# ROLE OF HDAC INHIBITION AND ENVIRONMENTAL CONDITION IN ALTERING PHASES OF AMPHETAMINE SELF-ADMINISTRATION

by

DAVID L. ARNDT

B.S., University of Kentucky, 2010
M.S., Kansas State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Psychological Sciences
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

# Abstract

Gene-environment interactions play a significant role in drug abuse and addiction. Epigenetics (the study of how environmental stimuli alter gene expression) has gained attention in recent years as a significant contributor to many behavioral phenotypes of drug addiction. The current study sought to determine if differential rearing conditions can alter a specific epigenetic mechanism, histone deacetylase (HDAC), and how HDAC inhibition can affect drug-taking and drug-seeking behaviors differently among enriched, isolated, or standard-housed rats. Ninety male Sprague-Dawley rats were reared for 30 days in enriched (EC), isolated (IC), or standard (SC) conditions prior to amphetamine (0.03, 0.05, 0.1 mg/kg/infusion, i.v.) self-administration, extinction, or reinstatement sessions. Trichostatin A (TsA; 0.3 mg/kg, i.v.), an HDAC inhibitor, was injected 30 min prior to drug-taking or drug-seeking sessions. Results indicated that EC rats self-administered less amphetamine (0.03 mg/kg/infusion) than IC rats. No significant effects of TsA administration were found on general self-administration for any of the three amphetamine doses. While enrichment facilitated the extinction of active lever pressing, there was also a mild facilitation of extinction in IC-TsA rats compared to IC-vehicle counterparts. Lastly, TsA administration decreased cue-, but not drug-induced reinstatement, with IC-TsA rats exhibiting significantly attenuated cue-induced reinstatement compared to IC-vehicle rats. These findings suggest that differential rearing can alter HDAC mechanisms that can change drug-seeking behaviors, particularly in rats reared in isolated conditions. While TsA-induced HDAC inhibition may be less protective against general amphetamine self-administration, it may decrease drug-seeking tendencies during relapse that are induced by the reintroduction of contextual environmental cues heavily associated with drug reward.

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Major Professor
Dr. Mary E. Cain

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# Acknowledgements

First and foremost, I would like to thank Kansas State University and my advisor, Dr. Mary Cain, for facilitating my scholarly achievements and for providing me so much support and guidance throughout my graduate career. I would also like to thank my committee members – Dr. Kim Kirkpatrick, Dr. Stephen Kiefer, Dr. Eva Horne, and Dr. Bradley Olson for their invaluable advice and contributions. I would like to thank my fellow graduate student, Erik Garcia, for the five-year friendship we have maintained throughout graduate school and for his surgical expertise that was needed for proper data collection in both experiments. I would also like to thank the other two graduate students in our lab, Michele Ulmer and T.J. Wukitsch, and the following undergraduate research assistants who provided vital assistance with daily experimental procedures and animal care – Julie Andazola, Allison Beesley, Greg Erickson, Wade Gutierrez, Morgan Hammes, Jacie Harris, Emily Jorgensen, Lauren Komer, Maria Martinez-Rosales, Key Parks, Jenny Russell, and Luke Sprick. Lastly, I would like to thank my family, especially my wife, Dr. Chelsea Schnabelrauch Arndt, who keeps me modest, possesses excellent proofreading and APA-formatting skills, and who continues to provide loving encouragement and support throughout all of my personal, educational, and professional endeavors.

# Chapter 1 - Introduction

## Overview

The following dissertation studies the preclinical operant paradigm of drug self-administration, and how this translational model of drug abuse is utilized to test the overarching hypothesis that rearing rats in different environmental conditions elicits epigenetic modifications that may alter several phases of drug-taking and drug-seeking behaviors. The current literature is discussed, linking preclinical evidence of rearing-induced histone modification to addictive behaviors, and the role that different rearing environments can have on altering this specific aspect of the epigenome to change the propensity to self-administer amphetamine as well as the likelihood to exhibit relapse of drug-seeking behaviors. Results of two large self-administration experiments are presented, concluding with a discussion of the literature, limitations, and ideas for future directions in this field of study.

## Rat Model of Intravenous Drug Self-Administration

Human drug-taking behaviors can be modeled using preclinical animal paradigms. The fundamental behavioral concept behind the rodent model of self-administration is operant conditioning, or operant responding, which was popularized by the work of B.F. Skinner (1938). Within this behavioral paradigm, organisms exhibit a response, such as a lever press inside an operant chamber. This response is followed by the presentation or infusion of a reinforcer (food or drug) that in-turn influences the occurrence of subsequent operant responses. Over the years, this paradigm has been developed to study the effects of an abundance of psychoactive substances, using rats and mice as the primary subjects (Yokel, 1987). Due to its predictive and translational value, along with its high validity, the self-administration model has provided

researchers with a robust method for studying drug abuse liability and addiction (Brady et al., 1987).

To investigate drug abuse liability, drug-taking patterns, the effects of altering lever-press contingencies on subsequent drug intake, and the effects of various pharmacological manipulations on neural substrates of reinforcement, researchers can alter the response requirements (schedules of reinforcement) within the operant chamber. By altering the schedule of reinforcement, it allows researchers to investigate the multifaceted behaviors of drug self-administration. For example, under a fixed-ratio (FR-1) schedule of reinforcement, the rodent needs to exhibit one lever response on the 'active' lever to receive one infusion of the drug under study. Active lever press behavior under an FR-1 schedule is presumably a measure of drug reward sensitivity (Richardson & Roberts, 1996).

Measuring the rate of FR-1 responding for amphetamine provides a valid method to investigate the acquisition and maintenance of drug-taking behavior. Additionally, in the extinction/reinstatement paradigm, after acquiring self-administration of the drug, a rat undergoes a period of extinction, where operant lever presses lead to no programmed response, and no drug reward. Eventually, the rat learns that drug is no longer available and active lever responding typically diminishes. Then, experimenters reintroduce various Pavlovian-conditioned cues (visual, auditory, and/or pharmacological) that were previously paired and associated with the self-administered drug, and active lever presses (drug-seeking behaviors) typically return, effectively modeling a state of relapse in drug-seeking behavior because the rat is actively seeking the drug reward that was once so highly reinforcing in earlier acquisition and maintenance phases. The extinction/reinstatement paradigm has been established as a valid method to study drug relapse in rat self-administration studies, and behavioral evidence gathered

in reinstatement studies with rats appears to correspond well with what is observed with drug relapse in humans (Shaham et al., 2003).

## Relationship between Epigenetics and Drug Abuse

Two organisms that share similar genomes, such as identical twins, can experience vastly different environmental stimuli throughout their lives. The different environmental stimuli that twins experience throughout early development and adolescence can have a direct impact on the behaviors they exhibit in adulthood. These changes in behavior as a result of different life experiences can reflect altered *epigenetic* expression that is independent of changes in DNA sequence. This environmentally-induced change to the structure of genes that does not depend on the sequence of DNA is the hallmark of epigenetics: the study of how life experiences change gene expression and subsequent behavior (Francis, 2011).

The relatively new field of epigenetics has emerged as a possible mechanism by which early-life experiences can have a significant impact on behavior later in adulthood. Epigenetic constructions can change during critical developmental periods, which can drastically affect behavior and alter responses to drugs of abuse. Thus, epigenetic expression is malleable and can be altered by environmental factors as well as drugs of abuse. For example, early life stressors and various environmental stimuli can modulate gene expression within drug reward regions of the brain that are highly integrated and involved within the mesocorticolimbic reward pathway (LaPlant & Nestler, 2011). The altered gene expression in these areas can result in differential drug-taking behaviors (Lewis & Olive, 2014).

Additionally, certain psychostimulants, such as cocaine, can alter the structure of chromatin, which is found in cells containing DNA, protein, and RNA (Renthal & Nestler, 2009). This change in chromatin structure can play a pivotal role that may serve as a prerequisite

to many addictive behaviors that manifest during symptoms of withdrawal, tolerance, and relapse (Adachi & Monteggia, 2009). Chromatin remodeling is a complex product resulting from the dynamic epigenetic process of histone modification and DNA methylation. DNA methylation and histone modification are the primary mechanisms underlying epigenetic change. Structurally speaking, histones are constructed into an octamer made up of two copies of the histones H2A, H2B, H3, and H4 (Luger & Richmond, 1998). These histone proteins, in response to environmental stimuli, undergo dynamic processes of compacting and expanding the chromatin to which they are attached, resulting in the inaccessibility and accessibility, respectively, of DNA and its related genes to various transcriptional machinery mechanisms (Renthal & Nestler, 2008). Histone modification plays a key role in gene expression, and by removing acetyl groups from histones, histone deacetylases (HDACs) allow histones to wrap DNA more tightly, primarily repressing genetic expression. Both DNA and histones are covered by chemical tags that comprise the epigenome. The epigenome can change the physical shape of the genome (DNA) that can alter genetic expression, which can in turn result in the manifestation of different behaviors.

In general, histone acetylation increases transcription and gene expression. As such, the administration of histone deacetylase inhibitors results in hyperacetylation of histones, greater transcription, and ultimately changes to many drug-induced behaviors and neuroplasticity (Sanchis-Segura, Lopez-Atalaya, & Barco, 2009). There are several classes of HDACs. The majority of research examining the role of HDACs in response to drugs of abuse has focused on Class I and Class IIa HDACs. Inhibiting HDAC1 and HDAC2 has been implicated in antagonizing the behavioral properties of cocaine (Kennedy et al., 2013). Inhibiting Class I HDACs can decrease motivation to consume alcohol and reduce the likelihood of relapse in

alcohol-preferring rats (Jeanblanc et al., 2015). Furthermore, prefrontal cortex (PFC) expression of both HDAC1 and HDAC2 has been shown to decrease after acute methamphetamine exposure (Li et al., 2014). These findings all suggest that inhibiting HDACs can result in decreased behavioral responses to many commonly abused drugs.

Current research suggests these effects are also due to modifications of HDAC function within the nucleus accumbens (NAcc). The NAcc and the prefrontal cortex (PFC) are two brain areas within the mesocorticolimbic pathway. This pathway is highly dopaminergic, and as such plays a significant role in many aspects of addiction and behavioral responses to drugs of abuse (Koob, 1992). Chronic administration of cocaine can decrease HDAC function in the NAcc. This leads to increased transcription of HDAC5 genes (Renthal et al., 2007), suggesting that administration of psychostimulants can change epigenomes in the NAcc. Overexpression of HDAC5 in the NAcc can inhibit cocaine-induced CPP, or conditioned place preference (Renthal et al., 2007). A rat exhibits CPP for a drug when it chooses to spend more time in a chamber in which the rat was previously administered the drug versus the chamber it did not previously receive drug, effectively modeling drug reward. Other work has shown that chronic psychostimulant-induced histone hyperacetylation in the NAcc can significantly increase the motivation to self-administer cocaine, and that H3 acetylation in the NAcc plays a key role in driving the operant responses to receive drug infusions (Wang et al., 2010).

From a behavioral standpoint, Class I HDAC inhibition can prevent or reverse amphetamine-induced locomotor behavior (Frey et al., 2006; Schroeder et al., 2013). Research also suggests that Class I HDAC inhibition can attenuate the maintenance of amphetamine-induced behavioral sensitization (Kalda et al., 2007), while Class I and Class II HDAC inhibition can attenuate the acquisition of methamphetamine-induced behavioral sensitization (Coccurello

et al., 2007). The Class I HDAC inhibitor, sodium butyrate (NaB), can decrease alcohol self-administration in dependent rats (Simon-O'Brien et al., 2015). Other HDAC inhibitors, such as Trichostatin A (TsA), which broadly inhibits many Class I and Class II HDACs, can dose-dependently reduce cocaine self-administration (Romieu et al., 2008). In addition, overexpression of Class II HDAC5 in the NAcc is reported to inhibit cocaine-induced CPP, (Renthal et al., 2007). This research suggests that HDAC inhibition can lead to altered drug-taking behaviors.

Furthermore, it has been shown that chromatin remodeling via Class I HDAC inhibition can lead to altered central nervous system plasticity that can change behavioral responses to drugs of abuse, and acute administration of morphine, ethanol, and cocaine leads to significant phosphorylation of HDAC3 in the dorsal striatum (Adachi & Monteggia, 2009; Sanchis-Segura, Lopez-Atalaya, & Barco, 2009). Therefore, epigenetic markers such as HDACs are gaining interest in the drug abuse literature as possible target mechanisms involved in mediating the rewarding properties of drugs of abuse (Romieu et al., 2008, 2011). While it is known that life experiences can have profound effects on epigenetic markers throughout the brain, it is unclear if there is an interaction between environmental conditions and HDAC inhibition that may significantly alter the sensitivity to psychostimulants.

## Rearing-Induced Differences in Drug-Taking Behavior and Neurobiological Mechanisms

In the preclinical setting, rodent models have been developed to investigate the influence of various environmental and genetic factors that underlie addiction. Altering the rearing environment of rodents during the post-weaning period leads to neurobiological changes that affect subsequent behaviors in adulthood (Bell & Carrillo, 2007; Greenough, Black, & Wallace,

1987; Renner & Rosenzweig, 1987). Rats reared in an enriched condition (EC) display less amphetamine-induced hyperactivity (Arndt et al., 2014; Bardo et al., 1995) and self-administer less amphetamine at low unit doses compared to rats reared in isolated (IC) conditions (Bardo et al., 2001; Green et al., 2002). Enriched rats also display decreased vulnerability to psychomotor stimulants compared to rats reared in isolated or standard conditions (Stairs et al., 2011).

While the behavioral effects of differential rearing on altering responses to psychostimulants are well-known, the neurobiological mechanisms responsible for the protective effects of enrichment are less clear. Exposure to environmental enrichment increases the density of dendritic spines in the striatum and NAcc, particularly for the Type 1 spiny neurons (Comery et al., 1996; Kolb et al., 2003). EC rats also have reduced functioning of the dopamine transporter (DAT) in the medial prefrontal cortex (mPFC) and NAcc, so that dopamine is available in the synapse for a longer period of time (Abi-Dargham et al., 1998; Darna et al., 2015). The repeated stimulation offered by novel objects, handling, and conspecifics may result in repeated dopaminergic stimulation and a compensatory decrease in dopamine reuptake (Stairs & Bardo, 2009; Zhu et al., 2004). Interestingly, differential rearing environments generally do not alter basal levels of dopamine (Bardo et al., 2995; Bardo & Hammer, 1991; Darna et al., 2015; Heidbreder et al., 2000; Solinas et al., 2009), suggesting the effects are specific to functional activity and turnover.

## Rearing-Induced Differences in Epigenetic Histone Modification

As noted above, DNA methylation and histone modification are the primary mechanisms underlying epigenetic change. The current literature suggests that differential rearing, specifically environmental enrichment, can lead to changes in how DNA is methylated. One epigenetic marker, methyl CpG binding protein 2 (MeCP2), has been implicated in

psychostimulant self-administration and brain reward circuitry (Lewis et al., 2013). Mutations in the X-linked gene for MeCP2 have been associated with Rett Syndrome (Kondo et al., 2008), a developmental disorder characterized by abnormal childhood motor and cognitive deficits. Recent research suggests that environmental enrichment can attenuate symptoms of Rett Syndrome. Specifically, the effects of environmental enrichment have been tested on mice harboring a MeCP2 mutation. Findings indicate the ability of enrichment to improve motor coordination in female rats carrying the mutated MeCP2 gene (Kondo et al., 2008). Further behavioral and brain volume assessments indicate that enrichment can also reduce ventricular volumes in mice with MeCP2 mutations, correlating with improved locomotor behavior (Nag et al., 2008). These studies support additional findings that environmental enrichment can significantly improve various phenotypes associated with MeCP2 mutation (Kerr et al., 2010). While these studies are promising in showing the ability of enrichment to alter epigenetic DNA methylation in a way that reverses the cognitive and motor deficits seen in Rett Syndrome, evidence also suggests that differential rearing can lead to histone modification.

EC and IC rats differ significantly in regard to drug-induced dopaminergic signaling and other functions at the neurotransmitter level (Fone & Porkess, 2008). Interestingly, previous studies have revealed a link between dopaminergic receptor signaling and epigenetic histone modification, such that administration of D1 dopamine agonists can induce behavioral sensitization to cocaine while simultaneously inducing phosphoacetylation of histone H3 (Schroeder et al., 2008). Epigenetic histone acetylation is associated with active gene transcription (Fischer et al., 2010), and increased histone acetylation facilitates memory formation (Whittle & Singewald, 2014). While the neural mechanisms driving the beneficial outcomes of enrichment are still being parsed, studies suggest that enrichment may affect

learning and memory through epigenetic mechanisms (Sweatt, 2009). Following the induction of neurodegeneration, rats exposed to environmental enrichment exhibit an increase in dendritic spine density, greater histone acetylation, and ultimately show the preservation of learning and memory, as well as the restoration of lost memory. Non-enriched rats that are administered HDAC inhibitors following the induction of neurodegeneration also exhibit similar benefits to learning and memory (Patel, 2012). This suggests that environmental enrichment and HDAC inhibition may be acting through common mechanisms, particularly those that involve long-term potentiation induced by both synaptogenesis and histone hyperacetylation.

Other research suggests that both environmental enrichment and histone acetylation can work in tandem to boost memory consolidation and increase brain derived neurotrophic factor (BDNF) in the dentate gyrus, while effectively protecting against the loss of neurogenesis (Fontán-Lozano et al., 2008; Kuzumaki et al., 2011; Segovia et al., 2006). BDNF is a protein involved in the epigenetic regulation of many psychiatric disorders (Mitchelmore & Gede, 2014). Expression of this specific protein differs between enriched and isolated rats in several brain areas (Ickes et al., 2000) and is known to be highly regulated by psychostimulants (Russo et al., 2009).

Interestingly, altered chromatin structure and function induced by HDAC inhibition can result in improvements in learning and memory and can significantly improve memory consolidation (Alarcón et al., 2004; Korzus, Rosenfeld, & Mayford, 2004). Increased histone acetylation has also been observed in the hippocampus of environmentally-enriched mice (Fischer et al., 2007). Whether the positive outcomes of enrichment on learning and memory, as well as the benefits of HDAC inhibition on learning and memory are mechanistically-related, remains to be determined.

Because epigenomes react and adapt to various environmental stimuli, it is plausible that the epigenome of enriched rats is vastly different than the epigenome of rats reared in isolated or standard conditions. EC rats are exposed to daily novel stimulation, cohort interaction, and experimenter handling. This routine novel stimulation differs significantly from the lifetime stimuli (or lack thereof) experienced by rats reared in isolated conditions. These enriched experiences lead to changes in cortical weight (Bennett, Rosenzweig, & Diamond, 1969), learning and memory (van Pragg et al., 2000), and a plethora of other neurobiological differences (Fone & Porkess, 2008). EC and IC rats also differ in psychostimulant-induced mesocorticolimbic neurotransmission (Kenny & Markou, 2004), a pathway in the brain heavily implicated in drug reward.

As Fischer and colleagues (2007) demonstrated, environmental enrichment may be partially mediated by hyperacetylation of histones H3 and H4, and the relationship between enrichment and histone hyperacetylation (via HDAC inhibition) may improve many aspects of learning and memory. Interestingly, HDAC5 is associated with downregulation of BDNF in social defeat paradigms, which can be reversed after the administration of an HDAC inhibitor (Tsankova et al., 2006). This Class I HDAC inhibition, in combination with environmental enrichment, further attenuates symptoms of social defeat stress in mice (Covington et al., 2011). It has also been posited that changes in chromatin remodeling (by inhibiting Class I HDACs) mimic the same epigenetic changes elicited by environmental enrichment, especially in regard to learning, memory, and even depression (Dash, Orsi, & Moore, 2009; Fischer et al., 2007; Tsankova et al., 2006). While this literature suggests that HDAC inhibition and differential rearing may be affecting common epigenetic mechanisms, it is unknown if HDAC inhibition and differential rearing can interact, or work in tandem to influence behavior in drug extinction and

reinstatement models that involve learning and memory. HDAC inhibition (combined with isolation or enrichment) could alter cue-induced reinstatement for example, because cue-induced reinstatement relies so heavily on previously learned associations between drug reward and environmental stimuli (tone or light previously paired with drug administration; Sanchis-Segura & Spanagel, 2006). These studies all suggest that differential rearing, specifically environmental enrichment and isolation, can alter certain epigenetic mechanisms such as DNA methylation and histone modification. In turn, this alteration can lead to many of the same neurological consequences observed following HDAC inhibition in regard to learning and memory capabilities. Notably, learning and memory play significant roles in the development of drug abuse and liability for relapse, which can be modeled in extinction/reinstatement studies described below.

## Extinction/Reinstatement Model: Role of Environmental Condition and HDAC Inhibition

While it is important to study the behavioral mechanisms involved in the acquisition and maintenance of drug-taking behavior (i.e. why people start using and keep abusing drugs), it is equally important to study the mechanisms underlying periods of withdrawal (extinction) and the reasons for which people relapse or reinstate their drug-seeking tendencies. During drug-taking sessions, people readily associate the environmental cues around them with the reinforcing properties of their drug of choice through classical conditioning (Hyman & Malenka, 2001). The conditioned cues involve complex learning and memory processes that can be modeled in preclinical animal models such as reinstatement (Shaham et al., 2003). This reinstatement can be induced from either cues or from drugs. Cue-induced reinstatement involves the presentation of visual or auditory cues (after a period of extinction and withdrawal) that were once paired with

the reinforcing properties of the drug of abuse under study. These Pavlovian conditioned cues can evoke an increase in drug-seeking behavior (active lever presses) because the rat so heavily associates the conditioned cues to the previously rewarding effects of the drug. During drug-induced reinstatement, the rat is systemically injected (after a period of extinction/withdrawal) with the drug of abuse under study, and then placed back into the environment where the drug was previously self-administered. Typically, the systemic injection, much like the cues, evokes an increase in drug-seeking behavior, indicated by an increase in active lever presses (the active lever in the operant chamber once led to a programmed drug infusion, where the inactive lever never led to a programmed response).

Research has shown that environmental enrichment leads to improved performance in learning and memory tasks, as well as increased dendritic growth and density in regions of the brain implicated in learned processes (van Praag, Kempermann, & Gage, 2000; Leggio et al., 2005). As previously mentioned, learning and memory play key roles in extinction and reinstatement models of drug addiction. Compared to models of acquisition and maintenance of drug self-administration, which primarily reflect drug reward sensitivity and discrimination, research shows that the neuronal mechanisms involved in these earlier stages differ from those in later stages of addiction (Kalivas & Volkow, 2014), such as periods of withdrawal and relapse (Shaham et al., 2003). Research suggests that differential-rearing can significantly modify not only the extinction (learning that an active lever press that previously resulted in drug reinforcement no longer leads to the programmed rewarding drug outcome), but also the propensity to exhibit the reinstatement of drug-seeking behaviors (previously established active lever presses for drug). For example, it has been shown that rats reared in enriched conditions show enhanced extinction for amphetamine compared to rats reared in isolation (Stairs, Klein, &

Bardo, 2006). Similarly, rats exposed to environmental enrichment display less cocaine-seeking during extinction sessions and less cocaine-induced reinstatement (Green et al., 2010). This extinction effect is also elicited by switching non-enriched rats to enrichment well after the post-weaning phase, and can also decrease the likelihood of displaying cue-induced reinstatement of psychostimulant-seeking (Hofford et al., 2014; Thiel et al., 2010). The effect of enrichment on increasing extinction and decreasing the likelihood of reinstatement also extends to alcohol (Li et al., 2014) and nicotine (Ewin, Kangiser, & Stairs, 2015). These studies, as well as findings by Alvers and colleagues (2012) with the psychostimulant methylphenidate, suggest that rats reared in enriched conditions have an enhanced ability to learn that previously-active lever presses no longer result in psychostimulant reinforcement. These interesting findings also suggest that environmental enrichment may be protecting against the etiology of addiction by altering the saliency and ability of Pavlovian conditioned stimuli to drive drug-seeking behaviors.

As noted previously, epigenetic chromatin remodeling via histone acetylation has been implicated in tolerance, withdrawal, and relapse (Adachi & Monteggia, 2009). HDAC inhibition has been investigated in only a few extinction and reinstatement studies, but the majority of these studies have focused on the model of CPP to study cue-induced relapse. Malvaez et al. (2013) found that the specific inhibition of HDAC3 enhanced long-term memory, facilitated extinction, and prevented reinstatement of cocaine CPP.

While these previous findings demonstrate a relationship between HDAC3 hyperacetylation and its ability to change conditioned behavioral responses to drugs of abuse, these findings need to be extended and studied in more refined, translational models of addiction, such as intravenous self-administration, and little is currently known about HDAC's role in altering the extinction and reinstatement of amphetamine self-administration. Literature shows

that Class I inhibition of histone deacetylases (via the HDAC inhibitor, sodium butyrate) can facilitate extinction and attenuate the reinstatement of nicotine self-administration in rats, and chromatin remodeling has been shown to be important for the formation of long-term extinction memories associated with drug use (Castino, Cornish, & Clemens, 2015). This suggests that histone hyperacetylation may enhance the consolidation of extinction memories. Importantly, Castino and colleagues (2015) found no effect of HDAC inhibition on sucrose responding, consistent with other groups investigating the effects of the HDAC inhibitor, Trichostatin A (TsA), on attenuating operant motivation and relapse for cocaine while having no effect on sucrose administration (Romieu et al., 2008; 2011). This indicates that the effects of HDAC inhibition are drug-specific and do not influence the appetitive nature of other reinforcers, such as sucrose solution.

Low doses of various HDAC inhibitors facilitate extinction of cocaine-induced CPP, while higher doses weaken extinction (Raybuck et al., 2013). TsA also reduces cocaine-seeking induced by the combination of a cocaine injection together with the exposure to a light cue previously associated with cocaine self-administration (Romieu et al., 2011). Just as certain HDAC isoforms have been implicated in acquisition and maintenance models, other findings indicate a shifting role of specific HDAC isoforms during other stages of addiction. Interestingly, Li and colleagues (2014) observed that HDAC2 expression in the prefrontal cortex was decreased after both acute and chronic methamphetamine exposure, while HDAC4 and HDAC5 were decreased only after withdrawal, with overall HDAC activity increased. This indicates a dynamic involvement of Class I HDACs to Class II HDACs during periods of withdrawal. If certain HDAC involvement varies at different stages of drug abuse, then the efficacy of TsA (which acts primarily on HDACs 1, 3, 4, 6, and 10) on attenuating drug-taking

behaviors may depend on whether it is administered concurrently with the drug of abuse or during periods of withdrawal and relapse (extinction and reinstatement). Different brain areas (NAcc, basolateral amygdala, mPFC; Everitt & Robbins, 2016) and neurotransmitter systems each play unique roles in not only different stages of addiction, but also between cue- and drug-induced reinstatement. Thus, it is important to study the effects of HDAC inhibition and environmental condition in both models of relapse. Because HDAC inhibition has the ability to alter psychostimulant-induced CPP, which so heavily relies on contextual cues, we sought to determine if HDAC inhibition will also affect cue-induced reinstatement.

Taken together, it appears that Class I and Class II HDACs may be more heavily involved in the psychostimulating effects of commonly abused drugs. However, research investigating the effects of HDAC inhibition on altering the rewarding effects and behavioral responses to psychostimulants is still in its infancy and it is not yet clear which specific HDACs differential rearing may be altering. In regard to the current dissertation, because Trichostatin A (TsA) inhibits a wide variety of HDAC isoforms (both Class I and Class II), studying this specific HDAC inhibitor allowed us to measure the effects of general HDAC inhibition and its interaction with environmental condition to alter drug-taking and drug-seeking behaviors.

The literature discussed above indicates a link between histone modification and the behavioral responses to drugs of abuse. There is also evidence of differences between enriched and isolated rats and how they extinguish and reinstate drug-taking behaviors. Epigenetic changes elicited from the rearing phase may be a contributor to many of the behavioral differences observed between enriched and isolated rats in regard to drug-taking and drug-seeking. Gene-environment interactions can hold clues as to why these differences exist, and studying one component of the epigenome via HDAC inhibition can give us insight into how

different lifetime experiences (such as differential rearing) interact with histone acetylation to influence different phases of amphetamine self-administration.

## Summary

While the protective effects of enrichment on attenuating the behavioral responses to drugs of abuse are well-documented, the neurobiological mechanisms remain unclear. Environmental enrichment may induce epigenetic chromatin modification (specifically histone hyperacetylation), which may in turn serve as one of the primary mechanisms responsible for reduced drug-taking and drug-seeking typically observed in enriched rats. The two experiments discussed below investigated how HDAC inhibition and differential rearing interact to alter amphetamine self-administration, extinction, and reinstatement. By rearing rats in enriched, isolated, or standard conditions, we sought to determine the importance of different environmental experiences, and how those experiences could alter a key epigenetic mechanism that can change drug-taking and drug-seeking behaviors.

## Experimental Plan and Hypotheses

The overarching objective of the current dissertation was to investigate how gene-environment interactions influenced the sensitivity to drug reward and the likelihood to relapse drug-taking behaviors after a period of no drug exposure.

### Experiment I: Acquisition and Lower-Unit Dose Amphetamine Self-Administration

The effect of TsA, a histone deacetylase inhibitor, was examined to determine the influence of rearing condition and HDAC inhibition pretreatment on altering the acquisition of drug-taking (0.1 mg/kg/infusion) and the self-administration of lower-unit dose amphetamine (0.03 and 0.05 mg/kg/infusion).

#### Hypotheses

**1.** Trichostatin A (TsA), a histone deacetylase inhibitor, should generally decrease amphetamine self-administration for all three doses under a FR-1 schedule of reinforcement.

**2.** EC-TsA rats should exhibit less attenuation than IC-TsA rats due to their already-increased histone acetylation.

**3.** The attenuation of amphetamine self-administration should be more pronounced in IC rats compared to EC rats due to IC rats' deficient histone acetylation.

## Experiment II: Extinction and Reinstatement of Amphetamine Self-Administration

The effect of TsA, a histone deacetylase inhibitor, was examined to determine the influence of rearing condition and HDAC inhibition on altering the extinction learning and likelihood to exhibit relapse (reinstatement) of drug-taking behaviors.

### Hypotheses

**1.** TsA should facilitate extinction in IC rats vs. IC-vehicle rats, but EC rats should still exhibit enhanced extinction learning due to EC rats and their preexisting enhancements in learning and memory.

**2.** Histone deacetylase inhibition via TsA should generally attenuate the reinstatement of amphetamine self-administration in SC rats.

**3.** TsA should attenuate cue- and amphetamine-induced reinstatement. This attenuation (difference between vehicle and TsA treatment) should be more pronounced in IC rats compared to EC rats due to IC rats' deficient histone acetylation.

#### *Justification of Alternative Hypotheses*

Previous research has shown that Class I HDAC inhibition via sodium butyrate (NaB), when administered concurrently with amphetamine, can enhance the maintenance and acquisition of behavioral sensitization (Kalda et al., 2007), a model for drug sensitivity.

However, Kalda et al. (2007) also found that eight daily injections of NaB prior to amphetamine exposure can attenuate the maintenance of behavioral sensitization. Furthermore, it has been observed that administration of the Class I HDAC inhibitor, butyric acid, can enhance the acquisition and maintenance of behavioral sensitization to methamphetamine (Harkness et al., 2013). Other studies have shown that chronic psychostimulant-induced histone hyperacetylation in the NAcc can significantly increase the motivation to self-administer cocaine, and that H3 acetylation in the NAcc plays a key role in driving operant responses to receive drug infusions (Wang et al., 2010).

Therefore, because HDAC inhibition (when administered concurrently with a drug of abuse) may lead to increased behavioral effects of the psychostimulant, and the observation that HDAC inhibition leads to facilitated extinction and attenuated reinstatement, we alternatively hypothesized that HDAC inhibitors may be more effective in protecting against the occurrence of relapse than protecting against drug reward sensitivity reflected in acquisition and maintenance models. This notion is supported by the aforementioned finding that rats pretreated with HDAC inhibitors following the induction of neurodegeneration exhibit significant benefits to learning and memory (Patel, 2012). Because relapse involves learned drug-associated cues and stimuli, we hypothesized that HDAC inhibition via TsA may be more effective in decreasing the occurrence of active-lever pressing after the reintroduction of associated cues (light and tone) that were once paired with the rewarding effects of amphetamine.

In sum, due to the evidence suggesting a link between histone acetylation and learning and memory, as well as some inconsistencies in the literature regarding HDAC inhibition and drug sensitivity measures, we alternatively hypothesized that inhibiting HDAC function via TsA

pretreatment would be more effective at altering extinction and reinstatement behaviors instead of general amphetamine self-administration behaviors.

***Alternative Hypothesis 1***

HDAC inhibition (histone hyperacetylation) should lead to increased active lever responding for amphetamine infusions at any dose on a FR-1 schedule of reinforcement.

***Alternative Hypothesis 2***

HDAC inhibition should not be effective at attenuating the acquisition of amphetamine self-administration at any dose, but should still facilitate extinction and significantly attenuate cue-induced reinstatement.

# Chapter 2 - General Methods

## Method and Materials

### Subjects

90 male Sprague-Dawley rats (Charles River, Portage, MI, USA) were reared and housed in one of three environmental conditions: enriched (EC), isolated (IC), or standard (SC). Rats were given free access to food and water throughout the entire experiment, with the exception of lever press training. The colony room was operated on a 12-hr light-dark cycle and was maintained at approximately 22° C, with humidity ranging from approximately 30-45%. All behavioral tests were conducted during the light portion of the cycle. All procedures conducted and research reported was in accordance with the Institutional Animal Care and Use Committee at Kansas State University, and complied with NIH guidelines and standards (Institute for Laboratory Animal Research, 2011).

## Environmental Conditions

Rats arrived in the lab at exactly 21 days of age and were randomly assigned to one of the three environmental conditions. EC rats lived with several other cohorts and were housed in a large metal cage (60 x 120 x 45 cm) lined with paper bedding. To maintain novelty and to provide further enrichment, EC rats were handled daily by experimenters for approximately one minute each. Fourteen objects (small children's toys and PVC pipe) were regularly rotated and replaced inside the cage. IC rats reared individually in hanging wire cages (17 x 24 x 20 cm). IC cages were composed of wire mesh on the front and bottom, with solid sides. The IC rats were not handled throughout the 30-day rearing phase and were not exposed to novel objects or paper bedding. SC rats were housed in pairs in standard shoebox cages (20 x 43 x 20 cm). SC rats were exposed to the same bedding as EC rats but did not have any novel objects in their cage and were only handled during the weekly cage change. The inclusion of the SC group of rats was not intended to control for differences between EC and IC rats, but rather to provide a known laboratory standard for comparison. Rats remained in their respective environmental conditions for the 30-day rearing period and remained in their housing conditions for the entire duration of the experiment.

## Lever Press Training

Temporary food restriction to 85% of rats' free-feeding weight was required to ensure rats were motivated to learn how to lever press. After each active lever press during lever press training, a 0.1 ml, 20% sucrose solution was presented to the rat in a recessed food receptacle inside a magazine. Rats experienced four additional, daily 30-min sessions of FR-1 responding for 20% sucrose solution.

## Apparatus: Operant Chambers

Lever press training and amphetamine self-administration sessions were conducted inside operant conditioning chambers (ENV-001, Med Associates, St. Albans, VT; see Figure 1). Each chamber was enclosed in a sound-attenuating compartment and was operated by a computer interface. Two metal response levers were located on either side of the food tray 7.3 cm above the metal grid floor. A 28-V, 3-cm diameter white cue light was centered above each response lever. A house light was equipped in each operant box but was only on during the magazine training and sucrose shaping phase of the experiment. The same active lever (left or right) was maintained for each rat for both the sucrose training and amphetamine self-administration phases of both experiments. For Experiment II, an 80 dB, 3000-Hz tone generator was utilized to accompany the cue light after active lever pressing during non-extinction sessions.

## Surgery and Self-Administration

Following sucrose training, rats were allowed to return to free-feeding weight for the remainder of the experiment. After returning to free-feeding weight, rats were deeply anesthetized with ketamine (80 mg/kg; 1 mg/ml, i.p.) and diazepam (5 mg/kg; 1 mg/ml, i.p.) prior to jugular catheter implantation. Polyurethane catheters measured approximately 12 cm in length and 0.2 mm in internal diameter (SAI Infusion Technologies) and were inserted through a dorsal incision on the rat's back that led up under the skin and around into the rat's left jugular vein. Catheter tubing from the jugular vein was connected subcutaneously to a 22-gauge back-mounted cannula (Plastics One; Roanoke, VA) secured and sutured to surgical mesh (Biomedical Structures; Warwick, RI; see Figure 2). A stainless steel bolt covered the catheter cannula cap to prevent damage to the back mount. To maintain patency and to protect against infection, catheters were flushed daily with infusions of heparinized saline (10–30 IU/ml; 0.1 ml, i.v.,

before self-administration and 0.1 ml, i.v., after self-administration) and cefazolin (50 mg/ml; 0.1 ml, i.v., after self-administration).

Amphetamine infusions were administered via a syringe pump (PHM-100, Med Associates) connected to a 10-ml syringe holding the correct amphetamine concentration based on each rat's body weight. Amphetamine concentrations from the syringe pump were infused through a leash and swivel arrangement comprised of a polyethylene supply tube encased in vinyl tubing with a captive collar to secure the unit to the cannula (Plastics One; Roanoke, VA). Each amphetamine infusion lasted 5.9 seconds at a dose of 0.03, 0.05, or 0.1 mg/kg/infusion at a volume of 100-µl. An 80 dB, 3000-Hz tone accompanied the cue lights after an active lever press during the FR-1 amphetamine training phase and the cue-induced reinstatement test in Experiment II.

**Drugs: Self-Administration Testing**

Trichostatin A (TsA; 0.3 mg/kg; 1.0 mg/ml; ApexBio) was dissolved in sterile saline and 10% DMSO. TsA was stored in frozen (-20° C) 2.0 ml centrifuge tubes. On test days, TsA was thawed and injected intravenously (i.v.) through the indwelling jugular catheters thirty minutes prior to amphetamine self-administration, extinction, or reinstatement test sessions. Injecting TsA no more than 30 minutes prior to drug-taking sessions was imperative given the short half-life of the drug. Sanderson and colleagues (2004) reported a half-life of 6.3 minutes following i.p. administration of TsA in mice at a dose of 0.5 mg/kg. Notably, repeated i.v. injections of TsA at 0.3 mg/kg for at least four days have been shown to result in significant decreases of HDAC activity in the NAcc, at levels of approximately 40% below their vehicle counterparts (Romieu et al., 2008).

D-amphetamine (Sigma Aldrich, MO, USA) was dissolved in 0.9% sterile saline and was self-administered intravenously (0.03, 0.05, 0.1 mg/kg/infusion). All rats acquired amphetamine self-administration at the 0.1 mg/kg/infusion dose, with rats in Experiment I self-administering 0.03 and 0.05 mg/kg/infusion amphetamine, counterbalanced, after the acquisition phase. Catheter patency was verified by infusing Brevital (10 mg/ml; 0.1-0.15 ml, i.v.).

## Specific Behavioral Procedures

### Experiment I: Acquisition and Lower-Unit Dose Amphetamine Self-Administration

Table 1 illustrates the timeline of Experiment I corresponding to the rats' postnatal days in which the behavioral tests occurred. Rats reared in their respective environmental conditions for 30 days (enriched (EC), isolated (IC), and standard (SC)) prior to a temporary deprivation of food to 85% free-feeding weight, and lever-press shaping and training for 20% sucrose reinforcement. Rats experienced four additional sessions of FR-1 responding for sucrose solution during 30 min sessions.

Following lever press training, rats regained access to unlimited food and water prior to undergoing surgery to implant indwelling jugular catheters. EC rats were pair-housed with one toy in a shoe box cage the night following surgery. The surgery recovery period lasted 5-7 days. Following surgery recovery, patency checks were conducted and only patent rats were randomly assigned to receive TsA or vehicle pretreatment for the duration of experiment. Non-patent rats were excluded from the study. Daily TsA or vehicle pretreatment started three days before the start of amphetamine self-administration sessions. This was done in light of literature suggesting that attenuation of drug-self-administration via HDAC inhibition is not evident until after three to four days of consecutive TsA pretreatment (Romieu et al., 2008; 2011). Rats trained to lever press for amphetamine at 0.1 mg/kg/infusion (100-µl infusion over 5.9 seconds) after receiving

i.v. injections of TsA or vehicle 30-min prior to drug-taking sessions. After each active lever press, a 20-sec timeout period commenced, signaled by the illumination of both cue lights immediately after each amphetamine infusion. Drug-taking sessions were 1 hr in duration. Rats received vehicle (10% DMSO in saline) or Trichostatin A (TsA) pretreatment at a dose of 0.3 mg/kg, i.v. (Host et al., 2010; Romieu et al., 2008; 2011). Drug pretreatment (HDAC inhibition) lasted for 20 consecutive days (three days TsA or vehicle pretreatment prior to initial amphetamine exposure, seven days TsA or vehicle pretreatment during 0.1 mg/kg amphetamine self-administration sessions, and six total days of pretreatment during lower-unit dose amphetamine self-administration sessions, with 4 additional days of TsA/vehicle pretreatment during 0.1 mg/kg/infusion sessions in between and after the counterbalanced lower-unit dose amphetamine self-administration sessions). For lower-unit dose amphetamine self-administration, amphetamine dose was counterbalanced, such that half of the rats self-administered 0.03 mg/kg/infusion first, and half of the rats self-administered 0.05 mg/kg/infusion first, with amphetamine training dose sessions (0.1 mg/kg/infusion) occurring between each counterbalanced lower-unit amphetamine session (Table 1). Patency checks were administered after the final self-administration session and non-patent rats were excluded from data analyses. A total of 42 rats were patent and were included in the data analysis for Experiment I (Table 3).

## Experiment II: Extinction and Reinstatement of Amphetamine Self-Administration

Table 2 illustrates the timeline of Experiment II corresponding to the rats' postnatal days in which the behavioral tests occurred. Rats experienced identical procedures and methods as Experiment I through the surgery recovery phase. Following surgery recovery, rats were trained to lever press for amphetamine at 0.1 mg/kg/infusion. These sessions included both a cue light and tone paired with drug infusions. Both the cue light (which turned on after active lever

presses only) and the tone turned on during the 5.9 second infusion. The light and tone turned off and the house light illuminated during the 20-second time out period in which no amphetamine infusion was possible. FR-1 amphetamine self-administration training continued for eight sessions. Rats exhibiting ten or more active lever presses per session and a greater than 2:1 active: inactive ratio of lever presses were then moved to the extinction phase. All rats met this criterion. Patency checks were administered after the last day of amphetamine training and non-patent rats were excluded from subsequent testing and analyses.

After eight FR-1 amphetamine sessions, extinction sessions commenced. The extinction sessions were also 1 hr in duration. All previously associated drug cues (light, tone, house light, and pump) were off during extinction. The levers remained in the chamber and lever presses resulted in no programmed consequence. TsA or vehicle injections began on Day 1 of extinction and occurred 30 min prior to operant sessions. TsA or vehicle injections continued through the cue- and drug-induced reinstatement tests. After ten extinction sessions, rats were moved to the cue-induced reinstatement phase of the experiment if the rats were under 20% of their peak FR-1 response rates (Bastle et al., 2012). Specifically, at the end of the tenth extinction session, the rats must have exhibited a decrease in active lever pressing to 20% of their peak response rate that occurred during extinction sessions. All rats met this criterion before moving on to the cue-induced reinstatement test.

All rats experienced the cue-induced reinstatement test first. Rats still received a TsA or vehicle injection 30 min prior to the session. The reinstatement sessions began with a 3-sec non-contingent cue presentation (80 dB, 3000-Hz tone accompanied with cue lights). After this cue presentation, subsequent active lever presses were paired with presentation of the cues. Rats then experience another round of extinction prior to the amphetamine (drug) - induced

reinstatement test. During this second extinction phase, active lever responses returned to the previously set extinction criteria described above (Bastle et al., 2012). This required four additional extinction sessions, after which all rats again met criterion. For the amphetamine-induced reinstatement test, 0.25 mg/kg amphetamine, s.c., was injected 15 min prior to the 1 hr reinstatement test. This specific amphetamine dose for drug-induced reinstatement has been shown to elicit robust reinstatement in rats reared in isolation (Stairs, Klein, & Bardo., 2006), and has resulted in sufficient drug-induced reinstatement rates in our lab's previous amphetamine self-administration studies with EC, IC, and SC rats (data not shown - Garcia et al., 2015). TsA or vehicle pretreatment injections continued 30 min prior to this reinstatement test. Any lever press resulted in no programmed consequence. Catheter patency checks were conducted after the drug-induced reinstatement test and non-patent rats were excluded from data analyses. A total of 34 rats were patent and were included in the data analysis for Experiment II (Table 3).

Testing for both drug-induced reinstatement and cue-induced reinstatement was important to measure the effects of both visual and auditory conditioned stimuli (tone, light, pump noise) vs. pharmacological stimuli (systemic amphetamine injection). This was conducted to parse the effects of both environmental condition and HDAC inhibition on different stimuli and essentially multiple risk factors for relapse.

## Data Analyses

### Experiment I: Acquisition and Lower-Unit Dose Amphetamine Self-Administration

#### Acquisition

To determine the effects of both environmental condition and HDAC inhibition (via TsA pretreatment) on rats' acquisition of amphetamine self-administration over the first five acquisition sessions, two separate mixed-factorial ANOVAs including the between-groups

factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor, acquisition session, were conducted on rats' active and inactive lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects and simple effect analyses were conducted to probe significant interaction effects.

***Acquisition time course***

Each 1-hr self-administration session was split into twelve, 5-min bins. To investigate the effects of environmental condition and HDAC inhibition (via TsA pretreatment) on rats' acquisition of amphetamine self-administration over twelve 5-minute bins for each of the first two acquisition sessions, two separate mixed-factorial ANOVAs including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor bin, were conducted on rats' active lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects.

**Lower-unit dose amphetamine self-administration**

Following the acquisition phase of five consecutive sessions, rats underwent two additional self-administration sessions prior to starting the lower-unit amphetamine dose test sessions. Rats were counterbalanced so that half of the rats responded for 0.03 mg/kg/infusion amphetamine first and half of the rats responded for 0.05 mg/kg/infusion amphetamine first. To help ensure that there were no undesired order effects, three-way ANOVAs including the between-groups factors environmental condition (EC vs. IC vs. SC), drug pretreatment (TsA vs. vehicle), and amphetamine dose order (0.03 mg/kg vs. 0.05 mg/kg) were conducted on rats' average active and inactive lever responding for the 0.03 and 0.05 mg/kg/infusion sessions.

After ensuring that no order effects were present, the effects of environmental condition and drug pretreatment on active and inactive lever pressing during lower-dose amphetamine

sessions (0.03; 0.05 mg/kg/infusion) were investigated by conducting two separate analyses of variance (ANOVAs) including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle). Specifically, one ANOVA was conducted using the average active lever presses across the three 0.03 mg/kg/infusion amphetamine dose sessions, and another ANOVA was conducted using the average active lever presses across the three 0.05 mg/kg/infusion amphetamine dose sessions. Identical analyses for inactive lever presses were also conducted to ensure neither environmental condition nor drug pretreatment led to nonspecific differences in general lever press behavior. Bonferroni multiple comparisons were conducted to probe significant main effects.

#### *Lower-unit dose amphetamine self-administration time course*

To investigate the effects of environmental condition and HDAC inhibition (via TsA pretreatment) on rats' amphetamine self-administration over twelve 5-min bins for both of the lower-unit dose amphetamine self-administration sessions (0.03 and 0.05 mg/kg/infusion), two separate mixed-factorial ANOVAs including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor bin, were conducted on rats' active lever presses. One ANOVA was conducted on the average of rats' active lever responses for the three 0.03 mg/kg/infusion sessions and one ANOVA was conducted on the average of rats' active lever responses for the three 0.05 mg/kg/infusion sessions.

## Experiment II: Extinction and Reinstatement of Amphetamine Self-Administration

### Acquisition

To ensure that EC, IC, and SC rats did not significantly differ in their acquisition of amphetamine self-administration (as measured by their active and inactive lever responding

during the eight acquisition sessions), two separate mixed-factorial ANOVAs including the between-groups factor environmental condition (EC vs. IC vs. SC) and the within-groups factor acquisition session were conducted on rats' active and inactive lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects.

**Extinction – changes in active lever presses**

To determine the effects of both environmental condition and HDAC inhibition (via TsA pretreatment) on rats' extinction of active lever pressing over the ten extinction sessions, two separate mixed-factorial ANOVAs including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor, extinction session, were conducted on rats' active and inactive lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects and simple effect analyses were conducted to probe significant interaction effects.

***Extinction – changes in active lever presses time course***

To further investigate the effects of environmental condition and HDAC inhibition (via TsA pretreatment) on rats' extinction of amphetamine self-administration over twelve 5-min bins for each of the first, second, and tenth extinction sessions, three separate mixed-factorial ANOVAs including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor bin, were conducted on rats' active lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects and simple effect analyses were conducted to probe significant interaction effects.

**Extinction – percent change**

To further illustrate the effects of drug pretreatment and environmental condition on extinction of active lever pressing, an additional mixed-factorial ANOVA including the between-

groups factors environmental condition (EC vs. IC. vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor, extinction session, was also conducted on the percent change of rats' active lever presses across extinction sessions. Bonferroni multiple comparisons were conducted to probe significant main effects.

**Cue-induced reinstatement**

To measure the effects of both environmental condition and HDAC inhibition on cue-induced reinstatement, two separate analyses of variance (ANOVAs) including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle) were conducted on rats' lever presses during the cue-induced reinstatement test session. Specifically, one ANOVA was conducted on the active lever presses during the cue-reinstatement session, and another ANOVA was conducted on the inactive lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects and simple effect analyses were conducted to probe significant interaction effects.

***Cue-induced reinstatement time course***

To further investigate the effects of drug pretreatment and environmental condition on the cue-induced reinstatement of active lever pressing over twelve 5-min bins for the cue-induced reinstatement session, a mixed-factorial ANOVA including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor bin, was conducted on rats' active lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects.

**Drug-induced reinstatement**

To determine the effects of both environmental condition and drug pretreatment on rats' lever presses on the drug-induced reinstatement test session, two separate analyses of variance

(ANOVAs) including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle) were conducted on rats' lever presses. Particularly, one ANOVA was conducted on the active lever presses during the drug-induced reinstatement test session, and another ANOVA was conducted on the inactive lever presses.

***Drug-induced reinstatement time course***

To further investigate the effects of drug pretreatment and environmental condition on the drug-induced reinstatement of active lever pressing over twelve 5-min bins for the drug-induced reinstatement session, a mixed-factorial ANOVA including the between-groups factors environmental condition (EC vs. IC vs. SC) and drug pretreatment (TsA vs. vehicle), and the within-groups factor bin, was conducted on rats' active lever presses. Bonferroni multiple comparisons were conducted to probe significant main effects and simple effect analyses were conducted to probe significant interaction effects.

# Chapter 3 - Results

## Experiment I: Acquisition and Lower-Unit Dose Amphetamine Self-Administration

### Acquisition

When investigating the effects of both environmental condition and HDAC inhibition (via TsA pretreatment) on rats' acquisition of amphetamine self-administration over the first five acquisition sessions, results revealed that the only significant effect was a main effect of acquisition session ($F(4, 144) = 34.71$, $p < .001$, $\eta^2 = .49$), suggesting that rats' acquisition of amphetamine self-administration differed across the acquisition sessions. The acquisition of amphetamine (0.1 mg/kg/infusion) self-administration was affected by neither environmental condition ($F(2, 36) = 1.00$, $p = .378$, $\eta^2 = .05$) nor HDAC inhibition ($F(1, 36) = 0.55$, $p = .464$, $\eta^2$

=.02). Additionally, there were no significant two-way interactions (acquisition session x environmental condition: $F(8, 144) = 0.70$, $p = .694$, $\eta^2 = .04$; acquisition session x drug pretreatment: $F(1, 144) = 0.09$, $p = .987$, $\eta^2 < .01$; environmental condition x drug pretreatment: $F(2, 36) = 0.62$, $p = .543$, $\eta^2 = .03$) or a three-way interaction (acquisition session x environmental condition x drug treatment: $F(8, 144) = 0.40$, $p = .918$, $\eta^2 = .02$; Figure 3A). These non-significant main effects of the between-groups factors of environmental condition and HDAC inhibition, and lack of significant interactions, indicate that rats' active lever presses were similar regardless of environmental condition and HDAC inhibition.

An identical mixed-factorial ANOVA was conducted on inactive lever presses which revealed a significant main effect of acquisition session ($F(4, 144) = 3.85$, $p = .005$, $\eta^2 = .10$), and a significant acquisition session x environmental condition interaction ($F(8, 144) = 2.27$, $p = .026$, $\eta^2 = .11$). Upon further analyses probing the significant acquisition session x environmental condition interaction, rats' inactive lever pressing on each separate acquisition session revealed no significant environmental condition differences ($F$s(2, 36) = 0.56–2.98, $p$s = .064–.576, $\eta^2$s = .03–.14). Furthermore, there was no significant main effect of TsA pretreatment ($F(1, 36) = 0.07$, $p = .789$, $\eta^2 < .01$), and no significant two-way interactions between acquisition session and drug pretreatment ($F(4, 144) = 1.07$, $p = .372$, $\eta^2 = .03$), or between environmental condition x drug pretreatment ($F(2, 36) = 0.40$, $p = .671$, $\eta^2 = .02$). Similarly, the three-way interaction between acquisition session, environmental condition, and drug pretreatment on inactive lever presses was not significant ($F(8, 144) = 0.62$, $p = .761$, $\eta^2 = .03$), suggesting that TsA pretreatment did not alter inactive lever press behavior during acquisition (Figure 3B).

**Acquisition time course**

TsA has a relatively short half-life in comparison with other HDAC inhibitors such as sodium butyrate (Siavoshian et al., 2000), with Sanderson and colleagues (2014) noting a half-life of 6.3 minutes following a low dose systemic injection (0.5 mg/kg, i.p.) and a 9.6 minute half-life following a high dose systemic injection (80 mg/kg, i.p.) in mice. Therefore, to examine active lever responding within the acquisition sessions to determine if TsA was more effective earlier in the session, analyses were conducted on Acquisition sessions 1 and 2 to investigate the effects of drug pretreatment within each environmental condition on active lever presses. For both Acquisition session 1 and 2, there were significant main effects of bin (Acquisition session 1: $F(11, 396) = 44.71$, $p < .001$, $\eta^2 = .55$; Acquisition session 2: $F(11, 396) = 55.98$, $p < .001$, $\eta^2 = .61$), but no significant main effects of environmental condition (Acquisition session 1: $F(2, 36) = 0.14$, $p = .870$, $\eta^2 = .01$; Acquisition session 2: $F(2, 36) = 1.38$, $p = .265$, $\eta^2 = .07$) or drug pretreatment (Acquisition session 1: $F(1, 36) = 0.15$, $p = .704$, $\eta^2 < .01$; Acquisition session 2: $F(1, 36) = 0.69$, $p = .509$, $\eta^2 = .04$). Furthermore, there was no significant environmental condition x drug pretreatment interaction effect for either acquisition session (Acquisition session 1: $F(2, 36) = 0.80$, $p = .459$, $\eta^2 = .04$; Acquisition session 2: $F(2, 36) = 0.69$, $p = .509$, $\eta^2 = .04$). Results revealed no significant drug pretreatment x bin interaction effect on either Acquisition session 1 ($F(11, 396) = 0.63$, $p = .805$, $\eta^2 = .02$) or Acquisition session 2 ($F(11, 396) = 0.57$, $p = .857$, $\eta^2 = .02$). Additionally, there were no significant three-way interactions between drug pretreatment, environmental condition, and bin on either Acquisition session 1 ($F(22, 396) = 1.26$, $p = .195$, $\eta^2 = .07$) or Acquisition session 2 ($F(22, 396) = 0.93$, $p = .559$, $\eta^2 = .05$). These results suggest that while TsA has a short half-life, we did not observe a significant TsA-induced early-session decrease in active lever responding during the early acquisition sessions for 0.1 mg/kg/infusion amphetamine. Figure 4 illustrates the active lever presses across

the twelve 5-minute bins for Acquisition session 1. Acquisition session 2 revealed a similar trend (data not shown).

## Lower-Unit Dose Amphetamine Self-Administration

While EC and IC rats do not differ in the self-administration of 0.1 mg/kg/infusion amphetamine, EC rats do differ from IC rats at lower unit doses, with less self-administration of amphetamine at lower unit doses in EC rats than IC rats (Arndt et al., 2015; Bardo et al., 2001; Green et al., 2002). Thus, we sought to determine whether HDAC inhibition via TsA pretreatment would differentially affect active lever pressing between EC, IC, or SC rats at lower unit doses of amphetamine to ensure any possible TsA-induced attenuation of amphetamine self-administration was not washed out by the reinforcing properties of a higher, 0.1 mg/kg/infusion unit dose.

### 0.03 mg/kg/infusion amphetamine self-administration

The presentation of the 0.03 and 0.05 mg/kg/infusion doses of amphetamine were counterbalanced across animals. Results revealed that there were no order effects on rats' 0.03 mg/kg/infusion lever responding for either the active or inactive lever (*p*s > .05). Furthermore, there were no significant interactions between rats' amphetamine dose order and rats' drug pretreatment (*p*s > .05) or between rats' amphetamine dose order and rats' environmental condition (*p*s > .05), indicating that for both vehicle and TsA pretreated rats, and for EC, IC, and SC rats, there were no significant dose order effects on rats' active and inactive lever responding during the 0.03 mg/kg/infusion amphetamine self-administration sessions.

Order analyses revealed no significant differences. Thus, the effects of environmental condition and drug pretreatment on amphetamine (0.03 mg/kg/infusion) self-administration were assessed. For the average active lever presses of all three of the 0.03 mg/kg/infusion

amphetamine sessions, results revealed a significant main effect of environmental condition ($F(2, 36) = 4.06$, $p = .026$, $\eta^2 = .18$), such that EC rats exhibited significantly fewer active lever responses for amphetamine than IC rats ($p < .05$). Results also revealed no significant main effect of drug pretreatment ($F(1, 36) = 0.28$, $p = .602$, $\eta^2 = .01$) or environmental condition x drug pretreatment interaction ($F(2, 36) = 0.60$, $p = .554$, $\eta^2 = .03$; Figure 5A). Together, these results suggest that while environmental enrichment attenuated low unit dose amphetamine self-administration, HDAC inhibition does not appear to differentially attenuate amphetamine self-administration between differentially-reared rats at the low (0.03 mg/kg/infusion) unit dose.

For inactive lever pressing, neither HDAC inhibition nor environmental condition had a significant effect on inactive lever presses. Specifically, results revealed rats' inactive lever presses were not significantly different based on their environmental condition ($F(2, 36) = 1.99$, $p = .151$, $\eta^2 = .10$) or their drug pretreatment ($F(1, 36) = 0.55$, $p = .465$, $\eta^2 = .02$). Additionally, there was no significant interactions between environmental condition and drug pretreatment ($F(2, 36) = 0.39$, $p = .678$, $\eta^2 = .02$; Figure 5B). Therefore, the effect of rats' drug pretreatment condition (TsA vs. vehicle) on their inactive lever pressing did not significantly depend on the rats' environmental rearing condition.

***0.03 mg/kg/infusion amphetamine self-administration time course***

Due to the short half-life of TsA, an ANOVA was conducted on the average of the three 0.03 mg/kg/infusion self-administration sessions to determine if TsA was more effective earlier in the session. Results revealed a significant main effect of bin ($F(11, 396) = 47.44$, $p < .001$, $\eta^2 = .57$) and a significant main effect of environmental condition ($F(2, 36) = 3.98$, $p = .027$, $\eta^2 = .18$). There was, however, no significant main effect of drug pretreatment ($F(1, 36) = 0.25$, $p = .620$, $\eta^2 = .01$) nor was there a significant environmental condition x drug pretreatment

interaction ($F(2, 36) = 0.61$, $p = .547$, $\eta^2 = .03$). Results also revealed no significant drug pretreatment x bin interaction effect ($F(11, 396) = 0.70$, $p = .742$, $\eta^2 = .02$) and no significant three-way drug pretreatment x environmental condition x bin interaction effect ($F(22, 396) = 1.25$, $p = .202$, $\eta^2 = .07$; Figure 6). These results suggest that while TsA has a short half-life, there was not a significant TsA-induced early-session decrease in active lever responding for 0.03 mg/kg/infusion amphetamine.

**0.05 mg/kg/infusion amphetamine self-administration**

As previously noted, the presentation of the 0.03 and 0.05 mg/kg/infusion doses of amphetamine were counterbalanced across animals. Results revealed that there were no order effects on rats' 0.05 mg/kg/infusion lever responding for either the active or inactive lever (*p*s > .05). Furthermore, there were no significant interactions between rats' amphetamine dose order and rats' drug pretreatment (*p*s > .05) or between rats' amphetamine dose order and rats' environmental condition (*p*s > .05), indicating that for both vehicle and TsA pretreated rats, and for EC, IC, and SC rats, there were no significant dose order effects on rats' active and inactive lever responding during the 0.05 mg/kg/infusion amphetamine self-administration sessions.

Order analyses revealed no significant differences. Thus, the effects of environmental condition and drug pretreatment on amphetamine (0.05 mg/kg/infusion) self-administration were assessed. Unlike the results for the ANOVA conducted on the average of all three 0.03 mg/kg/infusion amphetamine self-administration sessions, the results of the ANOVA conducted on the average of all three 0.05 mg/kg/infusion amphetamine sessions revealed no significant main effect of environmental condition ($F(2, 36) = 2.26$, $p = .119$, $\eta^2 = .11$), no significant main effect of drug pretreatment ($F(1, 36) < 0.01$, $p = .999$, $\eta^2 < .01$), and no significant environmental condition x drug pretreatment interaction ($F(2, 36) = 2.12$, $p = .135$, $\eta^2 = .11$). The effect of

environmental condition on rats' active lever presses did not depend on whether rats were administered TsA or vehicle before drug-taking sessions (Figure 7A). These results suggest that there was not a significant TsA-induced decrease in active lever responding for 0.05 mg/kg/infusion amphetamine.

For inactive lever pressing, neither HDAC inhibition nor environmental condition had a significant effect on inactive lever presses. Specifically, results revealed rats' inactive lever presses were not significantly different based on their environmental condition ($F(2, 36) = 1.96$, $p = .155$, $\eta^2 = .10$) or their drug pretreatment ($F(1, 36) < 0.01$, $p = .953$, $\eta^2 < .01$). Additionally, the effect of rats' drug pretreatment condition (TsA vs. vehicle) on their inactive lever pressing did not significantly depend on rats' environmental rearing condition ($F(2, 36) = 0.13$, $p = .881$, $\eta^2 = .01$; Figure 7B).

***0.05 mg/kg/infusion amphetamine self-administration time course***

Due to the short half-life of TsA, an ANOVA was conducted on the average of the three 0.05 mg/kg/infusion self-administration sessions to determine if TsA was more effective earlier in the session. Results revealed a significant main effect of bin ($F(11, 396) = 78.12$, $p < .001$, $\eta^2 = .69$), but no significant main effects of environmental condition ($F(2, 36) = 2.26$, $p = .119$, $\eta^2 = .11$) or drug pretreatment ($F(1, 36) < 0.01$, $p = .999$, $\eta^2 < .01$). In addition to the non-significant main effects of environmental condition and drug pretreatment, there was not a significant interaction between the two between-groups factors ($F(2, 36) = 2.12$, $p = .135$, $\eta^2 = .11$). Results further revealed no significant drug pretreatment x bin interaction effect ($F(11, 396) = 0.74$, $p = .701$, $\eta^2 = .02$) and no significant three-way drug pretreatment x environmental condition x bin interaction effect ($F(22, 396) = 0.95$, $p = .527$, $\eta^2 = .05$; Figure 8). These results suggest that

while TsA has a short half-life, there was not a significant TsA-induced early-session decrease in active lever responding for 0.05 mg/kg/infusion amphetamine.

## Experiment II: Extinction and Reinstatement of Amphetamine Self-Administration

### Acquisition

The ANOVA testing whether EC, IC, or SC rats differed in responding on the first eight days of amphetamine self-administration revealed that despite the significant main effect of acquisition session ($F(7, 224) = 24.18$, $p < .001$, $\eta^2 = .43$) on rats' active lever responding during acquisition, there was no significant main effect of environmental condition ($F(2, 32) = 3.20$, $p = .054$, $\eta^2 = .17$) nor a significant acquisition session x environmental group interaction ($F(14, 224) = 1.16$, $p = .308$, $\eta^2 = .07$) on rats' active lever responding, indicating that EC, IC, and SC rats did not differ in their active lever responding during the acquisition of amphetamine self-administration (Figure 9A). This lack of difference in active lever pressing during acquisition between EC, IC, and SC rats for 0.1 mg/kg/infusion amphetamine is in accordance with previous findings of our lab (Arndt et al., 2015) and others' (Bardo et al., 2001; Green, Gehrke, & Bardo, 2002).

Nearly identical to the non-significant acquisition effects for rats' active lever responding, rats' inactive lever responding for the eight acquisition sessions showed only a significant main effect of acquisition session, ($F(7, 224) = 3.16$, $p = .003$, $\eta^2 = .09$). There was no significant main effect of environmental condition ($F(2, 32) = 2.09$, $p = .140$, $\eta^2 = .12$), nor was there a significant acquisition session x environmental condition interaction ($F(14, 224) = 0.51$, $p = .926$, $\eta^2 = .03$), indicating that EC, IC, and SC rats did not differ in their inactive lever responding during the acquisition of amphetamine self-administration (Figure 9B).

## Extinction – Changes in Active Lever Presses

The results of the mixed-factorial ANOVA investigating the effects of both environmental condition and HDAC inhibition (via TsA pretreatment) on rats' active lever responding across the extinction sessions revealed a significant main effect of extinction session ($F(9, 261) = 73.37$, $p < .001$, $\eta^2 = .72$), such that rats overall decreased their active lever responses across the extinction sessions. There was also a significant main effect of environmental condition ($F(2, 29) = 18.51$, $p < .001$, $\eta^2 = .56$). In addition to the significant main effects of extinction session and environmental group, there was a significant session x environmental condition interaction ($F(18, 261) = 3.71$, $p < .001$, $\eta^2 = .20$). Probing this interaction using simple effects revealed that for all of the extinction sessions, there were significant differences in active lever pressing between the environmental conditions ($F$s(1, 21) = 5.10-32.45, $p$s < .05). For all of the extinction sessions, IC rats exhibited significantly more active lever presses than EC rats. IC rats also consistently pressed the active lever more than SC rats, and IC and SC rats consistently pressed the active lever more than EC rats. These differences in active lever pressing between environmental conditions differed across the extinction sessions (Figure 10A).

There was no significant main effect of TsA pretreatment ($F(1, 29) = 0.81$, $p = .375$, $\eta^2 = .03$) on rats' active lever responding over the ten extinction sessions. There was also no significant interaction between environmental condition and TsA pretreatment ($F(2, 29) = 1.24$, $p = .303$, $\eta^2 = .08$), and no significant interaction between extinction session and TsA pretreatment ($F(9, 261) = 1.23$, $p = .278$, $\eta^2 = .04$). Furthermore, the three-way interaction between extinction session, environmental condition, and TsA pretreatment was not significant ($F(18, 261) = 1.09$, $p = .358$, $\eta^2 = .07$; Figure 10B). These results suggest that drug pretreatment

(TsA or vehicle) did not appear to play a significant role in the extinction of active lever pressing for any one of the three environmental conditions across the ten extinction sessions.

**Extinction – changes in active lever presses time course**

Analyses were conducted on Extinction sessions 1, 2, and 10 to determine if TsA was more effective earlier in the 1 hr session during initial and later extinction days (due to the short half-life of TsA as previously discussed). Results revealed significant main effects of bin for all three extinction sessions (Extinction session 1: $F(11, 319) = 29.77$, $p < .001$, $\eta^2 = .51$; Extinction session 2: $F(11, 319) = 13.43$, $p < .001$, $\eta^2 = .32$; Extinction session 10: $F(11, 319) = 14.91$, $p < .001$, $\eta^2 = .34$) and significant main effects of environmental condition for all three extinction sessions (Extinction session 1: $F(2, 29) = 9.97$, $p = .001$, $\eta^2 = .41$; Extinction session 2: $F(2, 29) = 12.72$, $p < .001$, $\eta^2 = .47$; Extinction session 10: $F(2, 29) = 3.52$, $p = .043$, $\eta^2 = .20$). For all three extinction sessions, however, there were no significant main effects of drug pretreatment (Extinction session 1: $F(1, 29) = 0.07$, $p = .795$, $\eta^2 < .01$; Extinction session 2: $F(1, 29) = 1.59$, $p = .217$, $\eta^2 = .05$; Extinction session 10: $F(1, 29) = 0.01$, $p = .921$, $\eta^2 < .01$) nor any significant environmental condition x drug pretreatment interactions (Extinction session 1: $F(2, 29) = 0.12$, $p = .891$, $\eta^2 = .01$; Extinction session 2: $F(2, 29) = 1.70$, $p = .201$, $\eta^2 = .11$; Extinction session 10: $F(2, 29) = 0.65$, $p = .530$, $\eta^2 = .04$). There were no significant drug pretreatment x bin interaction effects on either Extinction session 1 ($F(11, 319) = 0.30$, $p = .985$, $\eta^2 = .01$), Extinction session 2 ($F(11, 319) = 1.04$, $p = .413$, $\eta^2 = .04$), or Extinction session 10 ($F(11, 319) = 0.25$, $p = .993$, $\eta^2 = .01$). Additionally, there were no significant three-way interactions between drug pretreatment, environmental condition, and bin on either Extinction session 1 ($F(22, 319) = 1.03$, $p = .422$, $\eta^2 = .07$; Figure 11) or Extinction session 2 ($F(22, 319) = 0.74$, $p = .794$, $\eta^2 = .05$; Figure 12). Results did reveal, however, a significant drug pretreatment x

environmental condition x bin three-way interaction for Extinction session 10 ($F(22, 319) = 2.17$, $p = .002$, $\eta^2 = .13$). Simple effects analyses on Extinction session 10 revealed that with the exception of bin 1, TsA did not significantly inhibit rats' active lever pressing in comparison to their vehicle counterparts. For the first bin, however, TsA inhibited IC rats' active lever pressing compared to IC-vehicle counterparts ($F(1, 9) = 11.09$, $p = .009$; Figure 13B) but did not inhibit EC or SC rats' active lever pressing in comparison to their vehicle counterparts ($p$s = .513-.516; Figures 13A and 13C). These results suggest that for later extinction sessions, particularly in Extinction session 10, TsA facilitated early within-session extinction in IC rats more so than EC or SC rats.

## Extinction – Percent Change

As a result of baseline differences in active lever responding during extinction between EC and IC rats observed in the current study, any possible effect of drug pretreatment (TsA vs. vehicle) on rats' extinction of active lever pressing was examined through additional mixed-factorial ANOVAs investigating the effects of both environmental condition and HDAC inhibition (via TsA pretreatment) on rats' percent changes in active lever pressing over the first 10 extinction sessions. Results revealed a significant main effect of extinction session ($F(8, 232) = 9.29$, $p < .001$, $\eta^2 = .24$), such that in comparison to rats' active lever pressing on the first session of extinction, rats' active lever responding decreased more drastically during earlier sessions of extinction rather than later sessions of extinction. There was also a significant main effect of environmental condition ($F(2, 29) = 3.94$, $p = .031$, $\eta^2 = .21$), such that regardless of drug pretreatment, and collapsed across all extinction sessions, EC rats expectedly exhibited a greater reduction in their active lever pressing in comparison to their first session of extinction than did IC or SC rats. These differences between environmental conditions did not change

significantly across the extinction sessions ($F(16, 232) = 1.63$, $p = .062$, $\eta^2 = .10$). There was no significant main effect of drug pretreatment ($F(1, 29) = 1.69$, $p = .204$, $\eta^2 = .06$), nor was there a significant environmental group x drug pretreatment interaction ($F(2, 29) = 2.17$, $p = .133$, $\eta^2 = .13$). Additionally, there was no significant extinction session x drug pretreatment interaction ($F(8, 232) = 1.10$, $p = .362$, $\eta^2 = .04$), and the three-way extinction session x environmental condition x drug pretreatment interaction was also not significant ($F(16, 232) = 1.25$, $p = .230$, $\eta^2 = .08$; Figure 14). These results suggest that while enriched rats extinguished active lever pressing more than IC rats, drug pretreatment (TsA or vehicle) did not appear to play a significant role in the extinction of active lever pressing for any one of the three environmental conditions.

## Cue-Induced Reinstatement

The ANOVA conducted on active lever presses during the cue-induced reinstatement test session revealed a significant main effect of environmental condition on rats' active lever pressing ($F(2, 28) = 17.10$, $p < .001$, $\eta^2 = .55$). Interestingly, there was a significant main effect of drug pretreatment on rats' active lever presses, ($F(1, 28) = 6.72$, $p = .015$, $\eta^2 = .19$) such that rats that received TsA injections prior to the cue-induced reinstatement test pressed the active lever significantly less than rats that received vehicle pretreatment, indicating a general TsA-induced decrease in drug-seeking.

Furthermore, results revealed a marginally-significant environmental condition x drug pretreatment interaction ($F(2, 28) = 3.14$, $p = .059$, $\eta^2 = .18$). Due to the aforementioned a priori hypotheses regarding possible differences in the ability of TsA to reduce reinstatement differently in isolated rats compared to enriched rats, this interaction was probed. Simple effects revealed that for both EC and SC rats, there were no significant differences in active lever

pressing between TsA or vehicle pretreated rats (EC: $F(1, 28) = 0.06$, $\eta^2 = .01$, SC: $F(1, 28) = 2.03$, $\eta^2 = .15$, $p$s $> .05$). However, IC-vehicle rats exhibited significantly more active lever presses than IC-TsA rats ($F(1, 28) = 9.94$, $p < .05$, $\eta^2 = .56$; Figure 15A). Together, this finding suggests that HDAC inhibition can attenuate cue-induced reinstatement in IC rats, but not EC or SC rats. This suggests that differential rearing conditions (particularly isolated environments) may alter epigenetic HDAC mechanisms that can change the likelihood of relapsing drug-seeking behaviors when exposed to conditioned contextual cues heavily associated with drug reward.

The ANOVA conducted on rats' inactive lever presses during the cue-induced reinstatement test session revealed no significant differences between EC, IC, and SC rats' inactive lever presses ($F(2, 28) = 1.43$, $p = .256$, $\eta^2 = .09$). The ANOVA also revealed no significant differences between TsA and vehicle rats' cue-induced reinstatement of inactive lever presses ($F(1, 28) = 0.32$, $p = .578$, $\eta^2 = .01$). Results further revealed a non-significant environmental condition x drug pretreatment interaction ($F(2, 28) = 0.41$, $p = .665$, $\eta^2 = .03$; Figure 15B), suggesting that the observed TsA-induced attenuation of lever pressing was specific to active lever responding only, and did not affect nonspecific, general lever press behavior.

**Cue-induced reinstatement time course**

Due to the short half-life of TsA, as previously discussed, an ANOVA was conducted on the cue-induced reinstatement session to determine if TsA was more effective earlier in the session. Results revealed a significant main effect of bin ($F(11, 319) = 38.21$, $p < .001$, $\eta^2 = .57$), a significant main effect of environmental condition ($F(2, 29) = 16.79$, $p < .001$, $\eta^2 = .54$), and a marginally significant main effect of drug pretreatment ($F(1, 29) = 3.94$, $p = .057$, $\eta^2 = .12$). The interaction between environmental condition and drug pretreatment was not, however,

significant ($F(2, 29) = 1.71$, $p = .199$, $\eta^2 = .11$). Results further revealed no significant drug pretreatment x bin interaction effect ($F(11, 319) = 1.24$, $p = .257$, $\eta^2 = .04$) and no significant three-way drug pretreatment x environmental condition x bin interaction effect ($F(22, 319) = 1.10$, $p = .342$, $\eta^2 = .07$); however, given the significant main effect of drug pretreatment ($F(1, 28) = 6.72$, $p = .015$, $\eta^2 = .19$) and the marginally significant environmental condition x drug pretreatment interaction ($F(2, 28) = 3.14$, $p = .059$, $\eta^2 = .18$) for the cue-induced reinstatement test session, simple effects analyses probing this three-way interaction comparing TsA vs. vehicle within each environmental group across the 12 bins revealed that with the exception of bin 1 and bin 9, TsA did not significantly inhibit rats' lever pressing in comparison to their vehicle counterparts. For the first and ninth bin, however, TsA inhibited IC rats' active lever pressing in comparison to IC-vehicle rats' active lever pressing ($F$s(1, 9) = 5.52 – 6.01, $p$s = .037-.043; Figure 16B) but did not inhibit EC or SC rats' active lever pressing in comparison to their vehicle counterparts ($p$s > .05; Figures 16A and 16C). These results suggest that TsA attenuated within-session active lever pressing in IC rats more so than EC or SC rats during the cue-induced reinstatement test, particularly during bins 1 and 9.

## Drug-Induced Reinstatement

For the ANOVA conducted on active lever presses during the drug-induced reinstatement test session, results revealed no significant main effect of environmental condition on rats' active lever pressing ($F(2, 29) = 1.59$, $p = .222$, $\eta^2 = .10$), or drug pretreatment on rats' active lever presses during the drug-induced reinstatement test session ($F(1, 29) = 1.41$, $p = .244$, $\eta^2 = .05$). Further, there was not a significant environmental condition x drug pretreatment interaction ($F(2, 29) = 0.02$, $p = .981$, $\eta^2 < .01$; Figure 17A), suggesting that the effect of environmental condition

on rats' drug-induced reinstatement of active lever pressing does not differ depending on whether rats received TsA or vehicle pretreatment.

Additionally, the ANOVA conducted on rats' inactive lever presses during the drug-induced reinstatement test session revealed no significant differences between EC, IC, and SC rats' inactive lever presses ($F(2, 29) = 1.45$, $p = .250$, $\eta^2 = .09$). However, the ANOVA revealed significant differences between TsA and vehicle rats' drug-induced reinstatement of inactive lever presses ($F(1, 29) = 5.33$, $p = .028$, $\eta^2 = .16$), such that rats pretreated with TsA exhibited fewer inactive lever presses than rats pretreated with vehicle injections. Results further revealed a non-significant environmental condition x drug pretreatment interaction ($F(2, 29) = 1.29$, $p = .290$, $\eta^2 = .08$; Figure 17B).

Taken together, when comparing the effects of environmental condition and HDAC inhibition from the cue-induced reinstatement to the drug-induced reinstatement, these results suggest that HDAC inhibition is more likely to attenuate cue-induced reinstatement, with a greater attenuation in IC rats compared to EC or SC rats. When investigating the effects of pharmacologically-driven reinstatement of drug-seeking behavior, both environmental condition and HDAC inhibition play a lesser role in decreasing the likelihood of exhibiting relapse of drug-seeking behaviors.

**Drug-induced reinstatement time course**

Due to the short half-life of TsA, as previously discussed, an ANOVA was conducted on lever responding within the drug-induced reinstatement session to determine if TsA was more effective earlier in the session. Results revealed a significant main effect of bin ($F(11, 319) = 28.00$, $p < .001$, $\eta^2 = .49$), no significant main effects of environmental condition ($F(2, 29) = 1.59$, $p = .222$, $\eta^2 = .10$) and drug pretreatment ($F1, 29) = 1.41$, $p = .244$, $\eta^2 = .05$), and no

significant environmental condition x drug pretreatment interaction ($F(2, 29) = 0.02$, $p = .981$, $\eta^2 < .01$). Results revealed no significant drug pretreatment x bin interaction effect ($F(11, 319) = 0.43$, $p = .943$, $\eta^2 = .02$) and a marginally significant three-way drug pretreatment x environmental condition x bin interaction effect ($F(22, 319) = 1.52$, $p = .066$, $\eta^2 = .10$). Simple effects analyses to probe this interaction revealed no significant differences between TsA pretreated rats' active lever responding and vehicle rats' active lever responding within each environmental group for any of the bins ($p$s > .05; Figure 18A-C). These results suggest that while TsA attenuated within-session active lever pressing in IC rats during the cue-induced reinstatement test, TsA pretreatment did not affect active lever pressing for any environmental condition during any bin of the drug-induced reinstatement test.

# Chapter 4 - Discussion

## Overview

This study supports current literature suggesting a protective effect of environmental enrichment against low unit-dose psychostimulant self-administration (Arndt et al., 2015; Bardo et al., 2001; Green, Gehrke, & Bardo, 2002). Additionally, while environmental enrichment alone facilitated the extinction of active lever presses for amphetamine (0.1 mg/kg/infusion), HDAC inhibition did not accentuate or diminish this effect in any of the environmental conditions. A similar null-TsA effect was observed in both EC and SC conditions for both the cue- and drug-induced reinstatement tests, suggesting that HDAC inhibition has no effect in facilitating the extinction or reducing the reinstatement of previous drug-seeking behaviors in rats housed in enriched or standard conditions. However, in IC conditions, HDAC inhibition significantly facilitated later-session extinction responding and attenuated cue-induced reinstatement, which suggests that isolated rats that receive TsA pretreatment are less likely to

exhibit a relapse in drug-seeking behaviors that are elicited by conditioned cues heavily associated with drug reward.

Our behavioral findings indicate that isolated rearing environments may alter epigenetic HDAC function, and HDAC inhibition may help reduce the high reinstatement behaviors typically observed in isolated rats (Green et al., 2010). These findings and their theoretical implications, along with future directions for related research are discussed in detail in the sections below.

## Acquisition and Lower-Unit Dose Amphetamine Self-Administration

## Discussion

Environmentally-enriched rats self-administer less amphetamine at low unit doses compared to rats reared in isolated conditions (Arndt et al., 2015; Bardo et al., 2001; Green, Gehrke, & Bardo, 2002). However, this 'protective' effect of enrichment in reducing amphetamine self-administration diminishes in rats lever-pressing for higher unit doses of amphetamine. In the current study, rats acquired amphetamine self-administration at a dose in which enriched rats do not differ in FR-1 active lever responding compared to isolated- or standard-housed rats, and their behavior during this phase was consistent with our previous findings (Arndt et al., 2015). As anticipated, there were no behavioral differences observed in active lever pressing for amphetamine (0.1 mg/kg/infusion) between any of the three environmental conditions during the acquisition phase. This lack of difference in baseline active lever pressing between EC, IC, and SC rats allowed us to confidently assess any effect of Trichostatin A (TsA) pretreatment in altering acquisition rates of amphetamine self-administration without the presence of preexisting differences in active lever responding induced by environmental condition alone..

## Dose-Dependency and Half-Life Factors

Due to the dose-dependent neurochemical differences following psychostimulant self-administration, and the observation that enrichment is less protective against higher doses of amphetamine, we questioned whether the ability of TsA to decrease amphetamine self-administration was also amphetamine dose-dependent. Therefore, in addition to studying the effects of both environmental condition and TsA pretreatment on amphetamine (0.1 mg/kg/infusion) self-administration, we also studied how environmental condition and TsA pretreatment affected self-administration for lower-unit amphetamine doses (0.03 and 0.05 mg/kg/infusion). This was conducted primarily to ensure any possible TsA-induced attenuation of amphetamine self-administration was not washed out by the more reinforcing effects of a higher, 0.1 mg/kg/infusion dose, and to measure how TsA affected responding for multiple doses of amphetamine. As previously mentioned, reports by Bardo et al. (2001), as well as Green and colleagues (2002), were supported by our findings in that EC rats self-administered less amphetamine (0.03 mg/kg/infusion) than IC rats. However, TsA again had little effect in altering active lever responding for either of the lower-unit amphetamine doses in any of the environmental conditions. The interaction between HDAC inhibition and psychostimulant dose-dependent effects has not been investigated with drugs such as cocaine or methamphetamine, but results of the current study suggest the ability of TsA to reduce amphetamine self-administration in differentially-reared rats does not appear to be amphetamine dose-dependent.

In addition to amphetamine dose-dependent issues, we also investigated the time course of amphetamine self-administration to determine when exactly any possible TsA-induced effect on active lever responding was taking place within each one hour session. With the relatively short half-life of TsA (Sanderson et al., 2004; Siavoshian et al., 2000) compared to other HDAC

inhibitors, we questioned whether TsA pretreatment decreased active lever pressing early on in drug-taking sessions, and whether any possible TsA effect was more apparent during lower unit-dose amphetamine self-administration sessions. Time course analyses revealed no evidence of TsA-pretreatment affecting early-session amphetamine self-administration at any dose, which further suggests that any TsA-induced changes in amphetamine self-administration in differentially-reared rats is not amphetamine dose-dependent.

## Role of Plasticity and BDNF

We did not observe robust TsA-induced decreases in active lever responding during the acquisition phase in any environmental group, and thus did not replicate the TsA findings of Romieu et al., (2008) in our differentially-reared rats. We actually observed that EC-TsA rats exhibited slightly more active lever pressing in than SC-TsA rats (Figure 3). While this effect was not significant, and the direct cause is unclear, part of the reason may be due to a neuroplasticity-induced change in TsA efficacy that may be age-, strain-, or drug-dependent. Romieu and colleagues (2008) found that TsA pretreatment reduces the self-administration of cocaine during acquisition sessions. Their route of administration for TsA pretreatment and operant procedures were similar to the current study, but the specific postnatal day in which the behavioral assessments occurred is unclear and they did not investigate the effects of TsA in differentially-reared rats. Furthermore, Romieu and colleagues (2008; 2011) studied active lever pressing for cocaine by Wistar rats, whereas the current study investigated active lever pressing for amphetamine in Sprague-Dawley rats. The rats in the current study were also fairly young during the acquisition phase of the experiment (postnatal day 75). Evidence suggests that HDAC inhibitors, such as sodium butyrate, valproic acid, and TsA can induce neuronal differentiation in cell cultures of rat cortical tissue (Hsieh et al., 2004). Additionally, it has been found that HDAC

inhibitor-induced cell proliferation and differentiation requires BDNF–tyrosine kinase B signaling (Kim, Leeds, & Chuang, 2009).

As mentioned previously, BDNF is involved in the epigenetic regulation of many psychiatric disorders (Mitchelmore & Gede, 2014), its expression differs between enriched and isolated rats in several brain areas (Ickes et al., 2000), and it is known to be highly regulated by psychostimulants (Russo et al., 2009). Furthermore, it has been observed that early-life stress can evoke age-dependent changes in hippocampal neurogenesis and BDNF expression (Suri et al., 2013). Given that environmental enrichment alters neuroplasticity and neurogenesis (Tanti et al., 2013), and that social isolation is associated with an up-regulation of BDNF expression (Kumari et al., 2016), it is possible that rearing-induced, age-dependent epigenetic regulatory mechanisms, such as BDNF, may have altered the ability of TsA to attenuate the acquisition of amphetamine self-administration in the current study.

## Differences in Psychostimulant-Induced Transporter Function

In addition to strain and age-dependent plasticity factors, there is an abundance of literature investigating the behavioral effects of cocaine in operant self-administration paradigms, while less is known about the psychostimulating properties of amphetamine. Although cocaine and amphetamine produce similar physiological and behavioral effects in rodents (Ritz & Kuhar, 1989), these two commonly abused psychostimulants, when self-administered by rodents in operant settings, result in differential dose-dependent increases in extracellular dopamine concentrations in reward regions of the brain, such as the nucleus accumbens (Moghaddam & Bunney, 1989).

Furthermore, while cocaine and amphetamine both increase dopamine release in the synapse, they both achieve this by uniquely interacting with dopamine transporter (DAT) and

vesicular monoamine transporter (VMAT) function (Fleckenstein & Hanson, 2003; Godino, Jayanthi, & Cadet, 2015). Amphetamine primarily functions as a 'dopamine releaser', releasing synaptic dopamine via amphetamine-induced disruptions to pH gradients that redistribute vesicular dopamine into the cytoplasm (Sulzer et al., 1995). As a result of this redistribution, amphetamine-induced cytoplasmic dopamine levels rise, and dopamine exits the neuron via reverse DAT activity, which leads to significant increases in synaptic dopamine (Sulzer & Rayport, 1990; Kahlig et al., 2005; Sulzer et al., 1995). Cocaine on the other hand functions more as a dopamine reuptake blocker (Riddle, Fleckenstein, & Hanson, 2005). While cocaine and amphetamine both affect VMAT function, cocaine primarily blocks DAT. Amphetamine also blocks DAT, but has a much higher affinity for VMAT than cocaine (Howell & Kimmel, 2008). Previous studies have also suggested differences in the way cocaine and amphetamine alter the subcellular localization of VMAT-containing synaptic vesicles, and the mechanistic functions of dopamine reuptake blockers (cocaine) and dopamine releasers (amphetamines) can differ depending on drug dose, or whether the drug is administered acutely vs. chronically (Riddle, Fleckenstein, & Hanson, 2005).

## Link between DAT, VMAT, and HDAC Function

Various chromatin and DNA modifiers change in response to acute vs. repeated psychostimulant exposure, but little is known about the intracellular signaling pathways through which amphetamine affects dopaminergic receptors that lead to global changes in histone acetylation (Nestler, 2014). However, histone deacetylase (HDAC) inhibition has been shown to increase DAT mRNA expression in vitro (Green et al., 2015). Also, HDAC inhibition via TsA administration can increase the uptake of the monoamine, norepinephrine, via the norepinephrine transporter (Martiniova et al., 2011) and can also lead to the induction of dopaminergic

transcription factors that correspond with increased coexpression of both DAT and VMAT (Rössler, Boddeke, & Copray, 2010). Cocaine has been shown to recruit HDACs through the binding of sigma-1 receptors, and also suppresses the gene expression of monoamine oxidase B in the nucleus accumbens without affecting DAT function (Tsai et al., 2015). Taken together, these findings suggest that cocaine (as was studied by Romieu et al., 2008; 2011) and amphetamine (investigated in the current study) affect VMAT and DAT function differently, and these different alterations to transporter function may reflect unique changes to histones and HDAC mechanisms in the nucleus accumbens that may lead to TsA-induced reductions of cocaine self-administration (Romieu et al., 2008), while having lesser TsA-induced effects on amphetamine self-administration (current findings). The specific HDAC subtypes that may be interacting with DAT or VMAT function should also be taken into consideration, as different HDAC isoforms could have varying roles in interacting with transporter function to change the behavioral responses to psychostimulants (Robison & Nestler, 2011).

## Role of Various Drug Models and the Interaction with HDAC and Gene Expression

Our primarily-null TsA findings during acquisition and lower-unit dose drug-taking sessions contradict the behavioral findings of others using different models of drug reward. Specifically, research suggests HDAC inhibition can prevent or reverse amphetamine-induced locomotor behavior (Frey et al., 2006; Schroeder et al., 2013) and attenuate the maintenance of amphetamine-induced behavioral sensitization (Kalda et al., 2007). Previous findings also suggest that Class I and Class II HDAC inhibition can attenuate the acquisition of methamphetamine-induced behavioral sensitization (Coccurello et al., 2007) and decrease alcohol self-administration in dependent rats (Simon-O'Brien et al., 2015). Our findings suggest a lesser ability of HDAC inhibition to attenuate amphetamine self-administration.

To date, very few studies have investigated HDAC and genetic expression during or after the self-administration of amphetamine, or its analog, methamphetamine. However, Cadet et al. (2015) found that during chronic methamphetamine self-administration and shortly thereafter, the majority of methamphetamine-induced striatal genes are up-regulated. However, when those same genes are measured one month into methamphetamine withdrawal, only about 12% show up-regulation. Thus, dynamic gene regulation patterns appear to exist in withdrawal phases compared to earlier stages of drug-taking. Interestingly, acute methamphetamine administration can increase expression of HDACs in the NAcc (Martin et al., 2012) and dorsal striatum (Jayanthi et al., 2014), further indicating complex and dynamic genetic processes after acute vs. repeated psychostimulant exposure in brain regions relevant to the current study. While the findings by Cadet et al. (2015) were observed following the administration of methamphetamine, it is plausible there would be similar down-regulation and up-regulation patterns of genes after the self-administration of amphetamine because both amphetamine and methamphetamine similarly affect monoamine transporter functions (VMAT; Godino, Jayanthi, & Cadet, 2015).

Our observations also suggest that TsA may be less effective at attenuating the behavioral responses to amphetamine in more translational models of drug abuse, such as self-administration, and that the therapeutic benefits of HDAC inhibitors in reducing the rewarding properties of drugs may depend on the behavioral model as well as the specific drug of abuse. Specificity and binding affinity of various HDAC inhibitors may have played a role as well, as TsA inhibits a broad range of HDACs (HDACs 1, 3, 4, 6 and 10), while other HDAC inhibitors, such as sodium butyrate and valproic acid, are more specific, inhibiting HDAC2 while TsA does not (Kim & Bae, 2011). TsA also displays an in vitro $IC_{50}$ (a measure of drug efficacy) in the nanomolar range (Yoshida et al., 1990), whereas other HDAC inhibitors, such as phenylbutyrate,

display an affinity in the micromolar range (Butler & Bates, 2006). Behaviorally, these affinity and efficacy issues appear to play a role in differentially affecting the responses to drugs of abuse, as the administration of valproic acid, sodium butyrate, or Trichostatin A can have different effects on the psychostimulating properties of amphetamine or methamphetamine depending on the HDAC inhibitor used, frequency of administration, or the model investigated (Godino, Jayanthi, & Cadet, 2015).

While HDAC inhibitor specificity may have played a role, our findings in Experiment I coincide with our alternative hypothesis in that pretreatment with HDAC inhibitors may be more effective at protecting against the occurrence of relapse instead of protecting against drug reward sensitivity as measured by FR-1 responding for amphetamine. Possible reasons for this difference in TsA efficacy between the two experiments in the current study are discussed in detail below.

## Extinction and Reinstatement Discussion

After acquiring amphetamine self-administration in Experiment II, rats were moved to the extinction phase in which active lever presses no longer led to a programmed amphetamine infusion paired with tone + light cues. Previous studies have shown that during the extinction phase, environmentally-enriched rats extinguish their lever pressing for psychostimulants more readily than isolated rats (Alvers et al., 2012; Green et al., 2010; Stairs, Klein, & Bardo, 2006). This enrichment-induced facilitation of extinction compared to isolated rats has also been observed in other animal models of drug reward, such as nicotine conditioned place preference (Ewin, Kangiser, & Stairs, 2015). Furthermore, facilitated extinction of active lever pressing for psychostimulants can also be achieved by placing non-enriched rats into enriched conditions (Thiel et al., 2010), which suggests the effects of enrichment on extinction does not require a full

post-weaning rearing of animals in enrichment, but rather post-adolescent exposure to enrichment later in life can also lead to a decrease in drug-seeking when drug reward is no longer available.

In the current study, we provide support for the literature suggesting that enriched (EC) rats display greater extinction of active lever presses for amphetamine than SC or IC rats. Independent of drug pretreatment, we observed a robust environmental condition-induced gradient in active lever pressing during extinction, with EC rats displaying the greatest extinction, followed by SC rats, then IC rats. This supports previous research and further implies that enriched environments, compared to isolated conditions, facilitate the reduction of active lever pressing during sessions in which active lever responding, which once led to a programmed drug infusion, no longer result in drug reward. These preclinical findings provide support for a facilitating role of environmental enrichment on extinction of drug-seeking and the potential remediating impact enrichment can have on reducing drug craving through Pavlovian, extinction-based, behavioral therapeutic techniques (Everitt & Robbins, 2016).

### TsA-Induced Facilitation of Extinction in IC Rats

We found some evidence to support the hypothesis that TsA would facilitate the extinction of active lever pressing in IC rats compared to EC or SC rats. For the most part, HDAC inhibition via TsA pretreatment did not facilitate the learning that active lever presses, which once led to a programmed drug infusion, no longer resulted in drug reward. However, further investigation of time course analyses revealed that TsA facilitated early-session extinction in IC, but not EC or SC rats during the final extinction session prior to the cue-induced reinstatement test, providing some support that TsA reduces active lever responding during extinction in IC rats more so than standard or enriched counterparts. Preclinical learning and

memory findings suggest that HDAC inhibition and environmental enrichment can improve learning and memory mechanisms (Fischer et al., 2007; Gräff & Tsai, 2013; Sweatt, 2009). Furthermore, while Malvaez et al. (2010; 2013) found that HDAC inhibition facilitates the extinction of cocaine conditioned place preference in standard rats, we are the first to measure the effects of HDAC inhibition on the extinction of amphetamine self-administration.

Only one study (Castino, Cornish, & Clemens, 2015) has investigated the effects of Class I HDAC inhibition (sodium butyrate) on the extinction of the self-administration of a psychostimulant. Castino and colleagues observed that sodium butyrate significantly facilitated extinction of nicotine self-administration across sessions. However, one important methodological difference between this study and the current one is that their HDAC inhibition occurred *after* the extinction sessions, whereas we administered our HDAC inhibitor, TsA, *prior* to operant sessions. Comparing their effects of HDAC inhibition to our null-effect of TsA on extinction responding in our SC group suggests that HDAC inhibition may alter the consolidation of extinction memory for psychostimulants differently depending on if the HDAC inhibitor is administered before vs. after extinction sessions. Here, we provide some evidence that broad Class I/II HDAC inhibition prior to extinction responding may be more effective at facilitating extinction in rats reared in isolated conditions, while being less effective in standard or enriched rats. Because the nucleus accumbens is so heavily involved in the extinction of psychostimulant self-administration (Ranaldi et al., 1999; Roberts et al., 1980), our TsA time course findings during extinction may suggest that isolated rats have altered accumbal HDAC function that may be important for learning and memory mechanisms associated with drug reinforcement.

## HDAC-Inhibitor-Induced Differences in Cue- vs. Drug-Induced Reinstatement

In addition to the role of HDAC inhibition in extinction behaviors, we anticipated that IC rats' cue-induced reinstatement of active lever pressing would be more attenuated by TsA than EC rats' reinstatement of active lever pressing. Our hypotheses were supported, such that during the cue-induced reinstatement test, IC rats that received TsA pretreatment exhibited significantly less cue-induced reinstatement than IC rats that received vehicle pretreatment, with no TsA-induced differences observed in SC or EC rats compared to their vehicle counterparts. This suggests that the isolated rearing condition alters epigenetic HDAC mechanisms that may change the likelihood of drug relapse when exposed to conditioned contextual cues heavily associated with drug reward. Perhaps epigenetic changes can interact with rearing conditions to alter Pavlovian-instrumental transfer (Everitt & Robbins, 2016), and our results may suggest that HDAC inhibition, in isolated rats only, can blunt the saliency of conditioned stimuli, effectively reducing the expression of instrumental behavior geared toward seeking out drug reinforcement.

Furthermore, even though TsA significantly attenuated cue-induced reinstatement, particularly in IC rats, TsA pretreatment resulted in no difference in active lever pressing during the drug-induced reinstatement test. Our findings suggest that HDAC inhibition is more important for alleviating the relapse potential induced by contextual and conditioned stimuli alone rather than the pharmacological effects of drugs of abuse. Research studying the effects of HDAC inhibition on cue- and/or drug-induced reinstatement is lacking, and while Romieu et al. (2011) concluded that TsA pretreatment can attenuate reinstatement, it is important to note they did not implement an extinction period, but rather a withdrawal period of three weeks prior to TsA pretreatment and reinstatement. Additionally, they implemented a simultaneous cue + drug-induced reinstatement test for cocaine, and did not conduct the reinstatement tests separately

following an extinction period as we did in the current study with amphetamine. Our findings extend Romieu et al. (2011) and suggest that of the two parameters (cue- vs. drug-induced reinstatement), HDAC inhibition appears to affect cue-induced reinstatement more so than drug-induced reinstatement. Thus, these methodological factors (extinction sessions vs. no extinction sessions) and conducting the reinstatement tests separately vs. together, combined with the use of different psychostimulants (amphetamine vs. cocaine), are important to consider in the design of future experiments, and may lead to differential TsA-induced effects on cue- or drug-induced reinstatement measures.

In either case, further work is needed to determine the specific role that environmental condition may play in these measures of relapse that involve complex learning and memory mechanisms. Interestingly, Levenson and colleagues (2004) observed that systemic injections of the HDAC inhibitor, sodium butyrate, enhanced the formation of contextual fear memory, which suggests that HDAC inhibition can alter learning and memory mechanisms that affect behavioral phenotypes known to differ between enriched and isolated rats (Barbelivien et al., 2006; Fone & Porkess, 2008; Lukkes et al., 2009; Nikolaev et al., 2002).

## Neurobiological Mechanisms of Extinction and Reinstatement

The differences in TsA efficacy we observed between the two reinstatement tests following extinction, compared to Romieu et al. (2011) who did not implement extinction sessions, suggests the involvement of different neurobiological mechanisms (Pickens et al., 2011) driving the behaviors during extinction sessions leading up through the cue- or drug-induced reinstatement tests that may have impacted the ability of TsA to alter drug-seeking behaviors. Indeed, Romieu et al. (2011) acknowledge being back in the context of the operant chamber following a non-extinction withdrawal period of three weeks probably accounted for

some of the reinstatement behaviors they observed in rats under any treatment condition. It is plausible that this methodological difference prior to reinstatement (extinction learning vs. no extinction learning) initiated different neural mechanisms that govern drug-seeking behaviors.

**Role of mesocorticolimbic regions**

While the specific brain regions and circuits responsible for our observed TsA effect during cue-induced reinstatement in isolated rats compared to enriched rats remain unclear, it is plausible that differences within the mesocorticolimbic reward system (Fone & Porkess, 2008; Stairs & Bardo, 2009; Zakharova et al., 2009) played a significant role. Interestingly, rats that self-administer cocaine display reduced HDAC activity in the prefrontal cortex and nucleus accumbens (Romieu et al., 2008), two regions within the mesocorticolimbic reward system that are not only involved in extinction and reinstatement behaviors, but also function differently between isolated and enriched rats (Del Arco & Mora, 2009; Fone & Porkess, 2008; Zhu et al., 2005). These studies suggests a role of HDAC-inhibitor-induced chromatin modification and its effects on altering genetic expression in regions of the brain responsible for drug-seeking behaviors (McQuown & Wood, 2010; Walker et al., 2015), and also in brain regions that are known to differ between enriched and isolated rats and their response to drugs of abuse.

Research by Kalivas, Peters, and Knackstedt (2006) have shown that projections from the ventral prefrontal cortex to the nucleus accumbens shell may form a circuit involved in reducing the reinstatement of drug-seeking, and this reduction in reinstatement can be further strengthened by prior extinction sessions. They also observed that continuous extinction sessions can not only affect glutamate signaling in the nucleus accumbens, but can also affect projections from the ventral prefrontal cortex that underlie reinstatement behaviors. Thus, following drug self-

administration, it appears that behavior during reinstatement, and its corresponding neural mechanisms, can be greatly influenced by the presence or absence of prior extinction learning.

The neural mechanisms governing drug craving appear to be associated with time-dependent, cue-induced psychostimulant-seeking that primarily resides within the mesocorticolimbic dopamine pathway (Shalev, Grimm, & Shaham, 2002). Specifically, contextual cue-induced increases in neuronal activation have been observed within the core of the nucleus accumbens (Hollander & Carelli, 2007), as well as the medial prefrontal cortex (mPFC; Koya et al., 2009). Research also suggests that there may be a neurobiological interplay between HDAC inhibition and differential rearing within the mesocorticolimbic dopamine system (Belin et al., 2009; Feltenstein & See, 2008).

At the neurotransmitter level, dopamine release in the dorsal prefrontal cortex appears to play an initiating role in the mesocorticolimbic circuit that mediates drug-induced cocaine-seeking (McFarland & Kalivas, 2001). This sheds some light on the neural circuits responsible for the initiation of drug-seeking behaviors and how craving and drug-seeking can be instigated by dopaminergic neurotransmission in the mesocorticolimbic pathway. Previous work has found a major role of the ventral tegmental area (VTA) in driving drug-induced reinstatement behaviors (Kalivas & McFarland, 2003).

Our findings, taken together with previous research and in comparison with similar studies (Romieu et al., 2011), suggest that the additional implementation of extinction sessions up through the cue-induced reinstatement test and continuing on before the drug-induced reinstatement test may have altered the ability of TsA to attenuate drug-induced reinstatement. The ability of TsA pretreatment to reduce cue-induced drug-seeking in IC rats suggests that in IC, and not SC or EC rats', HDAC function may have been altered by their specific rearing

condition. The IC-induced HDAC alterations may primarily reside within the NAcc or mPFC, rather than the VTA, but future work is needed to elucidate the specific function of dopaminergic projections throughout the mesocorticolimbic pathway and how these projections can be altered by environmental condition and HDAC inhibition.

Our results suggest a difference in HDAC function between enriched and isolated rats during the cue-induced reinstatement test. It is plausible that some of these rearing-induced differences in histone acetylation and HDAC function reside in the mesocorticolimbic pathway, because previous research suggests that chronic cocaine exposure increases H3 acetylation within the nucleus accumbens (Wang et al., 2010), and decreases accumbal HDAC5 expression (Schmidt et al., 2013) that alters the motivation for drug reinforcement.

Interestingly, Grimm and colleagues (2003) observed time-dependent increases in BDNF protein levels within the mesocorticolimbic pathway following cocaine withdrawal. Furthermore, BDNF injections into the shell of the nucleus accumbens during cocaine self-administration can increase both extinction responding and cue-induced reinstatement following two weeks of withdrawal (Graham et al., 2007). As mentioned previously, HDAC inhibitor-induced neurogenesis requires BDNF signaling (Kim, Leeds, & Chuang, 2009). While BDNF is highly regulated by psychostimulants (Russo et al., 2009), and its expression differs between enriched and isolated rats in several brain areas (Ickes et al., 2000), it is currently unclear if differential rearing can lead to BDNF-dependent changes in histone mechanisms within reward pathways of the brain that may in turn affect the reinstatement of drug-seeking behaviors.

These findings suggest that brain regions within the mesocorticolimbic pathway, which are known to significantly differ in function between enriched and isolated rats (Green, Gehrke, & Bardo, 2002; Neugebauer et al., 2004; Stairs & Bardo, 2009), are heavily involved in the

extinction and reinstatement of drug-seeking, but their projections, and the specific neurotransmitters involved, may differ depending on whether drug-craving is driven by extinction sessions, contextual cues, or the stimulating properties of the drug itself. Future studies directed at addressing these issues would further our understanding of environmental condition-induced differences in drug craving that may be driven by various epigenetic mechanisms in target brain regions known to play critical roles in the cue-induced reinstatement of psychostimulant-seeking, such as the core of nucleus accumbens, medial prefrontal cortex, or basolateral amygdala (BLA).

**Role of the basolateral amygdala (BLA) and hippocampus**

Notably, work by Kalivas & McFarland (2003) has indicated a major role of the basolateral amygdala (BLA) in driving cue-induced reinstatement (Kalivas & McFarland, 2003). Previous research has also shown that TsA injections directly into the BLA can increase BDNF expression and enhance morphine context-associated memory (Wang et al., 2015). Research has also shown that environmental enrichment can boost learning and memory through increased histone acetylation in the hippocampus and amygdala (Sweatt, 2009). Rats exposed to environmental enrichment exhibit an increase in dendritic spine density, greater global histone acetylation, and ultimately show the preservation of learning and memory. Similar research has shown that enriched rats possess greater memory restoration abilities, and non-enriched rats that are administered HDAC inhibitors following the induction of neurodegeneration also exhibit similar benefits to learning and memory (Patel, 2012). This suggests that environmental enrichment and HDAC inhibition may be acting through common mechanisms, particularly those that involve long-term potentiation induced by synaptogenesis and histone hyperacetylation. These mechanisms may have played an important underlying role in the

differences we observed between enriched and isolated rats following TsA pretreatment, especially in the behavioral phases of the experiment that directly involved Pavlovian learning and memory processes, such as cue-induced reinstatement.

Fischer and colleagues (2007) also noted increased histone acetylation in the hippocampus of environmentally-enriched mice. Interestingly, there is evidence to suggest that the dorsal hippocampus plays a critical role in contextual-, but not cocaine-induced reinstatement (Fuchs et al., 2005), and electrical stimulation of the hippocampus elicits cocaine seeking in rats following the extinction of cocaine self-administration (Vorel et al., 2001). Whether the positive outcomes of enrichment on learning and memory, as well as the benefits of HDAC inhibition on learning and memory are mechanistically-related, remains to be determined.

The current study does not allow us to conclude the exact effect differential rearing had on specific HDAC mechanisms and the brain areas involved, but the results do suggest that administration of HDAC inhibitors can reduce cue-induced relapse of amphetamine-seeking in isolated rats to levels comparable to those displayed by standard-housed rats, and based on previous research, these behaviors may be a manifestation of rearing-induced epigenetic function in hippocampal, mesocorticolimbic, or amygdala regions.

## Findings In Comparison With Other Drug Models

Our results showing that TsA pretreatment attenuated cue-induced drug-seeking for amphetamine are consistent with reports investigating relapse to other psychostimulants. Specifically, research has shown a similar HDAC-inhibitor-induced attenuation in cocaine-seeking and extinction of cocaine conditioned place preference (Malvaez et al., 2010; 2013; Peterson, Abel, & Lynch, 2014). Peterson and colleagues (2014) also observed that environmental enrichment through exercise via a running wheel can protect against cocaine

relapse by blocking the epigenetic modifications in BDNF in the prefrontal cortex that occur following chronic cocaine administration and abstinence. While the enriched condition in the current study did not include a running wheel, it is plausible that environmental enrichment, in combination with exercise, may result in an even greater decrease in cue-induced reinstatement and perhaps the beneficial effects of both enrichment and exercise may also alleviate drug-craving during other phases of addiction, such as acquisition and maintenance. It would be interesting to determine if the ability of TsA to reduce the drug-taking or drug-seeking behaviors measured in the current study could be boosted if supplemented by exercise.

Overexpression of HDAC4 and HDAC5 in the nucleus accumbens attenuates cocaine conditioned place preference, and TsA-induced HDAC inhibition in the BLA facilitates extinction and reduces the reinstatement of morphine-induced CPP (Wang et al., 2015). It is important to note however, as previously mentioned, that different neural and behavioral mechanisms are involved following morphine or cocaine administration compared to the administration of amphetamine or its analog, methamphetamine. Research has indicated that histone acetylation can influence the behavioral effects of methamphetamine (Shibasaki et al., 2011), and increased DNA methylation of histone H3 is associated with increased methamphetamine-induced conditioned place preference, whereas decreased DNA methylation of histone H3 is related to decreased CPP (Aguilar-Valles et al., 2014). Similar studies investigating amphetamine are lacking, but these findings suggest that epigenetic alterations can cause different behaviors in drug-related associative learning models that may be drug-specific (morphine vs. methamphetamine vs. cocaine), and it is possible that these behaviors may be a manifestation of long-term drug- or rearing-induced epigenetic modifications (Cadet, McCoy, & Jayanthi, 2016).

We extend the previous epigenetic, reinstatement, and differential rearing literature by showing that HDAC inhibition significantly attenuated cue-induced reinstatement in isolated rats compared to rats reared in standard or enriched conditions. We also support and extend the previous work by Malvaez and colleagues (2010; 2013) and Peterson et al. (2014) that suggest a role of HDAC inhibition in decreasing the conditioned and rewarding effects of psychostimulants that are facilitated by associative learning. Moreover, we show that the ability of HDAC inhibitors to decrease the cue-induced reinstatement of drug-seeking can depend upon non-pharmacological life experiences, such as differential rearing environments. However, our findings contradict previous CPP conclusions by Itzhak and colleagues (2013) who observed a strengthening in the expression of cocaine-associated contextual memory that was elicited by sodium butyrate-induced HDAC inhibition. This may suggest that HDAC inhibitor-induced changes to Pavlovian learning and memory processes relevant to addictive behavior may depend on either specific (sodium butyrate) or more global (Trichostatin A) HDAC inhibition, as well as the specific drug of abuse under study (cocaine vs. amphetamine) and the model employed (CPP vs. self-administration).

Our results suggest differences in epigenetic histone function between differentially-reared rats. HDAC mechanisms in isolated rats, and how they affect drug-taking and drug-seeking behaviors, are poorly understood, but the current findings provide support that HDAC inhibition can alleviate the propensity of isolated rats to exhibit cue-induced reinstatement. Part of the reason for this effect may be a result of increased histone acetylation in isolated rats following TsA pretreatment. Early life stress has been shown to decrease mRNA levels for multiple HDACs in the cortex (Levine et al., 2012; Lewis & Olive, 2014). Perhaps isolation rearing, serving as a form of early-life stress, decreases general HDAC expression. The fact that

enriched rats may have already been in a histone hyperacetylated state (Fischer et al., 2007; Patel, 2012; Sweatt, 2009), may explain why we did not observe a robust TsA effect in enriched rats compared to their vehicle counterparts. For SC-TsA rats, we observed a slight decrease in cue-induced reinstatement compared to SC-vehicle rats, but this difference was not significant. As such, the ability of HDAC inhibition to prevent cue-induced reinstatement may be most evident in IC rats, followed by SC rats, then EC rats, and this observation may reflect rearing-induced differences in HDAC function that can change cue-induced relapse behaviors.

The literature discussed here and in previous sections indicates an important role of epigenetic regulatory mechanisms within the mesocorticolimbic pathway, hippocampus, and basolateral amygdala, and how these epigenetic mechanisms can shape drug-taking and drug-seeking behaviors. Previous research has shown that after psychostimulant administration in various models of drug reward, activity within these brain regions can differ depending on whether a rat is reared in enriched, standard, or isolated conditions. HDAC inhibition may induce histone hyperacetylation within the nucleus accumbens and/or medial prefrontal cortex that may subsequently affect associative learning and other processes that govern conditioned and contextual cue-induced reinstatement. Further work is needed to answer these specific research questions, and avenues like these for future research are explained in the sections below.

## Limitations and Future Directions

The findings presented in these experiments help illustrate the role of both rearing condition and epigenetic alteration in both drug-taking and drug-seeking behaviors, and suggest that isolation rearing can impact HDAC mechanisms, because HDAC inhibition reduces the cue-induced reinstatement of active lever pressing for amphetamine in isolated rats more than enriched or standard rats. This study also suggests that HDAC inhibition affects cue-induced

reinstatement of drug-seeking more so than behaviors in earlier stages of addiction such as acquisition, maintenance, or extinction. Importantly, as evident in a recent review discussing the role of epigenetics and drug addiction, more is currently known about the epigenetic mechanisms of nicotine, alcohol, opiates, cocaine, and methamphetamine than is known about the epigenetic mechanisms of amphetamine (Cadet, McCoy, & Jayanthi, 2016). The current study adds to the amphetamine epigenetic literature and illustrates the importance of differential lifetime experiences in changing the epigenetic (HDAC) mechanisms involved in drug-seeking behaviors. Nonetheless, a few limitations remain, and possible avenues for future research coinciding with these limitations are discussed in detail below.

## Single Dose of HDAC Inhibitor

Previous research studying the effects of Trichostatin A on changing the behavioral responses to drugs of abuse have primarily used the dose of 0.3 mg/kg, i.v. (Host et al., 2011; Romieu et al., 2008; 2011), the dose used in the current study. Previous studies have investigated the effects of TsA at lower doses (0.03 mg/kg, i.v.; Romieu et al., 2008), as well as different routes of administration (100-1000 µg, i.c.v.) but significant decreases in psychostimulant self-administration are most robust at 0.3 mg/kg, i.v. Interestingly, Kumar and colleagues (2005) found that TsA at a dose of 0.2 mg/kg, administered systemically (i.p.) resulted in an increase in cocaine conditioned place preference, which suggests that the ability of Trichostatin A to decrease the conditioned and rewarding effects of drugs of abuse may not only be dose dependent, but may also yield different effects based on the route of administration. Further research should be conducted using various doses of TsA to gain a more comprehensive view of the role of TsA-induced alterations in drug-taking and drug-seeking behaviors. There may be dose-dependent effects occurring with TsA-induced HDAC inhibition during any of the

four phases of addiction studied in the current dissertation – acquisition, maintenance, extinction, or reinstatement. Specifically, while TsA was effective at attenuating cue-induced reinstatement in IC rats at 0.3 mg/kg TsA, perhaps a different dose is necessary to capture effects in the drug-induced reinstatement phase, or in earlier acquisition and extinction phases.

However, when considering the use of higher doses of HDAC inhibitors, it is important to ensure that higher doses do not significantly affect general locomotor activity, which may confound the operant data gathered in self-administration studies. Previous behavioral findings indicate that TsA, at a dose of 0.3 mg/kg TsA, i.v., can decrease the psychostimulating locomotor effects of cocaine, while having no effect on general locomotion (Romieu et al., 2008). We utilized the same dose as Romieu and colleagues (2008; 2011), but to help control for any possible TsA-induced locomotor effect, we conducted a small pilot test investigating the locomotor effects of 0.3 mg/kg, i.v. TsA. We also found no significant TsA-induced differences in locomotor activity compared to vehicle counterparts (data not presented). It is important to note that literature investigating locomotor activity following TsA pretreatment at doses greater than 0.3 mg/kg, i.v. in rats is lacking and should be included if subsequent studies of higher doses of TsA are to be used.

## Temporal and Causal Factors in HDAC Changes

In addition to the behavioral questions investigated in the current study, one important neurobiological question left unanswered involves the specific temporal and causal changes to epigenetic (e.g. HDAC) markers that may have occurred throughout the entire experimental period. Epigenetic constructions and functions can change in response to a plethora of environmental stimuli. Epigenetic markers can also transform during critical developmental periods (Sweatt, 2009), which can drastically affect behaviors later in adulthood. It is difficult to

conclude based on the presented behavioral data as to whether any rearing- or drug-induced changes in HDAC function were a result of differential rearing condition, amphetamine exposure, or both. Exposure to drugs of abuse is known to alter histone function (Renthal & Nestler, 2008), and environmental enrichment has also been found to alter histone mechanisms regarding learning and memory (Patel, 2012). Thus, it can be difficult to conclude when, and to what extent, differential rearing may be impacting HDAC mechanisms and whether chronic amphetamine exposure plays an equal or greater role in changing the HDACs that TsA is also affecting. Furthermore, any behavioral effect of HDAC inhibitors might be the result of alterations in acetylation of a wide variety of intracellular mechanisms, such as lysine amino acid side chains and other molecular targets in various signaling pathways (Sweatt, 2009). Thus, in addition to collecting behavioral data, future studies could pair behavioral findings with biochemical evidence to draw stronger conclusions about the specific neurobiological mechanisms responsible for differences in drug-taking and drug-seeking between enriched and isolated rats following HDAC inhibition.

**Biochemical assays**

Common biochemical assays that measure relative protein expression, as well as the distribution and localization of cellular markers, such as Western blotting and immunohistochemistry, respectively, may serve as useful tools in parsing rearing-induced vs. amphetamine-induced HDAC changes. Due to the role of the mesocorticolimbic pathway discussed in previous sections, and how activity within this system can be altered by both drugs of abuse and differential rearing, future research should primarily target brain regions within this system when investigating the various temporal and causal factors in HDAC changes and how these alterations can alter psychostimulant-driven behaviors.

Additionally, future work in this area should focus on a wide variety of HDACs, as evidence suggests varying roles of different HDAC isoforms in the response to drugs of abuse. Cocaine self-administration, for example, causes increased HDAC1 and HDAC2 expression in the nucleus accumbens (Host et al., 2011). Cocaine self-administration also causes decreased HDAC5 dephosphorylation in the nucleus accumbens (Taniguchi et al., 2012). HDAC3 and HDAC4 expression have been shown to change following psychostimulant administration (Kennedy et al., 2013), with HDAC3 negatively regulating cocaine-context-associated memory (Rogge et al., 2013). Acute methamphetamine administration has also been shown to decrease histone H3 acetylation (Martin et al., 2012), while increasing H4 acetylation in the striatum (Harkness et al., 2013). These studies suggest a complex and dynamic role of many different HDAC isoforms that may be drug- or brain region-dependent, so future studies can help identify the specific molecular mechanisms driving addictive behaviors. Also, while much is known about the role of drug-induced VMAT and DAT function, research into how HDAC inhibitors can alter the function of these monoamine transporters is lacking. Investigating the effects of HDAC inhibition on DAT and VMAT function would provide evidence of epigenetic regulation of monoamine transporters and their role in altering drug-taking and drug-seeking behaviors.

In addition to studies utilizing Western blotting and immunohistochemical techniques, future studies could use more advanced biochemical assessments such as genome-wide expression profiling through chromatin immunoprecipitation (ChIP) analyses to provide further insights into the neural and behavioral adaptations in the nucleus accumbens (Chakravarty & Kumar, 2012). Regardless of the specific biochemical technique used, literature suggests that future studies measuring rearing- and/or drug-induced changes to various HDAC mechanisms should be directed toward either Class I or Class II HDACs within the mesocorticolimbic

dopamine system. By implementing these techniques and supplementing biochemical evidence with behavioral data, future work could gain a clearer understanding of malleable epigenetic markers, and whether they change primarily as a function of differential rearing, amphetamine exposure, or both.

## Schedule of Reinforcement

The FR-1 schedule of reinforcement was the sole reinforcement schedule employed in the present study. FR-1 self-administration data are typically interpreted as measures of reward sensitivity because a rat needs to exhibit just one active lever press to experience the rewarding effects of the drug under study. However, the operant response of lever pressing for drug reinforcement is not what traditional behaviorists would use to estimate reinforcer magnitude. Rather, the goal of traditional behaviorists is to explore changes in behavior due to alterations in stimulus-response contingencies. Thus, the underlying principles in the drug self-administration paradigm were not built on theories to quantify the reinforcing efficacy of drugs of abuse (Arnold & Roberts, 1997). Also, it is often unclear if a lower active lever response rate reflects a less reinforcing drug, or if the animal has reached satiation of the drug and is choosing not to lever press because the rat no longer has the immediate desire to do so.

To better quantify the motivation to self-administer drugs in preclinical operant settings, utilizing various schedules of reinforcement (FR-10 and/or progressive-ratio (PR) schedules) may be beneficial in future studies investigating the effects of HDAC inhibition on either drug-taking or various Pavlovian incentive motivational processes (Everitt & Robbins, 2016). However, it is important to note that altering the schedule of reinforcement by increasing the response requirement of the rat to receive drug reward can result in significant baseline differences between enriched and isolated rats. Specifically, environmentally-enriched rats

display very low response rates for psychostimulants on progressive-ratio schedules of reinforcement (Alvers et al. 2012; Arndt et al., 2015; Bardo et al. 2001; Green, Gehrke, & Bardo, 2002), suggesting that enriched rats are less motivated to work for drug reinforcement than isolated rats. Therefore, for future studies to confidently assess the effects of HDAC inhibition in altering drug self-administration in enriched and isolated rats, more demanding schedules of reinforcement should be used with caution due to the preexisting baseline differences between EC and IC rats in the motivation to self-administer amphetamine. For these reasons, the FR-1 schedule seemed most appropriate for the current study.

## Statistical Approach

The statistical method most frequently used to analyze behavioral self-administration data centers around the analysis of variance (ANOVA), which was the analysis conducted in the current study. The ANOVA is the primary statistical model used in the literature, which effectively analyzes the differences between experimental group means. However, it should be noted that comparing group means via ANOVAs aggregates group data and consequently cannot investigate and capture significant individual differences that may exist within each treatment condition. Because individual differences inherently exist in both human and rodent drug-taking and drug-seeking behaviors, more advanced statistical models (e.g., linear mixed-effects modeling, multiple regression, multilevel modeling, etc.; Dingemanse & Dochtermann, 2013; Snijders, 2011) can allow for more statistical power and can more effectively detect individual slope differences in acquisition, maintenance, extinction, or reinstatement rates of active lever pressing. In addition to the better measurement of individual variation in drug-taking and drug-seeking behaviors, in the current study, a possible reason for some of the insignificant ($p > .05$) interactions presented may have been a result of low statistical power given the relatively low

sample sizes in some of the treatment conditions (see Table 3). By integrating and utilizing some of these more advanced statistical approaches, such as linear-mixed effect modeling, some of the interesting trends presented here may have been more likely to yield statistically significant results.

For example, these types of advanced analytical methods may help boost one's statistical model by treating each active lever response as its own event in the drug-taking or drug-seeking session. This results in more degrees of freedom and a more robust, advanced statistical approach. While individual differences in drug-taking and drug-seeking were not the primary focus of the current study, future research aimed at investigating the individual variation in drug-taking and drug-seeking behaviors using these statistical methods (described in detail by Cohen et al., 2013) may be valuable in effectively parsing individual differences that can alter the efficacy of therapeutic interventions intended to treat addictive behaviors.

## Summary

The results of this study have furthered our understanding of how different environmental conditions can alter an aspect of the epigenome, and how this alteration manifests itself in the operant drug abuse model of intravenous self-administration. These results support recent literature suggesting a role of HDAC inhibition in changing the behavioral responses to psychostimulants, and how the function of HDAC inhibition can depend on both the rearing condition and the specific phase of addiction. Ultimately, this study provides support for how different life experiences, modeled through differential rearing, can alter an aspect of the epigenome, changing the ability of HDAC inhibitors to decrease the relapse of conditioned, contextual drug-seeking.

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Table 1.

*Timeline of Behavioral Phases for Experiment I. Rats arrived to lab at postnatal day 21 (PND 21) and were differentially-reared in enriched, isolated, or standard conditions and remained in those conditions for the entire duration of the experiment. Trichostatin A (TsA; 0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections were administered 30 minutes prior to operant sessions (PND 75) and continued through the final day of experimentation.*

| PND 21-50 | PND 51-59 | PND 60-74 | PND 75-77 | PND 78-84 |
|---|---|---|---|---|
| Differentially-Rear in Environmental Conditions | Begin Food Restriction and Lever Press Training | Regain Weight, Surgery, and Surgery Recovery | Begin Trichostatin A (TsA) or Vehicle Injections | Acquisition of Amphetamine (0.1 mg/kg/infusion) Self-Administration |

| PND 85-87 | PND 88-89 | PND 90-92 | PND 93-94 |
|---|---|---|---|
| Amphetamine (0.03 or 0.05 mg/kg/infusion) Self-Administration | Return to Training Dose (0.01 mg/kg/infusion) Self-Administration | Amphetamine (0.05 or 0.03 mg/kg/infusion) Self-Administration | Return to Training Dose (0.01 mg/kg/infusion) Self-Administration |

Table 2.

*Timeline of Behavioral Phases for Experiment II. Rats arrived to lab at postnatal day 21 (PND 21) and were differentially-reared in enriched, isolated, or standard conditions and remained in those conditions for the entire duration of the experiment. Trichostatin A (TsA; 0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections were administered 30 minutes prior to operant sessions (PND 88) and continued through the final day of experimentation.*

| PND 21-50 | PND 51-62 | PND 63-79 | PND 80-87 |
|---|---|---|---|
| Differentially-Rear in Environmental Conditions | Begin Food Restriction and Lever Press Training | Regain Weight, Surgery, and Surgery Recovery | Acquisition of Amphetamine (0.1 mg/kg/infusion) Self-Administration |

| PND 88-97 | PND 98 | PND 99-102 | PND 103 |
|---|---|---|---|
| Begin TsA or Vehicle Treatment and Extinction Sessions | Cue-Induced Reinstatement Test Day | Additional Operant Extinction Sessions | Drug-Induced Reinstatement Test Day |

Table 3.

*Sample Size and Treatment Condition Breakdown after Final Patency Checks*. Catheter patency checks were conducted to ensure rats were properly receiving i.v. TsA or vehicle pretreatment, and to ensure proper amphetamine self-administration. Non-patent rats were excluded from the studies and only patent rats were included in the data analyses. The numbers of final patent rats included in the data analyses are indicated in the table below.

*Experiment I – Acquisition; Low-dose tests*

| **Environmental Condition** | **Drug Treatment** | |
|---|---|---|
| | Vehicle | TsA |
| Enriched | 5 | 8 |
| Isolated | 7 | 8 |
| Standard | 6 | 8 |

*Experiment II – Extinction; Reinstatement*

| **Environmental Condition** | **Drug Treatment** | |
|---|---|---|
| | Vehicle | TsA |
| Enriched | 5 | 7 |
| Isolated | 4 | 6 |
| Standard | 5 | 7 |

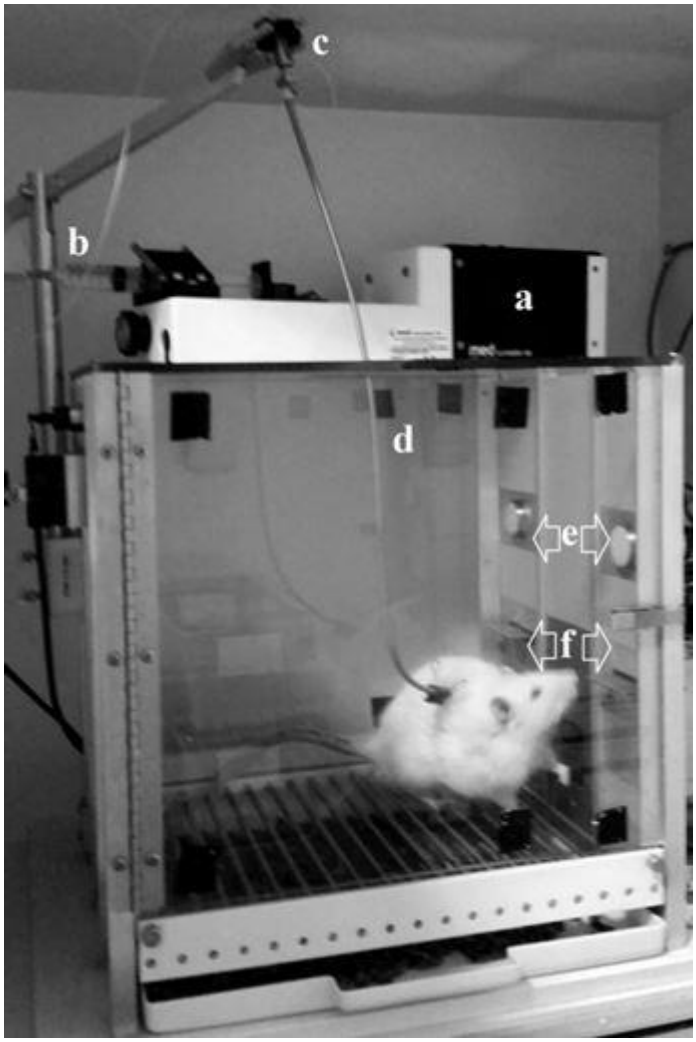


*Figure 1*. Operant setup used for rodent drug self-administration sessions. (A) Self-administration pump that systematically infuses the drug through the plastic drug infusion line. (B) Drug reservoir (10 ml syringe shown). (C) Swivel that rotates when the rat moves about the operant chamber. (D) Leash that protects the plastic drug infusion line. (E) Cue lights that can serve as conditioned stimuli associated with the operant responses for drug reinforcement. (F) Levers. Typically, one lever is 'active', which when pressed activates the pump to infuse the drug into the infusion line leading directly into the indwelling jugular catheter for instant drug reinforcement. The other 'inactive' lever leads to no programmed response and no drug reinforcement.

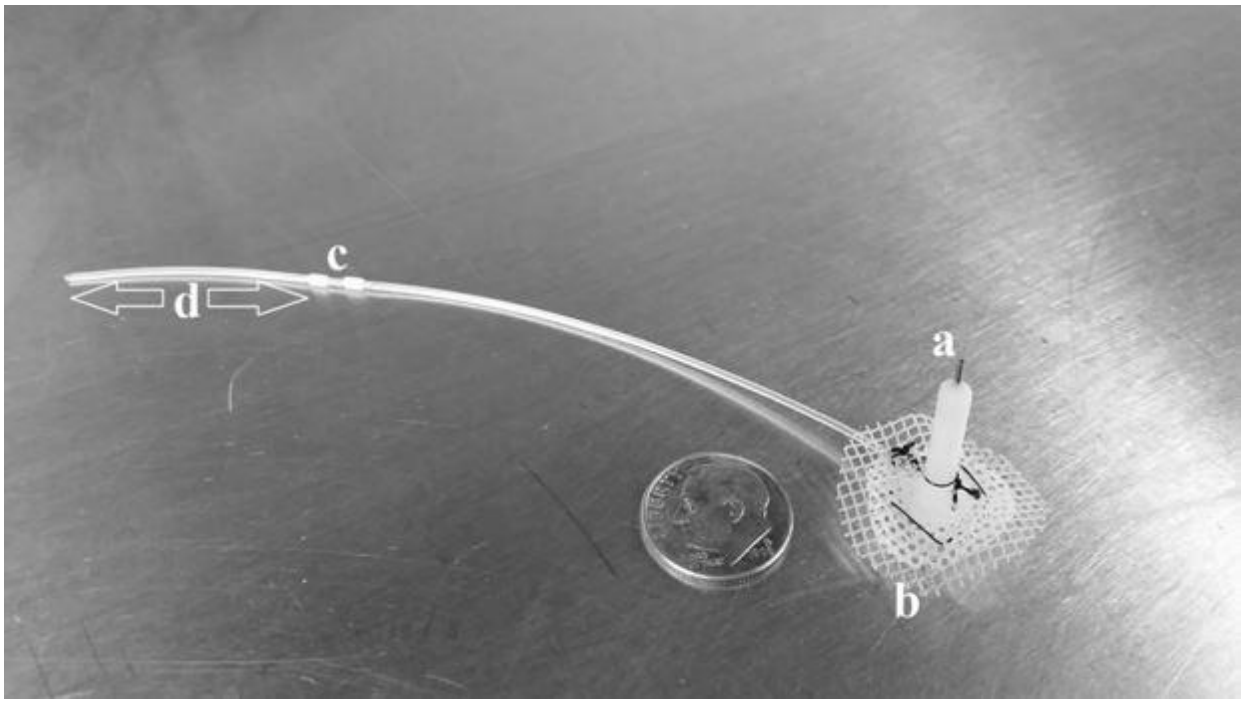


*Figure 2*. Indwelling jugular catheter used in the current study. (A) Entry point to the catheter which is attached to the leash and drug infusion line in the operant chamber. (B) Mesh material embedded subcutaneously to facilitate maximal tissue growth and stabilization of the catheter following surgical implantation. (C) Portion of the catheter in which sutures are tied around the jugular vein to help prevent the catheter from slipping out of the vein and atrium of the heart. (D) End portion of the catheter which is surgically inserted into the jugular vein and maintained in or near the atrium of the heart for the duration of the experiment. A United States dime is shown for size comparison.

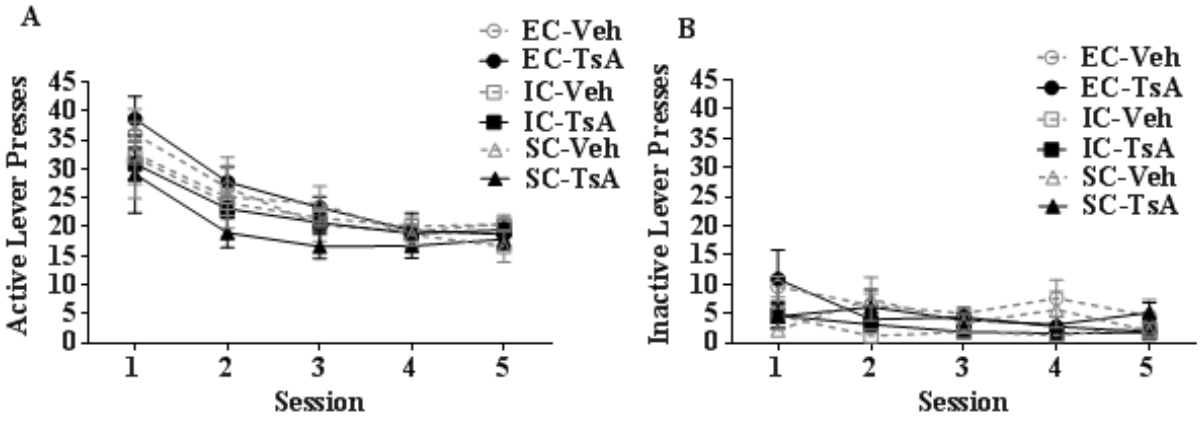


*Figure 3*. Mean ± SEM (A) active and (B) inactive lever presses during the acquisition of amphetamine (0.1 mg/kg/infusion, i.v.) self-administration between EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. When investigating all five acquisition sessions, rats' active lever presses were similar regardless of environmental condition or TsA pretreatment. There were also no significant differences found in inactive lever pressing between EC, IC, or SC rats in either drug treatment condition.

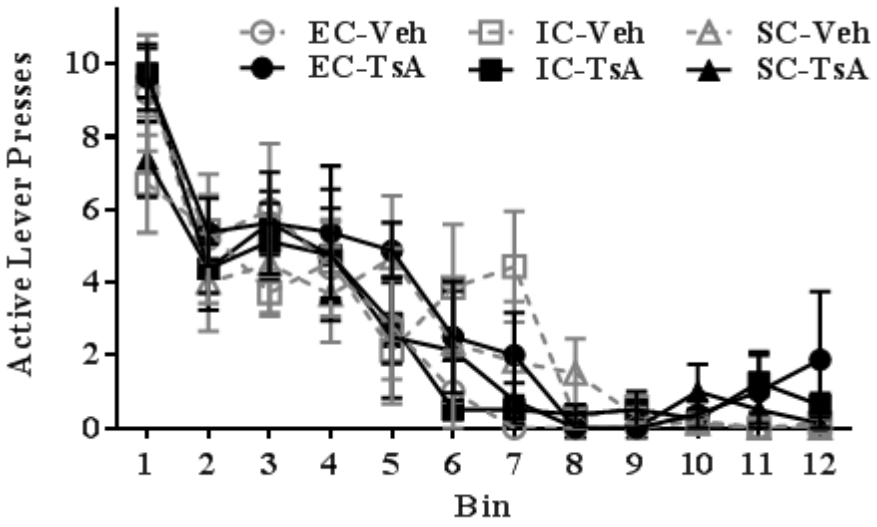


*Figure 4*. Mean ± SEM active lever presses across the twelve 5-min bins during Acquisition session 1 in EC, IC, and SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the twelve 5-min bins.

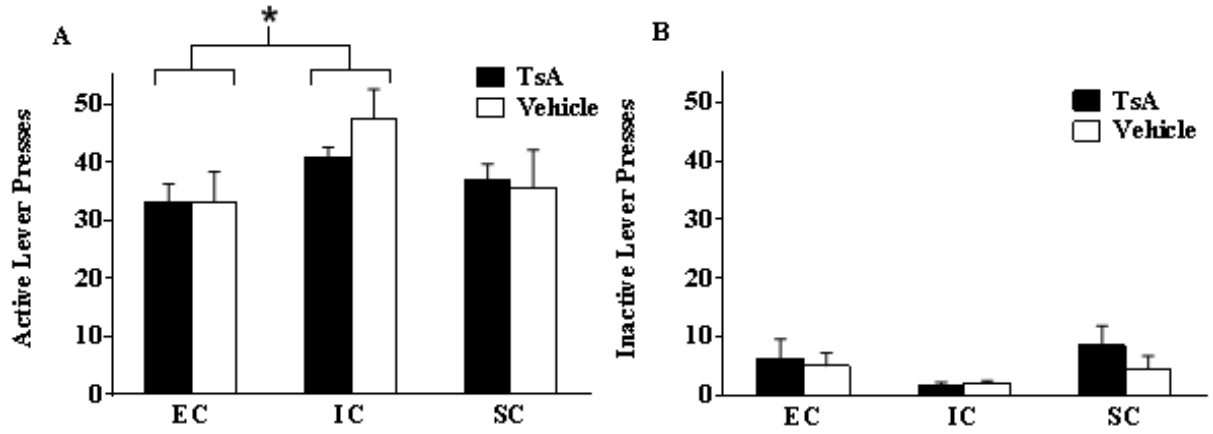


*Figure 5*. Mean ± SEM (A) active and (B) inactive lever presses for amphetamine (0.03 mg/kg/infusion, i.v.) between EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. Environmentally-enriched rats (EC) exhibited significantly fewer active lever responses for amphetamine than isolated (IC) rats ($p < .05$). There were no significant differences between any of the drug pretreatment conditions and there were no significant differences in inactive lever responding between EC, IC, or SC rats in any treatment condition.

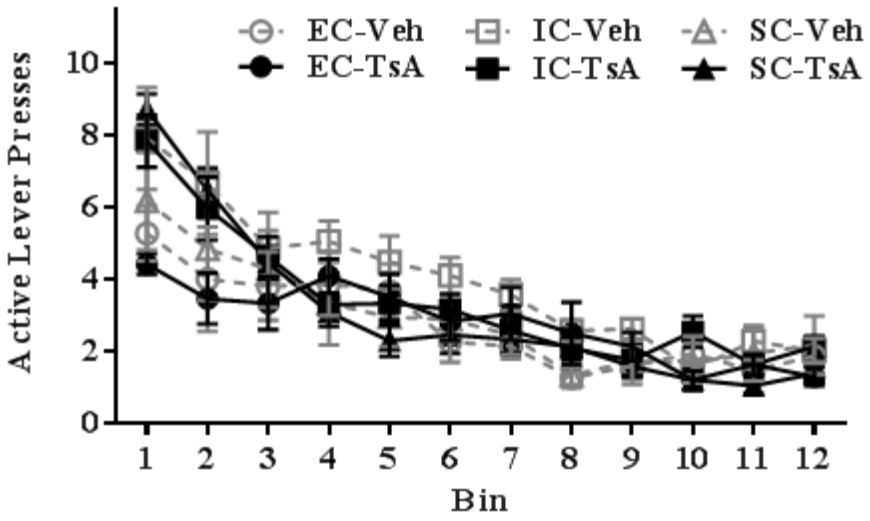


*Figure 6.* Mean ± SEM active lever presses across the twelve 5-min bins during active lever pressing for 0.03 mg/kg amphetamine in EC, IC, and SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the twelve 5-min bins.

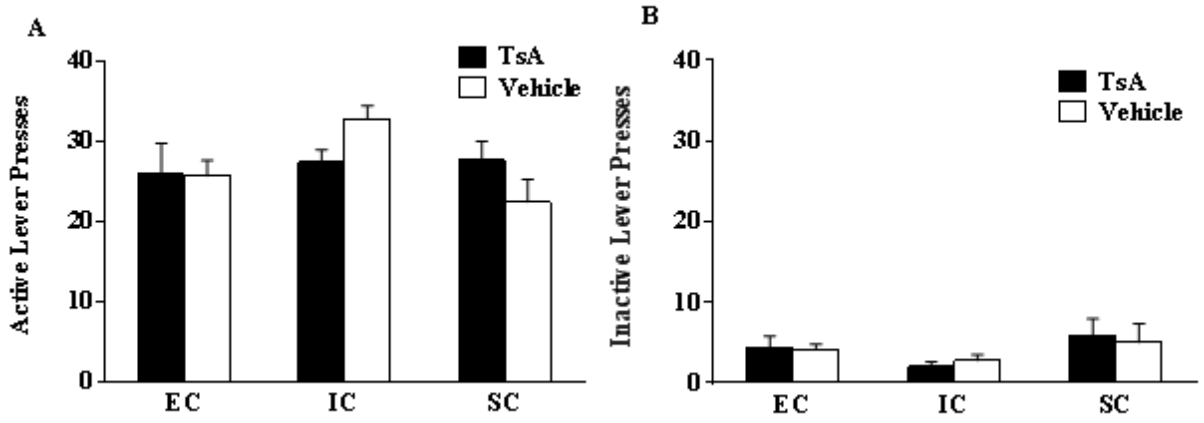


*Figure 7*. Mean ± SEM (A) active and (B) inactive lever presses for amphetamine (0.05 mg/kg/infusion, i.v.) between EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. There were no significant differences in active lever responding between any of the environmental conditions. Also, there were no significant differences between any of the drug pretreatment conditions and there were no significant differences in inactive lever responding between EC, IC, or SC rats in any treatment condition. There were no significant differences in inactive lever responding between EC, IC, or SC rats in any treatment condition.

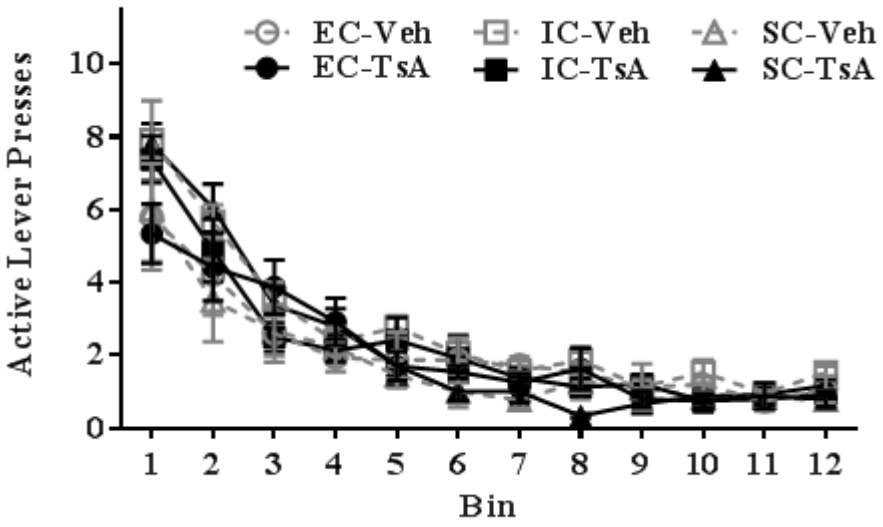


*Figure 8.* Mean ± SEM active lever presses across the twelve 5-min bins during active lever pressing for 0.05 mg/kg amphetamine in EC, IC, and SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the twelve 5-min bins.

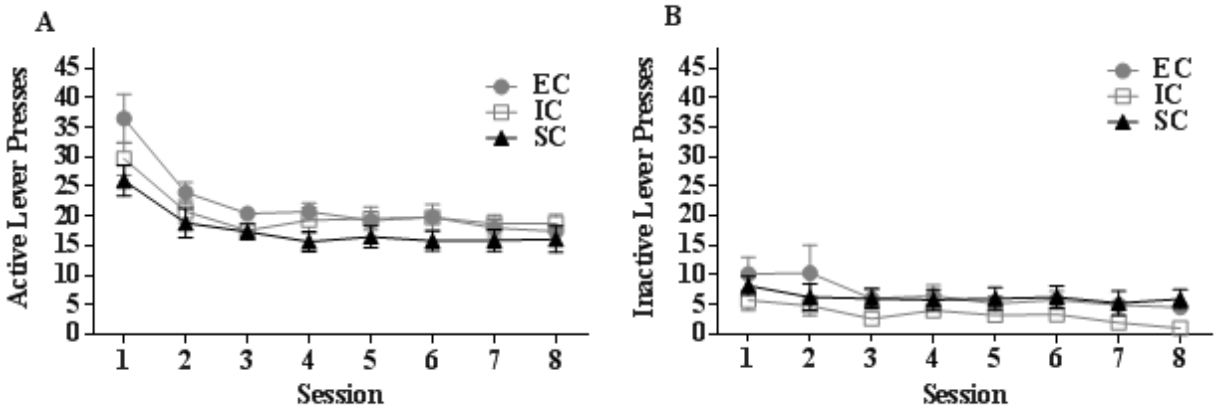


*Figure 9.* Mean ± SEM (A) active and (B) inactive lever presses during the first eight amphetamine (0.1 mg/kg/infusion, i.v.) self-administration sessions in Experiment II between EC, IC, and SC rats. When investigating all eight sessions, rats' active and inactive lever presses were similar regardless of environmental condition prior to starting TsA or vehicle pretreatment which initiated on the ninth day.

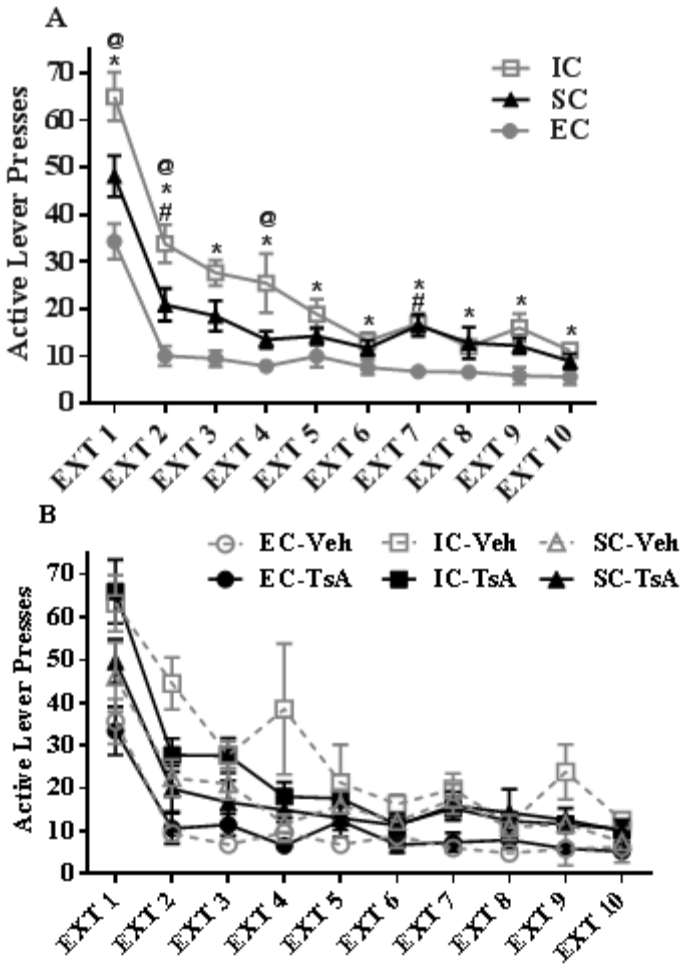


*Figure 10*. Mean ± SEM active lever presses during Extinction sessions 1-10 for (A) EC, IC, and SC, rats and (B) EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. (A) Asterisks (*) indicate that IC rats displayed greater active lever presses than EC rats (all *p*s < .05). The at symbols (@) indicate that IC rats displayed greater active lever presses than SC rats (all *p*s < .05), and the pound signs (#) indicate that SC rats exhibited significantly greater active lever presses than EC rats (all *p*s < .05). (B) There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the ten extinction sessions.

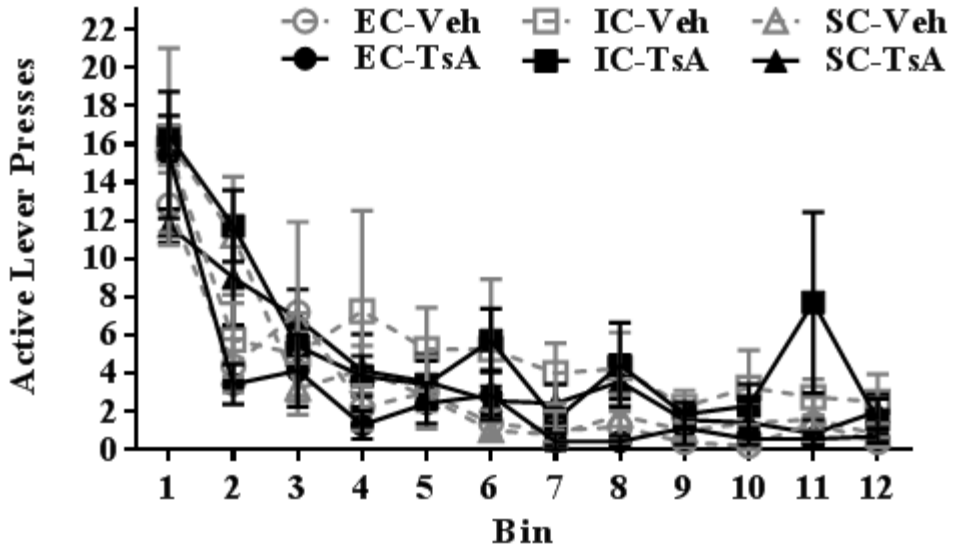


*Figure 11*. Mean ± SEM active lever presses across the twelve 5-min bins during active lever pressing during Extinction session 1 in EC, IC, and SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the twelve 5-min bins.

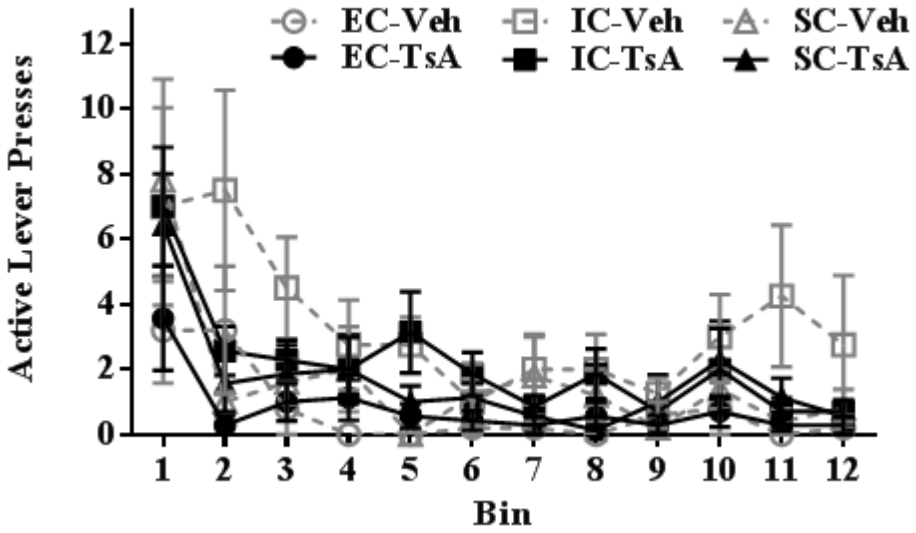


*Figure 12*. Mean ± SEM active lever presses across the twelve 5-min bins during active lever pressing during Extinction session 2 in EC, IC, and SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within any of the environmental conditions across the twelve 5-min bins.

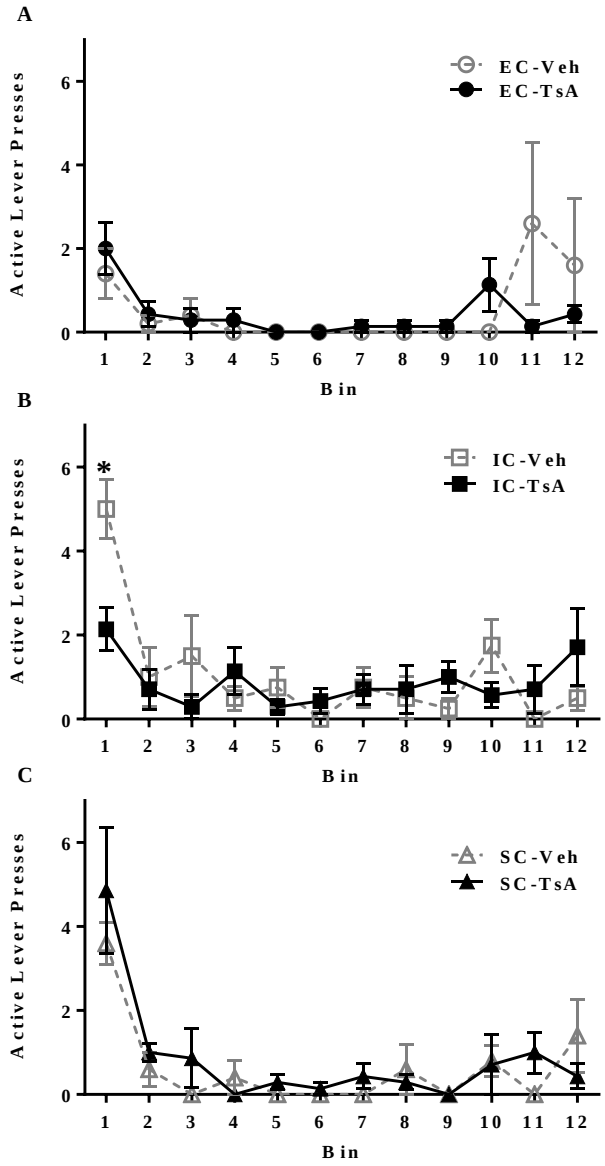


*Figure 13*. Mean ± SEM active lever presses across the twelve 5-min bins during Extinction session 10 in (A) EC, (B) IC, and (C) SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within (A) EC or (C) SC rats for any 5-min bin. (B) Asterisk (*) indicates that IC-TsA rats displayed significantly fewer active lever presses than IC-vehicle rats ($p$ = .009).

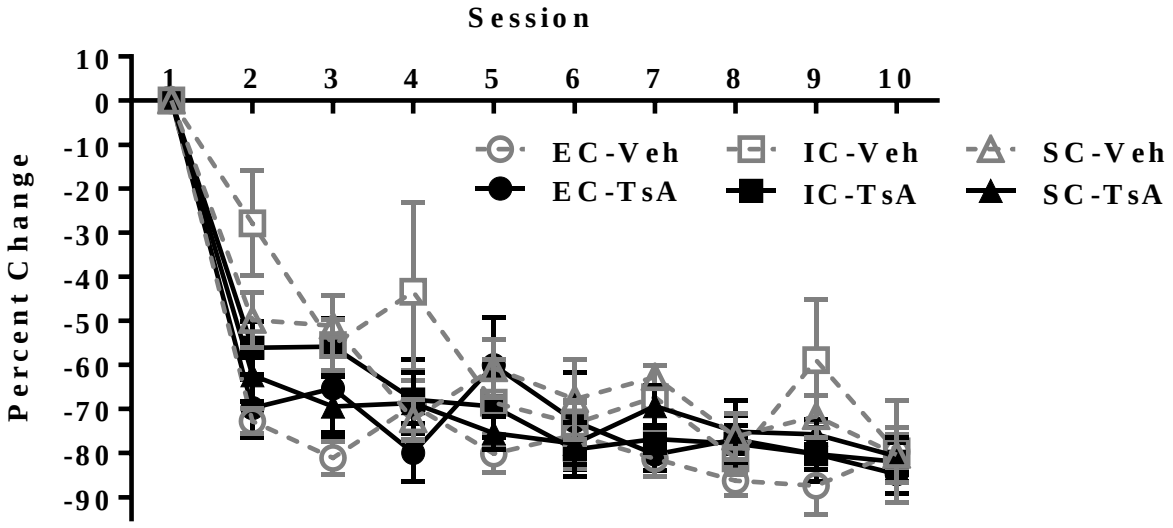


*Figure 14*. Mean ± SEM percent change in active lever presses during Extinction sessions 1-10 for EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. There were no significant environmental condition and drug pretreatment-induced differences in active lever presses across the ten extinction sessions.

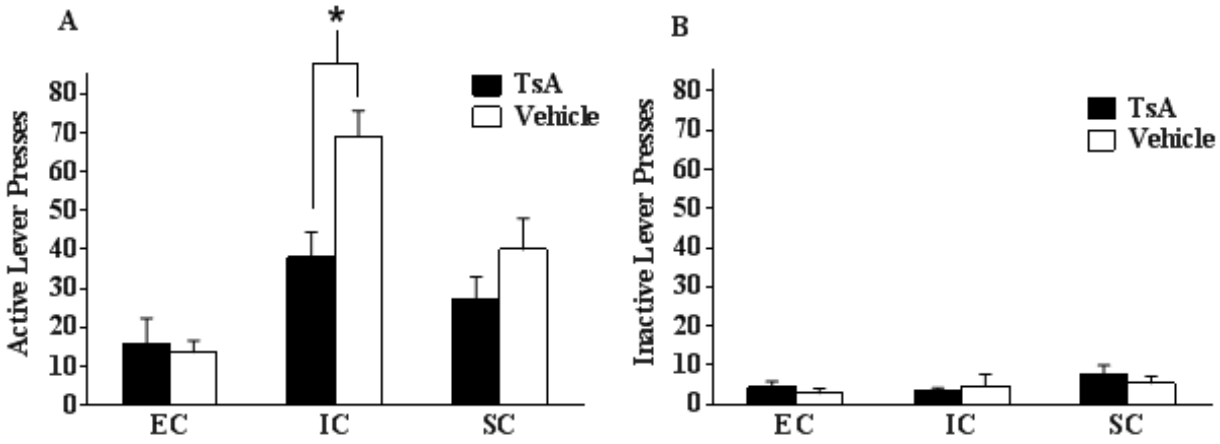


*Figure 15*. Mean ± SEM (A) active and (B) inactive lever presses during the cue-induced reinstatement test between EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. (A) Asterisk (*) indicates that IC-TsA rats exhibited significantly fewer active lever presses than IC-vehicle rats ($p < .05$). (B) There were no significant differences in inactive lever responding between EC, IC, or SC rats in any treatment condition.

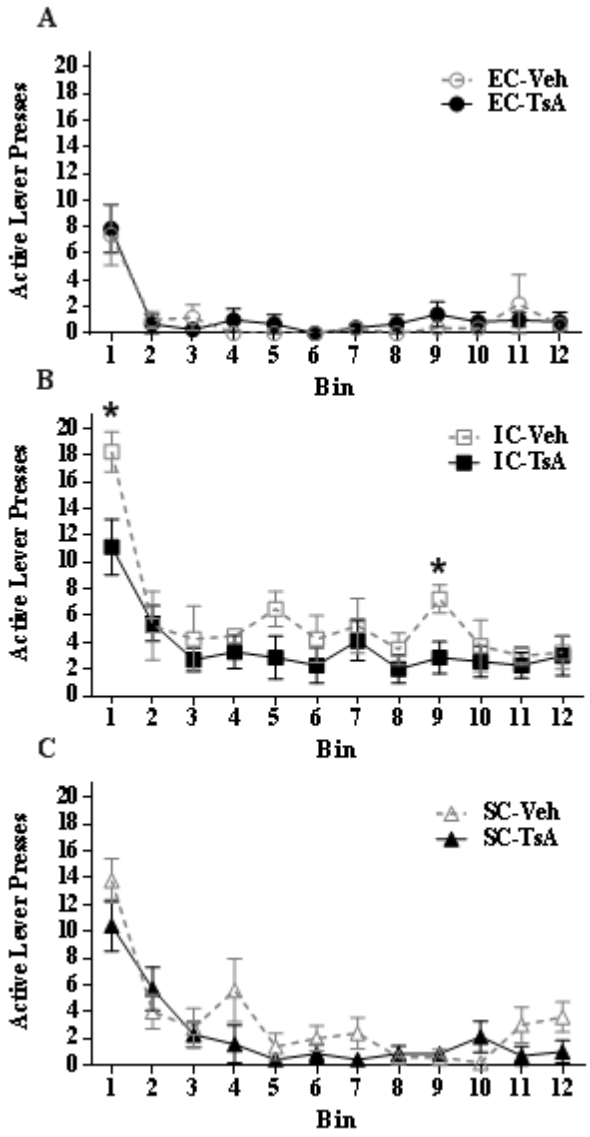


*Figure 16*. Mean ± SEM active lever presses across the twelve 5-min bins during during the cue-induced reinstatement test in (A) EC, (B) IC, and (C) SC rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within (A) EC or (C) SC rats for any 5-min bin. (B) Asterisks (*) indicate that IC-TsA rats displayed significantly fewer active lever presses than IC-vehicle rats during bin 1($p$ = .043) and bin 9 ($p$ = .037).

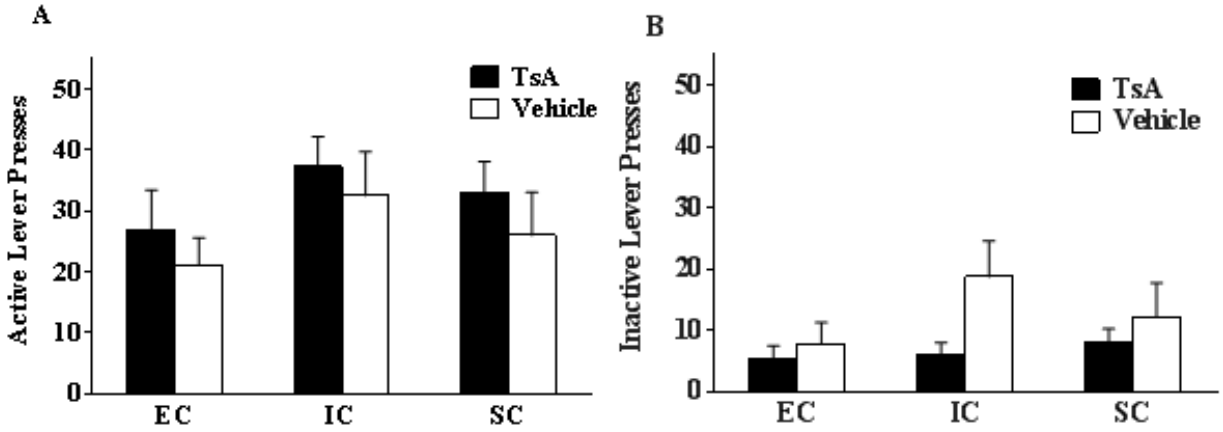


*Figure 17*. Mean ± SEM (A) active and (B) inactive lever presses during the drug-induced reinstatement test between EC, IC, and SC rats following Trichostatin A (TsA; 0.3 mg/kg, i.v.) or Vehicle (10% DMSO in saline) pretreatment. There were no significant differences in active lever pressing between any of the treatment conditions. For inactive lever responses, rats pretreated with TsA exhibited fewer inactive lever presses than rats pretreated with vehicle injections, but there was no significant environmental condition x drug treatment interaction.

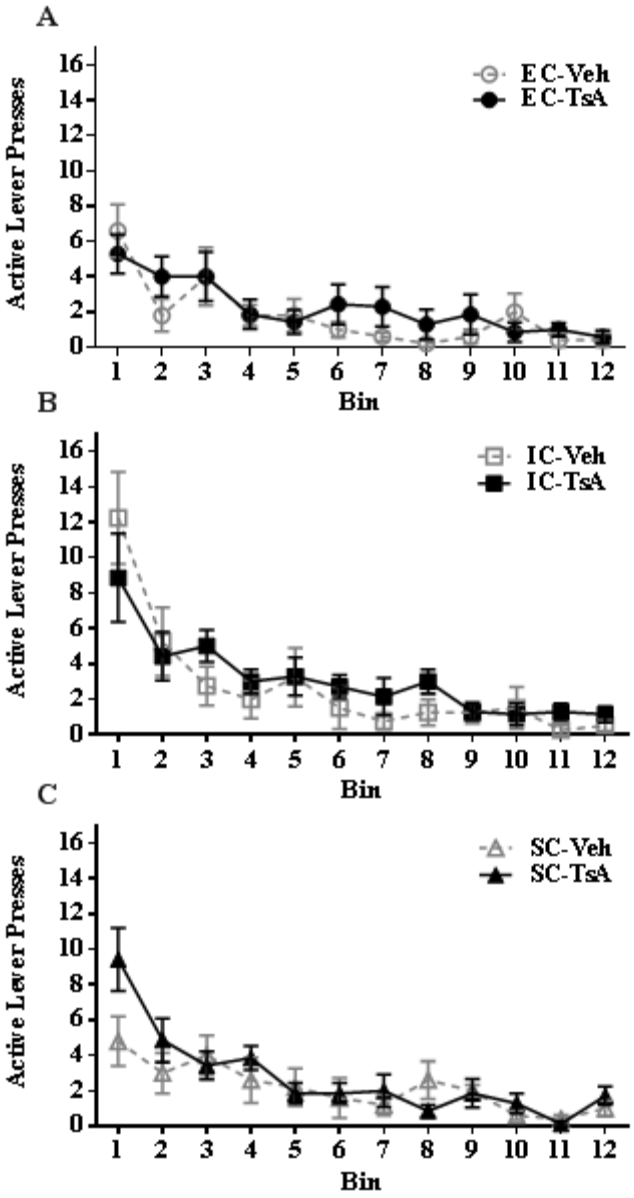


*Figure 18*. Mean ± SEM active lever presses across the 12 5-min bins within the 1-hr drug-induced reinstatement test session in (A) EC, (B) IC, and (SC) rats pretreated with TsA (0.3 mg/kg, i.v.) or vehicle (10% DMSO in saline) injections. There were no significant differences in active lever pressing between TsA-pretreated rats and vehicle-pretreated rats within (A) EC, (B) IC, or (C) SC rats for any 5-min bin.