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The Role of Neuron-Specific Nucleosome Remodeling in Cocaine-Associated Memory and Synaptic Plasticity

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The Role of Neuron-Specific Nucleosome Remodeling in Cocaine-Associated Memory and Synaptic Plasticity

DISSERTATION

submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

André O. White

Dissertation Committee:

Associate Professor Marcelo A. Wood, Chair
Assistant Professor Christie D. Fowler
Associate Professor John F. Guzowski

2016
DEDICATION

To my parents – Heather and Courtney White

I owe my pursuit of knowledge to you. You both lived in different countries for years in order to support my education. You have taught me the value of sacrifice, perseverance and commitment. Your love and support created the ideal environment for me to learn, fail but ultimately flourish. I count you both, equally, as my first inspirations and greatest mentors.

To my wife – Sarah White

Thank you. You are the cornerstone that has made my blessed life possible. You truly are a gift from God. Thank you for constantly supporting me through my undergraduate and graduate studies. You are all that is wonderful in this world.

To my extended family
Whether related by blood, marriage or joyful moments, you keep me happy and grounded. Your true value to me cannot be measured. It lies, in part, in the strength and love you have given me.
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I would like to thank my committee members, Dr. John Guzowski and Dr. Christie Fowler for their support, thoughtful feedback, and advice.

In addition, a thank you to Dr. Dina Matheos, who introduced me to molecular biology, and whose pragmatism and dedication to research and life had lasting effect.
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ABSTRACT OF THE DISSERTATION

The Role of Neuron-Specific Nucleosome Remodeling in Cocaine-Associated Memory and Synaptic Plasticity

By

André O. White

Doctor of Philosophy in Biological Sciences

University of California, Irvine, 2016

Dr. Marcelo A. Wood, Chair

Recent evidence have implicated epigenetic mechanisms in drug-associated memory formation. A possible role for one major epigenetic mechanism, nucleosome remodeling, in drug-associated memory formation has been largely unexplored. The present dissertation examined mice with genetic manipulations targeting a neuron-specific nucleosome remodeling complex subunit, BAF53b. These mice displayed deficits in cocaine-associated memory that were more severe in BAF53b dominant-negative transgenic mice compared to BAF53b heterozygous knockout mice. Similar to the memory deficits, theta-induced long-term potentiation (theta-LTP) in the nucleus accumbens (NAc) was significantly impaired in slices taken from BAF53b transgenic mutant mice but not heterozygous knockout mice. Further studies indicated that theta-LTP in the NAc is dependent on TrkB receptor activation, and that BDNF rescues theta-LTP and cocaine-associated memory in BAF53b transgenic mice. My studies suggest a role for BAF53b in NAc neuronal function required for cocaine-associated memories. Furthermore, targeting BDNF/TrkB activation in the nucleus accumbens may overcome memory and plasticity deficits linked to BAF53b mutations.
**Introduction**

Drug addiction is characterized by compulsive drug-seeking behavior despite negative consequences (Hyman, 2005). In the field of substance abuse, there remains unanswered questions about how drugs of abuse drive the establishment of long-lasting changes in the brain that support robust drug-associated behaviors and memories. A central feature of drugs of abuse that distinguish them from non-addictive drugs is their ability to promote persistent changes in neuronal function in the reward pathway of the brain (Kalivas et al., 2004; Shen et al., 2008; Valjent et al., 2004). For example, cocaine is known to generate changes in histone modification patterns that result in aberrant gene expression profiles and synaptic plasticity in the nucleus accumbens that drive several cocaine-seeking behaviors (Kumar et al., 2005; Malvaez et al., 2011; Maze et al., 2010; Renthal et al., 2007; Rogge et al., 2013; Schroeder et al., 2008; Sun et al., 2008).

Increasing evidence has supported a role for epigenetic mechanisms in memory formation (for a review see: White and Wood, 2014; Kwapis and Wood, 2015). In particular, epigenetic mechanisms such as histone modification are integral to the formation of drug-associated memories and behaviors (Malvaez et al., 2009; Rogge and Wood, 2013). Several epigenetic mechanisms including: 1) post-translational histone modification, 2) DNA methylation and 3) non-coding RNA, serve as key regulators of chromatin structure and gene expression that are required for aspects of drug-associated behaviors (see reviews: Rogge and Wood, 2013; Jonkman and Kenny, 2013). The histone modification patterns associated with specific gene expression changes observed in drug-associated behaviors have led to increased examination of the epigenetic mechanisms that regulate access to DNA. For example, histone acetylation and methylation mechanisms are pivotal for cocaine-induced
changes in gene expression that underlie cocaine-induced behaviors as well as cocaine-context associated memories (Malvaez et al., 2011; Maze et al., 2010; Renthal et al., 2007; Rogge et al., 2013; Schroeder et al., 2008; Sun et al., 2008; Romieu et al., 2008; Kennedy et al., 2013). However, there is a fourth major epigenetic mechanism (ATP-dependent nucleosome remodeling) that regulates transcription by altering chromatin structure (Hargreaves and Crabtree, 2011). Currently, it remains unclear to what extent ATP-dependent nucleosome remodeling complexes are involved in drug-induced behaviors and drug-associated memories.

**My dissertation addresses the role of a neuron-specific nucleosome remodeling complex, nBAF, in the acquisition/consolidation of cocaine-associated memories as well as synaptic plasticity. Specifically, I examine the effect of altering the expression and structure of the BAF53b subunit of nBAF. My results indicate that BAF53b in the nucleus accumbens is critical for synaptic plasticity and cocaine-associated memories. Additionally, deficits stemming from BAF53b manipulations can be overcome using BDNF/TrkB activation.**

**Epigenetics in Learning and Memory**

*Epigenetic Regulation of Transcription*

One of the fairly recent efforts to understand how transcription is capable of regulating long-term memory formation involves exploiting the existing epigenetic machinery. With respect to the regulation of transcription underlying behavior, the field of neuroscience has given special focus to histone modification. The core histone octamer of chromatin canonically includes two of each of the following core histone proteins: H2A, H2B,
H3, and H4 (Kouzarides, 2007). Incorporated with the histone octamer are histone tails that covalently interact with the negatively charged phosphate backbone of DNA. Post-translational modifications can be made to these histone tails, by the addition or removal of specific chemical groups (acetylated, methylated, phosphorylated, etc). These modifications alter histone-DNA interactions to either compress or relax chromatin structure. Compressing chromatin structure represses transcription by preventing DNA binding proteins, RNA polymerase, transcription factors and various proteins, necessary for transcription, from accessing regulatory regions of DNA (ie. promoters, enhancers and repressors regions). Conversely, loosening chromatin structure can promote gene expression by enabling the binding of proteins necessary for transcription (Kouzarides, 2007; Barrett & Wood, 2008).

In addition to manipulating histone-DNA interactions, DNA can also be modified through the addition or removal of methyl groups. However, the results of DNA methylation on transcription is varied and complicated as discussed in Li et al. (2013). The number of methyl groups that are added can have varying effects on chromatin compaction (permissive and repressive). Histone variants can be integrated in the nucleosomes and are linked to altered gene expression profiles (reviewed in Maze et al. 2013). Discussed in detail later, nucleosome remodeling complexes (NRCs) can regulate the accessibility of DNA through the movement of nucleosomes (spaced approximately every 10 to 50 base pairs) along DNA (Clapier and Cairns, 2009; Hargreaves and Crabtree, 2011). Many of these aforementioned epigenetic mechanisms have been shown to be important to generating changes in gene expression profiles related to cell function and cell fate decision during development (cellular memory; Turner, 2002). In the next section, I will briefly discuss the ways in which
these epigenetic mechanisms are involved in the formation of long-term memory (this is not meant to be a comprehensive description).

**Epigenetic Regulation of Transcription Necessary for Learning and Memory**

Chromatin modifications are currently the best-studied epigenetic mechanisms that regulate transcription necessary for long-term memory, with histone acetylation receiving the most focus. Histone acetyltransferases (HATs) add acetyl groups that neutralize the positive charge on lysine residues of histone tails (for review, see Kouzarides, 2007; Day and Sweatt, 2011). Otherwise, these positive lysine charges would form ionic bonds with the negative DNA phosphate backbone, resulting in a repressed structure. The addition of acetyl groups to these histone tails, therefore, results in a relaxed chromatin structure, which is conducive to gene expression. In addition, it provides a recognition site for bromo-domain containing proteins (including some NRCs like nBAF), an interaction necessary to build large multi-protein complexes required for transcription initiation and elongation. Counter to the action of HATs, histone deacetylases (HDACs) remove acetyl groups from histone tails, which results in a compacted chromatin structure that represses gene expression (Kouzarides 2007). Long-term memory formation is a transcription-dependent process so HATs are predicted to subserve long-term memory formation by permitting the expression of memory-related genes. In contrast, HDACs are predicted to hinder long-term memory formation by repressing the transcription of memory-related genes. It would also be predicted that inhibiting the regular action of these enzymes would confer the opposite result (i.e. HAT inhibition should impair memory formation while HDAC inhibition would
enhance memory formation). It is important to note that HATs and HDACs are also known to have non-histone substrates such as proteins that are involved in regulating transcription.

Studies have primarily explored the role of these two chromatin-modifying enzymes in long-term memory formation and the results were consistent with the aforementioned predictions. Mice with genetically modified forms of a HAT, cyclic AMP responsive element-binding protein (CBP), have shown memory and plasticity impairments. Mice with a dominant-negative truncated CBP have impaired cued fear conditioning, passive avoidance and novel object recognition (Alarcon et al. 2004; Bourtchouladze et al. 2003; Oike et al. 1999). CBP knockouts and deletion of specific binding domains impair memory for contextual fear conditioning, novel object recognition, Morris water maze and late-LTP (Alarcon et al. 2004; Korzus et al. 2004; Oliveira et al. 2007; Wood et al. 2005; Wood et al. 2006). Mutations in p300 and a knockout PCAF, two HATs, showed similar memory deficits (Oliveira et al. 2007; Maurice et al. 2008). These studies suggest that a relaxed chromatin structure through histone acetylation supports long-term memory formation for a variety of tasks.

As predicted, HDAC inhibition enhances memory formation. Several HDAC inhibitors that are specific to class I HDACs have generated enhancement in several forms of long-term memory. HDAC inhibitors valproic acid (VPA), trichostatin A (TSA) and sodium butyrate (NaB) have been shown to enhance fear potentiated startle (Yeh et al. 2004), contextual fear conditioning (Fischer et al. 2007; Levenson et al. 2004; Vecsey et al. 2007), Morris water maze (Fischer et al. 2007) and the extinction of cued and context fear memory (Bredy et al. 2007, 2008; Lattal et al. 2007). Of note is the fact that HDAC inhibition is also coupled with increased acetylation (as discussed in McQuown et al., 2011). This observation supports the
idea that relaxed chromatin, through acetylated histones, facilitates memory formation by permitting transcription whereas compressed chromatin represses transcription necessary for memory formation.

The idea that HDACs and associated corepressors serve to suppress gene expression necessary for long-term memory formation was addressed in McQuown and Wood (2011) in the proposal of the “molecular brake pad” hypothesis. Briefly, this hypothesis posits that HDACs and associated co-repressor complexes (the molecular brake pads) act to inhibit transcription of genes required for long-term memory formation and maintenance. However, an activity-dependent signal of sufficient strength is able to transiently remove the brake pads to permit gene expression required for stable forms of memory. Direct predictions of this hypothesis were that HDAC inhibition would lower the threshold for long-term memory formation and also contribute to the persistence of long-term memory by manipulation of the gene expression dynamics during memory consolidation. Described below are some studies that examine the effect of non-selective HDAC inhibition and specific HDAC knockouts on threshold effects for long-term memory formation as well as effects on the persistence of long-term memory.

The molecular brake pad hypothesis raises the question of how exactly HDAC inhibition regulates gene expression that enhances long-term memory processes. Vecsey et al. (2007) addressed one aspect of this question. They showed that the effect of TSA was dependent on the interaction between the transcription factor CREB and the CBP. Both CREB and CBP have well-established roles in long-term memory formation.

Interestingly, out of 12 cyclic-AMP response element (CRE; DNA sequence that CREB binds) containing genes examined (see Vecsey et al., 2007 for list), TSA administration only
led to an increase in the expression of 2 (Nr4a1 and Nr4a2) in the hippocampus after fear conditioning. These enhancements in gene expression were only observed when TSA was paired with fear conditioning and not when TSA was administered absent training. Perhaps the most surprising finding of the Vecsey et al. (2007) study was that TSA not only transformed a transcription-independent form of LTP (induced by a single tetanus) into a transcription-dependent form of LTP (as demonstrated by blocking with actinomycin-D). Earlier studies had shown that HDAC inhibition could enhance LTP (Levenson et al., 2004; Alarcón et al., 2004), but the transcription-dependent aspect of the enhancement had not been examined. Additionally, Vecsey et al. (2007) demonstrated that the enhancement of LTP by TSA required the interaction between CREB and CBP. Together, these results indicate that HDACs can regulate the temporal dynamics of gene expression during memory consolidation (Vecsey et al., 2007; McQuown et al., 2011) and also even be permissive (perhaps not instructive) in supporting gene expression for stabilizing potentiation (as in the LTP experiments; Vecsey et al., 2007). These studies also highlight that histone acetylation and the resulting relaxation of chromatin structure is involved in the formation of a variety of memories.

**Epigenetics in Drug addiction**

*Associative Memory in Drug Addiction*

Do the aforementioned results from epigenetic manipulations observed in fear memories, object location and object recognition long-term memory extend to drug-associated memories? The findings that HDAC inhibitors could enhance memory led researchers to examine the role of epigenetic mechanisms in drug addiction. Some
researchers contend that drug addiction is a disease that involves the usurpation of long-term memory system and plasticity (Hyman 2005). Therefore, it is possible that the persistence of drug-associated memories can be explained by their ability to hijack mechanisms that regulate the transcription of genes necessary for plasticity and memory formation.

Drug addiction is a disease characterized by compulsive drug-seeking behavior that increases despite negative consequences (Hyman et al., 2005; Kalivas et al., 2005; McLellan et al., 2000). These negative consequences can include failure to complete life goals, medical illness, mental illness and the increased risk of incarceration (Hyman, 2005). The profound strength of behavioral changes induced drugs of abuse is paralleled by drug-induced changes in plasticity in the reward pathway. Drugs of abuse can generate long-term potentiation in dopamine neurons as well as persistent changes in the structure of dendrites and spines (Ungless et al. 2001; Robinson and Kolb, 2004). Perhaps understanding the ways in which drugs of abuse induce changes at the molecular and cellular level will lead to better pharmacological treatments for addiction.

Drugs of abuse can generate a variety of homeostatic changes in the body that underlie drug tolerance, dependence as well as withdrawal (Anagnostaras and Robinson, 1996; Nestler and Aghajanian, 1997). However, the addictive properties of drugs of abuse extend beyond the physiological dependence that results from the influence of those drugs on the homeostatic system. Drug cravings and urges persist after addicts cease taking drugs of abuse and drug detoxification (Berke and Hyman, 2000; Hyman et al., 2005; O’Brien et al., 1998). Therefore, the avoidance of withdrawal symptoms alone does not seem to accounts for the persistent risk of relapse that outlasts drug use. If that were not the case, then
isolating addicts until drug detoxification would cure addiction. However, drug rehabilitation centers have had low rates of success even though they guarantee a drug-free environment for an extended period.

Therefore, what other factors contribute to the ability of drugs of abuse to generate such a long-lasting risk of relapse? Answering that question would provide a major tool in reducing the relapse rates of drug addicts. The mechanisms underlying the acquisition and consolidation of drug seeking behaviors and memories are not well understood but significantly contribute the addictive properties of drugs of abuse. Drug paraphernalia as well as people and places that were once associated with drugs of abuse are capable of triggering relapse (Berke and Hyman, 2000; Hyman et al., 2005; O’Brien et al., 1998). Imaging studies have shown that along with producing drug cravings, drug-associated cues are correlated with activation of brain regions implicated in stimulus-reward associations (Childress et al., 1999; Ehrman et al., 1992; Grant et al., 1996; Kilts et al., 2001). Also, several brain areas that undergo drug-induced changes in dendritic structure are regions implicated in learning and memory (Cardinal et al., 2002; Killcross and Coutureau, 2003; Cardinal and Everitt, 2004; Holland and Gallagher, 2004; Kelley, 2004). These results together suggest that learned associations formed during the course of consuming drugs are preserved in the brain and contribute to relapse, even after a prolonged period of drugs abstinence.

**Mechanisms Underlying Persistent Drug-Associated Memory**

Perhaps, the route to combatting drug addiction lies in elucidating the mechanisms that underlie the extremely resilient long-term associative memories addicts form while abusing addictive drugs. Among the changes induced by drugs of abuse are the
neurophysiological responses in addicts (Berke and Hyman, 2000). 1) Neuronal adaptation: the changes in the homeostatic environment due to enhanced stimulation of reward pathways by drugs of abuse. This change is believed to underlie the dependence and withdrawal symptoms encountered by addicts (Berke and Hyman, 2000). 2) Aberrant synaptic plasticity: changes at the synapse that arise from aberrant dopamine release and transcription-dependent plasticity. This change is thought to underlie the formation and persistence of drug-associated behaviors and memories (Berke and Hyman, 2000). Given the potential role of drug-associated cues in relapse, it is necessary to examine the aberrant changes in plasticity believed to subserve the association of drug stimuli and addictive behaviors.

One of the likeliest candidates for how associative memory is generated is through changes in synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) are both phenomena that have components that require synaptic plasticity. Both mechanisms are well studied in long-term memory formation in the hippocampus (Malenka and Bear, 2004; Martin et al., 2000), but not as well understood in other brain regions. In congruence with the notion that drug addiction is heavily dependent on associative memory processes, brain regions in the reward pathway such as the nucleus accumbens display LTP and LTD on medium spiny neurons (majority of neurons in this region) (Kombian and Malenka, 1994; Thomas et al., 2000). Maintenance of LTP in the hippocampus has been thoroughly studied and has been shown to involve gene expression and protein synthesis (Bourtchuladze et al., 1994; Nguyen et al., 1994; Frey et al., 1996; Nguyen and Kandel, 1996). Perhaps the regulation of transcription-dependent plasticity in the nucleus accumbens can
shed some light on the associative memory processes that link context and cues to drug reward.

Several studies have focused on the nucleus accumbens in particular because it is a sub-region of the striatum implicated in associative memory formation, reward and displays plasticity in response to drug intake (Berke and Hyman, 2000). Although the striatum receives afferents from brain areas including the hippocampus and amygdala, that are likely involved in linking the rewarding effects of drugs of abuse to particular contexts and cues (Berke and Hyman, 2000; Kincaid et al., 1998), the dopamine inputs received by the striatum have been the most instructive in addiction research. Dopamine is released from neurons originating in the substantia nigra pars compacta and ventral tegmental area. Psychostimulants such as cocaine induce dopamine release in the striatum resulting in altered gene expression profiles (Berke and Hyman, 2000; Carelli and Ijames, 2001; Carelli et al., 2000; Childress et al., 1999; Grant et al., 1996; Hyman et al., 2006). The rewarding properties of drugs of abuse are reduced when dopamine is blocked in the ventral striatum (Di Chiara and Imperato, 1988; Wise, 1996). A hallmark of various drugs of abuse is that they consistently increase dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988; Koob and Bloom, 1988; Kuczenski et al., 1991; Wise, 1996; Wise and Bozarth, 1987).

This dopaminergic input is coupled to synaptic plasticity mechanisms through D1 receptors (Berke and Hyman, 2000). D1 receptors, which are found in the core and shell of the nucleus accumbens, coupled to the cAMP/PKA/CREB pathway (Konradi et al., 1994; Hyman, 1996). However, it is important to note that although the cAMP/PKA/CREB pathway is activated after psychostimulant administration, it may not explain addiction. It has been shown that the overexpression of CREB in the nucleus accumbens attenuates the rewarding
effects of cocaine (Carlezon et al. 1998). Conversely, CREB reduction bolsters the rewarding effects of cocaine (Carlezon et al., 1998; Walters and Blendy, 2001). It is possible that CREB is involved in the homeostatic response, which counteracts the rewarding component of addiction.

In contrast, another transcription factor ΔFosB has been implicated in the sustenance of addiction-like behavior. An inducible over-expression of ΔFosB increased the rewarding effects of cocaine (McClung and Nestler, 2003). Nestler (2008) extensively reviews the proposed role for ΔFosB in the regulation of transcription that appears to underlie drug sensitivity and drug-seeking behavior. Among the genes targeted by ΔFosB are GluR2 (AMPA receptor subunit) and cyclin-dependent kinase-5 (signaling protein linked to cocaine-induced alterations at the synapse; Bibb et al., 2001; Norrholm et al., 2003). There is compelling support for the notion that drugs of abuse (in particular cocaine) induce changes in the expression of genes that are necessary for synaptic plasticity and the formation of persistent drug-associated behaviors and memories.

Epigenetic Mechanisms in Cocaine-Associated Memories and Behaviors

Do epigenetic enzymes regulate transcription necessary for drug-associated memory, plasticity, and behavior? Histone acetylation and methylation mechanisms have been shown to be pivotal for cocaine-induced changes in gene expression that underlie synaptic plasticity and cocaine-associated behaviors (Kumar et al. 2005; Malvaez et al. 2011; Maze et al. 2010; Renthal et al. 2007; Rogge et al. 2013; Schroeder et al. 2008; Sun et al. 2008; Romieu et al. 2008; Kennedy et al. 2013). In general, these results appear to mirror the results seen in object location and recognition memory insofar as HATs promote drug-memory formation.
while HDACs repress it (Schroeder et al., 2008; Sun et al., 2008; Malvaez et al., 2011; Rogge et al., 2013). It is important to note that others have reported HDACs as having various effects on cocaine-associated behaviors based on the class of HDAC as well as the interaction partners (Kennedy et al., 2013; Renthal et al., 2007). In addition, histone dimethylation is regulated by ΔFosB after cocaine administration (Maze et al., 2010). This regulation is coupled with changes in synaptic plasticity and underlies cocaine-seeking behaviors (Maze et al., 2010). These studies are not an exhaustive list, but support the majority view that epigenetic mechanisms regulate transcription necessary for drug-associated memory formation. However, despite the fact that epigenetic modifications help regulate transcription necessary for the formation of drug-associated behaviors as well various forms of memory, only one paper (Vogel-Ciernia et al. 2011) has shown a role for nucleosome remodeling in memory formation. In addition, to the best of my knowledge no paper has identified any specific nucleosome remodeler as having a role in drug-associated memories and behaviors.

**Nucleosome Remodelers**

*Nucleosome Remodeling Complexes*

Nucleosome remodeling complexes (NRCs) are evolutionarily conserved multi-subunit complexes that replace, eject, and alter nucleosomes to promote stable changes in gene expression (Nair and Kumar, 2012). Through their ATPase subunit, nucleosome remodeling complexes disrupt nucleosome-DNA contacts through ATP hydrolysis, thereby allowing the binding of the transcription machinery as well as associated transcriptional regulators (Hargreaves and Crabtree, 2011; Ho and Crabtree, 2010). Nucleosome
remodeling complexes fall into four major families defined by their ATPase: BAF (Brg1, hBrm), INO80/SWR1 (hINO80, Domino, SRCAP), ISWI or NURF (hSNF2H, hSNF2L), and CHD or NuRD (CHD1-9) (Hargreaves and Crabtree, 2011, reviewed in Vogel-Ciernia and Wood, 2014). My work focuses on the Brg1 Associated Factor (BAF) nucleosome remodeling family. Recently, these proteins have received attention due to their role regulating gene expression necessary for early development. Furthermore, findings implicate mammalian BAF and several of its subunits, with sporadic autism, sporadic mental retardation and syndromic intellectual disability (Ben-David and Shifman, 2013; Santen et al, 2012; Tsurusaki et al. 2012; Van Houdt et al. 2012). These human exome sequencing studies suggest mutations in BAF complex genes as a causal mechanism for the development of these intellectual disability disorders, but direct evidence demonstrating how a mutation in a BAF complex gene gives rise to cognition deficits, or even developmental deficits, remained unknown.

Mammalian BAF complexes contain the Brg1 or hBrm ATPase (Kadam and Emerson, 2003) as well as at least 14 other subunits that are encoded by approximately 30 genes (Ronan et al., 2013; Staahl et al., 2013). These subunits are added or subtracted throughout development and are combinatorially assembled (Ho and Crabtree, 2010). Combinatorial assembly means that the individual subunits included in each unique complex can determine NRC function (Ho and Crabtree, 2010). For example, a homozygous deletion of the Brg1 ATPase has different effects than a deletion of the hBrm ATPase. Mice with a deletion of hBrm appear developmentally normal (except for increased weight) while a homozygous deletion of Brg1 is embryonically lethal (Bultman et al., 2000). These differences in phenotype possibly stem from the preferential inclusion of Brg1 and not hBrm in embryonic stem cells.
(reviewed in Vogel-Ciernia and Wood, 2014). Therefore the specific ATPase (and other subunits) included in the complex is regulated to serve the function of the complex.

*Nucleosome Remodeling Complexes in Development*

The differential inclusion and exclusion of some BAF subunits is particularly pronounced during development and is related to the function of the complex. The coordinated exchange of BAF subunits is required when embryonic stem cells (ESCs) differentiate into neuronal precursors and ultimately into post-mitotic neurons. ESCs express esBAF that has Brg1, BAF155 (homodimer), and BAF60A or B subunits (Ho et al., 2009b). The unique combinations of esBAF subunits is involved in transcription regulation necessary for ESC pluripotency and self-renewal (Ho et al., 2009b, 2009a; 2011, Vogel-Ciernia and Wood, 2014).

The transition from esBAF to neuronal progenitor BAF (npBAF) is characterized by the replacement of one BAF155 subunit with BAF170 as well as the incorporation of BAF60 (a, b, or c), and Brm (not present in esBAF; Lessard et al., 2007; Staahl et al., 2013). In addition to those newly integrated subunits, npBAF is defined by subunits SS18, BAF45a/d and BAF53a. Manipulations that target BAF45a, BAF53a (Lessard et al., 2007), or SS18 (Staahl et al., 2013) result in attenuated neural stem/progenitor proliferation. In addition research by Lessard et al., (2007) suggests that npBAF is involved in regulation of gene expressed in the Notch signaling pathway as well the Sonic Hedgehog pathway during development. Together these findings support an important role for npBAF in regulating gene expression required for neuronal progenitor proliferation during development.
At embryonic day 13.5, there is subunit exchange that transitions npBAF into neuron-specific BAF (nBAF). This exchange in subunits, in part, drives the conversion of neuronal progenitor cells to post-mitotic neurons. During this stage of development several subunits are exchanged: BAF45a to BAF45b/c, BAF53a to BAF53b (Lessard et al., 2007; Wu et al., 2007), and SS18 to CREST (Staahl et al., 2013). There is evidence that in the case of BAF53a, its replacement with BAF53b is governed by the expression of microRNAs miR-9* and miR-124 (Yoo et al., 2009). miR-9* and miR-124 expression are repressed in neuronal progenitor cells until neural differentiation where their expression is up-regulated. The expression of those microRNAs in turn represses BAF53a and allows expression of BAF53b (Yoo et al., 2009). The BAF53b subunit is necessary for appropriate gene expression during neuronal differentiation (Lessard et al. 2007) and dendritic branching (Wu et al. 2007). In addition, even though BAF53a and b are highly homologous, BAF53a cannot rescue deficits observed in BAF53b knockout mice (Wu et al., 2007). The tightly orchestrated subunit exchanges that occur in BAF complexes along with the concomitant changes in gene expression profiles illustrate the importance of nucleosome remodeling as well as specific subunits during development.

**Nucleosome Remodeling in Long-Term Memory Formation**

The role of NRCs in regulating gene expression necessary for cell function is not limited to early development. Recently, genetic manipulations targeting the BAF53b subunit were used to explore the role of nucleosome remodeling in cognition in the adult rodent (Vogel-Ciernia et al 2013). Mice with a heterozygous knockout of BAF53b and transgenic mice expressing a dominant negative form of BAF53b both showed long-term memory
impairments for contextual fear, object location and novel object recognition (Vogel-Ciernia et al 2013). Neither type of BAF53b mutant mice exhibited impaired anxiety, locomotor activity, or short-term memory, suggesting that deficits were specific to long-term memory. Similarly, deficits were observed in hippocampal long-term potentiation stabilization. BAF53b is only found in the nBAF complex. The neuron specificity of nBAF therefore means that BAF53b is also only found in post-mitotic neurons (Wu et al. 2007; Olave et al. 2002). The unusual dedication of BAF53b to the nBAF complex, as well its neuronal specificity, made BAF53b the ideal target to explore the role of nucleosome remodeling in memory formation. Together, the results from the aforementioned study demonstrated (for the first time) a role for neuron-specific nucleosome remodeling in gene expression changes and synaptic plasticity underlying long-term memory formation in the adult rodent, independent of a role in development (Vogel-Ciernia et al 2013). It remains unclear, however, whether the role of BAF53b in synaptic plasticity and hippocampal dependent memory extends to drug-associated memories and synaptic plasticity outside the hippocampus.

**Nucleosome Remodeling in Drug Addiction**

Although histone modification and nucleosome remodeling can work in a coordinated manner to regulate gene expression, and the role of histone acetylation and methylation have been shown to have a critical role in cocaine action, until now there was no direct evidence linking a specific nucleosome remodeler in cocaine-associated behaviors. One previous study implicated the ATPase subunit Brg1 as being associated with cocaine-induced gene expression changes in rodents (Kumar et al., 2005). More specifically, Brg1 was shown to physically interact with the cyclin-dependent kinase 5 (Cdk5) gene during
chronic cocaine conditions (Kumar et al., 2005). This was an enticing demonstration that Brg1 and nucleosome remodeling may be involved in cocaine-induced regulation of gene expression. However, that study did not link changes in Brg1 association to changes in cocaine-associated behavior. Furthermore, targeting the role of Brg1 in cocaine-associated behaviors is confounded by the role of Brg1 as the ATPase in several different nucleosome remodeling complexes that are found in multiple cell types (Hargreaves and Crabtree, 2011). Brg1 has been shown to associate with transcription factors and histone-modifying enzyme complexes, WINAC (WSTF including nucleosome assembly complex), NUMAC (nucleosome methylation activation complex), NCoR-1 complex.

Is nucleosome remodeling critical for cocaine-associative memory formation? To answer this question, I targeted the neuron-specific nBAF subunit BAF53b, using mice that had either a deletion mutation or traditional heterozygous knockout of BAF53b (Chapter 1). Then, I investigated the role of BAF53b in cocaine-associated behavior (Chapter 2) and cocaine-associated memory (Chapters 3). The role of neuron-specific nucleosome remodeling factors in drug-associated memory formation and their influence on synaptic plasticity in the nucleus accumbens has not been previously explored.

Therefore, I extended my studies to examine the role of BAF53b in long-term potentiation in nucleus accumbens, as long-term potentiation a thought to be a candidate mechanism underlying long-term memory formation (Chapter 4). First, a stimulation protocol was established that induced LTP using theta stimulation (theta-LTP) in slices from wildtype mice and compared the level of stable potentiation to that achieved in slices from BAF53b mutant mice. Then, I investigated the possibility that 1) theta-LTP in the NAc requires TrkB receptor activation, and 2) whether the ligand for TrkB, brain-derived
neurotrophic factor (BDNF), could reverse deficits in theta-LTP and cocaine-associated memory, absent in BAF53b mutant mice. Together, these experiments provide the first evidence for a role of neuron-specific nucleosome remodeling complexes in cocaine-associated memory formation. Furthermore, these studies suggest targeting BDNF/TrkB activation as a potential mechanism for correcting related plasticity deficits in the nucleus accumbens.
Chapter 1

BAF53b Mutant Mice

Rationale

nBAF has an established role in regulating gene expression necessary for neuronal development in the brain. nBAF is a multi-subunit complex that is neuron specific (Fig.1a). It is defined, in part, by the BAF53b subunit (highlighted in yellow), which is only found in the nBAF complex (Wu et al., 2007; Vogel-Ciernia et al., 2013). BAF53b homozygous knockout mice (Baf53b−/−) do not survive into adulthood and have profound deficits in activity-dependent dendritic outgrowth, synapse formation, and axonal myelination (Wu et al., 2007). These deficits appear to stem from BAF53b’s role in targeting nBAF to specific promoter regions during gene expression (Wu, et al., 2007). BAF53b is necessary for neural stem cell differentiation into post mitotic neurons (Lessard, et al., 2007). Therefore, it is important that any BAF53b manipulations used in subsequent experiments avoid the role of BAF53b in development.

To avoid developmental confounds while studying the role of BAF53b in cocaine-associated memories and behaviors, we generated a deletion mutation of BAF53b (BAF53bΔHD) as well as examined a traditional heterozygous knockout of BAF53b (a generous gift from Gerald Crabtree, Stanford). We previously demonstrated that a similar deletion of the hydrophobic domain of BAF53a (the non-neuronal homolog of BAF53b) generates a dominant-negative form of BAF53a (Park et al., 2002). Given that BAF53a and BAF53b are 83% identical and 94% similar (Harata et al., 1999), we predicted a similar dominant-negative result by deleting the hydrophobic domain of BAF53b. The transgene in the BAF53bΔHD line is expressed via the CaMKIIα promoter, which regulates Baf53bΔHD
expression to forebrain excitatory neurons (Vogel-Ciernia et al., 2013; Mayford et al., 1996; Kojima et al., 1997). The rationale for targeting the hydrophobic domain (ΔHD) is that this domain is predicted to enable protein-protein interactions essential to nBAF’s role as a transcription regulator.

**Methods**

**Mice**

For all experiments, mice of either sex were between 8 and 15 weeks old at the time of behavioral testing. Mice had free access to food and water and lights were maintained on a 12:12h light/dark cycle, with all behavioral testing performed during the light portion of the cycle. Mutant mice were bred to wildtype C57BL/6J from Jackson Laboratories. The resultant mutant and wildtype littermates were then used for experiments. Mice were genotyped before experimentation by examining DNA from tail samples. Experimenters were blinded to genotype during behavioral. Additionally, mice were genotyped after testing using DNA from tail samples. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

**Immunoprecipitation and Western Blotting**

WT Baf53b and Baf53bΔHD were cloned into pLVX-EF1a-IRES-mCherry (Clontech, Mountain View, CA) creating plasmids MW95 and MW97 respectively. HT22 cells (Salk Institute, LaJolla, CA) were transfected with either the empty vector, pLVX-EF1a-IRES-mCherry, MW95 or MW97 using Lipofectamine LTX with Plus Reagent (ThermoFisher,
Cells were harvested 48hrs after transfection and lysed in TPER (ThermoFisher, Waltham, MA). Immunoprecipitations against Brg1 were performed using 1μg of antibody ab70558 (Abcam, Cambridge, MA). Westerns were probed for Baf53b (75-311) at a 1:1000 dilution (Neuromab, Davis, CA) and for Brg1 at a 1:1000 dilution using the same antibody as above.

**Immunofluorescence**

Immunofluorescence was performed as described in our previous studies (Vogel-Cibernia et al., 2013). Wildtype mice were euthanized via cervical dislocation and their brains removed and frozen by immersion into isopentane cooled to approximately -50°C on dry ice. Coronal sections were then cut at a thickness of 20-μm on a cryostat at the level corresponding to NAc (NAc; AP: +1.4 mm to +0.6 mm), thaw-mounted on glass slides and stored at −20°C until used for immunofluorescent staining. Immunofluorescence was carried out on the fresh frozen 20-μm thaw-mounted sections using the Tyramide Signal Amplification (TSA) Plus system (Perkin Elmer, NEL704A001KT). In brief, all slides were fixed using ice cold 4% paraformaldehyde, pH 7.4. (10 min), permeablisized in 0.01% Triton X-100 in 0.1 M PBS (5 min), and incubated in 2% hydrogen peroxide diluted in 0.1 M PBS (vol/vol) (15 min). Subsequently, all slides were then blocked (1 h) using the TSA blocking buffer and incubated overnight (4°C) in rabbit antibody to BDNF (1:500, Santa Cruz Biotech., # sc-546), washed (3 × 5 min in 0.1 M PBS with 0.05% Tween-20, vol/vol), and incubated for 1 h at 21–23 °C with HRP-conjugated goat antibody to rabbit IgG (1:500, 711-036-152, Jackson ImmunoResearch). Slides were again washed and the TSA reaction was performed.
to detect BDNF as described in the kit (TSA-Alexa Fluor 488 1:50). Slides were coverslipped in ProLong Gold antifade reagent with DAPI (P-36931, Invitrogen).

Data Analysis

For all experiments, no differences were observed with respect to sex so datasets were collapsed. Datasets were then analyzed by two-way repeated-measures analysis of variance (ANOVA). Bonferroni post hoc tests were performed when appropriate. Specific comparisons were made using Student’s t tests with alpha levels held at 0.05.

Results

Both wildtype BAF53b and the BAF53bΔHD mutant protein interact with Brg1, as demonstrated by co-immunoprecipitation of either wildtype or BAF53bΔHD with Brg1 (Fig. 1c). Using RT-qPCR, we verified transgene expression in the NAc (Fig. 1d). In addition to transgenic BAF53bΔHD mice, we also examined a BAF53b+/− heterozygous knockout mouse line (Wu et al., 2007; Vogel-Ciernia et al., 2013). BAF53b+/− heterozygous knockout mice have approximately 50% Baf53b mRNA and protein expression in the hippocampus (Vogel-Ciernia et al., 2013). RT-qPCR revealed that Baf53b mRNA expression in the nucleus accumbens of BAF53b+/− heterozygous home-cage mice was significantly reduced (approximately 50%) compared with their wildtype littermates. Both genetically modified mouse lines have previously been shown to be indistinguishable from wildtype littermates with respect to locomotor activity, anxiety, short-term memory and basal synaptic
transmission (Lessard et al., 2007). We observed that these animals bred normally and no physical defects were observed.

Figure 1.1. Characterization of mutant mice. (a) nBAF complex is combinatorially assembled, meaning that the individual subunits included in each unique complex can alter NRC function (20) containing the dedicated and neuron-specific subunit BAF53b (highlighted in yellow). (b) Wild-type Baf53b is illustrated with the hydrophobic domain (gray). Amino acids 323–333 in the hydrophobic domain were deleted to generate the BAF53bΔHD construct. This BAF53bΔHD mutant sequence was then cloned into a separate vector containing intron and exon sequences with splice sites and the SV40 intron and polyadenylation signal, which was then cloned downstream of the 8.5-kb mouse CamkIIα promoter (Vogel-Ciernia et al., 2013). This construct was then used to generate the BAF53bΔHD transgenic mice. (c) Immunoprecipitation and western blot showing that Brg1 co-immunoprecipitates with BAF53b as well as BAF53bΔHD (d) RT-qPCR revealed that the BAF53bΔHD transgene is only expressed in the BAF53bΔHD mutant mice and not their wild-type littermates. Data are presented as mean ± S.E.M. (e) Quantitative reverse transcription PCR (RT-qPCR) revealed that BAF53b+/− heterozygous knockout mice (n=8) have significantly reduced wild-type Baf53b expression in the NAc compared to their wild-type littermates (n=7; t(13)=10.25, p<0.0001)

Similar to the home-cage data (Fig 1.1), we examined whether administering cocaine in a novel context would alter Baf53b expression in BAF53b genetically mice. Mice were
given a 5mg/kg dose of cocaine then placed in a novel chamber. Thirty minutes after the injection, mice were removed from the chamber. Mice were sacrificed 30 minutes after being removed from the chamber. We observed that BAF53b+/− heterozygous knockout mice had approximately 50% less Baf53b expression in the nucleus accumbens compared to wildtype and BAF53bΔHD transgenic mice (Fig 1.2). Drug administration had no effect on Baf53b expression regardless of genotype. The results indicate that there is no Baf53b compensation in the nucleus accumbens of in BAF53b+/− knockout mice by the remaining allele after cocaine administration.

![Graph showing Baf53b mRNA change](image)

**Figure 1.2** BAF53b expression unaffected by cocaine. Quantitative RT-PCR revealed that wildtype Baf53b mRNA expression in the nucleus accumbens is significantly reduced in BAF53b+/− heterozygous mice compared to wildtype and BAF53bΔHD mice.
We also confirmed that BAF53b protein is expressed in the nucleus accumbens of wildtype mice. BAF53b colocalizes with DAPI staining, which illustrates that it is a nuclear protein (Fig 1.2). This localization is predicted for a protein implicated in nucleosome remodeling. The nucleus accumbens is a brain region is integral to several cocaine-associated behaviors as well as cocaine-associated memory formation. This brain region is targeted in our future cocaine-associated memory and synaptic plasticity studies.

Figure 1.3 BAF53b nuclear expression. BAF53b nuclear expression in the nucleus accumbens of wildtype mice. BAF53b (green) is colocalizes with nuclei, DAPI (blue).
Discussion

By targeting BAF53b, we circumvented several of these limitations that prevent elucidating the role of nucleosome remodeling in cocaine-associative memory formation. BAF53b is unique because it is currently thought to be exclusively found in the nBAF nucleosome remodeling complex and is neuron specific (Wu et al., 2007; Olave et al., 2002). Examining a homozygous deletion of BAF53b (BAF53b−/−) was not possible as those mice die shortly after birth (Wu et al., 2007). Cultured neurons from homozygous knockout mice display aberrant gene expression, synapse formation and activity-dependent dendritic arborization (Wu et al., 2007). Therefore, we used two independently derived mouse lines that avoid the lethality associated with BAF53b−/− knockout mice. To further account for unforeseen effects associated with having only one BAF53b allele (heterozygous knockout) throughout development, the BAF53bΔHD transgenic line expresses mutant BAF53b outside of BAF53b’s known role in embryonic development. The mutant BAF53bΔHD protein co-immunoprecipitates with the BRG1 ATPase, suggesting that the BAF53bΔHD mutation does not disrupt one of the complexes key interaction partners.

BAF53a is incapable of rescuing the function of nBAF after BAF53b manipulation (Wu et al. 2007). Also, there are also no known neuronal homologs of BAF53b that could compensate for the expression decrease observed in BAF53b−/− knockout mice. Therefore, we examined whether the loss of one allele resulted in a compensatory upregulation of endogenous Baf53b in BAF53b−/− knockout mice. We observed that cocaine paired with a novel context did not result in any Baf53b compensation that would confound future experiments. We paired cocaine with a novel context in order to mimic the conditions of future cocaine conditioned place preference memory tasks. Additionally, both heterozygous
knockout (BAF53b<sup>−/−</sup>) and BAF53bΔHD transgenic mice appear indistinguishable from wildtype littermates in development, anxiety, locomotor activity, birth weight, and short-term memory (Vogel-Ciernia et al., 2013). Given these data as well as the aforementioned described published data on these genetically modified mouse lines, we consequently studied the role of BAF53b in cocaine-associated behaviors and memories.
Chapter 2

BAF53b in Cocaine Sensitization

Rationale

We investigated the role of BAF53b in cocaine sensitivity. Repeated intermittent exposure to some drugs of abuse can result in tolerance to some effects of the drug but also sensitization to other effects (Robinson et al., 1993; reviewed in Hyman, 2005). This incentive sensitization model is thought to be reflected behaviorally in rodents as increasing locomotor response to repeated psychostimulants administration (Anagnostaras and Robinson, 1996). Sensitization to the locomotor activating effects of psychostimulants has been used to explain the increased ability of drug-associated cues to drive behavior (Robinson et al., 1993; Robinson and Berridge, 2003; Kalivas et al., 1991). We and other laboratories have shown that chromatin-modifying enzymes can play a role in cocaine-induced sensitization and responses to repeated cocaine administration (Malvaez et al., 2011; Maze et al., 2010; Kennedy et al., 2013; Renthal et al., 2009). Therefore, to test whether BAF53b plays a role in cocaine sensitivity, BAF53b+/− heterozygous and BAF53bΔHD mice were given acute or intermittent chronic cocaine treatments then their locomotor activity recorded and compared to wildtype littersmates that were given saline (Fig. 2a). A 10mg/kg cocaine dose, which generates robust sensitization, was used as in our previous experiments (Malvaez et al., 2011).
Methods

Mice

For all experiments, mice of either sex were between 8 and 15 weeks old at the time of behavioral testing. Mice had free access to food and water and lights were maintained on a 12:12h light/dark cycle, with all behavioral testing performed during the light portion of the cycle. Mutant mice were bred to wildtype C57BL/6J from Jackson Laboratories. The resultant mutant and wildtype littermates were then used for experiments. Mice were genotyped before experimentation by examining DNA from tail samples. Experimenters were blinded to genotype during behavioral and electrophysiological experiments. Additionally, mice were genotyped after testing using DNA from tail samples. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Cocaine Sensitization

This conditioning examines the locomotor activating effects of cocaine in animals due to acute or chronic cocaine administrations. The cocaine sensitization experiment was performed as described in our previous study (Malvaez et al., 2011). In brief, prior to any behavioral procedure, mice were handled for 1–2 min for 3 d and were habituated to the activity apparatus (Plexiglas open field with sawdust bedding; base 16 cm X 32cm) for 30 min/d for 2 consecutive days. Mutant mice and their respective wildtype littermates were randomized into 3 different treatment groups (Control, Acute, and Chronic) and locomotor
activity was monitored for 30 min after an intraperitoneal (i.p.) injection of cocaine-HCl (10 mg/kg; Sigma) or vehicle (0.9% saline). The Control group received saline injections days 1–5. The Acute group received saline injections days 1–4 and a single cocaine injection on day 5. The Chronic group received cocaine injections days 1–5. Locomotor activity (total distance traveled) was recorded each day with a video camera mounted above the activity apparatus and tracked automatically from MPEG videos using EthoVision 3.1 software (Noldus Technology, Leesburg, VA; see Malvaez et al., 2011).

Data Analysis

For all experiments, no differences were observed with respect to sex so datasets were collapsed. Datasets were then analyzed by two-way repeated-measures analysis of variance (ANOVA). Bonferroni post hoc tests were performed when appropriate. Specific comparisons were made using Student’s t tests with alpha levels held at 0.05.

Results

Heterozygous knockout of BAF53b did not affect baseline locomotor activity during habituation (Fig. Supplemental 2b). All mice, regardless of genotype, travelled similar distances on the first habituation day. Their locomotor activity then decreased significantly from habituation day 1 to habituation day 2. This decrease illustrates that all mice habituated to the context. After the first conditioning day, the locomotor activity of the chronic group became significantly increased compared to saline treated littermates, and remained significantly different for the remaining conditioning days. On conditioning days, both BAF53b+/− heterozygous and wildtype mice had similar responses to chronic cocaine
administration, with both groups showing a graded increase in their response to cocaine indicative of cocaine sensitization (Fig. 2.1b). No differences were observed between BAF53b+/− heterozygous and wildtype mice after acute cocaine administration (Fig. 2.1c). Therefore, we found that the loss of one allele of BAF53b did not alter cocaine sensitivity as BAF53b+/− heterozygous mice performed comparably to wildtype littermates.

**Figure 2.1.** BAF53b+/− heterozygous knockout and BAF53bΔHD mice displayed normal baseline locomotor activity during habituation. (a) Schematic representation of cocaine sensitization procedure. (b) BAF53b+/− heterozygous knockout and wildtype mice, regardless of future treatment, displayed similar locomotor activity throughout habituation (significant main effect of habituation day, $F_{1,24}=69.79, p<0.0001$; no main effect of genotype, $F_{2,24}=0.41, p=0.67$; no interaction, $F_{2,24}=0.91, p=0.41$). (c) BAF53bΔHD and wild-type mice displayed similar locomotor activity throughout habituation (significant main effect of habituation day, $F_{1,20}=37.08, p<0.0001$; no main effect of genotype, $F_{2,20}=2.02, p=0.16$; no interaction, $F_{2,20}=1.38, p=0.27$).
Similarly, we investigated whether the deletion mutant of BAF53b would affect cocaine sensitivity. During habituation day 1, we observed that BAF53bΔHD transgenic mice and wildtype littermates displayed similar baseline locomotor activity (Fig. Supplemental S1c). Reminiscent of the BAF53b heterozygous knockout mice, the locomotor activity of the BAF53bΔHD transgenic mice and wildtype littermates then decreased significantly from habituation day 1 to habituation day 2. Therefore, all mice in this experiment habituated to
the context regardless of genotype. On conditioning day, BAF53bΔHD mice were given acute or chronic cocaine treatments and their locomotor activity recorded in the same manner as the BAF53b+/− heterozygous mice. A 10mg/kg cocaine dose was used as in the previous experiment. Both BAF53bΔHD and wildtype mice had a similar response to chronic cocaine administration indicative of cocaine sensitization (Fig. 2d). Similarly, no differences were observed between BAF53bΔHD and wildtype mice after acute cocaine administration (Fig. 2e). Therefore, we found that the deletion mutation did not alter cocaine sensitivity as BAF53bΔHD mice performed comparably to wildtype littermates. Overall these results suggest that BAF53b is not required for cocaine sensitization.

Figure 2.2 Mutant mice have normal cocaine sensitization. (a) Schematic representation of cocaine sensitization procedure. (b) Throughout conditioning, chronic cocaine-treated BAF53b+/− heterozygous knockout mice (n=8) traveled the same mean distance ± S.E.M. as chronic cocaine-treated wild-type littermates (n=8; significant main effect of injection day, $F_{4,56}=13.90, p<0.0001$; no main effect of genotype, $F_{1,14}=0.24, p=0.6$; no interaction, $F_{4,56}=0.78, p=0.54$). (c) Acute cocaine-treated BAF53b+/− heterozygous knockout mice (n=9) traveled the same distance as acute cocaine-treated wild-type littermates (n=8; significant main effect of injection day, $F_{4,60}=11.78, p<0.0001$; no main effect of genotype, $F_{1,15}=2.70, p=0.12$; no interaction, $F_{4,60}=0.24, p=0.91$). (d) Both BAF53bΔHD and wild-type mice had a similar response to chronic cocaine administration with both groups showing a graded increase in their response to cocaine indicative of cocaine sensitization (Figure 3c significant main effect of injection day, $F_{4,52}=20.54, p<0.0001$; no main effect of genotype, $F_{1,13}=0.23, p=0.64$; no interaction, $F_{4,52}=0.76, p=0.56$). Similarly, no differences were observed between BAF53bΔHD and wild-type mice after acute cocaine administration (Figure 3d; significant main effect of injection day, $F_{4,52}=14.46, p<0.0001$; no main effect of genotype, $F_{1,13}=0.01, p=0.94$; no interaction, $F_{4,52}=0.78, p=0.54$).
### Table

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### Graphs

**Graph b.**
- Chronic Wt
- Chronic BAF53b+/−
- Saline Wt
- Saline BAF53b+/−

**Graph c.**
- Acute Wt
- Acute BAF53b+/−

**Graph d.**
- Chronic Wt
- Chronic BAF53b+/−
- Saline Wt
- Saline BAF53b+/−

**Graph e.**
- Acute Wt
- Acute BAF53b+/−
Discussion

We used two independently derived genetically modified mouse lines to determine the role of BAF53b in cocaine-associated behaviors. Specifically, we examined whether BAF53b played a necessary role in cocaine-associated locomotor sensitization. Our lab has previously shown that chromatin modifying enzymes in the nucleus accumbens play a role in cocaine sensitization (Malvaez et al., 2011). Therefore, we explored whether nucleosome remodeling had a similar role. It was first important to confirm that mutant mice had no baseline locomotor deficits, as reported in Vogel-Ciernia et al., (2013). In accordance with those previously reported findings, we did not see any differences between mutant mice and wildtype littermates with respect to baseline locomotor activity. Given that we did not observe any locomotor confounds, we examined a potential role for BAF53b in cocaine sensitization. However, we did not see an effect of BAF53b manipulation on cocaine sensitization nor acute cocaine administration. This suggests that BAF53b does not mediate the locomotor activating effects of cocaine.

Psychostimulants are believed to act on the ventral tegmental area, resulting in increased dopamine in the nucleus accumbens (Kalivas and Weber 1988; Vezina and Stewart, 1990). Direct administration of amphetamine into the ventral tegmental area (VTA) but not the nucleus accumbens produces locomotor sensitization to cocaine administered to the periphery (Kalivas and Weber 1988). Additionally, manipulation of CBP in the nucleus accumbens produces deficits in cocaine sensitization. Together, those studies suggest that sensitization is initiated in the VTA but expressed through the nucleus accumbens (Hyman,
In regards to our data, if BAF53b had a role in the initiation but not expression cocaine sensitization, it would be predicted that the heterozygous mice, who have BAF53b one allele throughout the brain (including VTA), would show a deficits in cocaine sensitization but the BAF53bΔHD mice would not. However, if BAF53b was involved in the expression of cocaine-sensitization through the nucleus accumbens then it would be predicted that both genetically modified mice would show a deficit in sensitization. The lack of any effect suggests that BAF53b is not involved in the initiation or expression of cocaine-sensitization.
Chapter 3

BAF53b in Cocaine-Associated Memory

Rationale

Drug-induced sensitization and drug-induced conditioned place preference share underlying brain circuitry (Wise and Bozarth 1987). However, there are reports that the effect of drugs on sensitization and conditioned place preference are dissociable (Shimosato and Ohkuma, 2000). Dong et al., (2004) showed that GluRA homozygous knockout mice displayed normal locomotor sensitization but deficits in conditioned place preference. Given these prior reports, we examined whether mutant mice, which had normal cocaine sensitization, had impairments in cocaine-associated memories. To examine the role of BAF53b in cocaine-associated memory formation, we used cocaine-associated conditioned place preference (CPP). The CPP paradigm involves animals associating the rewarding properties of cocaine with a distinct cocaine-paired context compared to a distinct vehicle-paired context (Fig. 3.1a). The strength of the association is then tested while the animal is in a drug-free state. Mice with strong cocaine-associated memories spend significantly more time in the cocaine-paired compartment compared to the vehicle-paired compartment.

Methods

Mice

For all experiments, mice of either sex were between 8 and 15 weeks old at the time of behavioral testing. Mice had free access to food and water and lights were maintained on a 12:12h light/dark cycle, with all behavioral testing performed during the light portion of the cycle. Mutant mice were bred to wildtype C57BL/6J from Jackson Laboratories. The
resultant mutant and wildtype littermates were then used for experiments. Mice were genotyped before experimentation by examining DNA from tail samples. Experimenters were blinded to genotype during behavioral and electrophysiological experiments. Additionally, mice were genotyped after testing using DNA from tail samples. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

**Conditioned Place Preference**

Place conditioning was performed as described previously in our studies (Malvaez et al., 2011; Rogge et al., 2013). Briefly, the experiments were conducted in a three-compartment apparatus which had two larger compartments (12.5 X 17cm; checkered and white) on either side of a smaller compartment (12.5 X 11.5cm) separated by guillotine doors. The larger compartments had distinct visual, tactile and olfactory cues. One of the larger compartments had white walls with bar flooring and pine shavings underneath. The other large compartment had checkered walls with grid flooring and cedar shavings underneath. The middle smaller compartment had a solid floor, two gray walls as well as one checkered and one white wall leading to the respective larger compartments.

All mice were handled for 1 min each day for 3 consecutive days prior to the experiment (days 1-3). Baseline preferences were assessed by placing the animals in the center compartment of the three compartment place preference apparatus with the guillotine doors open, allowing free access to three distinct compartments for 15 min (day 4). Time spent in each compartment was recorded. Conditioning was conducted over the
subsequent 4 days with the guillotine doors closed, thus confining animals to a specific compartment for 30 min (days 5-8). An unbiased paradigm was used such that half of the animals were injected with cocaine-HCl (5 or 10 mg/kg, i.p.; Sigma) prior to placement in the checkered compartment, and half were injected with cocaine prior to placement in the white compartment (CS+). The next day, mice were injected with 0.9% saline (1.0 ml/kg, i.p.) prior to placement in the alternate compartment (CS−). Injections were alternated for subsequent conditioning sessions. Forty-eight hours after the last conditioning session, preference (15 min, Posttest 1; day 10) was assessed in all animals as described above in a drug-free state. CPP score was calculated as the time (s) spent in cocaine-paired (CS+) minus saline-paired (CS-) compartments. Time spent in each chamber of the conditioned place preference apparatus was tracked automatically from MPEG videos using EthoVision 3.1 software (Noldus Technology, Leesburg, VA; see Malvaez et al., 2011).

Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was performed to examine gene expression. Tissue was collected as described in our previous studies (Malvaez et al., 2011; Rogge et al., 2013). Tissue from the NAc was stored in RNALater (Invitrogen, AM7020) at -80 °C until processed. RNA was isolated using RNeasy minikit (Qiagen). cDNA was made from 50-150ng of total RNA using the Transcriptor First Strand cDNA Synthesis kit (Roche Applied Science 04379012001). Primers were derived from the Roche Universal Probe Library. Gapdh probe is conjugated to Lightcycler Yellow555 and all other probes were conjugated to a reporter fluorophore. The non-overlapping dyes and quencher on the reference gene allow for multiplexing in the Roche LightCycle 480 II machine (Roche Applied Science). All values were
normalized to *Gapdh* expression levels. Analysis and statistics were performed using the Roche proprietary algorithms and REST 2009 software based on the Pfaffl method (Pfaffl, 2001; Pfaffl et al., 2002).

**Data Analysis**

For all experiments, no differences were observed with respect to sex so datasets were collapsed. Datasets were then analyzed by two-way repeated-measures analysis of variance (ANOVA). Bonferroni post hoc tests were performed when appropriate. Specific comparisons were made using Student’s t tests with alpha levels held at 0.05.

**Results**

In the first experiment, BAF53b*+/−* heterozygous mice and wildtype littermates were conditioned using 5mg/kg cocaine (Fig. 3b). Neither BAF53b*+/−* heterozygous mice nor wildtypes displayed an initial preference for either context prior to conditioning (pre-test). After conditioning with two pairings of cocaine, post-test results revealed that wildtype mice had a robust CPP score (time spent in cocaine-paired chamber minus saline-paired chamber). In contrast, BAF53b*+/−* heterozygous mice did not display a cocaine-associated CPP, spending similar time in both cocaine-paired and saline-paired chambers after conditioning. We examined the locomotor activity during the test days to assess whether there were any differences in the locomotor activating effect of cocaine in BAF53b*+/−* heterozygous mice compared to wildtype (Supplemental Fig S2b). We observed no differences in locomotor activity between BAF53b*+/−* heterozygous mice and wildtype controls on either test day. These results suggest that the loss of one allele of BAF53b
significantly disrupts cocaine CPP at 5mg/kg without altering overall locomotor performance.

We were interested in determining whether there was a dose dependent role for BAF53b in cocaine CPP. Therefore, we conducted a separate CPP experiment at 10mg/kg cocaine using a different cohort of BAF53b+/− heterozygous mice (Fig. 3c). Similar to the previous CPP experiment, neither BAF53b+/− heterozygous mice nor wildtype mice displayed an initial preference for either context. At post-test, both wildtype and BAF53b+/− heterozygous mice established a preference for the cocaine-paired context. Therefore, unlike the 5 mg/kg CPP experiment, one BAF53b allele is sufficient for cocaine CPP when using a 10mg/kg cocaine dose. We also examined the locomotor activity during the 10mg/kg experiment to assess whether there were any differences in the locomotor activating effect of cocaine in the BAF53b+/− heterozygous mice compared to wildtype at 10 mg/kg (Supplemental Fig S2c). However, no differences were observed between BAF53b+/− heterozygous and wildtype mice on test days. Together these results suggest that removal of one BAF53b allele disrupts cocaine CPP memory formation in a cocaine dose dependent manner (at 5mg/kg but not 10mg/kg). Importantly, all BAF53b+/− heterozygous mice traveled similar distances to their respective wildtype controls on test days, indicating no differential effects of cocaine on locomotor performance.

Figure 3.1. Mutant mice have impairments in cocaine-CPP. (a) Schematic representation of cocaine-CPP procedure. (b) Cocaine-CPP expression indicated by mean CPP score (CS+ minus CS−) ± S.E.M. At 5mg/kg cocaine dose, BAF53b+/− heterozygous knockout mice (n=10) exhibited significantly attenuated CPP score compared to wild-type littermates (n=8). A two-way repeated measures ANOVA revealed a significant main effect of genotype (F_{1,16}=6.99, p=0.02). No effect of conditioning was observed (F_{1,16}=2.05, p=0.17) but there was a significant interaction (F_{1,16}=4.93, p=0.04). Bonferroni post hoc analysis showed no initial preference for either context on the pretest (t= 0.46, p>0.05). However, there was a
significant difference between the wild-type and BAF53b\(^{+/-}\) heterozygous mice on the posttest ($t= 3.45, p<0.01$). (c) At 10mg/kg cocaine dose, BAF53b\(^{+/-}\) heterozygous knockout mice ($n=9$) exhibit similar CPP score to wild-type littermates ($n=8$). Using a two-way repeated measures ANOVA, we found a significant main effect of conditioning ($F_{1,17}=112.81, p<0.0001$) but not genotype ($F_{1,17}=0.24, p=0.63$) and no interaction ($F_{1,17}=0.14, p=0.71$). Bonferroni post hoc analysis showed no difference between the wild-type and the BAF53b\(^{+/-}\) heterozygous mice on the pretest ($t= 0.21, p>0.05$) or post-test ($t= 0.61, p>0.05$). (d) At 5mg/kg cocaine dose, BAF53bΔHD mice ($n=9$) exhibited significantly attenuated CPP score compared to wild-type littermates ($n=10$). Using a two-way repeated measures ANOVA, we found significant main effects on conditioning ($F_{1,17}=29.19, p<0.0001$) and genotype ($F_{1,17}=5.62, p=0.03$), but no interaction ($F_{1,17}=2.56, p=0.13$). Bonferroni post hoc analysis showed no differences between the preference of wild-type and BAF53bΔHD mice for either context on the pretest ($t= 0.83, p>0.05$) but a significant difference on the posttest ($t= 2.85, p<0.05$). (e) At a 10mg/kg cocaine dose, BAF53bΔHD mice ($n=10$) exhibited significantly attenuated CPP score compared to wild-type littermates ($n=7$). A two-way repeated measures ANOVA revealed significant main effects of conditioning ($F_{1,15}=47.37, p<0.0001$), genotype ($F_{1,15}=7.91, p=0.01$) and an interaction ($F_{1,15}=5.71, p=0.03$). Bonferroni post hoc analysis showed no differences between the preference of wild-type and BAF53bΔHD.
We then examined whether BAF53bΔHD mice had similar memory deficits to BAF53b+/− heterozygous mice using a CPP experiment (Fig. 3d). Neither BAF53bΔHD mice nor wildtype displayed an initial preference for either context on the pre-test. BAF53bΔHD mice and wildtype littermates were conditioned using 5mg/kg cocaine. After conditioning,
wildtype mice acquired a robust CPP, whereas BAF53bΔHD mice displayed a significantly reduced CPP. We observed no differences in locomotor activity between BAF53bΔHD mice and wildtype controls on either test day (Supplemental Fig. S2d). These findings further suggest that BAF53b is important for the formation of cocaine CPP at 5mg/kg.

Figure 3.2. Mutant mice have normal locomotion on test days during cocaine-CPP. (a) Schematic representation of cocaine-CPP procedure. (b) Cocaine-CPP expression indicated by mean CPP score (CS+ minus CS−) ± S.E.M. At 5mg/kg cocaine dose, BAF53b+/− heterozygous knockout mice (n=10) exhibited similar locomotion compared to wild-type littermates (n=8). A two-way repeated measures ANOVA revealed no main effect of genotype (F_{1,16}=0.14, p=0.72). No effect of conditioning was observed (F_{1,16}=0.09, p=0.77) and there was no interaction (F_{1,16}=0.69, p=0.49). (c) At 10mg/kg cocaine dose, BAF53b+/− heterozygous knockout mice (n=9) exhibit similar CPP score to wild-type littermates (n=8). Using a two-way repeated measures ANOVA, we found no main effect of conditioning (F_{1,17}=2.22, p=0.15) nor genotype (F_{1,17}=3.07, p=0.09) and no interaction (F_{1,17}=0.09, p=0.77). (d) At 5mg/kg cocaine dose, BAF53bΔHD mice (n=9) exhibited significantly attenuated CPP score compared to wild-type littermates (n=10). Using a two-way repeated measures ANOVA, we found significant main effects on conditioning (F_{1,17}=5.20, p=0.03) but not genotype (F_{1,17}=0.70, p=0.42), and no interaction (F_{1,17}=1.68, p=0.21). (e) At a 10mg/kg cocaine dose, BAF53bΔHD mice (n=10) exhibited significantly attenuated CPP score compared to wild-type littermates (n=7). A two-way repeated measures ANOVA revealed significant main effects of conditioning (F_{1,15}=0.08, p=0.78), genotype (F_{1,15}=0.05, p=0.83) and an interaction (F_{1,15}=1.30, p=0.27).
To examine whether there was a dose dependent role of BAF53b in cocaine CPP, we performed a 10mg/kg cocaine CPP experiment in a different cohort of BAF53bΔHD mice (Fig. 3e). Similar to the previous experiment, neither BAF53bΔHD mice nor wildtype displayed an initial preference for either context before conditioning. After conditioning, wildtype mice
displayed a robust preference for the cocaine-paired compartment. However, unlike the BAF53b+/− heterozygous mice at 10 mg/kg, BAF53bΔHD mice demonstrated a significantly reduced CPP at post-test. These results indicate that the BAF53bΔHD mutation disrupts the formation of cocaine-associated CPP in both doses of cocaine (at 5 and 10 mg/kg). No differences in locomotor activity were observed between BAF53bΔHD mice and their wildtype littermates during the 10mg/kg experiment (Supplemental Fig. 2e). Therefore CPP deficits observed in the BAF53bΔHD mice appear to be independent of any locomotor impairment. Together these data from the BAF53bΔHD and BAF53b+/− heterozygous mice suggest that BAF53b plays a key role in the acquisition/consolidation of cocaine-associated CPP. However, there remains the open question of what accounts for the more severe cocaine-memory impairments observed in the BAF53bΔHD mice compared to the BAF53b+/− heterozygous mice.

**Discussion**

Conditioned place preference is a task used to assess drug-associated memory strength. However, it is not a model of human addiction. As discussed by Sanchis and Spanagel (2006), CPP procedure results in animals receiving drugs independent of the will or behavior of the animal. It therefore does not mirror the manner in which humans abuse addictive drugs. However, while CPP does not model human drug use, it effectively tests drug-context associated memory strength. Through Pavlovian conditioning, the rewarding properties (unconditioned stimuli) of drugs become associated with the drug-paired environment and cues (conditioned stimuli), which results in animals deciding to spend significantly more time in the drug-paired compartment (conditioned response). The time
spent in the drug-paired environment compared to a control-paired environment provides valuable information about the strength of the association formed during conditioning, which requires an underlying drug-associated memory.

Unlike the cocaine-induced sensitization experiment, we observed profound deficits in cocaine-associated memories in mutant mice. Both genetically modified mice spent significantly less time in the cocaine-paired compartment compared to the saline-paired compartment. However, even though both mutant mouse lines displayed deficits in cocaine-associated memory formation, their responses to an increase in cocaine dose differed. Increasing the dose of cocaine from 5mg/kg to 10mg/kg resulted in a continued CPP impairment in the BAF53bΔHD, but not the BAF53b+/− heterozygous mice. The lack of impairment in the BAF53b+/− heterozygous knockout at 10mg/kg was not predicted. In addition, both the BAF53bΔHD and BAF53b+/− heterozygous mice traveled similar distances to their respective wildtype littermates at the 10mg/kg dose. This suggests that the differential CPP performance between both mutant mice at that dose is not due to a differential locomotor performance deficits induced by cocaine.

Overall, these results suggest that while cocaine sensitization and CPP share some neural circuitry, BAF53b’s importance to each behavior varies. Additionally, the observed differences in responses to cocaine dose by mutant mice likely underlie differences in neuronal function caused by their respective genetic manipulation. The differences observed between BAF53bΔHD and BAF53b+/− heterozygous mice possibly reflect the differences between the powerful effects of overexpressing a deletion mutation of BAF53b compared to the loss of one BAF53b allele. It is likely that a deletion mutant of BAF53b ultimately affects the nBAF complex in a persistent manner such that even higher cocaine doses are unable to
induce strong CPP. The hydrophobic domain is likely forming important protein-protein interactions, which are then disrupted in the transgenic BAF53bΔHD mice. However, this possibility remains open to further exploration.
Chapter 4

BAF53b in Synaptic Plasticity

Rationale

The observed differences in cocaine-associated memory between the BAF53bΔHD and BAF53b+/− heterozygous mouse lines led us to examine whether synaptic plasticity was differentially altered. One of the likeliest candidates for how associative memories are generated is through changes in synaptic plasticity and brain regions in the reward pathway such as the NAc display long-term potentiation (LTP) and long-term depression (LTD; Kombian and Malenka, 1994; Thomas et al., 2000). However, it remains unknown as to how disruption of BAF53b within the NAc influences long-term synaptic plasticity in that region. Thus, we conducted a study using parasagittal slices containing the NAc core from: (1) BAF53b+/− heterozygous, (2) BAF53bΔHD, and (3) wildtype littermate mice to examine BAF53b influences on long-term potentiation (Fig. 4a).

Methods

Mice

For all experiments, mice of either sex were between 8 and 15 weeks old at the time of behavioral testing. Mice had free access to food and water and lights were maintained on a 12:12h light/dark cycle, with all behavioral testing performed during the light portion of the cycle. Mutant mice were bred to wildtype C57BL/6J from Jackson Laboratories. The resultant mutant and wildtype littermates were then used for experiments. Mice were genotyped before experimentation by examining DNA from tail samples. Experimenters were blinded to genotype during behavioral and electrophysiological experiments.
Additionally, mice were genotyped after testing using DNA from tail samples. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

**Slice Preparation and Recording**

Parasagittal slices containing the NAc core were prepared from BAF53b+/−, CamKIIα-BAF53bHDLow, and wildtype mice (approximately 2 months of age). Following isoflurane anesthesia, mice were decapitated and the brain was quickly removed and submerged in ice-cold, oxygenated dissection medium containing (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 2.5 CaCl2, 26 NaHCO3, and 10 glucose. The cerebellum and lateral aspects of both hemispheres were removed. Parasagittal slices (320 µm) were cut from the blocked brain using a FHC vibrating tissue slicer (Model:OTS-5000) before being transferred to an interface recording containing preheated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 1.5 MgSO4, 2.5 CaCl2, 26 NaHCO3, 10 glucose and 10 μM picrotoxin to reduce feedforward inhibition (56-58). Slices were continuously perfused with this solution at a rate of 1.0-1.5 ml/min while the surface of the slices were exposed to warm, humidified 95% O2 / 5% CO2 at 31 ± 1°C. Recordings began following at least 1.5 hr of incubation.

Stimulation of glutamatergic afferent fibers within the NAc was achieved by placing a bipolar stainless steel stimulation electrode (25um diameter, FHC) just below the anterior commissure. Activation of field excitatory postsynaptic potentials (fEPSP) were recorded using a glass pipette (2-3 MΩ) positioned caudal or caudal-ventral to the stimulation
electrode (Fig 4a). Thus correct placement of electrodes within the NAc was confirmed by visual inspection of the slice and comparison with mouse brain atlas (Paxinos and Watson; 0.84-1.08 lateral to midline) (Pennartz et al., 1990; Pennartz et al., 1993; Li and Kauer, 2004). Two parasagittal slices/hemisphere containing a large portion of the NAc core were obtained for each animal. Pulses were administered at 0.05 Hz using a current that elicited a 30-40% maximal response. Measurements of fEPSP slope (measured at 10–90% fall of the slope) were recorded during a minimum 20 min stable baseline period at which time long-term potentiation (LTP) was induced by delivering 3-5 trains (intertrain interval of 1 min), each train containing 5 ‘theta’ bursts, with each burst consisting of four pulses at 100 Hz and the bursts themselves separated by 200 msec (i.e., theta burst stimulation or TBS). The stimulation intensity was not increased during the delivery of TBS. Data were collected and digitized by NAC 2.0 Neurodata Acquisition System (Theta Burst Corp., Irvine, CA) and stored on a disk. The electrophysiology experiments were conducted by Dr. Enikö A. Kramár.

Data in the text are presented as means ± SD. Data in figures on LTP were normalized to the last 10 min of baseline. The LTP experiment, and conventional measures of baseline synaptic transmission including paired-pulse facilitation and input/output curves, was analyzed using a 2-way repeated measure ANOVA.

**Results**

First, we established that the field excitatory postsynaptic potentials generated within the core following afferent stimulation of glutamatergic fibers that form synapses on neurons were blocked by AMPA and NMDA receptor antagonists, DNQX and D-APV, respectively (Fig. 4b). Low intensity single pulse stimulation of afferent fibers within the core
produced similar field responses to those described in previous studies (d’Alcantara et al., 2001; Schramm et al. 2002; Mishra et al. 2012). Then we generated input/output curves to determine whether the slope of the fEPSP responses across a range of stimulation currents differed between the two genetically modified mice relative to wildtype controls (Fig. 4c). No differences were found between groups. Next we tested for differences in transmitter release kinetics using paired-pulse facilitation. The BAF53b+/− heterozygous and BAF53bΔHD slices did not differ from wildtype slices (Fig. 4d). Taken together, these results indicated that genetic manipulation of BAF53b did not alter conventional measures of baseline synaptic transmission in the NAc core.

We then examined long-term potentiation through theta burst stimulation (TBS). Trains of 5 theta bursts, which has been previously shown to be the threshold for inducing stable potentiation in mice (Vogel-Ciernia et al., 2013), produced a robust and immediate potentiation in wildtype slices, that briefly decayed over a 10-15 min period to stabilize at approximately 70% above the pre-TBS baseline (Fig. 4e). Slices containing NAc core from BAF53b+/− heterozygous mice exhibited normal short-term potentiation that was no different from wildtype values but the level of LTP 60 min post-TBS was slightly attenuated relative to controls. In contrast, TBS delivered to BAF53bΔHD slices produced a profound deficit in LTP. Short-term potentiation was comparable to wildtype controls, but the level of potentiation 60 min post-TBS was markedly reduced compared to wildtype controls. Collectively, these findings suggest that the BAF53b complex plays a pivotal role in the cellular processes that maximize synaptic strength in the NAc. This result is particularly pronounced in the BAF53bΔHD slices and correlates with the greater cocaine-associated memory impairment observed in this transgenic mutant line (Fig. 3).
Figure 4. Mutation of BAF53b disrupts LTP in NAc core slices. (a) Photomicrograph of a parasagittal slice containing NAc and the relative position of the stimulation (red arrow) and recording (green arrow) electrodes (1.08 lateral to mid-sagittal plane). Abbrev: aca – anterior commissure, AcbC – accumbens nucleus, core, CPu – caudate putamen, Hipp. – hippocampus. (b) Representative trace of glutamatergic transmission collected within the NAc (black line) that is blocked by AMPA and NMDA receptor antagonists, 20 µM DNQX and 50 µM D-APV, respectively (red line). Scale = 0.2mV/10ms (c) Left, Input/Output curves measuring the slope of the fEPSP response across a range of stimulation currents (10-80 µA) was comparable between BAF53b+/− (dark blue circles, n=9), BAF53bΔHD (light blue circles, n=9) and wild type (WT) (brown circles, n=9) mice, (F$_{2,24}$ = 0.15, $p = 0.81$). Right, representative traces collected during the generation of input/output curves from WT, BAF53b+/−, and BAF53bΔHD slices (scale = 0.2 mV, 5 ms). (d) Paired-pulse facilitation of the initial slope of the synaptic response was comparable (40, 100, and 200 ms inter-pulse intervals) in slices from BAF53b+/− (blue circles, n=9), BAF53bΔHD (light blue circles, n=10), and WT (brown circles, n=8) mice (F$_{2,24}$ = 0.16, $p = 0.96$). (e) Left, LTP induced using theta burst stimulation (black arrows) produced stable potentiation in slices from WT mice (brown circles). Short-term potentiation measured 5 min post-TBS was no different between groups (F$_{2,25}$ = 6.22, $p=0.11$). While potentiation measured 50-60 min post-TBS was marginally reduced in slices from BAF53b+/− (blue circles, n=9), LTP in slices from BAF53bΔHD mice (light blue circles, n=10) was significantly impaired relative to control WT slices (brown circles, n=9) (F$_{2,25}$ = 31.79, $p=0.0013$). Right, fEPSP traces collected during baseline (grey line) and 1 hr after TBS (colored line). Scale: 0.2 mV/5ms.
Discussion

To further elucidate the underlying mechanisms that might account for the differences observed between the BAF53bΔHD and BAF53b+/− heterozygous knockout, we developed a theta stimulation protocol to induce long-term synaptic changes at glutamatergic synapses in NAc slices. This work was pioneered and performed by Dr. Enikő A. Kramár in our lab. Theta driven wave patterns are a natural occurrence in the rodent and occur during learning (Otto et al., 1991). We found that the level of potentiation in slices from BAF53bΔHD mice was significantly reduced, whereas BAF53b+/− heterozygous knockout showed a slight attenuation in the level of LTP with respect to the wildtype group. The deficits in BAF53bΔHD mice were confined to LTP maintenance, as we did not observe any differences in short-term potentiation (LTP-induction) or baseline synaptic response. These results parallel previous observations that these mice have normal short-term memory and behaviors outside of long-term memory (Vogel-Ciernia et al., 2013). These results support the interpretation that the deletion mutation of BAF53b (BAF53bΔHD) leads to a significant LTP impairment compared to the heterozygous knockout and this profile was consistent with the cocaine dose response differences observed between the two genetically modified mouse lines at 10 mg/kg in the CPP. These results are also a departure from studies exploring BAF53b’s role in synaptic plasticity in the hippocampus. Vogel-Ciernia et al. (2013) previously reported deficits in hippocampal theta-LTP in BAF53b+/− heterozygous knockout mice.
CHAPTER 5

Rescuing deficits in BAF53b mutant mice

Rationale

Having observed significant deficits in BAF53bΔHD mutant mice, we performed the following rescue experiments. In an effort to restore deficits in both cocaine memory and synaptic plasticity, we explored the role of brain-derived neurotrophic factor (BDNF) in the NAc. BDNF is a potent positive neuromodulator in the brain and has been reported to play an important role in influencing addictive-type behaviors (see reviews; Post and Kalivas, 2013; Russo et al., 2009). Therefore, we examined whether BDNF administration into NAc would rescue previously observed deficits in CPP. Additionally, given that BDNF is released from terminals following TBS (Balkowiec and Katz, 2002; Aicardi et al., 2004), and effectively facilitates LTP (Figurov et al., 1996; Kramar et al., 2004; Patterson et al., 1996), we tested the possibility that increasing exogenous levels of BDNF in the slice would reverse the impairment in LTP in BAF53bΔHD slices. Together, these experiments evaluate the role of BDNF in LTP in the NAc and BAF53b-mediated CPP.

Methods

Mice

For all experiments, mice of either sex were between 8 and 15 weeks old at the time of behavioral testing. Mice had free access to food and water and lights were maintained on a 12:12h light/dark cycle, with all behavioral testing performed during the light portion of the cycle. Mutant mice were bred to wildtype C57BL/6J from Jackson Laboratories. The
resultant mutant and wildtype littermates were then used for experiments. Mice were genotyped before experimentation by examining DNA from tail samples. Experimenters were blinded to genotype during behavioral and electrophysiological experiments. Additionally, mice were genotyped after testing using DNA from tail samples. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

**Slice Preparation and Recording**

Parasagittal slices containing the NAc core were prepared from BAF53b+/−, CamKIα-BAF53bHDLow, and Wildtype mice (approximately 2 months of age). Following isoflurane anesthesia, mice were decapitated and the brain was quickly removed and submerged in ice-cold, oxygenated dissection medium containing (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 2.5 CaCl2, 26 NaHCO3, and 10 glucose. The cerebellum and lateral aspects of both hemispheres were removed. Parasagittal slices (320 µm) were cut from the blocked brain using a FHC vibrating tissue slicer (Model:OTS-5000) before being transferred to an interface recording containing preheated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 1.5 MgSO4, 2.5 CaCl2, 26 NaHCO3, 10 glucose and 10 µM picrotoxin to reduce feedforward inhibition (56-58. Slices were continuously perfused with this solution at a rate of 1.0-1.5 ml/min while the surface of the slices were exposed to warm, humidified 95% O2 / 5% CO2 at 31 ± 1°C. Recordings began following at least 1.5 hr of incubation.
Stimulation of glutamatergic afferent fibers within the NAc was achieved by placing a bipolar stainless steel stimulation electrode (25um diameter, FHC) just below the anterior commissure. Activation of field excitatory postsynaptic potentials (fEPSP) were recorded using a glass pipette (2-3 MΩ) positioned caudal or caudal-ventral to the stimulation electrode (Fig 4a). Thus correct placement of electrodes within the NAc was confirmed by visual inspection of the slice and comparison with mouse brain atlas (Paxinos and Watson; 0.84-1.08 lateral to midline). Two parasagittal slices/hemisphere containing a large portion of the NAc core were obtained for each animal. Pulses were administered at 0.05 Hz using a current that elicited a 30-40% maximal response. Measurements of fEPSP slope (measured at 10–90% fall of the slope) were recorded during a minimum 20 min stable baseline period at which time long-term potentiation (LTP) was induced by delivering 3-5 trains (intertrain interval of 1 min), each train containing 5 ‘theta’ bursts, with each burst consisting of four pulses at 100 Hz and the bursts themselves separated by 200 msec (i.e., theta burst stimulation or TBS). The stimulation intensity was not increased during the delivery of TBS. Data were collected and digitized by NAC 2.0 Neurodata Acquisition System (Theta Burst Corp., Irvine, CA) and stored on a disk.

Data in the text are presented as means ± SD. Data in figures on LTP were normalized to the last 10 min of baseline. The LTP experiment, and conventional measures of baseline synaptic transmission including paired-pulse facilitation and input/output curves, was analyzed using a 2-way repeated measure ANOVA.
Reagents

The AMPA receptor antagonist, DNQX (Tocris), and human recombinant form of BDNF (Chemicon) were prepared fresh in aCSF, whereas the TrkB receptor antagonist, ANA12 (Tocris), stock was dissolved in DMSO and then diluted to working concentration in aCSF (DMSO < 0.01%). BDNF virus (AAV2-CAG2-mCherry-2A-mBDNF-WPRE; AAV-253926) and control virus (AAV2-CAG2-mCherry-WPRE) were generous donations from Dr. Mathew Blurton-Jones.

Conditioned Place Preference

Place conditioning was performed as described previously in our studies (Malvaez et al., 2011; Rogge et al., 2013). Briefly, the experiments were conducted in a three-compartment apparatus which had two larger compartments (12.5 X 17cm; checkered and white) on either side of a smaller compartment (12.5 X 11.5cm) separated by guillotine doors. The larger compartments had distinct visual, tactile and olfactory cues. One of the larger compartments had white walls with bar flooring and pine shavings underneath. The other large compartment had checkered walls with grid flooring and cedar shavings underneath. The middle smaller compartment had a solid floor, two gray walls as well as one checkered and one white wall leading to the respective larger compartments.

All mice were handled for 1 min each day for 3 consecutive days prior to the experiment (days 1-3). Baseline preferences were assessed by placing the animals in the center compartment of the three compartment place preference apparatus with the guillotine doors open, allowing free access to three distinct compartments for 15 min (day 4). Time spent in each compartment was recorded. Conditioning was conducted over the
subsequent 4 days with the guillotine doors closed, thus confining animals to a specific compartment for 30 min (days 5-8). An unbiased paradigm was used such that half of the animals were injected with cocaine-HCl (5 or 10 mg/kg, i.p.; Sigma) prior to placement in the checkered compartment, and half were injected with cocaine prior to placement in the white compartment (CS+). The next day, mice were injected with 0.9% saline (1.0 ml/kg, i.p.) prior to placement in the alternate compartment (CS−). Injections were alternated for subsequent conditioning sessions. Forty-eight hours after the last conditioning session, preference (15 min, Posttest 1; day 10) was assessed in all animals as described above in a drug-free state. CPP score was calculated as the time (s) spent in cocaine-paired (CS+) minus saline-paired (CS−) compartments. Time spent in each chamber of the conditioned place preference apparatus was tracked automatically from MPEG videos using EthoVision 3.1 software (Noldus Technology, Leesburg, VA; see Malvaez et al., 2011).

Results

It was critical to first establish whether LTP in the NAc was BDNF/TrkB activation-dependent. We used a novel TrkB receptor antagonist, ANA12 (Carzola et al., 2011), to test whether blocking TrkB receptor activation would inhibit theta-LTP. As summarized in Figure 5a, the compound had no measureable influence on baseline synaptic transmission elicited by stimulation of afferent glutamatergic fibers, but effectively blocked theta burst-induced LTP. Following a 60 min washout of the drug, a second set of theta stimulation produced stable LTP, suggesting that the compound is fully reversible. These data suggests that TrkB activation has a role in the consolidation of LTP in the NAc.
Figure 5. Infusions of BDNF reverse LTP deficit in BAF53bΔHD slices. (a) Bath application of 500 nM ANA12 blocked theta burst-induced LTP (TBS1) in NAc (n=6) in slices from WT mice. A 60 min period of ANA12 washout restored LTP after a second bout of theta stimulation (TBS2) in the same stimulated pathway. Inset, Representative traces collected during baseline (grey line), 60 min post-TBS1 (blue line), and 60 min post-TBS2 (dark blue line). Scale = 0.4 mV, 5 ms. (b) Infusion of BDNF (2 nM) began after a 15 min stable baseline recording. BDNF produced a rapid increase in glutamatergic transmission (~ 35%) within the NAc in slices from both WT (n=6) and BAF53bΔHD (n=5) mice. Prior to delivery of theta bursts, stimulation intensity was reduced to reset baseline to pre-drug levels to evaluate the effects of BDNF on the magnitude of LTP (dotted downward arrow). Five theta trains (5 theta bursts each, separated by 1 min) delivered 5 min after reestablishing stable baseline recordings (upward arrow) produced robust and stable LTP in WT slices, and completely reversed the LTP impairment previously seen in slices from BAF53bΔHD mice (see figure 4, panel E). Inset, Representative traces collected during baseline (grey line), 30 min after start of BDNF infusion (blue line), and 60 min post-TBS (dark blue line). Scale = 0.4 mV, 5 ms. (c) Pretreatment with 500 nM ANA12 completely blocked BDNF-induced increases in baseline synaptic transmission. The delivery of theta burst stimulation produced a brief short-term potentiation that rapidly returned toward baseline levels (n=6). Inset, Representative traces collected during baseline (grey line) and 60 min post-TBS (black line). Scale = 0.5 mV, 5 ms.
We then examined whether increasing exogenous levels of BDNF in the slice preparation would alter baseline synaptic transmission and reverse LTP impairment in BAF53bΔHD slices. Administration of recombinant BDNF generated a rapid increase in fEPSP slope in NAc slices from wildtype and BAF53bΔHD mice (Figure 5b). This effect was significantly above baseline within 30 min after the start of BDNF infusion. Stimulation intensity was then adjusted to pre-drug baseline levels and TBS was applied to determine the magnitude of LTP in the presence of BDNF for both BAF53bΔHD and wildtype controls. Both groups displayed robust potentiation that remained stable for the remainder of the recording period. In a follow-up experiment, we were interested in determining whether the BDNF-induced increase in synaptic transmission and LTP were dependent on TrkB receptor activation. Slices from wildtype mice were pretreated with the ANA12 compound 20 min prior to a 1 hr co-application of BDNF followed by TBS. As shown in Figure 5c, the TrkB receptor antagonist blocked BDNF-induced increases in baseline synaptic transmission and theta-LTP. Taken together, BDNF plays a critical role in the potentiation of glutamatergic synaptic transmission in the NAc and that TrkB activation is required for restored theta-LTP in BAF53bΔHD slices to levels observed in wildtype slices. These data indicate that exogenous application of BDNF is sufficient to overcome deficits in theta-LTP in BAF53bΔHD mice.

Given that exogenous recombinant human BDNF was sufficient to rescue theta-LTP deficits in BAF53bΔHD slices, we then examined whether viral delivery of human BDNF into the NAc would rescue the CPP deficits (Fig. 6a). We infused either AAV2-mCherry-BDNF or AAV2-mCherry (control) into the NAc of wildtype (WT+BDNF; WT+mCherry) and BAF53bΔHD (BAF53bΔHD+BDNF; BAF53bΔHD+mCherry) mice. After a two week recovery
period, we evaluated each group's capacity for cocaine-associated memory formation using CPP (Figure 6b). No group displayed a significant chamber preference on the pre-test. At post-test, both WT+BDNF and WT+mCherry groups displayed a robust CPP. The BAF53bΔHD+mCherry group failed to establish a CPP, as predicted. In contrast, the BAF53bΔHD+BDNF group displayed a preference for the cocaine-paired chamber comparable to the wildtype groups. These results suggest that viral delivery of human BDNF rescued the CPP impairments in this mutant line.

**Figure 6.** Viral overexpression of BDNF reverses CPP and LTP deficits in BAF53bΔHD mice. (a) Immunohistological image showing viral expression in the NAc using mCherry (red), BDNF (green) and DAPI (blue). Schematic representation of cocaine-CPP procedure. (b) Cocaine-CPP expression indicated by mean CPP score (CS+ vs. CS−) ± S.E.M. At 5mg/kg cocaine dose, WT+mCherry (n=15) and WT+BDNF (n=16) robust CPP. BAF53bΔHD+BDNF (n=12) established a CPP comparable to wildtype groups. BAF53bΔHD+mCherry (n=14) exhibited significantly attenuated CPP score compared to wild-type groups. A two-way repeated measures ANOVA revealed a significant main effect of group (F3,53=3.08, p=0.035) and conditioning (F3,53= 95.10, p<0.0001) There was a significant interaction (F3, 53=2.90, p=0.043). Bonferroni post hoc analysis showed no initial preference for either context on the pretest (t= 2.861, p<0.05). (c) Left, The initial expression of LTP was significantly greater in WT+BDNF slices (n=7) relative to WT+mCherry (t15=2.32, P=0.018) slices (n=10), as was the level of potentiation 60 min after theta burst stimulation (TBS) in the former group (t15=6.95, P<0.0001). In contrast, slices (n=9) from BAF53bΔHD+BDNF mice produced a similar level of short-term potentiation as found in slices (n=8) from BAF53bΔHD+mCherry mice (t15=1.14, P=0.13), however, viral expressing BDNF in BAF53bΔHD mice rescued the impairment in LTP found in slices from mice expressing the control mCherry-linked virus (t15=6.10, P<0.0001). There was no significant difference in the level of potentiation at 60 min post-TBS between WT+mCherry and BAF53bΔHD+BDNF slices (t17=, P=0.09). Right, representative traces collected during initial baseline recordings (grey line) and 60 min post-TBS (black line) for each group. Scale = 5 ms / 0.5 mV. (d) Left, Virally expressing BDNF caused a significant increase in paired-pulse facilitation (PPF) at the shortest interval tested (40 ms) in slices from both WT and BAF53bΔHD mice (F3,30=13.8, P=0.025). Right, Representative examples of PPF recorded at 40 msec interstimulus interval for each group tested. Traces of the first (solid black line) and second (dotted black line) fEPSPs are superimposed. Scale = 5 ms / 0.5 mV. (e) Input/Output curves measuring the slope of the fEPSP response across a range of stimulation currents (10-80 µA) was comparable among all groups (F3,30 = 0.25, P=0.87).
We later examined the effects of the BDNF virus on synaptic function in the NAc using a subset of mice from each group tested in CPP. We found that theta-induced potentiation in slices from control mice (WT+mCherry) was similar to that found in slices from wildtype mice without any virus (see Fig. 4, panel e), however, slices from BDNF expressing control mice (WT+BDNF) produced a dramatic increase in potentiation minutes following LTP induction as well as 60 min after TBS (Fig. 6c). We then examined slices from BAF53bΔHD+mCherry group, and as predicted, LTP was significantly reduced 60 min post-induction. In accordance with the behavioral results, LTP was rescued in slices taken from the BAF53bΔHD+BDNF group (Fig. 6c). We were also interested in determining whether chronic exposure to BDNF may have altered presynaptic release. As shown in figure 6d, both groups injected with the BDNF virus (WT+BDNF and BAF53bΔHD+BDNF) showed a significant increase in paired-pulse facilitation at the shortest interpulse interval tested as compared to their respective control vehicle groups (WT+mCherry and BAF53bΔHD+mCherry). Finally, we also measured any changes in neuronal excitability between groups by generating an input/output curve. We found no difference between groups across a range of stimulation intensities (Fig. 6e). The results of our electrophysiological and behavioral tests indicate that viral infusion of human BDNF is sufficient to rescue cocaine-associated memory and synaptic function in BAF53bΔHD mutant mice. Collectively, our results suggest that manipulation of BDNF/TrkB activation can overcome the cocaine-memory and plasticity deficits caused by deleting the hydrophobic domain of BAF53b.
Discussion

BDNF is a neuromodulator released from terminals following theta burst stimulation and supports LTP (Aicardi et al., 2004; Figurov et al., 1996; Kramar et al., 2004; Patterson et al., 1996; Tyler et al., 2002). In addition, BDNF/TrkB signaling has been implicated in drug-context memory and drug-associated behaviors in the NAc (Bahi et al., 2008; Crooks et al., 2010; Graham et al., 2007; Hall et al., 2003; Lobo et al., 2010). Therefore, even though BDNF/TrkB signaling has an established role in theta-LTP (in the hippocampus; Patterson et al., 1996; Korte et al., 1996; Korte et al., 1995) and drug-associated memories (in the NAc), there were open questions as to whether BDNF/TrkB receptor activation is 1) required for synaptic plasticity in the NAc and 2) capable of ameliorating LTP and CPP deficits induced by a BAF53b mutation. The current study determined that TrkB receptor activation is required for theta-LTP in the NAc using ANA12, a TrkB receptor antagonist (Carzola et al., 2011). We observed that infusions of ANA12 blocked theta-LTP in a reversible manner. Bath applied BDNF generated a rapid increase in synaptic transmission in slices from both wildtype and BAF53bΔHD mice. This BDNF-induced increase in synaptic transmission was blocked by ANA12 indicating that BDNF effects were being modulated through the TrkB receptor.

Upon examining the effects of bath-applied human BDNF on both WT and mutant slices we found that BDNF restored the ability of BAF53bΔHD slices to maintain a similar level of potentiation as found in WT slices. Given that wildtype slices did not display potentiation above that of BAF53bΔHD slices after bath BDNF application, we interpret this to mean that BDNF influence on the stabilization of LTP in the mutants likely involves events that are initiated after induction which is consistent with our observation that short-term
potentiation was unaffected in these mice. As described in an earlier report, BAF53b appears to be involved in activity-induced actin signaling in hippocampus CA1 pyramidal cells (Vogel-Ciernia et al., 2013). Additional morphological and cell signaling studies should reveal whether the same holds true for BAF53b in NAc neurons. Taken together, our results suggest that BDNF/TrkB receptor activation is integral to theta-LTP in the NAc and it is possible that there is a role for BAF53b in BDNF related signaling cascades. The interaction between BAF53b and BDNF/TrkB signaling needs to be further explored, however, this effort is currently hampered by the unavailability of a ChIP-grade BAF53b antibody.

The successful rescue of LTP in slices from mutant mice using a relatively acute application of BDNF human recombinant BDNF led us to explore a possible rescue of the CPP impairments in these mutant mice. In order to deliver a sustained amount of BDNF that could last over the course of a CPP experiment, we used an adeno-associated virus overexpressing human BDNF. Delivery of this virus into the striatum has previously been shown to ameliorate the cognitive deficits in a transgenic model of dementia while rescuing motor deficits (Goldberg et al., 2015). We pursued a rescue of the BAF53bΔHD mice, because they displayed the most severe LTP and CPP deficits. Our results showed that overexpressing BDNF in the NAc was sufficient to overcome CPP deficits in BAF53bΔHD mice. This result suggests that the deficits initially observed in BAF53bΔHD mice were not due to developmental confounds because the BAF53bΔHD mice with overexpressing BDNF were capable of establishing a CPP.

Consistent with their behavioral data, viral overexpression of BDNF in BAF53bΔHD mice was able to restore theta-LTP to potentiated levels found in slices from WT+mCherry mice. Notably, the level of potentiation was dramatically increased following TBS in slices
from WT+BDNF mice when compared to WT+mCherry mice. Furthermore, WT+BDNF groups slice displayed potentiation significantly above that of the BAF53bΔHD+BDNF group. This result was not predicted as we observed that potentiation was comparable in slices taken from wildtype and mutant mice after bath-application of BDNF. This discrepancy in the level of LTP is possibly due to the differential effects of acute (bath-applied) versus chronic (viral overexpressed) human BDNF exposure. It is possible that a brief application of BDNF in the NAc has limited utility in enhancing synaptic function, that is, once BDNF triggers a chemical form of LTP, the delivery of TBS saturates the synapse by increasing the level of potentiation to its maximum ceiling, but also engages downstream signaling mechanisms involved in consolidating LTP. In addition, viral overexpression of BDNF led to an enhancement of paired-pulse facilitation. This result suggests that BDNF in increasing presynaptic processes, possible increase vesicle binding. Paired-pulse facilitation was not examined in the acute BDNF experiment, it will therefore be important to compare the differences observed in a variety of measures depending on the method of BDNF administration. These results highlight the importance of evaluating the effect of BDNF/TrkB activation on LTP using a variety of experimental approaches. Collectively, the behavioral and electrophysiological results provide a platform for future studies to extend our knowledge on the underlying mechanism(s) affected by BAF53b mutations.
Chapter 6

Conclusion

Drug addiction is a disease that costs the United States approximately $700 billion dollars annually due to healthcare costs, crime and loss productivity (National Institute on Drug Abuse). One of the major obstacles to treating substance abuse is the persistent risk of relapse during drug abstinence, long after the cessation of withdrawal symptoms. One prevailing belief among drug addiction researchers is that persistent drug-associated memories partially underlies the sustained risk of relapse. Drug-associated memories are formed when the rewarding effects of addictive drugs are paired with a context. To those ends, drugs of abuse exploit the existing associative memory systems to facilitate the formation of drug-associated behaviors and memories. Perhaps understanding the mechanisms that underlie or contribute to persistent drug-associated memory and behavior will provide a viable avenue to combatting substance abuse.

Epigenetic mechanisms are involved in the acquisition and consolidation of long-term memory formation, including drug-associated memories. However, to date, research into the role of epigenetics in drug-associated memories and behaviors have focused on histone modifications and DNA methylation. Those studies have made inroads into understanding the mechanisms underlying changes in cellular function thought to underlie persistent drug-associated memory and behavior. But, they ignore one powerful epigenetic mechanism, nucleosome remodeling. My dissertation explored the role of one specific nucleosome remodeling complex on the formation of cocaine-associated memory and behavior as well as synaptic plasticity.
Recent studies have implicated the BAF nucleosome remodeling complex and its subunits in severe intellectual disability disorders and more recently, the neuron-specific and dedicated subunit of the nBAF complex, BAF53b, has been shown to be critical for long-lasting forms of synaptic plasticity and long-term memory. Considering the dynamic interplay between histone modification mechanisms, which have a key role in regulating gene expression induced by cocaine and other drugs of abuse, and nucleosome remodeling complexes, it was important to investigate the role of nucleosome remodeling complexes in cocaine-associated behaviors and memories. The studies presented in this dissertation extends the previous research by showing for the first time that 1) neuron-specific nucleosome remodeling is critical for cocaine-associated memory formation and synaptic plasticity 2) suggests studying BDNF/TrkB activation as a potential target to relieve BAF53b-associated deficits. Nucleosome remodeling complexes provide an additional layer of complexity to the epigenetic mechanisms that regulate gene expression induced by drugs of abuse, and potentially provide novel possibilities for therapeutic avenues.

The results presented in this dissertation represent a significant advance in the current knowledge of the epigenetic mechanisms involved in cocaine-associated memory formation. However, there are still outstanding questions. It is unclear whether BAF53b directly interacts with BDNF or TrkB signaling pathway to support LTP. Vogel-Ciernia et al., (2013) showed through RNA sequencing that BAF53b appears to target genes that play a role in synaptic structure. That result coupled with the failure of cultured cells, from BAF53b homozygous knockout mice, to show proper activity-dependent dendritic outgrowth suggest that BAF53b regulates gene expression required for synaptic structure and function (Wu et al., 2007). To date, however, neither RNA-sequencing nor RT-qPCR studies have linked
BDNF’s role in synaptic structure and function to any BAF53b manipulation. It is possible that the BDNF manipulations that rescue cocaine memory and synaptic plasticity are operating through a pathway that is independent of BAF53b’s influence. In order to examine whether BAF53b and BDNF operate together directly, indirectly or independently of each other, we would need to examine possible interactions between BAF53b and BDNF. This effort is currently hampered but the lack of a ChIP-grade BAF53b antibody.

The role of BAF53b in regulating chromatin structure remains unexplored. This research is not only lacking from this dissertation but the field of neuroscience. Utilizing chromosome confirmation capture (3C) and related techniques to analyze the spatial and structural organization of chromatin before and after nucleosome remodeling could yield valuable information about nBAF’s function. Additionally, it is important to correlate changes on chromatin structure with altered gene expression. Chromatin structure and spatial profiles may provide insight into the genes required for cocaine-associated memory formation as well as changes in neuronal function. The current theories of how nucleosome remodeling operates to support transcription suggest that gene loci that are necessary for memory formation will become unraveled or exposed after a learning event by nucleosome remodelers.

In regards to addiction and compulsive drug-seeking research, it will be important to examine the role of BAF53b in operant learning tasks that model compulsive drug-seeking behavior. A self-administration task would extend the research currently presented in my dissertation to reveal whether nucleosome remodeling complexes play a role in compulsive operant drug-seeking behavior. Together, the outstanding questions highlight the need for further exploration of this oft ignored epigenetic mechanism.
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