of synaptic activity. For example, EPSP propagation and properties of the action potential (e.g., threshold, amplitude, width, and adaptation) are not dependent on synaptic input. Intrinsic excitability is modulated by experience (Woody and Black-Cleworth, 1973; Alkon, 1974; Disterhoft et al., 1986; Moyer et al., 1996) and by neuromodulators, including NE (Madison and Nicoll, 1982, 1986b, a; Pedarzani and Storm, 1993). In hippocampal and infralimbic mPFC pyramidal neurons, NE reduces the slow afterhyperpolarization (sAHP) and limits spike frequency adaptation, effects that are blocked by β-AR antagonists (Madison and Nicoll, 1982, 1986a, b; Pedarzani and Storm, 1993; Mueller et al., 2008). Thus, β-AR activation

enhances the intrinsic excitability of hippocampal and infralimbic mPFC pyramidal neurons. However, the effect of β-AR activation on the excitability of PL-mPFC pyramidal and GABAergic neurons was unknown.

Using patch-clamp electrophysiology, we determined the effects of β-AR activation on PL-mPFC pyramidal cell and GABAergic interneuron excitability. Pyramidal neurons were identified based on the presence of an apical dendrite, and this morphology was subsequently confirmed via immunohistochemistry (see Figure 17A). Following baseline recordings, action potentials were evoked by brief current pulses (see Figure 17B). Application of NE increased the number of evoked action potentials (see Figure 17C). Moreover, NE, caused membrane depolarization, decreased rheobase, and reduced action potential latency (see Table 2), all of which indicate enhanced intrinsic excitability. These changes were not present in neurons that were treated with both propranolol and NE, indicating that NE increases the intrinsic excitability of PL-mPFC pyramidal neurons via β-AR activation. Finally, although NE had no effect on the fast AHP (fAHP) in pyramidal neurons, NE transformed the sAHP to a slow afterdepolarization (see Figure 17D). As the sAHP limits AP frequency (Wu et al., 2004), reversal of the sAHP is a likely mechanism by which β-AR activation enhances the number of evoked action potentials. Consistent with this, propranolol prevented NE from reversing the sAHP (see Figure 17E).

Although the primary output neurons of the PL-mPFC are pyramidal neurons, these neurons function within a network of GABAergic interneurons. Thus, we next evaluated the effects of β-AR activation on the intrinsic excitability of GABAergic interneurons. GABAergic interneurons were identified by morphology (lack of apical

dendrite), electrophysiological characteristics (see Table 1), and by streptavidin and GAD67 co-immunoreactivity (see Figure 18A). Similar to pyramidal neurons, application of NE increased the number of evoked action potentials (see Figure 18C). Moreover, NE caused membrane depolarization, decreased rheobase, and reduced action potential latency (see Table 3), all of which indicate enhanced intrinsic excitability. These changes were not present in GABAergic interneurons that were treated with both propranolol and NE, indicating that NE increases the intrinsic excitability of these neurons via β-AR activation. Unlike pyramidal neurons, NE had no effect on the sAHP of GABAergic interneurons (see Table 3). This finding is consistent with data revealing that these neurons lack a sAHP (McCormick et al., 1985). In contrast, NE reduced the fAHP, (see Figure 17D), and this effect was blocked by co-application of propranolol (see Figure 17E). Thus, β-AR activation may enhance the intrinsic excitability of GABAergic interneurons by reducing the fAHP.

The data described reveal that β-AR activation strengthens synaptic activity within the mPFC (e.g., STDP and NMDAr currents) and enhances intrinsic excitability of PL-mPFC pyramidal and GABAergic interneurons. Below I describe a possible mechanism by which these changes may maintain memory retrieval.

Table 1

Neuron	R_N (MΩ)	V_m (mV)	AP_{width} (ms)	AP_{amp} (mV)	fAHP (mV)	sAHP (mV)
Pyramidal	125 ± 11	-69 ± 2	2.6 ± 0.2	67 ± 2	-6.4 ± 0.6	-0.7 ± 0.1
GABA	310 ± 6***	-66 ± 1	2.1 ± 0.1**	61 ± 2*	-13.6 ±0.8***	-0.0 ± 0.1***

Table 1. Basic membrane properties of PL-mPFC pyramidal and GABA neurons. PL-mPFC GABAergic neurons had larger input resistance and fAHP, but smaller AP width and sAHP as compared with pyramidal neurons. AP_{width}, action potential width; AP_{amp}, action potential amplitude; R_N, input resistance; V_m, Resting membrane potential; fAHP, fast afterhyperpolarization; sAHP, slow afterhyperpolarization. ***$p < 0.001$ and *$p < 0.05$ as compared with pyramidal neurons (Otis et al., 2013).

Figure 17

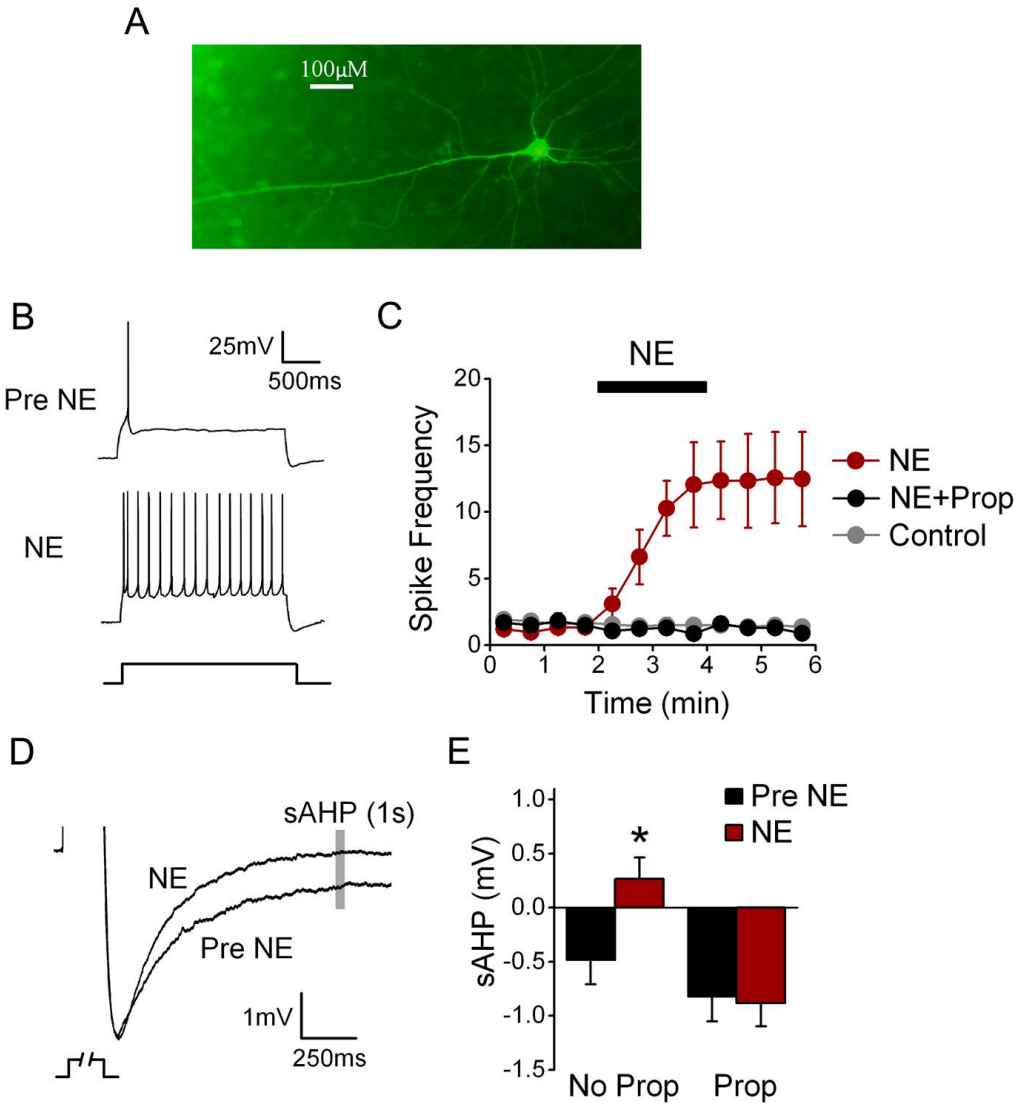

Figure 17. β-AR blockade prevents NE from enhancing intrinsic excitability of PL-mPFC pyramidal neurons. (A) Example photomicrograph of biocytin-filled PL-mPFC pyramidal neuron. (B) Example traces revealing that NE increased the number of evoked action potentials. (C) Grouped data reveal that NE-induced enhancement of evoked action potentials was blocked by propranolol. (D) Example traces revealing that NE decreased the sAHP. (E) Grouped data reveal that NE-induced reduction of sAHP was blocked by propranolol (Otis et al., 2013). Prop, propranolol; *$p < 0.05$.

Figure 18

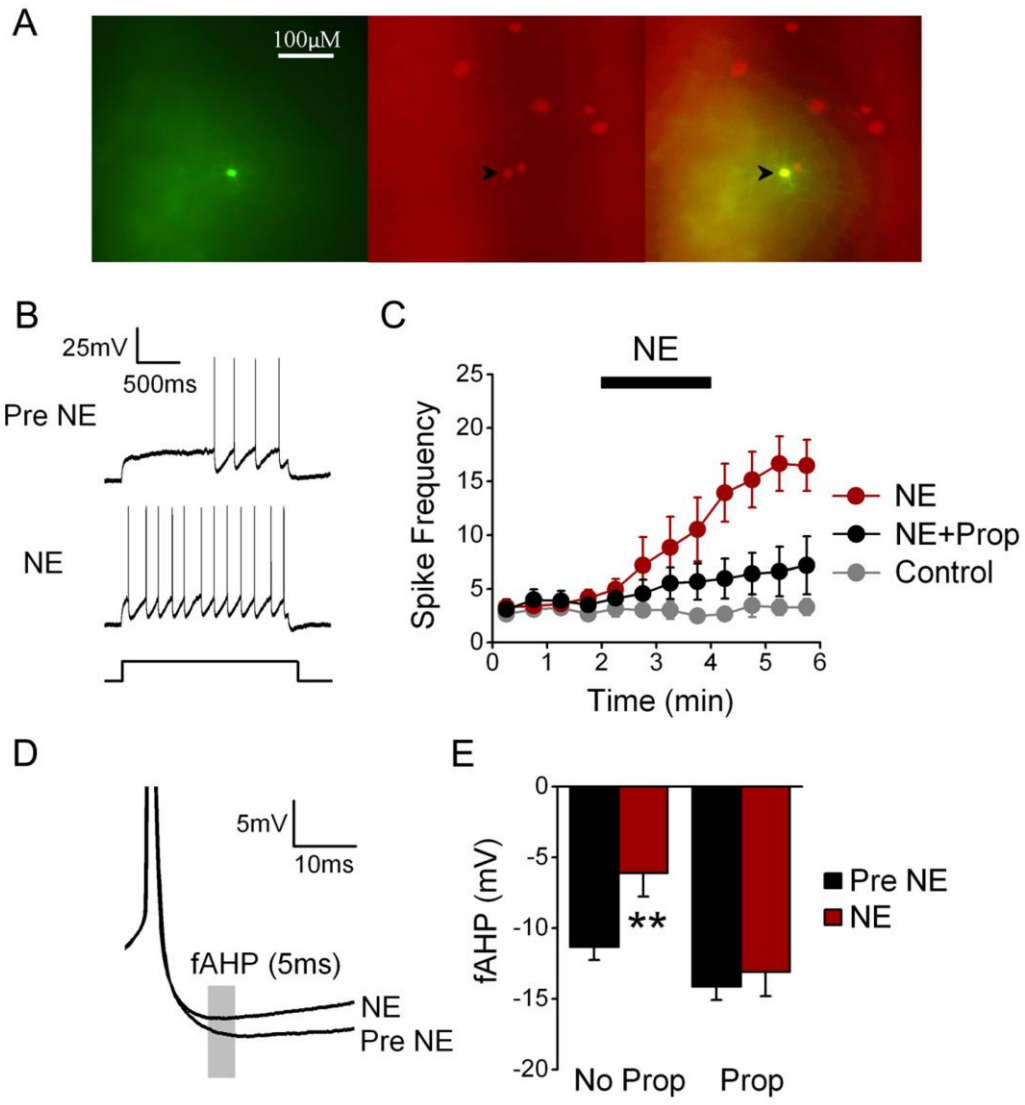

Figure 18. β-AR blockade prevents norepinephrine from enhancing intrinsic excitability of PL-mPFC GABAergic interneurons. (A) Example photomicrograph of biocytin-filled PL-mPFC GABAergic interneuron. (B) Example traces revealing that NE increased the number of evoked action potentials. (C) Grouped data reveal that NE-induced enhancement of evoked action potentials was attenuated by propranolol. (D) Example traces revealing that NE decreased the fAHP. (E) Grouped data reveal that NE-induced reduction of fAHP was blocked by propranolol (Otis et al., 2013). Prop, propranolol; ***p* < 0.01.

Table 2

Drug	Time	R_N (MΩ)	V_m (mV)	Rheo (pA)	AP_{thresh} (mV)	$AP_{latency}$ (ms)	fAHP (mV)	sAHP (mV)
NE	Pre	121± 24	-70 ± 3	223 ± 75	-40 ± 2	118 ± 18	-7.3 ± 0.7	-0.5 ± 0.2
	Post	124 ± 30	-65 ± 3**	173 ± 74**	-39 ± 1	53 ± 8**	-7.7 ± 1.1	0.3 ± 0.2*
Prop + NE	Pre	132 ± 13	-69 ± 4	93 ± 11	-41 ± 5	130 ± 28	-5.6 ± 1.3	-0.9 ± 0.3
	Post	149 ± 12	-68 ± 4	88 ± 18	-41 ± 4	102 ± 29	-4.5 ± 1.6	-0.9 ± 0.3

Table 2. Effects of NE and propranolol on intrinsic excitability of PL-mPFC pyramidal neurons. NE, norepinephrine; Prop, propranolol; R_N, input resistance; V_m, resting membrane potential; Rheo, rheobase; AP_{thresh}, action potential threshold; $AP_{latency}$, action potential latency; fAHP, fast afterhyperpolarization; sAHP, slow afterhyperpolarization. *$p < 0.05$, **$p < 0.01$ as compared with before NE application (Otis et al., 2013).

Table 3

Drug	Time	RN (MΩ)	Vm (mV)	Rheo (pA)	AP_{thresh} (mV)	$AP_{latency}$ (ms)	fAHP (mV)	sAHP (mV)
NE	Pre	285 ± 56	-65 ± 2	74 ± 15	-42 ± 2	127 ± 35	-11 ± 0.9	0.0 ± 0.1
	Post	231 ± 39	-60 ± 2**	31 ± 9**	-44 ± 2	32 ± 10**	-6.1 ±1.6**	0.0 ± 0.2
Prop + NE	Pre	333 ± 52	-69 ± 3	58 ± 9	-44 ± 2	148 ± 44	-14.1 ± 1.0	-0.2 ± 0.2
	Post	365 ± 53	-69 ± 3	39 ± 9*	-46 ± 2	83 ± 23	-13.1 ± 1.7	0.3 ± 0.4

Table 3. Effects of NE and propranolol on intrinsic excitability of PL-mPFC GABAergic interneurons. NE, norepinephrine; Prop, propranolol; R_N, input resistance; V_m, resting membrane potential; Rheo, rheobase; AP_{thresh}, action potential threshold; $AP_{latency}$, action potential latency; fAHP, fast afterhyperpolarization; sAHP, slow afterhyperpolarization. *$p < 0.05$, **$p < 0.01$ as compared with before NE application (Otis et al., 2013).

Proposed mechanism of memory retrieval deficits: PL-mPFC synaptic depression

Salient cue exposure induces NE release, leading to β-AR activation (Cassens et al., 1980). Thus, cocaine- or fear-associated cue exposure is likely to induce β-AR activation, which is known to increase synaptic currents and intrinsic excitability of PL-mPFC neurons (Ji et al., 2008; Otis et al., 2013). In support of this, exposure to a fear-associated CS increases the firing rate of PL-mPFC neurons (Burgos-Robles et al., 2009; Sotres-Bayon et al., 2012), whereas spontaneous PL-mPFC activity is reduced by systemic injections of propranolol (Rodriguez-Romaguera et al., 2009). In addition to noradrenergic potentiation of PL-mPFC neurons, PL-mPFC activity during cue presentation is dependent on glutamatergic input from the hippocampus and amygdala (Sotres-Bayon et al., 2012). Taken together, PL-mPFC neurons are more responsive to glutamate input during retrieval (due to NE-induced enhancement of EPSCs and intrinsic excitability), increasing the likelihood that EPSCs will induce action potentials. On the other hand, presynaptic input in the absence of synchronous postsynaptic action potentials can induce synaptic depression (Froemke and Dan, 2002; Froemke et al., 2005). Thus, PL-mPFC β-AR activation may maintain synchronous presynaptic and postsynaptic activity during retrieval, whereas disruption of this synchrony likely weakens PL-mPFC synapses. Consistent with this hypothesis, NE prevents spike-timing dependent depression (STDD) at cortical synapses (Salgado et al., 2012). Thus, β-AR blockade during retrieval may induce PL-mPFC synaptic depression, resulting in long-term retrieval impairments.

Dissertation Goal and Aims

Abnormally strong memories underlie drug addiction and fear disorders such as PTSD. Preventing retrieval of these memories would alleviate these disorders. Above, I describe three publications revealing that cocaine-associated CPP memory retrieval is susceptible to persistent disruption (Otis and Mueller, 2011; Otis et al., 2013; Otis et al., 2014a). Moreover, recent studies using humans reveal that β-AR blockade can induce persistent disruption of visual and emotional memory retrieval (Kroes et al, 2010; Kroes et al., 2012 *SfN Abstracts*). Although the human studies do not completely rule out memory reconsolidation effects, such experiments indicate that β-AR blockade-induced memory retrieval impairments may not be limited to cocaine CPP memories. Thus, the goal of this dissertation is to characterize the mechanism of memory retrieval deficits and determine whether retrieval of other memories is maintained by β-AR activity. This will be accomplished in the two following aims.

Aim 1: Determine whether β-AR activation is a fundamental mechanism for maintenance of memory retrieval. Here I focus on the necessity of PL-mPFC β-AR activity for maintaining retrieval of fear memories. Specifically, I determine the effects of PL-mPFC β-AR blockade on retrieval of 1) a contextual fear memory, 2) a delay fear memory, and 3) a trace fear memory.

Aim 2: Evaluate the underlying mechanisms of memory retrieval impairments. Next, I determined the underlying mechanism by which memory retrieval impairments

occur. Using patch-clamp electrophysiology, I characterized the intrinsic and synaptic properties of PL-mPFC neurons from adult rats that have retrieval impairments as compared with rats from appropriate control groups.

Methods

Subjects

Adult male Long-Evans rats weighing 300-325 grams were housed individually in clear plastic cages with access to standard laboratory rat chow (Harlan Laboratories) and water *ad libitum* unless otherwise noted. Rats were maintained on a 14 hour light/10 hour dark cycle (lights on at 7am) and were weighed and handled daily. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee in accordance with National Institutes of Health guidelines.

Cannula surgery

PL-mPFC cannula surgeries were performed to allow PL-mPFC β-AR blockade. Rats were anesthetized with ketamine/xylazine (90 mg/kg, 10.5 mg/kg, i.p.). Following anesthetization, double-barrel guide cannula (26 gauge; Plastics One, Roanoke, VA) were implanted within PL-mPFC (AP, +2.8; ML, ±0.6; DV, -2.9 mm relative to bregma). Cannula were fixed into place with 3 stainless steel skull screws and grip cement. Following surgery, rats were treated with an antibiotic (penicillin g procaine, 75,000 units in 0.25 ml, s.c.) and an analgesic (carprofen, 5.0 mg in 0.1 ml, s.c.). Rats were given a minimum of 7 days for recovery following surgeries, during which behavioral experiments were not conducted. Stylets remained within the guide cannula following surgery to maintain patency until microinfusions were performed.

Drugs and microinfusions

Cocaine HCl (National Institute of Drug Abuse) was dissolved in sterile 0.9% saline and was systemically administered (10 mg / kg). PL-mPFC β-AR blockade was induced via infusions of nadolol (1.2 µg / 0.6 µl saline; Sigma-Aldrich) at 0.3 µl / side over a 2 minute time period. Microinfusion injectors were left in place for a minimum of 1 minute following microinfusions.

Fear conditioning chamber

Fear conditioning was conducted within sound-attenuating chambers (MED Associates, St. Albans,VT) containing an electrifiable floor of rods (23 4.8-mm stainless steel bars spaced 1.3 cm apart), clear Plexiglas (front/back) and aluminum (side) walls, a tone generator, and a house light. The conditioning chamber was cleaned with 10% ethanol before conditioning. An alternative context was used for delay and trace fear testing, and this context was equipped with infrared lighting, smooth, black fiberglass floors, and striped black and white Plexiglas walls (front/back). The alternative context was cleaned with ammonium hydroxide before each test.

Place conditioning apparatus

Place conditioning and testing were conducted in a 3-chamber apparatus containing 2 distinguishable conditioning chambers (13” x 9” x 11.5”) separated by a smaller center chamber (6” x 7” x 11.5”). One of the conditioning chambers contains wire mesh flooring with white walls, whereas the other conditioning chamber has gold-grated flooring with a black wall. The smaller center chamber has aluminum sheeting as

flooring and white walls. Each of the larger chambers contains two infrared photobeams separated by 3". If the beam furthest from the center chamber was broken, then the rat was determined to be in the larger chamber. If only the beam closest to the center chamber was broken, then the rat was determined to be in the center chamber.

Methods: Aim 1

Aim 1 was completed to determine whether β-AR activation is a fundamental mechanism of memory retrieval.

Aim 1a: Contextual fear conditioning

Rats underwent foreground contextual fear conditioning, during which the conditioning context was paired with an aversive foot shock. Rats were exposed to the conditioning context for a 6 minute baseline period, followed by 4 presentations of a 1 second shock (0.8 mA). The shocks were separated by a 240 ± 20 second inter trial interval followed by a 4 minute post-conditioning period.

Rats were next given daily context fear retrieval tests. To determine the necessity of PL-mPFC β-AR activation for memory retrieval, 1 or 30 days after conditioning PL-mPFC microinfusions of saline or nadolol were administered. Fifteen minutes later rats were given a 3 minute context fear memory retrieval test within the original conditioning chamber (Corcoran and Quirk, 2007). Following the microinfusion test, daily infusion-free tests continued to determine the long-lasting effects of β-AR blockade on context fear memory retrieval. Following multiple unreinforced retrieval tests, rats undergo extinction (Quirk and Mueller, 2008), allowing us to assess reinstatement of the fear

memory. To induce reinstatement, rats were given a normal context retrieval test, but at the end of the test a 1 second shock (0.8 mA) was presented. The rats were then tested for reinstatement of fear the following day by giving an identical context test in the absence of shock.

Aim 1b: Delay fear conditioning

Rats were trained to associate a white noise CS with a 1 second shock UCS. Specifically, rats were exposed to the training context for a 6 minute baseline period, followed by 4 pairings of the CS and UCS. The white noise (72 dB) CS was played for 10 seconds and co-terminated with the 1 second shock UCS (1 mA). The CS-UCS pairings were presented with a 240 ± 20 second inter trial interval followed by a 4 minute post-conditioning period.

Rats were next given daily delay fear retrieval tests. To determine the necessity of PL-mPFC β-AR activation for memory retrieval, 1 or 30 days after conditioning PL-mPFC microinfusions of saline or nadolol were administered. Fifteen minutes later rats were exposed to the alternative context. Following a 2 minute baseline period, the CS was presented for 2 minutes, followed by a 2 minute post CS period. Freezing during and after the CS was quantified separately. Following the microinfusion test, daily infusion-free tests continued to determine the long-lasting effects of β-AR blockade on delay fear memory retrieval. Finally, following extinction of delay fear rats were represented with the shock UCS (1s; 1mA) immediately following a normal delay fear test. The rats were then tested for reinstatement of fear the following day by giving an identical retrieval test in the absence of shock.

Aim 1c: Trace fear conditioning

Rats learned to predict the presentation of an aversive footshock UCS following presentation of a tone CS. Rats were exposed to the training context for a 6 minute baseline period, followed by 6 pairings of the CS and UCS. Specifically, a white noise (72 dB) CS was presented for 10 seconds, followed by a 20 second interval, and finally a 1 second shock UCS (1 mA). The CS-UCS pairings were presented with a 240 ± 20 second inter trial interval followed by a 4 minute post-conditioning period.

Rats were next given daily trace fear retrieval tests. To determine the necessity of PL-mPFC β-AR activation for memory retrieval, 1 or 30 days after conditioning PL-mPFC microinfusions of saline or nadolol were administered. Fifteen minutes later rats were exposed to the alternative context. Following a 2 minute baseline period, the CS was presented for 2 minutes, followed by a 2 minute post CS period. Freezing during the and after the CS was quantified separately. Following the microinfusion test, daily infusion-free tests continued to determine the long-lasting effects of β-AR blockade on delay fear memory retrieval. Finally, following extinction of trace fear rats were represented with the shock UCS (1s; 1mA) immediately following a normal trace fear test. The rats were then tested for reinstatement of fear the following day by giving an identical retrieval test in the absence of shock.

Figure 19

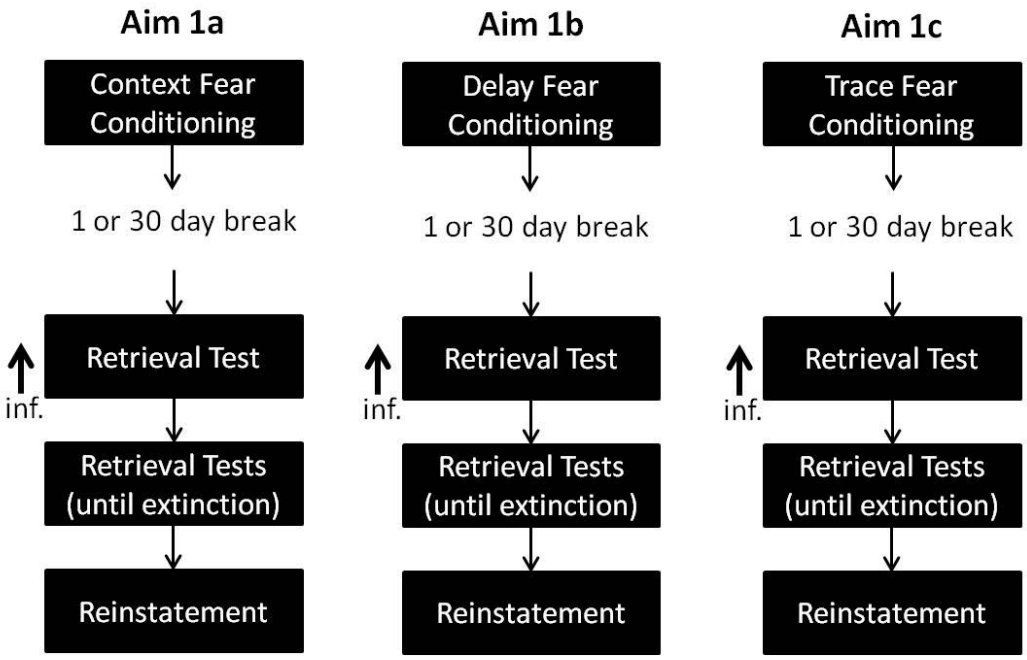

Figure 19. Summary of behavioral experiments as described in aim 1. Arrows represent microinfusions 15 minutes before behavioral testing.

Methods: Aim 2

Aim 2 was completed to determine the mechanism of memory retrieval impairments.

Place conditioning

Baseline preferences were assessed by placing the rats into the center of the CPP apparatus with full access to all 3 chambers 15 minutes. We previously demonstrated that rats spend equivalent time within the larger conditioning chambers before conditioning (Otis and Mueller, 2011). Thus, following baseline testing rats were conditioned to associate one chamber, but not another, with cocaine in a pseudorandom and counterbalanced fashion over 8 days. Injections of saline or cocaine were administered immediately before each 20 minute conditioning session, during which rats were confined to the appropriate chamber.

Experimental manipulations

Following conditioning, rats were given systemic injections of saline (CPP-S) or propranolol (CPP-P) as previously described (Otis and Mueller, 2011). Twenty minutes later each rat was given a CPP memory retrieval test, during which full access to all 3 chambers was allowed for 15 minutes. A final control group of rats received post-test propranolol injections (CPP-PP), allowing us to determine assess the effects of propranolol on CPP memory reconsolidation. The next day, rats were given a second CPP retrieval test in the absence of saline or propranolol injections. To determine the mechanism of propranolol-induced memory retrieval impairments, rats were sacrificed

for electrophysiological recordings 1 hour following the second memory retrieval test (Pattwell et al., 2012).

Patch-clamp electrophysiology

Patch-clamp recordings were established as previously described (Otis et al., 2013; Otis et al., 2014b). Rats aged 3-6 months were anesthetized with pentobarbital, and brains were quickly removed and transferred into ice-cold (0-2˚C) oxygenated (95% O_2 / 5% CO2) artificial cerebral spinal fluid (aCSF) composed of the following (in mM): 124 NaCl, 2.8 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 20 dextrose. Coronal slices 300 µm thick containing prefrontal cortex were taken using a vibratome (Leica VT1200). Slices recovered in warm aCSF (32˚C) for approximately 30 minutes, followed by incubation in room temperature aCSF for 0.5-8 hours. Next, slices were transferred into a recording chamber and continuously perfused with aCSF (2 ml / min). PL-mPFC layer V neurons were visualized with differential interference contrast using a 60X water-immersion lens mounted on an upright Eclipse FN1 microscope (Nikon Instruments). Pyramidal neurons were identified based on the presence of an apical dendrite, and GABAergic interneurons were identified based on the lack of an apical dendrite (Otis et al., 2013). Whole cell recordings of pyramidal neurons were then obtained using borosilicate glass pipettes with low resistance tips (2-4 MΩ) containing a potassium gluconate-based internal solution composed of the following (in mM): 110 K-gluconate, 20KCl, 10 HEPES, 2 $MgCl_2$, 2 ATP, 0.3 GTP, 10 phosphocreatine, 0.2% biocytin, 7.3 pH, 280 mOsm. Synaptic recordings were obtained with voltage clamp, whereas intrinsic excitability recordings were obtained with current clamp using the

MultiClamp 700B amplifier connected to a Digidata 1440A digitizer (Molecular Devices). The liquid-liquid junction potential (measured as 13 mV) was compensated for throughout all recordings. All electrophysiological data were analyzed using Clampfit (Molecular Devices).

The intrinsic excitability of PL-mPFC pyramidal neurons was investigated by recording under current clamp. Neurons were held at -70 mV via direct somatic stimulation to control for differences in resting membrane potential. Next, a series of 1 second depolarizing steps were applied (0 to 500 pA; 50 pA steps), and the number of evoked action potentials was recorded. To quantify neuronal excitability, the rising slope of action potentials (excitability slope) was quantified. Specifically, excitability slope was measured as the number of action potentials by input (from 0 pA to the level of somatic stimulation that induced the maximum number of action potentials). Furthermore, the maximum number of action potentials evoked by a single depolarizing step was measured for each neuron. These alternative intrinsic excitability indices correct for differences in input resistance, as high input resistance can result in a large decrease in action potentials when high intensities of somatic stimulation is applied. Finally, the excitability protocols were also conducted in neurons held at resting membrane potential to determine if differences in resting membrane potential may induce modifications in intrinsic neuronal excitability.

Monosynaptic glutamatergic currents were recorded from PL-mPFC pyramidal neurons in voltage-clamp mode. First, spontaneous EPSCs (sEPSCs) were recorded to allow identification of both presynaptic (sEPSC frequency) and postsynaptic (sEPSC amplitude) modifications in PL-mPFC. To record sEPSCs, neurons were held at -80 mV

in the presence of the $GABA_A$ receptor antagonist picrotoxin for a minimum of 60 seconds. Next, EPSCs were evoked using presynaptic stimulation applied with a bipolar concentric microelectrode placed within 250 µm of the recording electrode. Considering that AMPAr EPSCs but not NMDAr EPSCs are detectable at -80 mV, we first measured the maximum monosynaptic AMPAr EPSCs for each neuron at -80 mV via stepwise presynaptic input. After identifying the maximum presynaptic input for monosynaptic AMPAr EPSCs, we induced and averaged a minimum of 8 AMPA EPSCs (0.067 Hz) using that intensity of presynaptic stimulation. Next, neurons were depolarized to -35 mV. Both AMPA and NMDA receptors contribute to the total excitatory current at -35 mV (Figure 21), and the relative amount of AMPAr current between -80 mV and -35 mV is linear even if AMPArs are inward-rectifying (Clem and Huganir, 2010). Thus, a minimum of 8 EPSCs were evoked (0.067 Hz) at -35 mV to assess the relative amount of NMDAr current as compared with AMPAr current (AMPA:NMDA ratio). The AMPA:NMDA ratio was then calculated by dividing the peak of averaged EPSCs at -80 mV by the peak at -35 mV. To confirm that this was an accurate calculation of the AMPA:NMDA ratio, we recalculated the AMPA:NMDA ratio in a subset of neurons by applying the NMDAr antagonist APV (50 µM) while recording evoked EPSCs at -35 mV (0.067 Hz). The following equation was then used as the second index of the AMPA:NMDA ratio:

$$\frac{\text{EPSC after APV (AMPA)}}{\text{EPSC before APV (AMPA+NMDA)} - \text{EPSC after APV (AMPA)}}$$

Presynaptic plasticity was also characterized via the paired-pulse ratio (PPR). Neurons were held at -80 mV in the presence of picrotoxin, and 2 presynaptic pulses were applied with an inter-stimulation interval of 250 ms (4.0 Hz). The peak of the second EPSC was then divided by the peak of the first EPSC (P2/P1) for quantification of the PPR.

Figure 20

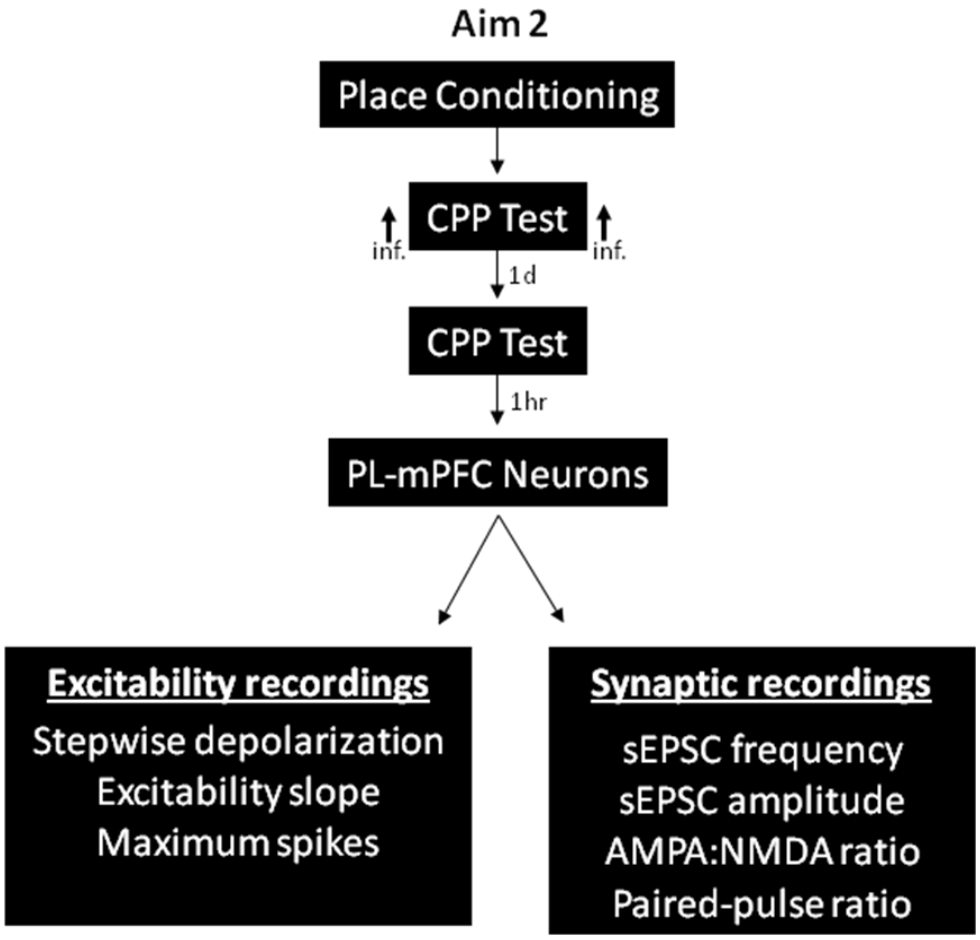

Figure 20. Summary of experimental design as described in aim 2. Arrows represent microinfusions 15 minutes before or immediately after behavioral testing. CPP, conditioned place preference; sEPSC, spontaneous excitatory postsynaptic current.

Figure 21

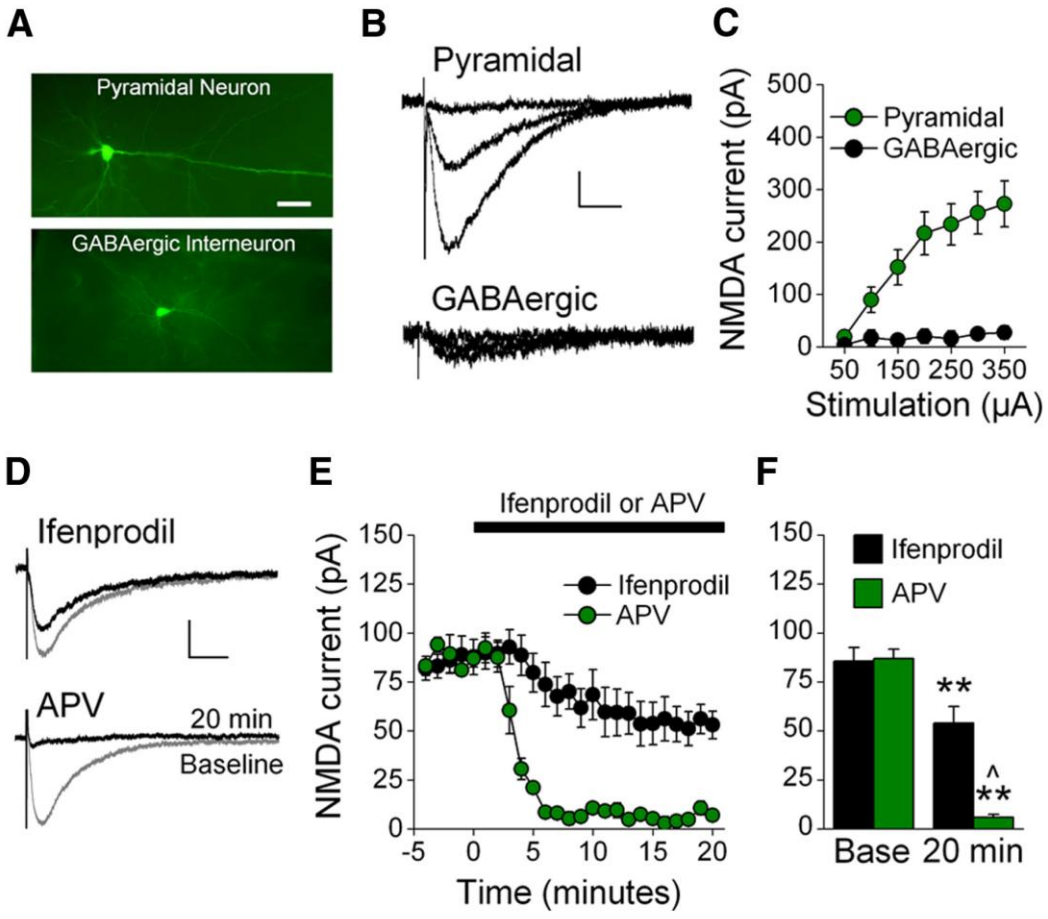

Figure 21. Focal stimulation induces monosynaptic, glutamate receptor-specific postsynaptic currents in mPFC neurons. (A) Photomicrographs of biocytin-filled mPFC pyramidal and GABAergic neurons. Scale bar represents 100 μm. (B) Example traces of evoked EPSCs (50, 150, and 350 μA) in the presence of the $GABA_A$ antagonist picrotoxin (100 μM) and AMPAr antagonist DNQX (10μM). (C) Evoked EPSCs were larger in mPFC pyramidal neurons (n = 15) as compared with GABAergic interneurons (n = 5). (D-F) Evoked EPSCs were glutamatergic, as application of the selective GluN2B-containing NMDAr antagonist ifenprodil (3 μM) and nonselective NMDAr antagonist APV (25 μM) abolished the EPSCs (Otis et al., 2014b). **p < 0.01 compared with baseline. ^ p < 0.01 compared with ifenprodil-treated neurons.

Results

Aim 1

The effects of PL-mFPC β-AR blockade on retrieval and subsequent reinstatement of 1) contextual fear memories, 2) delay fear memories, and 3) trace fear memories was first investigated.

Context fear conditioning

The necessity of PL-mPFC β-AR activation for recent contextual fear memory retrieval was first examined. One day following contextual fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 6) or nadolol (n = 4) before the first test only. Nadolol reduced freezing during the first test (t_8 = 2.65, p = 0.03; Figure 22a-c), but not during subsequent nadolol-free tests (all t-values < 1, all p-values > 0.05). Thus, PL-mPFC β-AR activation is required for fear expression during a recent context fear memory retrieval test, but disruption of this activity does not induce persistent fear memory impairments.

Next, the requirement of PL-mPFC β-AR activation for remote contextual fear memory retrieval was assessed. Thirty-one days following contextual fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 6) or nadolol (n = 7) before the first test only. Nadolol reduced freezing during the first test (t_{11} = 2.35, p = 0.04; Figure 22d-f), but not during subsequent nadolol-free tests (all t-values < 1, all p-values > 0.05). Thus, PL-mPFC β-AR activation is required for fear expression during recent and remote context fear memory retrieval tests, but disruption of this activity does not induce persistent fear memory impairments.

Figure 22

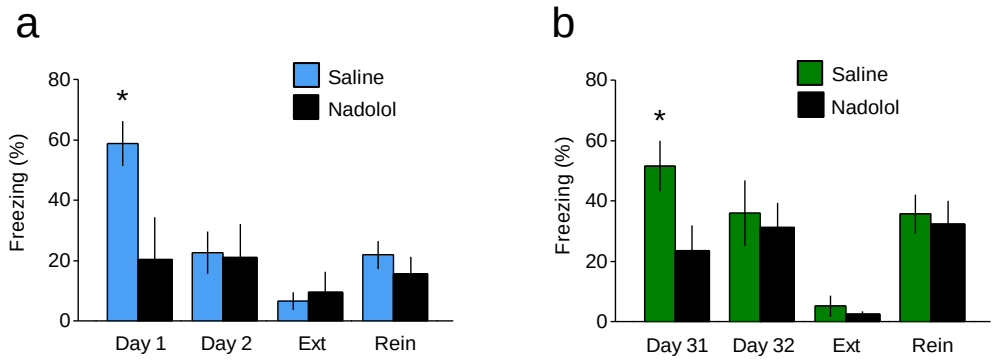

Figure 22. PL-mPFC β-AR blockade prevents retrieval of recent and remote contextual fear memories. (a) PL-mPFC microinfusions of nadolol before a recent context retrieval test (day 1) reduced freezing, but did not have any persistent effects on freezing during subsequent drug-free tests. **(b)** PL-mPFC microinfusions of nadolol before a remote context retrieval test (day 31) reduced freezing, but did not have persistent effects on freezing during subsequent drug-free tests. *$p < 0.05$.

Delay fear conditioning

The involvement of PL-mPFC β-AR activation for recent delay fear memory retrieval was investigated. One day following delay fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 7) or nadolol (n = 8) before the first test only. Nadolol prevented recent fear memory expression, but did not have long-lasting effects on fear expression. During the first test, nadolol did not significantly reduce freezing during the baseline period ($t_{13} = 1.60$, $p = 0.13$) or post CS period ($t_{13} = 2.08$, $p < 0.06$; Figure 23a-c). However, nadolol reduced freezing during presentation of the CS ($t_{13} = 3.23$, $p = 0.007$), indicating that recent delay fear memory expression was reduced. In contrast, nadolol had no effect on freezing during subsequent nadolol-free tests (all *t*-values < 1.3, all *p*-values > 0.05). Thus, PL-mPFC β-AR activation is required for fear expression during a recent delay fear memory retrieval test, but disruption of this activity does not induce persistent fear memory impairments.

Next, the requirement of PL-mPFC β-AR activation for remote delay fear memory retrieval was assessed. Thirty-one days following delay fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 10) or nadolol (n = 8) before the first test only. Nadolol had no effect on freezing during the first test or during subsequent nadolol-free tests (all *t*-values < 2.0, all *p*-values > 0.05; Figure 23d-f). Thus, β-AR activation is necessary for fear expression during a recent, but not remote, delay fear memory retrieval test

Figure 23

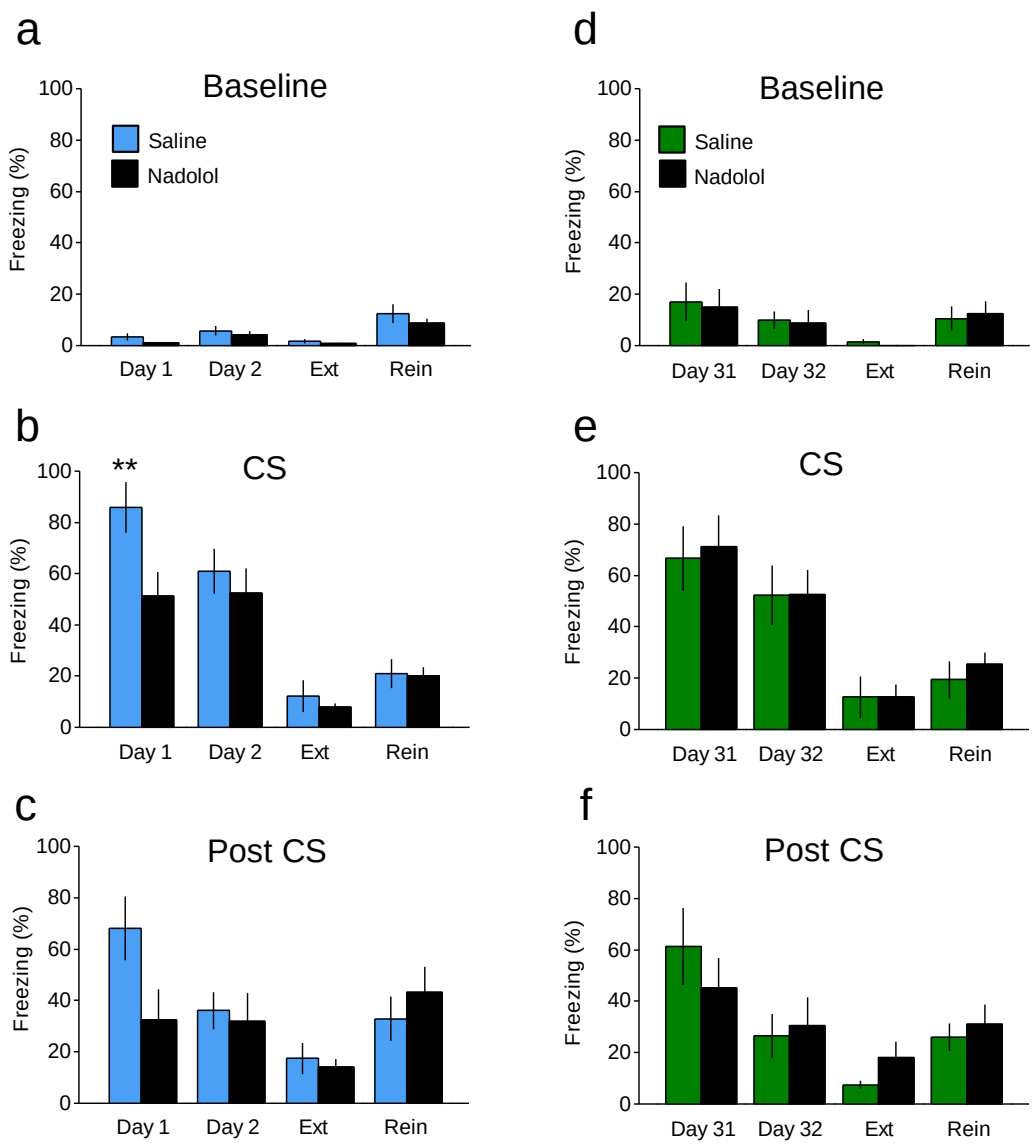

Figure 23. PL-mPFC β-AR blockade prevents retrieval of recent but not remote delay fear memories. (a-c) PL-mPFC microinfusions of nadolol before a recent delay fear memory retrieval test (day 1) reduced freezing during CS presentation, but did not have persistent effects on freezing during subsequent drug-free tests. **(d-f)** PL-mPFC microinfusions of nadolol before a remote delay fear memory retrieval test (day 31) did not reduce freezing during that test or during subsequent drug-free tests. $^{**}p < 0.01$.

Trace fear conditioning

The involvement of PL-mPFC β-AR activation for recent trace fear memory retrieval was investigated. One day following trace fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 5) or nadolol (n = 5) before the first test only. Nadolol had no effect on freezing during the first test or during subsequent nadolol-free tests (all *t*-values < 2.0, all *p*-values > 0.05; Figure 24a-c). Thus, β-AR activation is not required for fear expression during a recent trace fear memory retrieval test.

Next, the requirement of PL-mPFC β-AR activation for remote trace fear memory retrieval was assessed. Thirty-one days following trace fear conditioning, rats were given daily memory retrieval tests with PL-mPFC microinfusions of saline (n = 5) or nadolol (n = 4) before the first test only. Nadolol prevented trace fear memory expression during this test and during a subsequent UCS-induced reinstatement test (Figure 24d-f). During the first test, nadolol had no effect on freezing during the baseline period ($t_8 = 0.92$, $p = 0.39$). However, nadolol reduced freezing during presentation of the CS ($t_8 = 3.52$, $p = 0.01$) and during the post-CS period ($t_8 = 11.32$, $p = 0.000009$), indicating nadolol reduced remote trace fear memory expression. In contrast, nadolol had no effect on freezing during the next nadolol-free test or during the final nadolol-free extinction test (all *t*-values < 1.3, all *p*-values > 0.05). During the nadolol-free reinstatement test, however, previous nadolol treatment significantly reduced freezing during the baseline period ($t_8 = 3.52$, $p = 0.01$) and during the post CS period ($t_8 = 3.18$, $p = 0.02$). However, freezing during presentation of the CS was not significantly reduced ($t_8 = 3.52$, $p = 0.01$). Taken together, β-AR activation is necessary for fear expression during a remote, but not

recent, trace fear memory retrieval test. Moreover, disruption of β-AR activity during remote trace fear memory retrieval prevents subsequent reinstatement of the memory.

Figure 24

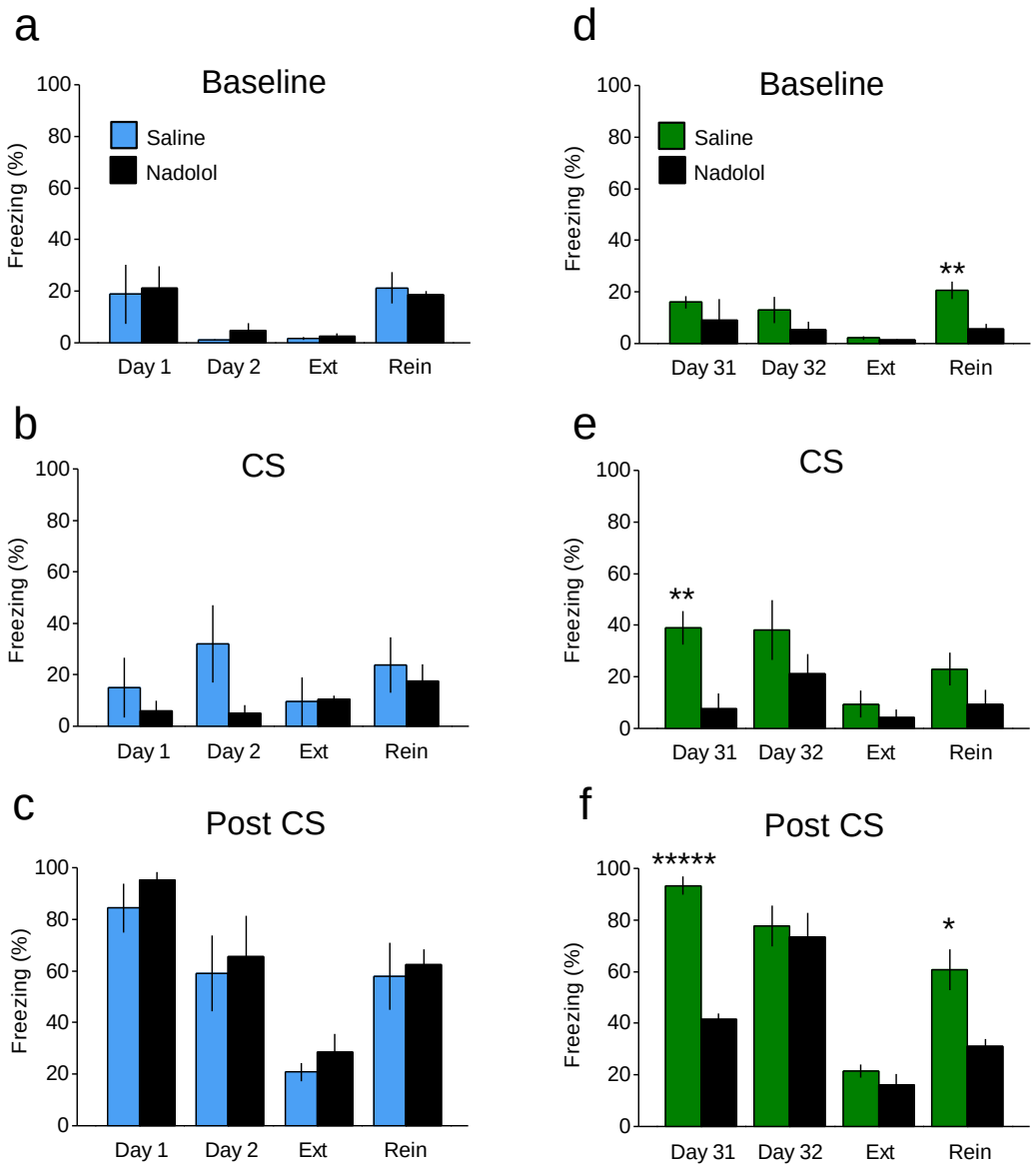

Figure 24. PL-mPFC β-AR blockade prevents retrieval and subsequent reinstatement of remote trace fear memory. (a-c) PL-mPFC microinfusions of nadolol before a recent trace fear retrieval test (day 1) did not reduce freezing during that test or during subsequent drug-free tests **(d-f)** PL-mPFC microinfusions of nadolol before a remote trace fear memory retrieval test (day 31) reduced freezing during that test and during a subsequent UCS-induced reinstatement. *$p < 0.05$, **$p < 0.01$, *****$p < 0.00001$.

Aim 2

The mechanisms of cocaine-associated memory retrieval impairments were next investigated. Following conditioning, rats were given two daily CPP tests, with systemic injections of saline (n = 15) or propranolol (n = 6; 10 mg/kg) before the first test only. Similar to previous investigations, propranolol prevented CPP expression during the CPP test and during a subsequent propranolol-free CPP test (Figure 21a). Repeated measures ANOVA revealed an effect of group ($F_{1,19} = 11.74$, $p = 0.003$), and *post-hoc* comparisons revealed that CPP-S rats had significantly higher CPP scores as compared with CPP-P rats during the first and second CPP tests (ps < 0.01). Thus, propranolol persistently impaired retrieval of the cocaine-induced CPP memory, consistent with previous findings (Otis and Mueller, 2011; Otis et al., 2013).

Intrinsic excitability (-70 mV)

β-AR blockade before the first CPP test induced a CPP memory retrieval impairment, but whether CPP conditioning or β-AR blockade modifies the intrinsic excitability of PL-mPFC neurons is unknown. Thus, one hour following the second CPP test, rats were sacrificed and electrophysiological recordings were obtained from PL-mPFC neurons held at a voltage of -70 mV. We found subtle potentiation in the excitability of PL-mPFC neurons after CPP conditioning, and this enhancement was not modified by propranolol treatment (Figure 26a-d). First, no differences in the number of evoked action potentials were found overall in neurons taken from naïve (n = 14), CPP-S (n = 36), and CPP-P rats (n = 23). ANOVA revealed no input by group interaction ($F_{20,700} = 1.23$, $p = 0.22$), suggesting that the number of evoked action potentials were similar across groups. Despite this, further analysis revealed that the excitability slope

(rising slope of spikes by level of somatic depolarization) for CPP-S and CPP-P may have increased following CPP conditioning. One-way ANOVA revealed a trend between groups ($F_{2,70} = 2.70$, $p = 0.07$), and *post-hoc* analysis revealed that neurons from CPP-S and CPP-P rats had a significantly higher excitability slopes as compared with neurons from naïve rats (ps < 0.05). In contrast, no differences were found between CPP-S and CPP-P rats ($p = 0.65$). Furthermore, no overall differences between groups were found for the maximum number of evoked spikes (one-way ANOVA: $F_{2,70} = 2.04$, $p = 0.14$), although direct comparison between neurons taken from naïve and both CPP-s and CPP-P rats revealed an increase in the maximum number of spikes for PL-mPFC neurons following CPP conditioning (Figure 22d, $t_{71} = 2.04$, $p < 0.05$). These data indicate that CPP conditioning may induce a subtle potentiation in the excitability of PL-mPFC pyramidal neurons.

We next examined whether the excitability of PL-mPFC pyramidal neurons changed in all rats, or specifically in rats showing CPP memory retrieval. Rats were split into groups expressing CPP scores below the mean (< 335 seconds, N = 3) versus those expressing CPP scores above the mean (>335 seconds, N = 8). Rats with CPP scores above the mean (high retrieval, HR) had significantly elevated CPP scores as compared with rats expressing CPP scores below the mean (low retrieval, LR; Figure 26e). Repeated measures ANOVA revealed a between groups effect ($F_{1,9} = 6.34$, $p = 0.03$), and *post-hoc* analyses confirmed a significant increase in CPP for HR rats as compared to LR rats during the second CPP test ($p = 0.0005$), although no significant difference during the first test ($p = 0.24$). Thus, HR rats expressed higher CPP scores as compared to LR rats. Next, we analyzed the excitability of PL-mPFC neurons from these rats while

holding the neurons at -70 mV. PL-mPFC pyramidal neurons from HR rats (n = 22) had increased excitability as compared with those from both LR rats (n = 14) and naïve rats (n = 14; Figure 26f-h). Repeated-measures ANOVA indicated an input by group interaction for the number of evoked spikes ($F_{20,470} = 3.54$, $p = 0.000001$), revealing that the excitability of PL-mPFC neurons was different between groups. One-way ANOVA further confirmed that excitability slope was different between groups ($F_{2,47} = 8.07$, $p < 0.001$) and maximum spikes was different between groups ($F_{2,47} = 8.33$, $p < 0.001$). Finally, *post-hoc* analysis revealed that neurons from HR rats had significantly higher excitability slope (ps < 0.003) and maximum spikes (ps = 0.001) as compared with neurons from naïve and LR rats. These data reveal that the intrinsic neuronal excitability of PL-mPFC pyramidal neurons is increased in rats expressing high CPP memory retrieval, but not in rats expressing low CPP memory retrieval.

We also found that CPP memory retrieval positively correlated with intrinsic neuronal excitability (Figure 27). CPP scores from CPP-S rats correlated with excitability slope ($r_9 = 0.73$, $p = 0.01$) and maximum spikes ($r_9 = 0.77$, $p = 0.006$) when neurons were held at -70 mV. In contrast, CPP scores from CPP-P rats did not correlate with excitability slope ($r_4 = -0.61$, $p = 0.20$) or maximum spikes ($r_4 = -0.66$, $p = 0.16$) when neurons were held at -70 mV. Taken together, these data indicate that enhancement of PL-mPFC neuronal excitability supports CPP memory retrieval. Furthermore, inhibition of PL-mPFC β-AR activation, which limits PL-mPFC excitability (Otis et al., 2013), may prevent PL-mPFC neuronal excitability from supporting memory retrieval.

Figure 25

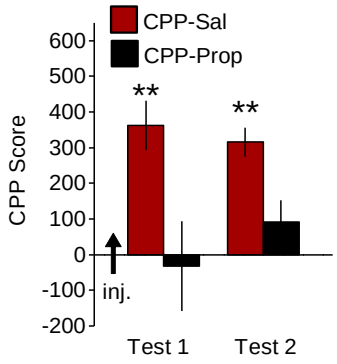

Figure 25. β-AR blockade persistently impairs expression of a cocaine CPP memory. Systemic injections of propranolol, but not saline, before the first CPP test prevented rats from expressing a cocaine CPP during the first test and during a subsequent propranolol-free test (similar to previous observations by Otis and Mueller, 2011). $^{**}p < 0.01$.

Figure 26

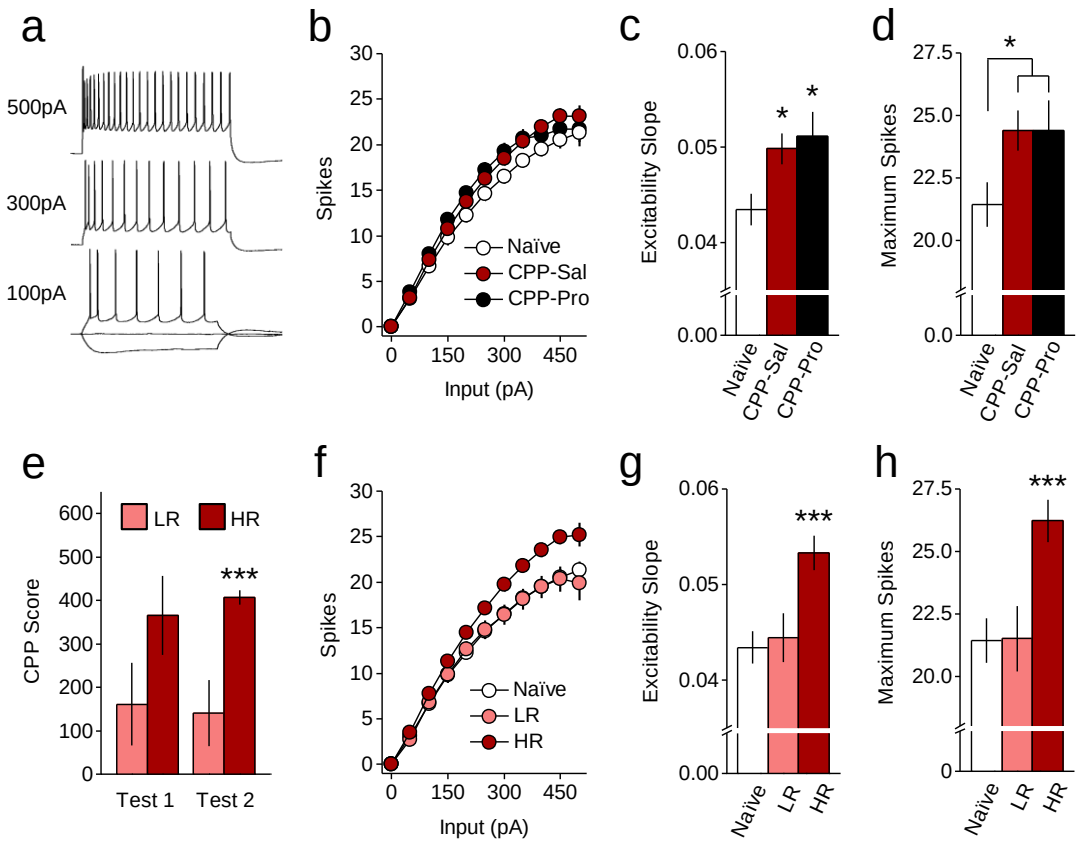

Figure 26. Cocaine conditioning increases the excitability of PL-mPFC pyramidal neurons (held at -70 mV) in rats expressing robust CPP memory retrieval. (a) Example waveforms revealing the number of action potentials evoked following somatic depolarization of PL-mPFC pyramidal neurons. **(b)** The number of evoked spikes was unchanged overall between naïve, CPP-S, and CPP-P rats. **(c, d)** The excitability slope and maximum number of spikes was increased in rats that underwent CPP conditioning. **(e)** Rats were split into groups expressing low and high retrieval. **(f-h)** The number of evoked spikes, excitability slope, and maximum spikes was increased in neurons taken from HR rats versus LR and naïve rats. LR, low retrieval; HR, high retrieval. $*p < 0.05$, $***p < 0.001$.

Figure 27

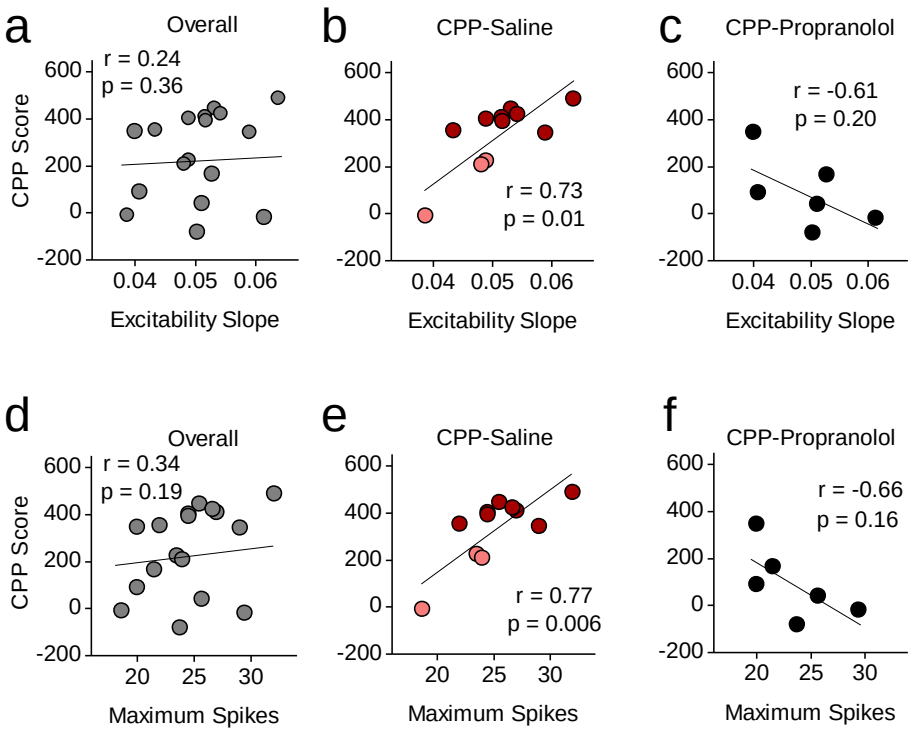

Figure 27. Excitability of PL-mPFC pyramidal neurons (held at -70 mV) positively correlates with CPP memory retrieval. **(a, d)** Overall, CPP scores from CPP-S and CPP-P rats do not correlate with excitability slope or maximum spikes of PL-mPFC neurons. **(b, e)** CPP scores from CPP-S rats positively correlate with excitability and maximum spikes of PL-mPFC neurons. **(c, f)** CPP scores from CPP-P rats do not positively correlate with excitability slope or maximum spikes of PL-mPFC neurons.

Intrinsic excitability (resting membrane potential)

Differences in PL-mPFC resting membrane potential could contribute to the cocaine conditioning-induced modifications in PL-mPFC intrinsic neuronal excitability. To assess this, we replicated excitability recordings in neurons held at resting membrane potential. These recordings yielded an identical pattern of results, indicating that CPP conditioning increases PL-mPFC neuronal excitability without modifying resting membrane potential (Figure 28).

Similar to recordings at -70 mV, no differences were found for the number of evoked action potentials in neurons taken from naïve (n = 15), CPP-S (n = 39), and CPP-P rats (n = 24; Figure 28a). ANOVA revealed no input by group interaction ($F_{20,750}$ = 1.23, p = 0.22), suggesting that the number of evoked action potentials were similar across groups. Despite this, further analysis revealed that the excitability slope for neurons taken from CPP-S and CPP-P rats may have increased following conditioning (Figure 28b). One-way ANOVA revealed a trend between groups ($F_{2,75}$ = 2.94, p = 0.06), and *post-hoc* analysis revealed that neurons from CPP-S and CPP-P rats had a trend toward higher excitability slopes as compared with neurons from naïve rats (ps < 0.07). Furthermore, direct comparison between naïve and cocaine-conditioned rats indicated that neurons taken from cocaine-conditioned rats had increased excitability slope (t_{76} = 2.30, p = 0.02). No overall differences between groups were found for the maximum number of spikes (one-way ANOVA: $F_{2,75}$ = 2.33, p = 0.10), although direct comparison between neurons taken from naïve and CPP rats revealed an increase in the maximum number of spikes for PL-mPFC neurons following CPP conditioning (t_{76} = 2.06, p = 0.04;

Figure 28c). These data provide further support that CPP conditioning increases the excitability of PL-mPFC neurons.

We next examined whether the excitability of PL-mPFC pyramidal neurons held at resting membrane potential was different in rats expressing high CPP memory retrieval versus those expressing low CPP memory retrieval. PL-mPFC pyramidal neurons from HR rats (n = 24) had increased excitability as compared with those from both LR rats (n = 15) and naïve rats (n = 15; Figure 28d-f). Repeated-measures ANOVA indicated an input by group interaction for the number of evoked spikes ($F_{20,510} = 3.34$, $p = 0.000002$), revealing that the excitability of PL-mPFC neurons was different between groups. One-way ANOVA further confirmed that excitability slope was different between groups ($F_{2,51} = 5.43$, $p = 0.007$) and maximum spikes was different between groups ($F_{2,51} = 5.27$, $p = 0.008$). Finally, *post-hoc* analysis revealed that neurons from HR rats had significantly higher excitability slope and maximum spikes (*p*s = 0.003) as compared with neurons from naïve rats. These data reveal that the intrinsic neuronal excitability of PL-mPFC pyramidal neurons held at resting membrane potential is increased in rats expressing high CPP memory retrieval, but not in rats expressing low CPP memory retrieval.

We also found that CPP memory retrieval positively correlated with intrinsic neuronal excitability when neurons were held at resting membrane potential (Figure 29). CPP scores from CPP-S rats correlated with excitability slope ($r_9 = 0.66$, $p = 0.03$) and maximum spikes ($r_9 = 0.74$, $p = 0.01$) when neurons were held at resting membrane potential. In contrast, CPP scores from CPP-P rats did not significantly correlate with excitability slope ($r_4 = -0.52$, $p = 0.29$) or maximum spikes ($r_4 = -0.73$, $p = 0.10$) when

neurons were held at resting membrane potential. Taken together, these data indicate that enhancement of PL-mPFC neuronal excitability supports CPP memory retrieval, and changes in resting membrane potential do not ameliorate this effect. Despite this, how β-AR activity maintains CPP memory retrieval remains to be determined.

Figure 28

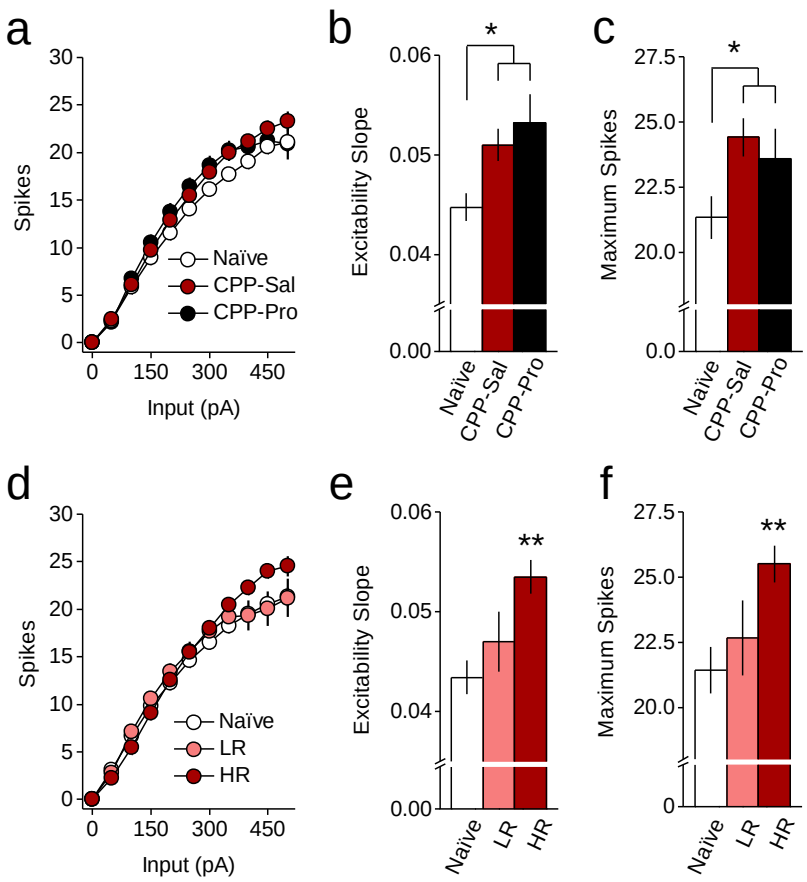

Figure 28. Cocaine conditioning increases the excitability of PL-mPFC pyramidal neurons (held at resting membrane potential) in rats expressing robust CPP memory retrieval. (a) The number of evoked spikes was unchanged overall between naïve, CPP-S, and CPP-P rats. **(b, c)** The excitability slope and maximum number of spikes was increased in rats that underwent CPP conditioning. **(d-f)** The number of evoked spikes, excitability slope, and maximum spikes was increased in neurons taken from HR rats versus LR and naïve rats. LR, low retrieval; HR, high retrieval. *$p < 0.05$, ***$p < 0.001$.

Figure 29

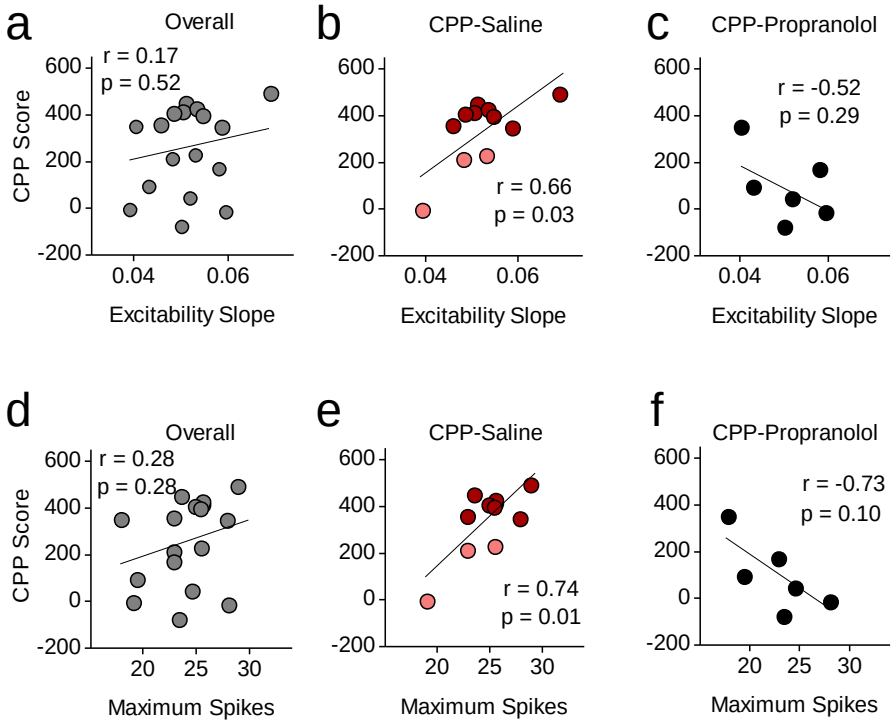

Figure 29. Excitability of PL-mPFC pyramidal neurons (held at resting membrane potential) positively correlates with CPP memory retrieval. **(a, d)** Overall, CPP scores from CPP-S and CPP-P rats do not correlate with excitability slope or maximum spikes of PL-mPFC neurons. **(b, e)** CPP scores from CPP-S rats positively correlate with excitability and maximum spikes of Pl-mPFC neurons. **(c, f)** CPP scores from CPP-P rats do not positively correlate with excitability slope or maximum spikes of PL-mPFC neurons.

Synaptic plasticity

CPP conditioning increases the excitability of PL-mPFC pyramidal neurons, and this excitability is associated with CPP memory retrieval. Despite this, how β-AR activation maintains CPP memory retrieval remains unclear. Thus, we recorded sEPSCs (-80 mV) from PL-mPFC neurons taken from naïve (n = 9), CPP-S (n = 27), and CPP-P rats (n = 17). Next, sEPSC amplitude was analyzed as a measure of postsynaptic plasticity, whereas sEPSC frequency was analyzed as a measure of presynaptic plasticity. Data reveal that the sEPSC amplitude and frequency were increased in cocaine-conditioned rats, and propranolol reversed this change (Figure 30). One-way ANOVA revealed a significant effect of group for sEPSC amplitudes ($F_{2,50} = 12.65$, $p = 0.00004$), and *post-hoc* analyses confirmed an increase sEPSC amplitude for neurons from CPP-S rats as compared with naïve and CPP-P rats (ps < 0.004). Similarly, one-way ANOVA revealed a significant effect of group for sEPSC frequency ($F_{2,50} = 5.37$, $p = 0.008$), and *post-hoc* analyses confirmed an increase in sEPSC frequency for neurons from CPP-S rats as compared with naïve and CPP-P rats (ps < 0.03). Thus, CPP conditioning increased the amplitude and frequency of sEPSCs in PL-mPFC pyramidal neurons, indicating postsynaptic and presynaptic potentiation, respectively. Moreover, β-AR blockade during memory retrieval reversed this plasticity.

The changes in sEPSC amplitude indicate postsynaptic modifications, possibly due to potentiation of AMPAr currents. To investigate this, AMPA:NMDA ratios were recorded. EPSCs were evoked at -80 mV (AMPAr currents) and -35 mV (NMDAr currents) in PL-mPFC neurons taken from naïve (n = 7), CPP-S (n = 16), and CPP-P rats (n = 12). Data reveal an increase in AMPA:NMDA ratio in neurons taken from cocaine-

conditioned rats, and propranolol reversed this plasticity (Figure 31a,b). One-way ANOVA revealed a significant effect of group ($F_{2,32} = 16.39$, $p = 0.00001$), and *post-hoc* analyses confirmed an increase AMPA:NMDA ratio in neurons taken from CPP-S rats as compared with naïve and CPP-P rats (ps < 0.001). Thus, CPP conditioning increased the AMPA:NMDA ratio, and this increase was reversed by propranolol. We confirmed these findings by re-calculating the AMPA:NMDA ratio by applying APV, the NMDAr antagonist, in neurons taken from naïve (n = 3), CPP-S (n = 5), and CPP-P rats (n = 3). Similar to the above findings, cocaine conditioning increased the AMPA:NMDA ratio, and this increase was reversed by propranolol injections before the first memory retrieval test (Figure 32a-b). One-way ANOVA revealed an effect of group ($F_{2,8} = 5.08$, $p = 0.04$), and *post-hoc* analyses confirmed an increase in AMPA:NMDA ratio for neurons taken from CPP-S rats as compared with naïve and CPP-P rats (ps < 0.05). Moreover, AMPA:NMDA ratios measured by voltage were positively correlated with those measured by APV in the same PL-mPFC neurons ($r_9 = 0.92$, $p < 0.00001$). Thus, CPP conditioning increased the AMPA:NMDA ratio, as measured using voltage clamp or APV, and this effect was reversed by propranolol. These findings indicate a long-term enhancement in AMPAr currents following cocaine CPP conditioning, and this synaptic plasticity is reversed by β-AR blockade during CPP memory retrieval.

Along with postsynaptic plasticity, the sEPSC frequency was increased in PL-mPFC neurons from cocaine-conditioned rats, indicating presynaptic plasticity. To further investigate this we recorded the PPR, a second marker of presynaptic plasticity, in neurons taken from naïve (n = 7), CPP-S (n = 18), and CPP-P (n = 17) rats. Cocaine conditioning increased the PPR in PL-mPFC neurons, an effect that was reversed by

propranolol (Figure 31c,d). One-way ANOVA revealed a significant effect of group ($F_{2,39} = 9.62$, $p = 0.00004$), and *post-hoc* analyses confirmed an increased PPR for neurons taken from CPP-S rats as compared with naïve and CPP-P rats ($ps < 0.003$). Thus, CPP conditioning induced paired-pulse facilitation in PL-mPFC neurons, indicating presynaptic potentiation. Moreover, this presynaptic plasticity was reversed by propranolol.

Figure 30

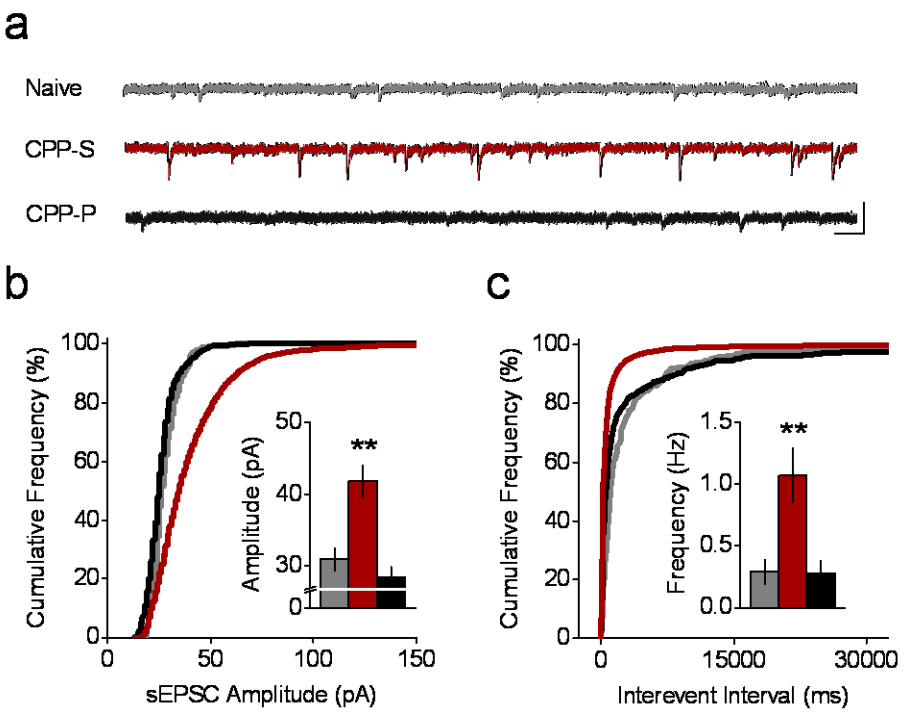

Figure 30. β-AR blockade during memory retrieval reverses cocaine conditioning-induced potentiation of PL-mPFC sEPSCs. (a) Example waveforms of PL-mPFC sEPSCs from PL-mPFC pyramidal neurons. Scale bars represent 50 pA (vertical) and 100 ms (horizontal). **(b)** Cumulative frequency distribution and means plot reveal an increase in the amplitude of sEPSCs in neurons from CPP-S rats versus CPP-P and naïve rats. **(c)** Cumulative frequency distribution reveals a decrease in the sEPSC inter-event interval, and the means plot confirms that the frequency of sEPSCs increased in neurons taken from CPP-S versus CPP-P and naïve rats. $^{**}p < 0.01$.

Figure 31

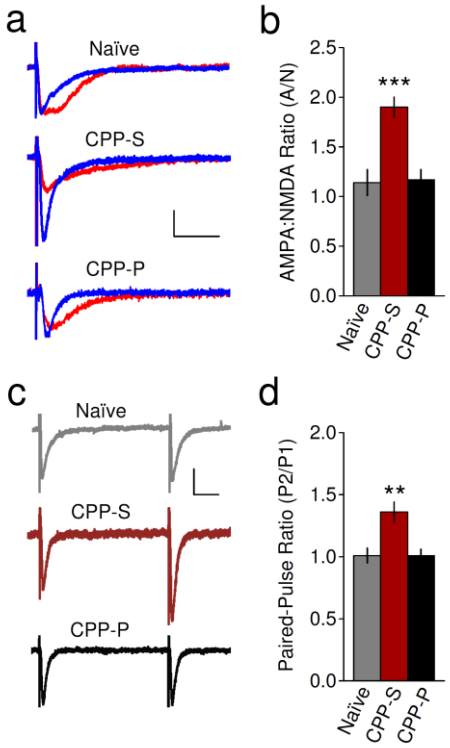

Figure 31. β-AR blockade reverses cocaine conditioning-induced AMPAr potentiation and paired-pulse facilitation in PL-mPFC neurons. (a) Example waveforms of evoked EPSCs at -80 mV (blue traces, AMPAr EPSCs) and -35 mV (red traces, NMDAr EPSCs) that were used to quantify AMPA:NMDA ratios. **(b)** AMPA:NMDA ratios were increased in neurons from CPP-S rats versus CPP-P and naïve rats. **(c)** Example waveforms revealing PPRs (P2/P1) in PL-mPFC neurons. **(d)** PPRs were increased in neurons from CPP-S rats versus CPP-P and naïve rats. Scale bars represent 50 pA (vertical) and 100 ms (horizontal). P2/P1, pulse 2 divided by pulse 1 $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure 32

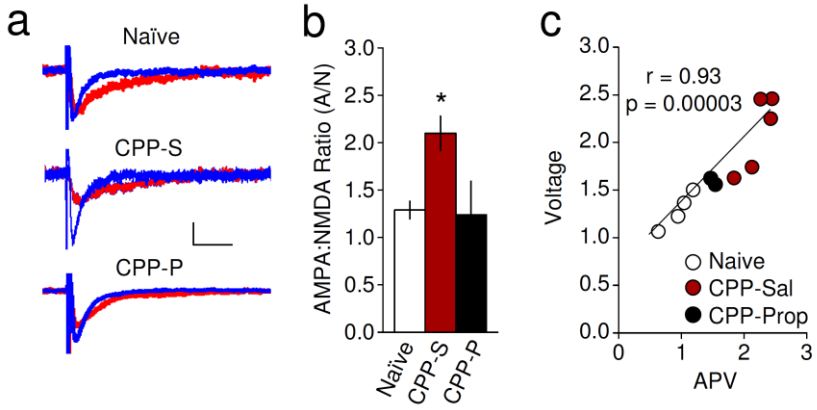

Figure 32. Confirmation that β-AR blockade reverses cocaine conditioning-induced AMPAr potentiation in PL-mPFC neurons. (a) Example waveforms of evoked EPSCs at -35 mV after APV application (blue, AMPAr EPSCs) and the calculated difference (red, NMDAr EPSCs). **(b)** AMPA:NMDA ratios recorded via APV application were increased in neurons from CPP-S rats versus CPP-P and naïve rats. **(c)** AMPA:NMDA ratios as recorded by voltage clamp positively correlate with AMPA:NMDA ratios recorded by APV application in the same PL-mPFC neurons.

Data reveal presynaptic and postsynaptic plasticity in PL-mPFC neurons following cocaine conditioning. Moreover, this plasticity is reversed by β-AR blockade during memory retrieval. Next we confirmed that this reversal was specific to β-AR blockade during CPP memory retrieval, and not due to nonspecific effects such as reconsolidation blockade. Following conditioning, rats were given two daily CPP tests with systemic injections of propranolol after the first test only (N = 5, CPP-PP, Figure 33a). One hour after the second test, rats were sacrificed for patch clamp electrophysiology. Overall, data reveal that posttest injections of propranolol did not reverse the increase in PL-mPFC synaptic potentiation (Figure 33b-h). First, sEPSC amplitudes were increased in neurons taken from CPP-PP rats (n = 5) as compared with neurons taken from naïve rats (n = 9; $t_{12} = 3.12$, $p = 0.009$). Similarly, sEPSCs were more frequent in neurons taken from CPP-PP rats (n = 5) as compared with those from naïve rats (n = 9; $t_{12} = 2.95$, $p = 0.01$). These indicate that propranolol has no effect on presynaptic and postsynaptic plasticity in PL-mPFC when given after memory retrieval. In further support of this, neurons taken from CPP-PP rats (n = 5) had increased AMPA:NMDA ratio (as measured by voltage) as compared with neurons taken from naïve rats (n = 7; $t_{10} = 2.19$, p = 0.05). Finally, the PPR was increased in neurons taken from CPP-PP rats (n = 7) as compared with neurons taken from naïve rats (n = 7; $t_{12} = 3.78$, $p = 0.003$). Taken together, β-AR blockade after CPP memory retrieval did not reverse the change in sEPSC amplitude, sEPSC frequency, AMPA:NMDA ratio, or PPR. Thus, these data confirm that β-AR activation during memory retrieval, but not after, maintains cocaine-related plasticity in PL-mPFC pyramidal neurons.

Figure 33

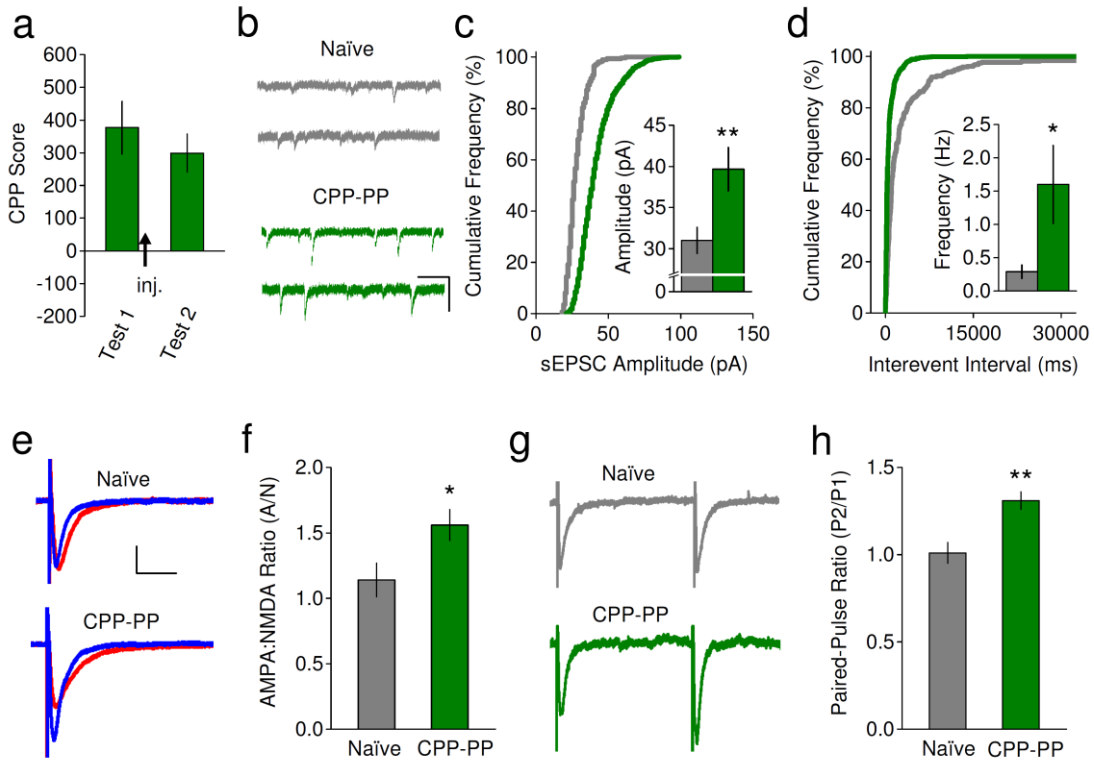

Figure 33. Post-retrieval propranolol does not reverse cocaine conditioning-induced presynaptic and postsynaptic plasticity in PL-mPFC neurons. (a) Post-test propranolol injections did not prevent CPP expression during a subsequent propranolol-free test. **(b-d)** sEPSC frequency and amplitude were increased in PL-mPFC neurons from CPP-PP rats versus naïve rats. **(e,f)** AMPA:NMDA ratios as measured by voltage clamp were increased in PL-mPFC neurons from CPP-PP rats versus naïve rats. **(g,h)** PPRs were increased in PL-mPFC neurons from CPP-PP rats versus naïve rats. $*p < 0.05$, $**p < 0.01$.

Discussion

This dissertation aims (1) to establish whether PL-mPFC β-AR activation is a fundamental mechanism for maintenance of memory retrieval and (2) to determine the mechanism of cocaine-associated memory retrieval impairments.

Results reveal that PL-mPFC β-AR activation is not a fundamental mechanism required for maintenance of fear memory retrieval. PL-mPFC infusions of the β-AR antagonist nadolol reduced fear during context, recent delay, and remote trace fear memory retrieval tests, but had no effect during a subsequent retrieval test. However, PL-mPFC β-AR blockade during the remote trace fear memory retrieval test reduced subsequent reinstatement, indicating some long-lasting effects of β-AR blockade on the fear memory. Next, results reveal the mechanism of cocaine-associated memory retrieval impairments. Cocaine conditioning increased intrinsic neuronal excitability of PL-mPFC neurons, particularly in rats expressing high CPP memory retrieval. Further, cocaine conditioning induced presynaptic and postsynaptic potentiation in PL-mPFC neurons. Finally, systemic injections of the β-AR antagonist propranolol during, but not after retrieval, reversed the synaptic plasticity in PL-mPFC neurons. Taken together, reversal of PL-mPFC synaptic potentiation by β-AR blockade induces long-lasting cocaine-associated memory retrieval impairments, but not long-lasting fear memory retrieval impairments.

Aim 1

Data reveal that PL-mPFC β-AR blockade reduced fear during recent and remote contextual fear memory retrieval tests. These findings are consistent with data revealing

PL-mPFC inactivation reduces contextual fear memory expression 24h after conditioning (Corcoran and Quirk, 2007; Laurent and Westbrook, 2009). Similarly, PL-mPFC lesions prevent context-dependent cued fear expression 8d after conditioning (Kim et al., 2013), supporting the idea that PL-mPFC regulates both recent and remote contextual fear memories. Unit recording data further reveal that PL-mPFC neurons exhibit context-dependent short-latency responses to a fear-conditioned cue (Kim et al., 2013). Taken together, PL-mPFC neurons may encode contextual information for fear expression. Our data further reveal that PL-mPFC β-AR activation promotes neural activity in PL-mPFC neurons for expression of recent and remote contextual fear memories.

PL-mPFC β-AR blockade reduced fear expression during a recent delay fear memory retrieval test, but not during a remote delay fear memory retrieval test. Inactivation of PL-mPFC also reduces recent delay fear memory expression (Corcoran and Quirk, 2007; Sierra-Mercado et al., 2011). In contrast, microstimulation of PL-mPFC increases fear expression when a delay fear conditioned cue is presented, whereas PL-mPFC microstimulation alone does not induce fear (Vidal-Gonzalez et al., 2006). Finally, PL-mPFC, neurons exhibit sustained tone responses during presentation of a recently fear-conditioned cue, and this firing correlates with delay fear expression (Burgos-Robles et al., 2009). Thus, these data are consistent with the idea that β-AR signaling in PL-mPFC neurons support the neural activity required for recent delay fear memory retrieval.

PL-mPFC β-AR blockade reduced fear expression during a remote trace fear memory retrieval test, but not during a recent trace fear memory retrieval test. Although much evidence supports the involvement of PL-mPFC for trace fear conditioning (Baeg

et al., 2001; Gilmartin and McEchron, 2005; Gilmartin and Helmstetter, 2010; Guimarais et al., 2011; Gilmartin et al., 2013), only one recent study has examined the necessity of PL-mPFC for expression of a recent and remote trace fear memories. Beeman and colleagues (2013) found that lesions made 30d following conditioning reduces freezing during a remote trace fear memory retrieval test. In contrast, lesions made 1d following conditioning had no effect on freezing during a recent trace fear memory retrieval test (Beeman et al., 2013). Overall, these data support the conclusion that PL-mPFC β-AR signaling is required for remote trace fear memory retrieval, but not recent trace fear memory retrieval. The mechanism by which this PL-mPFC β-AR activation allows subsequent reinstatement of the trace fear memory, however, is unclear.

Results reveal that fear memory retrieval is not susceptible to memory retrieval impairments by PL-mPFC β-AR blockade. However, unpublished data from several labs indicate that fear memories are susceptible to memory retrieval impairments. For example, oral administration of a β-AR antagonist persistently reduces cue-induced fear expression and subsequent reinstatement in humans (Kroes et al, 2012 *SfN Abstracts*). Furthermore, pharmacological or optogenetic inactivation of paraventricular thalamic neurons reduces delay fear memory retrieval in rodents during a retrieval test and during a subsequent manipulation-free test (Do-Monte et al, 2013 *SfN Abstracts*). Taken together, fear memories can be persistently impaired during retrieval. Despite this, future experiments need to be performed to determine the particular mechanisms that maintain fear memory retrieval.

Aim 2

Experiments described in aim 2 reveal the mechanism of memory retrieval impairments. Cocaine conditioning increased the intrinsic excitability of PL-mPFC pyramidal neurons, particularly in rats expressing high CPP memory retrieval. Furthermore, cocaine conditioning increased sEPSC frequency and induced paired-pulse facilitation in PL-mPFC pyramidal neurons, indicative of presynaptic potentiation. Cocaine conditioning also increased sEPSC amplitude and AMPA:NMDA ratios, indicative of postsynaptic potentiation. Finally, β-AR blockade during but not after a CPP test induced persistent impairments in CPP memory retrieval and reversed modifications in sEPSC frequency, sEPSC amplitude, PPR, and AMPA:NMDA ratios. In contrast, β-AR blockade during memory retrieval did not reverse modifications in intrinsic neuronal excitability. Taken together, CPP memory retrieval impairments are likely due to reversal of cocaine-related synaptic potentiation in PL-mPFC pyramidal neurons.

Intrinsic plasticity

Cocaine conditioning increased the excitability of PL-mPFC pyramidal neurons in rats expressing high CPP memory retrieval but not low CPP memory retrieval. Furthermore, PL-mPFC neuronal excitability positively correlated with CPP expression, unless the β-AR antagonist propranolol was administered during memory retrieval. These data are consistent with previous observations that cocaine increases the excitability of neurons in mPFC. Repeated systemic administration of cocaine decreases conductance of voltage-gated K^+ channels (mainly slowly inactivating (I_D) K^+ channels),

resulting in enhanced membrane excitability (Dong et al., 2005; Nasif et al., 2005b). Furthermore, repeated cocaine increases voltage-gated Ca^{2+} currents (I_C) via enhanced conductance of high-voltage activated (HVA) L-type Ca^{2+} channels (Nasif et al., 2005a; Ford et al., 2009). Unlike other HVA Ca^{2+} channels that control medium and slow-afterhyperpolarization, HVA L-type Ca^{2+} channels promote repetitive firing by reducing the interspike interval of neocortical pyramidal neurons (Pineda et al., 1998). Thus, enhanced L-type Ca^{2+} currents may contribute to the enhancement of PL-mPFC pyramidal neuron excitability following repeated cocaine exposure, particularly at high voltages which induces repetitive firing of these neurons. To assess this, future analysis should be completed to assess the interspike interval of PL-mPFC neurons in cocaine-conditioned rats versus naïve rats. If HVA L-type Ca^{2+} channels contribute to cocaine-induced enhancement of PL-mPFC neuronal excitability, the interspike interval of these neurons should be reduced in neurons from high retrieval rats but not low retrieval rats.

Synaptic plasticity

Cocaine conditioning induced presynaptic and postsynaptic potentiation in PL-mPFC pyramidal neurons. These data are consistent with investigations characterizing dendritic morphology of these neurons following exposure to psychostimulants. Systemic injections of amphetamine and cocaine, for example, increase dendritic length, dendritic branching, and dendritic spine density in dorsal mPFC pyramidal neurons for at least one month (Robinson and Kolb, 1997, 1999). Similarly, cocaine self-administration increased dendritic branching and dendritic spine density in dorsal mPFC pyramidal neurons (Robinson et al., 2001). These findings paralleled dendritic spine plasticity in

NAc medium spiny neurons (Robinson and Kolb, 1997, 1999; Robinson et al., 2001), which receive input from PL-mPFC neurons for expression of drug seeking (McFarland et al., 2003; Peters et al., 2009). Thus, long-lasting neuroadaptations within this corticolimbic circuit may mediate drug-associated memory retrieval for the persistence of drug seeking

Recent experiments using 2-photon microscopy *in vivo* confirm an increase in PL-mPFC dendritic spine gain, and reveal no change in dendritic spine loss, in layer V dorsal mPFC pyramidal neurons 2h and 96h following cocaine exposure (Munoz-Cuevas et al., 2013). Moreover, these experiments reveal that PL-mPFC dendritic spine gain positively correlates cocaine CPP expression. Thus, dendritic spine plasticity within layer V PL-mPFC pyramidal neurons is a likely mechanism for cocaine-associated memory retrieval. Data described here support this idea, revealing that cocaine conditioning induces both presynaptic and postsynaptic plasticity within layer V PL-mPFC pyramidal neurons. Moreover, we found that β-AR blockade during CPP memory retrieval reversed this plasticity and prevented drug-associated memory retrieval. Thus, dendritic/synaptic potentiation in PL-mPFC may be essential for cue-induced drug seeking. The mechanism by which β-AR blockade reverses this plasticity, however, is less clear.

Mechanism

β-AR blockade induced a persistent impairment in retrieval of a cocaine-induced CPP memory. This retrieval impairment could be in part due to transient limitation of PL-mPFC intrinsic neuronal excitability. Presentation of salient stimuli causes activation of locus coeruleus noradrenergic neurons (Sterpenich et al., 2006) and provokes

norepinephrine release (Cassens et al., 1980). Norepinephrine enhances the excitability of PL-mPFC pyramidal neurons, and this excitation is blocked by β-AR inhibition (Otis et al., 2013). Thus, β-AR blockade prevents norepinephrine-induced enhancement of PL-mPFC neuronal excitability during memory retrieval (Rodriguez-Romaguera et al., 2009), indicating that β-AR-dependent potentiation of intrinsic excitability may maintain retrieval. In support of this, unpublished data from our lab reveal that PL-mPFC protein kinase A (PKA)-induced inhibition of Ca^{2+}-activated K^{+} channels maintains retrieval (Fitzgerald et al, *unpublished*). This cascade is downstream of β-ARs, and is the mechanism by which β-AR activation increases neuronal excitability (Foehring et al., 1989; Mueller et al., 2008). Taken together, both CPP conditioning and cue-induced β-AR activation increase PL-mPFC pyramidal neuron excitability, and this neuronal excitation is required for CPP memory retrieval. Despite this, data here reveal that excitability of PL-mPFC neurons remain increased after β-AR blockade during retrieval. Thus, long-lasting cocaine CPP memory retrieval impairments are not due to long-lasting reversal of PL-mPFC intrinsic neuronal excitability.

Cocaine CPP conditioning induced intrinsic neuronal plasticity in PL-mPFC neurons, and this plasticity may function in unison with synaptic plasticity for the control of cocaine CPP memory retrieval. Previous research reveals dendritic spine growth in layer V PL-mPFC pyramidal following cocaine CPP conditioning, and this spine growth correlates with CPP memory retrieval (Munoz-Cuevas et al., 2013). Taken together with data shown here, PL-mPFC synaptic potentiation likely controls cocaine-associated memory retrieval. In further support of this, memory retrieval impairments induced by β-AR blockade were associated with reversal of synaptic potentiation in PL-mPFC. These

data indicate that β-AR activation during memory retrieval is critical for maintenance of retrieval-related synaptic plasticity. Despite this, the mechanism underlying reversal of synaptic plasticity by β-AR blockade during retrieval is unclear.

The data described here are consistent with the hypothesis that β-AR activation maintains cocaine-associated memory retrieval by synchronizing PL-mPFC synaptic input with postsynaptic activation of PL-mPFC pyramidal neurons. Specifically, intrinsic excitability of PL-mPFC neurons is increased during cocaine CPP memory retrieval, indicating that PL-mPFC neurons are more responsive to excitatory inputs. In contrast, presynaptic input in the absence of synchronous postsynaptic action potentials can induce synaptic depression (Froemke and Dan, 2002; Froemke et al., 2005). Thus, PL-mPFC β-AR activation may maintain synchronous presynaptic and postsynaptic activity during retrieval, whereas disruption of this synchrony likely weakens PL-mPFC synapses. Consistent with this hypothesis, NE prevents spike-timing dependent depression (STDD) at cortical synapses (Salgado et al., 2012). Thus, β-AR blockade during retrieval may induce PL-mPFC synaptic depression by causing neural asynchrony, resulting in long-term retrieval impairments.

Future Directions

Reversal of PL-mPFC synaptic potentiation underlies cocaine-associated memory retrieval deficits, although the neural circuits underlying memory retrieval are not well-defined. To assess the neural circuits of memory retrieval, genetic approaches can be used to tag and manipulate the retrieval circuit both *in vivo* and *in vitro.* For example, I plan to use *arc*-tTA transgenic mice (similar to Liu et al., 2014) along with

adenoantivirus transfections (AAV-Chr2-mCherry or AAV-eArchT-mCherry) to allow region-specific, activity-dependent tagging of neurons. Considering that channelrhodopsin or archaerhodopsin are inserted within the adenoantivirus, this technology would allow 1) tagging of neurons that become active during memory retrieval, 2) optogenetic manipulation of these neurons *in vivo*, and 3) electrophysiological characterization of these neurons *in vitro*. Thus, using a combination of behavioral, genetic, and electrophysiological approaches the neural circuits required for fear and drug-associated memory retrieval can be well defined.

Evidence described here indicates that PL-mPFC layer V pyramidal neurons regulate memory retrieval. However, activity of these neurons is not only influenced by synaptic inputs to PL-mPFC, but also by PL-mPFC interneurons. For example, convincing evidence from Cyril Herry's lab now indicate that PL-mPFC parvalbumin-positive interneurons coordinate theta oscillations for the control of fear expression (Courtin et al., 2014). Thus, monitoring the coordinated activity of many genetically-defined neurons would allow us to define how different types of neurons coordinate activity within particular brain regions for memory retrieval. Such experiments could be conducted using 2-photon ultrasensitive fluorescent calcium imaging (GCaMP6.0; Chen et al., 2013). Using this technique along with genetic labeling of particular neurons (e.g., parvalbumin versus somatostatin interneurons), the neuronal assemblies which coordinate activity within a given brain region for memory retrieval could be determined.

Although PL-mPFC β-AR blockade does not persistently impair fear memory retrieval, evidence indicates that fear memory retrieval is susceptible to persistent disruption (Kroes et al, 2012 *SfN Abstracts*; Do-Monte et al, 2013 *SfN Abstracts*). Thus,

future experiments should identify how fear memory retrieval is maintained. For example, Do-Monte and colleagues (2013, *SfN Abstracts*) found that optogenetic inhibition of pavaraventricular thalamic inputs to the central amygdala (PVT-CeA) persistently reduces remote delay fear memory retrieval. However, the mechanism by which the PVT-CeA pathway maintains retrieval is completely unknown. To solve this, experiments using optogenetic and electrophysiological approaches should be conducted. For example, fear memory retrieval impairments could be induced via optogenetic inhibition of the PVT-CeA pathway *in vivo.* Next, intrinsic and synaptic recordings of CeA neurons can be obtained *in vitro*, with EPSCs evoked via optogenetic stimulation of the PVT-CeA pathway. Based on the findings presented here, synaptic depotentiation of PVT-CeA synapses may account for fear memory retrieval impairments. Findings such as these would confirm that reversal of memory-related synaptic plasticity can occur during both fear memory retrieval and cocaine-associated memory retrieval.

Clinical Relevance

Presentation of drug-associated cues leads to cravings and relapse among addicts, whereas presentation of trauma-related cues can provoke anxiety and fear in PTSD patients. Disruption of fear or drug-associated memory retrieval would therefore alleviate these disorders. Data described here reveal for the first time that memory-related synaptic plasticity is maintained by neuronal activity during retrieval. Although future experiments should further elucidate the mechanisms and neural circuits that maintain synaptic plasticity during retrieval, these data provide the framework for development of therapies that could lead to elimination of cue-induced drug seeking and

fear. Currently, exposure therapy involves the repeated, unreinforced presentation of drug or fear-related cues, inducing retrieval and extinction learning. Although extinction leads to inhibition of cue-induced behaviors, spontaneous recovery and reinstatement of those behaviors is common. Data described here reveal that a more direct approach of reversing memory-related synaptic plasticity during retrieval (i.e., during exposure therapy) may be possible. Such effects would not only eliminate cue-induced behaviors, but would also provide long-lasting protection against spontaneous recovery and reinstatement. Taken together, our findings support the use of pharmacological adjuncts to exposure therapy, such as β-AR antagonists, for persistent impairment of fear and drug-associated memory retrieval.

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Otis, J.M., Fitzgerald, M.K., and Mueller, D. **(2014)**. Inhibition of hippocampal β-adrenergic receptors impairs retrieval but not reconsolidation of cocaine-associated memory and prevents subsequent reinstatement. ***Neuropsychopharmacology***, 39: 303-10. **(Impact Factor, 8.68).**

Otis, J.M., Dashew, K.B., and Mueller, D. **(2013)**. Neurobiological dissociation of retrieval and reconsolidation of cocaine-associated memory. ***Journal of Neuroscience***, 33: 1271-81. (**Impact Factor, 6.91).**

Otis, J.M. and Mueller, D. **(2011).** Inhibition of β-adrenergic receptors induces a persistent deficit in retrieval of a cocaine-associated memory providing protection against reinstatement. ***Neuropsychopharmacology***, 36: 1912-20. **(Impact Factor, 8.68).**

UNPUBLISHED MANUSCRIPTS

Otis, J.M., Werner, C.T., and Mueller, D. **(*in revision*)**. Noradrenergic regulation of fear and drug-associated memory reconsolidation.

Otis, J.M., Drake., M., and Mueller, D. **(*in preparation*)**. Prefrontal cocaine-related synaptic plasticity is reversible during memory retrieval.

Otis, J.M., Fitzgerald, M.K., Burkard, J., Drake., M., and Mueller, D. **(*in preparation*)**. Prelimbic beta-adrenergic receptor blockade during trace fear memory retrieval reduces fear and prevents reinstatement after extinction.

Otis, J.M. and Mueller, D. **(*in preparation*)**. Neurobiological mechanisms of memory retrieval.

Fitzgerald, M.K., **Otis, J.M.**, and Mueller D. ***(in preparation)***. Distinct roles for β1 and β2-adrenergic receptors in retrieval and reconsolidation of cocaine-associated memory.

HONORS AND AWARDS

2014	Association of Graduate Students in Psychology Award, UWM
2014	Graduate Students in Neuroscience Grant, UWM
2013-2014	Distinguished Dissertation Fellowship, UWM
2013	Summer Research Fellowship, UWM
2013	Psychology Graduate Research Award, UWM
2013	Society for Neuroscience Abstract Award (Hot Topic)
2013	Graduate Students in Neuroscience Grant, UWM
2013	Graduate Student Travel Award, UWM
2012	Invited Talk and Honorarium, UWS (Funded by NSF)
2012	Society for Neuroscience Abstract Award (Hot Topic)
2012	Graduate Students in Neuroscience Grant, UWM
2012	Graduate Student Travel Award, UWM
2011	Milwaukee Society for Neuroscience Presentation Award
2011	Graduate Students in Neuroscience Grant, UWM
2011	Graduate Student Travel Award, UWM
2010	Society for Neuroscience Abstract (1) Award (Hot Topic)
2010	Society for Neuroscience Abstract (2) Nomination (Hot Topic)
2010	Association of Graduate Students in Psychology Award, UWM
2010-2011	Chancellor's Graduate Student Award, UWM
2009-2010	Chancellor's Graduate Student Award, UWM

POSITIONS HELD

2014-2015	Post-Doctoral Research Associate, University of North Carolina
2014	Elected President, Graduate Students in Neuroscience, UW-Milwaukee
2013	Elected President, Graduate Students in Neuroscience, UW-Milwaukee
2012	Elected President, Graduate Students in Neuroscience, UW-Milwaukee
2011	Elected President, Graduate Students in Neuroscience, UW-Milwaukee
2008-2009	Intern, Planning and Research Quality Office (PARQ), UW-Stout

TEACHING EXPERIENCE

2012, 2013 Summer	Physiological Psychology (Lecturer)
2012 Spring	Physiological Psychology (Teaching Assistant)
2011, 2013 Spring	Advanced Physiological Psychology (Teaching Assistant)
2010, 2011, 2012 Fall	Psychological Statistics (Teaching Assistant)

MEMBERSHIPS

2013-Present	Member, Sigma Xi
2009-Present	Member, Society for Neuroscience
2009-Present	Member, Molecular and Cellular Cognition Society
2009-Present	Member, Graduate Students in Neuroscience, UW-Milwaukee
2008-2009	Member, C-NERVE, University of Wisconsin-Stout

SELECTED MEDIA RECOGNITION

2012 EurekAlert (NIH), Science Daily, UWM Post (cover)
2011 EurekAlert (NIH), Science Daily
2010 EurekAlert (NIH), Science Daily