DISRUPTING METHAMPHETAMINE ASSOCIATED MEMORY BY TARGETING SYNAPTIC DYNAMICS

By
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This thesis was prepared under the direction of the candidate’s research advisors Dr. Courtney Miller and Dr. Erica Young and the candidate’s thesis advisor, Dr. Nicholas Quintyne, and has been approved by the members of the supervisory committee. It was submitted to the faculty of The Honors College and was accepted in partial fulfillment of the requirements for the degree of Bachelor of Arts in Liberal Arts and Sciences.

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Abstract

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Title: Disrupting Methamphetamine Associated Contextual Memory by Targeting Synaptic Dynamics

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Methamphetamine (METH) is addictive and associated with a high rate of relapse. One relapse trigger is re-experiencing drug-associated contextual associations. Therefore it is possible that, by targeting METH-associated contextual memories, drug seeking behavior can be inhibited. Recent evidence has suggested that memory formation relies on actin polymerization, which allows dendritic spines to undergo structural and functional plasticity, key components of memory. To see if actin polymerization could be a target for the extinction of METH seeking memories we inhibited actin polymerization in animals that had been trained in either METH or food associated conditioned place preference. Pretest inhibition of actin cycling in the basolateral amygdala complex produced immediate and persistent extinction of METH seeking behavior. Additionally, inhibiting actin polymerization 24hrs before testing disrupted seeking behavior for METH but not food. These results indicate that METH-associated memories are selectively vulnerable to disruption through inhibition of actin dynamics.
Dedication

To Mom and Dad
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Introduction

Methamphetamine (METH) abuse has become one of the leading drug problems in the US. Each year METH addiction costs the country millions of dollars in state mandated rehabilitation, law enforcement, and other related areas (Nicosia et al., 2005; NACo, 2007; Nicosia et al., 2009). METH relapse after rehab treatment occurs in 9 out of 10 people within a year after leaving rehab. There are 3 main triggers for relapse: small doses of METH, stressful situations, and drug associated environmental cues (Sinha, 2011). In an effort to prevent relapse, rehab centers primarily utilize behavioral modification therapy which successfully helps addicts develop strategies for avoiding drugs and managing stress. However, relapse triggered by drug-associated environmental cues has proved difficult to treat with therapy due to their sometimes abstract nature. In order to help prevent these drug cravings, scientists are now looking into pharmacological treatments to try and attenuate the METH-related contextual memories. Presentation of these contextual associations not only elicits craving for METH leading to a relapse they also activate brain areas involved with emotional memory, which can hopefully be targeted to inhibit drug seeking (Childress, et al., 1999). The goal of the experiments presented here is to identify potential pharmacological therapies that would be combined with therapy to prevent drug relapses.

Many of the cognitive effects of psychostimulate drugs, including METH, are caused by the release of massive amounts of catecholamines, dopamine, norepinephrine and serotonin in the mesolimbic system of the brain (Routtenberg 1988). The mesolimbic system is comprised of the ventral tegmental area (VTA), nucleus accumbens (NAc),
amygdala (AMY), hippocampus (HIPP) and the bed nucleus of the stria terminalis and is involved in mediating behavioral drives of positive reinforcements, emotional moods, avoidance behavior, appetite drives, the processes of learning and memory, and sexual drives (Goldstein, 1989; Kalant, 1989; Watson et al., 1989). Once in the brain, METH causes a reversal of dopamine, serotonin, and norepinephrine transporters and blocks future reuptake of these molecules. Therefore there are elevated levels of catecholamines present in the synaptic cleft for a prolonged period of time. The elevated levels of dopamine are responsible for producing the feelings of euphoria associated with METH use (Thompson et al., 2004). The massive release of dopamine and serotonin in these mesolimbic areas also elicits a strong drive for METH seeking, which is one of the main characteristics of addiction. However, the body tries to maintain homeostasis, therefore the brain adapts to the massive release of catecholamines in the limbic system by reducing the amount dopamine receptors or producing less dopamine. This lessens the positive reward and euphoria associated with drug use and other rewarding activities (NIH, 2011). This forces addicts to seek more and more drug, since it is now the only way to feel the same level of euphoria (NIH, 2011). Because drug-related levels of catecholamines become the new baseline level withdrawal from drug can cause depression, anxiety, craving for the drug, and even suicide. In order to feel the euphoria associated with drug use and avoid withdrawal users continue to take the drug despite all the negative reinforcements (Wikler & Pescor, 1967; Hutcheson et al., 2001). An emerging view of addiction that is being adopted in hopes of providing new directions for development of therapeutic is that addiction can be considered a maladaptive memory (Koenigs & Grafman, 2009; Bellani et al., 2011).
Powerful associative memories are formed between environmental cues, such as context, sounds and smells, and positive reinforcements, such as a METH injection, to drive seeking behavior. One region necessary for the formation, maintenance and retrieval of associative memories is the AMY (Boeing, 2001; Koob & LeMoral, 1997; Nestler & Aghajanian, 1997; LeDoux, 1990; McGaugh 1966; LeDoux, 2000; Raganath, 2004). Many consider the main function of the AMY to be governing flight or fight responses to stressful or fearful situations. For example, people with a disease called Urbach-Wiethe, a disease with lesions in their basolateral amygdala, do not display the appropriate fear responses to innately fearful situations (Brand et al., 2007). Animal studies investigating the involvement of the AMY with aversive memories often consist of pairing neutral conditioned stimuli (CS), such as auditory tones and contextual cues, with an innately arousing unconditioned stimulus, such exposure to predators, or mild shocks to feet or eyelids, and then measuring the resulting fear expression when presented with shock-associated cues (McGaugh 1966; Blanchard & Blanchard, 1969; LeDoux, 1990; Hugues, 2004). Inputs about these stimuli and peripheral responses converge in the AMY during acquisition and consolidation (LeDoux, 2000). Additionally, lesions to lateral AMY following fear conditioning have led to an attenuation of fear expression when presented with the shock-paired auditory cue (LeDoux, 1990). While the basolateral complex (BLC) of the amygdala, which includes the basolateral and lateral amygdala, is mainly associated with aversive memory formation it is also plays key part of reward-stimulus memory formation. For example, BLC lesions prevent the acquisition and consolidation of drug-associated condition place preference (CPP) memories (Robinson & Berridge, 1993; Robinson & Berridge, 2008;
Kosten et al., 2005; Miller & Marshall, 2004). In addition to appetitive memory formation, the BLC is also necessary for drug associated reward-stimulus memory. Studies have shown that lesions in the BLC have disrupted the acquisition and consolidation of cocaine (Yun & Fields, 2003) and amphetamine (Hiori et al., 1991) contextual memories in rats. These results indicate that the BLC has a major role acquiring and consolidating all types of emotional memories, including fear, appetitive and drug associative memories (Schroeder & Packard, 1998; Hatfield et al., 1999). Additionally, it has also been seen in withdrawal studies in rats that the BLC also promotes drug seeking to prevent the side effects of withdrawal (Tran-Nguyen et al., 1997; Fuchs et al., 2006; Gussakov et al., 2006; Fuchs et al., 2007; Fuchs et al., 2009). It has been suggested that drug addiction can be treated as a maladaptive memory, therefore given the involvement of the BLC with emotional memory it is a great candidate for investigating drug-associated memories.

In order to investigate the mechanisms that underlie associative memory it can be broken into different stages: acquisition, consolidation, maintenance, retrieval and reconsolidation. Acquisition is the learning portion of memory formation where an organism learns to associate a previously neutral cue with an innately arousing cue (Johansen et al., 2011). After a learning event takes place the association undergoes consolidation to form a long term memory (LTM) (Morris, 2006). Consolidation consists of changes at the cellular and molecular level that are driven by protein synthesis to strengthen the formation of a memory (Dudai, 2002; Rodrigues et al., 2004; Morris, 2006). Consolidation is thought to occur minutes to hours after acquisition. One aspect on
consolidation that is gaining recognition for being critical for memory formation is synaptic plasticity. Long term potentiation (LTP), one of the defining characteristics of memory consolidation, has been shown to elicit plasticity in dendritic spines located in the AMY (Blair et al., 2001) and the Hippocampus (Bliss & Lomo, 1973). For example, following memory formation new dendritic spines have been observed within the learning centers of the brain, such as the AMY, HIPP, and NAc (Matus, 2000; Morris, 2006; Bourne & Harris, 2008; Russo et al., 2010). Acquisition and consolidation of memories has been shown to be critically dependent on plasticity mechanisms in synapses. There is some emerging evidence that after consolidation there are cellular mechanisms that maintain (Sikhar, 2011). When the brain retrieves the memories by re-exposure to memory-related cues the memory circuit formed during learning is reactivated, followed by reconsolidation during which the memory can be strengthened or weakened (Ranganath et al., 2004; Fuchs et al., 2009).

Plasticity is an important characteristic for excitatory synapses because it allows for the strengthening and weakening of connections during memory formation and extinction. Most excitatory synapses are located on dendritic spines in the basal ganglia, the cortex, and the cerebellum (Kasai, et al., 2010). Functional and volumetric changes in the dendritic spine are critical for long-term memory (Yang et al., 2009; Rex et al., 2010; Lai et al., 2012). Plasticity that occurs following LTP is dependent on actin polymerization in the dendritic head to extend the membrane increasing its volume (Matsuzaki, et al., 2004). The actin polymerization in the spines occurs immediately within 3min of LTP (Gustafsson & Wigstrom, 1990; Honkura et al., 2008; Rex et al.,
The spine enlargement facilitates functional changes that allow for the stabilization of the synapses through the insertion of receptors and synapse binding proteins, the main one being AMPA receptors (Kessels & Malinow, 2009). AMPA receptors incorporate into the plasma membrane by connecting to post synaptic density (PSD) proteins in an actin-dependent manner increasing overall PSD (Lisman & Zhabotinsky, 2001; Heine et al, 2008; Kato et al., 2008; Makino & Malinow, 2009). During LTP, receptors are added whereas during LTD AMPA receptors are removed via endocytosis from the plasma membrane causing a change in the size of the PSD of the dendritic spine (Kessels and Malinow, 2009). The change in protein expression on the spine leads to a functional change and a stronger synapse (Kasai, et al., 2010). These functional and morphological changes associated with synaptic plasticity are dependent on the polymerization of the actin cytoskeleton.

Since actin polymerization is such a crucial part of synaptic strengthening, the mechanisms mediating polymerization has been investigated as a method to disrupt memory retrieval. Actin polymerization is tightly regulated process within dendrites that starts with the exposure of the NMDA receptor to glutamate and glycine (Kleckner & Dingledine, 1988). Following NMDA receptor activation Ca^{2+} fluxes in dendritic spines which initiate series of Rho GTPases which in turn modulate actin polymerization mediators, such as profilin, cofilin and Arp2/3 (Murakoshi et al., 2001; Ethell & Pasquale, 2005). Actin polymerization is tightly regulated therefore there are many targets available for manipulation to disrupt memories, particularly drug-associated contextual memories.
This series of experiments was performed to determine the role of BLC spine dynamics during drug seeking and whether spine dynamics could be targeted to disrupt drug relapse. To this end we trained animals in either METH- or sucrose-associated CPP and then inhibited actin polymerization prior to testing. The goal of these experiments is to discover possible future targets for pharmacotherapies.
Materials and Methods

Animals

Adult male Sprague Dawley rats weighing 275-350 grams were obtained from Charles River Laboratories. Rats were housed under 12:12 light/dark cycles. Food and water were available ad libitum for animals assigned to methamphetamine CPP experiments. Animals assigned to the CPP for food reward were food restricted to 90% of their ad libitum weight. All animal procedures were performed in accordance with the Scripps Research Institute Animal Care and Use Committee and national regulations and policies. All animals were handled for 3-5 days prior to the behavioral conditioning.

Cannulae Implantation

Rats were anesthetized with dexmedetomidine (0.25mg/kg, IM) and ketamine (0.5mg/kg, IM) and secured in a Kopf stereotaxic apparatus during stereotaxic implantation of cannula. Bilateral stainless steel guide cannulae (26G, Plastics One) were targeted for placement 2mm above the basolateral complex of the amygdala (BLC; AP: -2.9mm, ML: ±5.0 relative to bregma; DV: -6.7 mm from skull; Paxinos and Watson, 2007). Immediately after surgery, buprenex (0.05ml, SC) was administered. Clearance through the guide cannulae was maintained with dummy cannulae (Plastics One) cut to project 1 mm beyond the tip of the guide. Animals were allowed 5d of recovery and handling before the start of any behavioral conditioning. Rats were habituated to dummy cannulae removal during this time.
Infusions

All infusions were delivered at a rate of 0.25 mL/min for 2 min. Infusers were left in place for 1 min following the infusion to allow for diffusion of the drug away from needle tips. To ensure accurate cannulae placement, brains were collected after completion of behavioral testing. Cannulated regions of the BLC were sliced into 40 mm sections using a microtome, and stained with cresyl violet to verify the location of the infusion needle tips. Only those animals with verified placement of infusion needle tips into the BLC were included in the statistical analyses of behavioral data (Figure 1).

Drugs

Methamphetamine hydrochloride (Sigma-Aldrich) was delivered IP at 1.0mg/kg. The concentration of Latrunculin A (Calbiochem) used for in vivo infusions was 25 ng/mL (12.5 ng of LatA per BLC). LatA was prepared in a 2% DMSO/saline vehicle at a concentration of 60 mM. 2% DMSO/saline was used as the vehicle for LatA experiments.

Behavioral Procedures

Conditioned Place Preference Apparatus

Animals were trained using an unbiased conditioned place preference (CPP) procedure. Conditioning took place in a three-chamber apparatus (Med Associates) consisting of two larger compartments (29 cm × 25 cm) separated by a smaller compartment (11 cm × 25 cm). Doors dropped in between the large and small compartments that sealed them off. The large containers had either black or white walls. The black wall compartment
contained a metal bar floor paired with a peppermint scent in Methamphetamine related experiments, and almond scent in food related experiments. The white container contains a metal grid floor paired with a scent of vanilla scent.

**Condition Place Preference Procedure**

The CPP protocol consisted of three phases: Pretesting, training and testing. Pretesting involved allowing the animals to freely roam in all three containers for fifteen minutes to get a baseline preference of the animals. The time spent in each container was recorded by a computer. During training, animals received a 1mg/kg METH injection prior to a 30min confinement to the CS+ chamber (counterbalanced between chambers within groups). On alternating days, rats were injected with an equivalent volume of 0.9% saline and confined to the opposite chamber for 30min. Training consisting of 3 METH pairings took place over the next 6 days. Additionally, the starting treatment (METH or saline) was also counterbalanced within groups. Intra-BLC infusions of LatA were administered 15min or 24hrs prior to testing. Testing began 48hrs after the final conditioning was performed and consisted of free access to the CPP apparatus for 15min. Animals underwent 1 to 4 days of testing but only received 1 intra-BLC infusion prior to the first test.

The food CPP protocol was consistent with the METH CPP except for the CS+ and CS-. The CS+ was 50 pieces of fruit loops in the lower right hand corner of the black chamber; while CS- did not have any food and was associated with the white chamber.
Additionally the black chamber was paired with almond instead of peppermint. LatA was administered to the animals 24hrs before the final preference test.

**Statistical analysis**

One-way analysis of variance and repeated measures ANOVAs were used to analyze all behavioral data, with post hoc tests performed when appropriate.
Results

These experiments were performed to determine whether dynamic actin in the amygdala is necessary for the expression of METH-associated memories. As mentioned earlier, exposure to environmental cues previously associated with drug use can elicit motivation to seek out that drug, through activation of multiple limbic brain regions including the amygdala (Jaffe, 1990; Tiffany, 1990; Robinson & Berridge, 1993; Grant et al., 1996; Childress et al., 1999; Kosten et al., 2005). In animals, one paradigm designed to examine context-induced drug seeking is condition place preference (CPP; Bardo & Bevins, 2000). An additional feature of CPP that made it attractive for use in these experiments is that CPP memories are supported by multiple areas of the brain that includes the BLC of the amygdala (Everitt et al., 1991; Hiroi & White, 1991; Brown & Fibiger, 1993; Miller & Marshall, 2004, 2005a). Therefore actin polymerization in the BLC was targeted for inhibition by Latrunculin A (LatA). LatA inhibits actin polymerization by binding to monomeric G-actin and prevents the monomer from being attached to F-actin (Coué, et al., 1987; Yarmola, 2000; Morton 2000). For CPP training rats were trained to associate one chamber of the CPP apparatus with I.P. METH injections (CS+), and the other with saline injections (CS-). Two days after the CPP training to associate METH with CS+ rats received an infusion of either vehicle or LatA in the BLC fifteen minutes before final CPP testing. The tests went on for four days, after the LatA infusion (Figure 2) The vehicle animals showed a significant preference for the CS+ chamber whereas the LatA animals, surprisingly, displayed an immediately disruption of CPP expression during Test 1 (Veh: Pretest vs T1, $P < 0.05$; LatA: Pretest
vs T1 \( P > 0.05 \)). This trend continued on throughout all training days (Overall: Treatment x Test \( F_{(4,17)} = 3.07, P < 0.05 \); Veh: \( F_{(4,9)} = 8.13, P < 0.0001 \); LatA: \( F_{(4,8)} = 0.45, P > 0.05 \)).

These data indicate that even though consolidation has taken place METH-associated memories are susceptible to immediate and lasting disruption by depolymerization of actin days after training. This was a surprising result since it has been shown that depolymerization of actin only disrupts fear expression when injected 30min prior to training (Rex et al., 2010; Gavin et al., 2012). When actin polymerization was inhibited prior to fear testing did not stop the retrieval of the auditory cued fear memory (Rex et al., 2010; Gavin et al., 2012). So the surprising part of this experiment was that LatA worked 15 minutes prior to testing, which could indicate that actin polymerization is necessary for the maintenance of METH-related memories.

To address whether expression of METH-associated memories are dependent on memory maintenance processes rather than retrieval we inhibited actin polymerization 24hrs prior to testing. Sacktor and colleagues (YEAR?) showed through inhibition studies of PKMζ, by ZIP, that cellular mechanisms are used to actively maintain memories. However, it has been shown that PKMζ null rats still have learning and memory so the targets of ZIP that disrupted memory have been called into question. Even though PKMζ might not have a hand in memory maintenance it is still possible that other cellular mechanisms underlying memory maintenance are affected by ZIP (Lee et al., 2013; Volk et al., 2013). The vehicle animals showed a significant difference in the time they spent in the CS+ chamber compared to the C- chamber whereas the LatA animals showed no preference for either chamber (Figure 3; \( F_{(3,24)} = 4.11, P < 0.05 \); Veh: CS- vs CS+, \( P < 0.005 \); LatA: CS- vs CS+, \( P > 0.05 \)). This result indicates that it is possible that
consolidated METH-associated memories causes actin to enter a state of continuous cycling without retrieval of the memory, pointing toward the possibility that actin polymerization is necessary during memory maintenance to support METH-associated memories.

One possible explanation for the effects of depolymerized actin on drug seeking is that the actin dynamics underlying memories for positive reinforcers may be different than those underlying fear memories. To test this, we inhibited actin polymerization during the memory maintenance phase and then tested the animals’ food seeking behavior. During food CPP training, we associated the CS+ with a high sucrose based food, fruit loops, and while the CS- container contained no food. After sucrose training, we infused either vehicle or LatA into the BLC 24 hours before accessing memory for food-associated place preference. When tested for expression of food-associated CPP both the Veh and the LatA animals showed a significant preference for the CS+ chamber (Figure 4; $F_{(3,20)} = 3.72, P > 0.05$; Veh: CS- vs CS+ $P < 0.05$; LatA: CS- vs CS+ $P < 0.05$). This result illustrates that even though food and METH are both positive reinforcers, expression of food-seeking memories is not dependent on actin polymerization in the BLC. These experiments seem to indicate that METH-associated memories are selectively vulnerable to disruption by depolymerization of actin whereas food- and fear-associated memories are not.
Discussion

Methamphetamine addiction boasts the highest relapse rate amongst drug addicts (Sinha, 2011), having almost a 90% relapse rate after 1 year of rehabilitation. This leads to many economic costs for the US such as government mandated rehabilitation and increased law enforcement (Nicosia, 2009). Currently the best treatment for METH addicts is behavioral modification therapy which focuses on development of strategies to avoid drug use, manage stress and avoid blatant drug-associated cues. However, drug craving elicited by exposure to drug-associated environmental cues remains difficult to treat due to the abstract nature of some contextual cues. Environmental cues can elicit drug craving by activating parts of the limbic system, such as the AMY (Childress, 1999). In this thesis we tested whether memory associated mechanisms that underlie drug-associated contextual memories could be targeted to prevent drug seeking behavior.

The AMY, specifically the BLC, plays a major role in storing emotional memories in the brain. Among other things the AMY mediates fight or flight responses (LeDoux, 2000; Brand et al., 2007), supports both associative memories for both positive and negative reinforcers (Robinson & Berridge, 1993; Kosten et al., 2006; Robinson & Berridge, 2008), and also drives drug seeking (Tran-Nguyen et al., 1997; Goussakov et al., 2006,). Lesions in the BLC have been shown to disrupt retrieval of contextual reward memories formed from cocaine (Yun & Fields, 2003), and amphetamine (Hitori et al. 1991). From the current literature it can be concluded that the BLC is necessary to consolidate and store not just arousing memories but also addiction memories.
There is converging evidence that during early memory encoding dendritic spines must undergo plasticity to allow successful consolidation to take place (Kasai, et al., 2010). Memory formation is dependent on the morphological changes in dendritic spines’ volume and density which is driven by actin polymerization (Gustafsson & Wigstrom, 1990; Honkura et al., 2008). Actin polymerization allows the spine to increase the number of AMPA and NMDA receptors and binding proteins present on its surface thus increasing synaptic strength. It has been shown that inhibition of actin polymerization during the first 3 min of LTP disrupts memory formation (Rex et al., 2010; Gavin et al., 2012). Furthermore actin polymerization appears to stabilize after that 3min post training window as seen by the lack of effect on fear expression when actin polymerization is inhibited either 30min following training or 30min prior to retrieval (Rex et al., 2010; Gavin et al., 2012). Since actin polymerization drives changes in synapse dynamics and the BLC is critically involved in associative memories a logical place to test whether inhibiting mechanisms that support drug-associated context memories disrupts drug seeking would be to depolymerize actin in the BLC.

The experiments presented here were performed to test whether extinction of METH-associated memories could be accelerated by inhibiting actin polymerization in the BLC. Animals first underwent CPP training to form consolidated METH-associated contextual memories and then received an infusion of LatA, to inhibit actin polymerization, 15min prior to testing. This single inhibition of actin polymerization resulted in immediate and persistent disruption of METH-seeking behavior. This finding indicates that expression of METH-associated memories is dependent on actin
polymerization. What was so surprising about this finding is that the same pretesting inhibition of actin polymerization had no effect on expression of fear expression (Gavin et al., 2012). Another surprising aspect of this finding is how immediate inhibition of actin polymerization was able to effect behavior. This finding points to the possibility that METH-associated memories are supported by different actin dynamics compared to fear-associated memories. One explanation is that METH memories could be maintained by constant actin cycling.

Our next experiment collaborate this theory. The CPP test followed the same guidelines as the first except BLC infusions were administered in the home cage one day prior to testing. This resulted in the same effect observed following the 15min pretest treatment mainly that vehicle animals displayed significant preference for the METH paired chamber, while 24hr pretreatment with LatA disrupted animals’ preference for the METH paired chamber. This result collaborated with the 15 minute experiment to provide evidence that actin was cycling in order to maintain METH seeking memories. This shows the first indication actin could be cycling in the maintance of a METH seeking memory in the BLC. This finding adds to the small number of studies investigating the molecular and cellular mechanisms that are active during maintenance (Cui et al., 2004; Pastalkova et al., 2006; Miller et al., 2010; Lee et al., 2013; Volk et al., 2013). With this surprising finding on memory maintenence we wanted to see if it was exclusive to METH related memories or if it applied to all contextual reward based memories.
To see if actin was continuing to cycle during the maintenance of other reward-associated memories, we performed another CPP experiment but the reinforcement was changed from METH to sucrose in the form of fruit loops. As with METH, CPP animals were able to form strong preferences for the sucrose reward. Injecting the rats with LatA 24 hours before the final test produced an interesting finding that food related memories were not maintained through actin cycling. The vehicle and LatA groups both showed significant preference for the METH-paired chamber. Therefore food-associated contextual memories were not disrupted by inhibiting actin polymerization. This indicates that F-actin polymerization is handled differently based on the unconditioned stimulus associated with the memory. This finding furthers the theory that non-METH associated memories, such as for fear and food, are not maintained by actin cycling whereas METH-associated memories are uniquely vulnerable to depolymerization of actin prior to retrieval.

The three experiments presented in this thesis provide evidence that actin dynamics in the BLC can be targeted after consolidation but before retrieval to disrupt METH seeking behavior. Future studies are needed to investigate whether dendritic actin in the BLC is continuously cycling causing METH-associated memories to be uniquely vulnerable., These studies do indicate that targeting actin polymerization could be developed into a possible therapeutic to prevent drug relapse. However, the inhibition of actin polymerization systemically proves fatal as it disrupts all actin function, including contraction of the heart. Thankfully actin polymerization is tightly modulated therefore there are many other regulators that can be targeted to cause depolymerization of actin. One possible upstream target is myosin IIb, a nonmuscle related motor protein, which has
been shown to drive actin polymerization in response to synaptic stimulation (Rex et al., 2010; Gavin et al., 2012). Myosin II motors are primarily involved in actin dynamics contained within neurons (Rex et al., 2010) and thus can be taken a tolerated systemically by the body (Si et al., 2010). Since myosin II primarily targets neuronal actin, it is now one of the leading candidates in future studies of addiction.

The findings in this paper seem to show disrupting METH seeking can occur through targeting actin polymerization in the BLC of the amygdala, while other reward memories like food-seeking are unaffected. This selectivity in the disruption of METH addiction memories offers hope for a possible treatment for not only drug addiction but also other disorders caused by maladaptive memories, such as PTSD. However, many more studies are needed to provide an actual treatment for these disorders.
References


Figures

Figure 1: Cannula Placement for all injection locations in the BLC. Representation of all needle injection sites in the BLC for all experiments. Some needle points are covered since they overlap.

Figure 2: Inhibition of actin polymerization disrupted METH-seeking behavior. Vehicle animals showed strong CS+ preference during all 4 tests whereas an infusion of LatA 15min prior to Test 1 disrupted CPP preference during all 4 tests. Error bars represent SEM.
Figure 3: When given a 24 hr before training injection LatA animals lost drug seeking preference. The Vehicle animals showed a significant preference in the time spent in the CS+ container compared to the CS-. The LatA animals showed no preference in time spent in the CS+ and CS- chambers between the chambers. Error bars represent SEM.
Figure 4: LatA injection did not disrupt food-seeking behavior. The both Veh and LatA groups showed significant preference for the CS+ container over the CS-. Error bars represent SEM.