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Epigenetic Mechanisms in Mood Disorders: Targeting Neuroplasticity

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Abstract

Developing novel therapeutics and diagnostic tools based upon an understanding of neuroplasticity is critical in order to improve the treatment and ultimately the prevention of a broad range of nervous system disorders. In the case of mood disorders, such as major depressive disorder and bipolar disorder, where diagnoses are based solely on nosology rather than pathophysiology, there exists a clear unmet medical need to advance our understanding of the underlying molecular mechanisms and to develop fundamentally new mechanism experimental medicines with improved efficacy. In this context, recent preclinical molecular, cellular, and behavioral findings have begun to reveal the importance of epigenetic mechanisms that alter chromatin structure and dynamically regulate patterns of gene expression that may play a critical role in the pathophysiology of mood disorders. Here, we will review recent advances involving the use of animal models in combination with genetic and pharmacological probes to dissect the underlying molecular mechanisms and neurobiological consequence of targeting this chromatin-mediated neuroplasticity. We discuss evidence for the direct and indirect effects of mood stabilizers, antidepressants, and antipsychotics, among their many other effects, on chromatin-modifying enzymes and on the epigenetic state of defined genomic loci, in defined cell types and in specific regions of the brain. These data, as well as findings from patient-derived tissue, have also begun to reveal alterations of epigenetic mechanisms in the pathophysiology and treatment of mood disorders. We summarize growing evidence supporting the notion that selectively targeting chromatin-modifying complexes, including those containing histone deacetylases (HDACs), provides a means to reversibly alter the acetylation state of neuronal chromatin and beneficially impact neuronal activity-regulated gene transcription and mood-related behaviors. Looking beyond current knowledge, we discuss how high-resolution, whole-genome methodologies, such as RNA-sequencing (RNA-Seq) for transcriptome analysis and chromatin immunoprecipitation-sequencing (ChIP-Seq) for analyzing genome-wide occupancy of chromatin-associated factors, are

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beginning to provide an unprecedented view of both specific genomic loci as well as global properties of chromatin in the nervous system. These methodologies when applied to the characterization of model systems, including those of patient-derived induced pluripotent (iPS) cell and induced neurons (iNs), will greatly shape our understanding of epigenetic mechanisms and the impact of genetic variation on the regulatory regions of the human genome that can affect neuroplasticity. Finally, we point out critical unanswered questions and areas where additional data are needed in order to better understand the potential to target mechanisms of chromatin-mediated neuroplasticity for novel treatments of mood and other psychiatric disorders.

2.2.1 Critical need for mood disorder research and new mechanism therapeutics

Remarkable advances have been made in understanding the complexity of the nervous system and its capacity to exhibit plasticity in response to a variety of stimuli both on short and long-term time scales. Continued advancement of our knowledge of the molecular mechanisms of these adaptive processes of neuroplasticity and the development of novel therapeutics based upon these findings is critical in order to improve the treatment and prevention of nervous system disorders. The mood disorders, major depressive disorder (MDD) and bipolar disorder (BPD), are both prevalent and costly. Both disorders are characterized by episodes of depressed mood and diminished interest and hedonic capacity, referred to as major depressive episodes. In addition to mood symptoms, individuals commonly experience changes in neurovegetative functions including sleep and appetite. Depressive episodes are typically recurrent, and may be chronic as well (Perlis et al., 2006). In bipolar disorder, individuals also experience periods of unusually elevated or irritable mood, referred to as manic episodes; when such episodes do not significantly impact functioning, they are considered to be hypomanic episodes. This mood elevation is generally associated with decreased need for sleep, increased physical activity, impulsive as well as goal-directed activity, and pressured speech. Notably, despite its name, bipolar disorder is not truly 'bipolar' in nature: manic and depressive symptoms can commonly co-occur or fluctuate rapidly, a phenomenon known as a mixed state. In both disorders the affective state (mood) of an individual may be influenced by external stimuli but responses are diminished or exaggerated. During mood episodes, individuals with MDD or BPD often experience cognitive dysfunction, particularly individuals with BPD who may experience residual cognitive symptoms between episodes even in the absence of other prominent mood symptoms (Zarate et al., 2000, Baune et al., 2010). Other psychiatric disorders, including anxiety disorders and substance use disorders, commonly co-occur with mood disorders and contribute to their morbidity.

Despite intensive basic research and clinical studies, our understanding of the etiology and pathophysiology of mood disorders is severely limited (Manji and Duman, 2001, Quiroz and Manji, 2002, Krishnan and Nestler, 2008, Pittenger and Duman, 2008). Consequently, mood disorders remain one of the leading causes of disability worldwide, with the World Health Organization projecting that depressive disorders will be the leading cause of disease burden by 2030 (World Health Organization, 2004). While defined by an episodic course, MDD and BPD symptoms may be chronic, and while periods of recovery are common, rates of recurrence remain high even with appropriate treatment (Rush et al., 2008, Bowden et al., 2012). In fact, such treatments may themselves be associated with significant adverse effects and safety concerns. Consequences of these disorders include functional impairment and poorer general health outcomes; it has been estimated that the economic burden of these disorders in the United States alone stretches into tens of billions of dollars each year (Greenberg et al., 1993).

In the case of MDD, there exists substantial experimental and clinical evidence dating back 6 decades implicating alterations in serotonin (5-hydroxytryptamine) and catecholamines such as dopamine and norepinephrine in pathophysiology as well as treatment, giving rise to the “monoamine theory” of MDD. However, despite the wealth of data supporting the role of aberrant monoaminergic system in the pathophysiology of MDD, directly targeting these mechanisms of synaptic transmission is neither necessary nor sufficient for clinical efficacy, as shown by the effectiveness of electroconvulsive therapy (Dierckx et al., 2012), the effects of glutamatergic antidepressants such as ketamine (Zarate et al., 2010), and the repeated observation that more than a third of individuals with MDD do not achieve symptomatic remission with monoaminergic antidepressants (Rush et al., 2009).

In the case of BPD, which involves both manic and depressive episodes, longitudinal studies indicate that patients with BPD I and II are burdened by significant depressive symptoms for much of their course despite standard treatments (Judd et al., 2002, Judd et al., 2003). These symptoms contribute to the substantial morbidity and mortality observed in bipolar disorder, including persistent functional impairment (Tohen et al., 2000) as well as suicide (Osby et al., 2001). National and international treatment guidelines recognize the challenges in treating bipolar depression (Hirschfeld et al., 2002, Keck et al., 2004). Specifically, standard antidepressants have repeatedly failed to show benefit in randomized, controlled trials (Nemeroff et al., 2001, Sachs et al., 2007). Lithium, considered a gold standard treatment in preventing recurrence by *all* major guidelines, nonetheless does not consistently show superiority to placebo for treatment of depression. Lamotrigine failed to separate consistently from placebo in large trials (Calabrese et al., 2008). Finally, the majority of subjects in atypical antipsychotic studies still fail to achieve euthymia (Tohen et al., 2003, Thase et al., 2006, Vieta et al., 2007, Weisler et al., 2008), metabolic risks are substantial (Newcomer, 2005), and rates of discontinuation are high (Scherk et al., 2007).

The identification of additional safe and effective treatment options for mood disorders would have a major impact clinically given the prevalence of the disorders and the limitations of existing treatments (Murrough and Charney, 2012). However, rational drug development for mood disorders has been hindered by a limited understanding of disease pathophysiology, which greatly limits the available treatment targets (Manji and Duman, 2001, Quiroz and Manji, 2002, Berton and Nestler, 2006, Pittenger and Duman, 2008, Murrough and Charney, 2012). For example, the focus on monoaminergic models of depression has led to a proliferation of treatments with very modest differences in benefit and adverse effect profile (Husain et al., 2009). Two areas of particular need are treatments for the substantial proportion of individuals who don't benefit from standard interventions, and treatments with more rapid onset of antidepressant effect: standard treatments may require a delay of several weeks before significant antidepressant response.

The basis for the delayed onset of antidepressant interventions is unknown, but may suggest that their mechanism involves adaptive alteration of variety of signaling pathways, including neurotrophic factor pathways affecting cell survival, stem cell proliferation and differentiation, as well as epigenetic changes that lead to the alterations of gene expression. Given the capacity for epigenetic mechanisms that regulate chromatin structure and gene expression to *integrate, process, and homeostatically respond* to the diverse range of intracellular signaling pathways modulated by neural activity over a range of time scales, a compelling notion emerging from research on the role of chromatin in diverse aspects of nervous system function is that gaining a deep understanding of the underlying molecular mechanisms will provide insight that will allow the future development of novel methods for therapeutic intervention in mood disorders (Berton and Nestler, 2006, Tsankova et al., 2007, Haggarty and Tsai, 2011, Houston et al., 2012).

2.2.2 Epigenetic mechanisms and chromatin-mediated neuroplasticity

The fundamental unit of chromatin that mediates epigenetic regulation of gene expression in all eukaryotic cells is that of the nucleosome, which is composed of 146 bp of DNA wrapped around two copies each of the core histones H2A, H2B, H3, histone H4, along with one copy of the linker histone H1 (Figure 1). By packaging DNA and thereby controlling access of other factors to that DNA, epigenetic mechanisms provide an important level of regulation of the genome and gene expression throughout development and in post-mitotic cells including neurons. Multiple lines of investigation of the biochemistry of chromatin in the past few decades have shown that gene expression states are coordinately regulated through the dynamic, and highly regulated, interplay of two major classes of multisubunit complexes: i) histone-modifying complexes, which posttranslationally modify the N-terminal tails of histone proteins through acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, glycosylation, and ribosylation; and ii) ATP-dependent remodeling complexes, which alter the position of nucleosomes to either increase or decrease transcription through allow access of transcription factors and RNA polymerase complexes (Racki and Narlikar, 2008, Gardner et al., 2011). These complexes can simultaneously engage variously modified histone tails in individual nucleosomes either as distinct entities, or by forming complexes with multiple types of enzymatic activities (Ruthenburg et al., 2007, Gardner et al., 2011). In addition to the modification of the structure of histones and the position of nucleosomes along DNA, there is also growing recognition that the structure of DNA itself is modified through methylation and other modifications that are dynamically regulated in the nervous system (Guo et al., 2011, Mikaelsson and Miller, 2011). Collectively, there is a growing body of evidence suggesting these histone and DNA-based mechanisms of epigenetic regulation may play a fundamentally important role in the pathogenesis of stress-related disorders and mood disorders and the long-term response to clinically effective therapeutics (Berton and Nestler, 2006, Tsankova et al., 2007, Borrelli et al., 2008, Haggarty and Tsai, 2011, Houston et al., 2012, Sun et al., 2012).

Of the various histone modifications that have been discovered to date and characterized in the context of the nervous system, the reversible acetylation and deacetylation of the ϵ -amino group of lysine side chains within the N-terminal tails of histones has emerged as an important regulator of neuronal gene expression and brain plasticity (Figure 1). The multiple enzymes responsible for histone acetylation of histones are collectively known as histone acetyltransferases (HATs), which are classified into various families based upon their sequence similarity, including the cAMP response element binding protein (CREB)-binding protein (CBP)/p300 family that has been more extensively investigated in its role in activity-dependent regulation of gene expression and neuroplasticity (Berndsen and Denu, 2008, Selvi et al., 2010). Once recruited to a promoter region of a gene, HAT-mediated acetylation of histone tails HATs may alter transcription in two ways. First, histone acetylation may create a more permissive 'open' conformation of chromatin due to loss of the electrostatic attraction of positively charged amino groups on the lysine side chains of histones and the negatively charged phosphodiester backbone on DNA. Alternatively, histone acetylation may also lead to the recruitment of additional chromatin-modifying and chromatin-remodeling factors that contain acetyl-lysine binding domains, such as bromodomains (Filippakopoulos and Knapp, 2012). The exact composition of complexes recruited to gene promoters in response to histone acetylation in the different cell types of the brain remains poorly understood, but is an active area of investigation. The molecular counterpart of HATs is provided by histone deacetylases (HDACs) that remove the acetyl group from the ϵ -amino group of lysine side chains in the N-terminal tails of histones (and other non-histone substrates) (de Ruijter et al., 2003). In doing so, HDACs induce a closed, repressive state of chromatin and the further recruitment of other transcriptional co-repressors that interact with

the hypo-acetylated histone tails that may also become methylated in certain cases to create a repressive state (e.g. histone H3K9). A total of 17 genes in the human genome have been identified that encode proteins with HDAC activity, which have been grouped into three classes (HDAC I, II, and III) based upon DNA sequence, cofactor specificity, and inhibitor sensitivity (de Ruijter et al., 2003, Bradner et al., 2010). Class I, II, and IV HDACs (HDAC1-11), which have been the focus of the majority of studies on HDACs in the nervous system, are metalloenzymes that rely on an active site zinc ion as part of the catalytic cycle, whereas Class III HDACs are the NAD⁺-dependent family of sirtuins (SIRT1-7)

There is a burgeoning body of evidence for the association between changes in histone acetylation and neuronal gene expression regulation, brain plasticity, and behavioral changes. The initial evidence for this came from studies of the role of chromatin in learning and memory (Guan et al., 2002, Alarcon et al., 2004, Korzus et al., 2004, Levenson et al., 2004, Levenson and Sweatt, 2005, Wood et al., 2005, Vecsey et al., 2007, Day and Sweatt, 2011). Although a requirement for transcription for new memory formation had been known for many years (Appel, 1965, Nguyen et al., 1994), one of the first demonstrations of a specific role for histone acetylation in regulating synaptic plasticity came from seminal work by Kandel and colleagues using sensory neurons from the invertebrate *Aplysia* to demonstrate that the excitatory input of serotonin induces transcription by activating the transcription factor CREB (cAMP response element-binding protein, leading to recruitment of the HAT CBP, whereas an inhibitory neurotransmitter represses transcription by recruitment of the transcriptional co-repressor HDAC5 (Guan et al., 2002). The importance of CBP-mediated HAT activity in humans, is underscored by the fact that Rubinstein-Taybi syndrome, a genetic disorder characterized by cognitive deficits and skeletal abnormalities, is caused by mutations in the gene encoding CBP. As demonstrated by a series of studies (Alarcon et al., 2004) (Wood et al., 2005), heterozygous *Cbp* mutant mice show impairments in histone acetylation, synaptic plasticity, and the late phase of hippocampal long-term potentiation (LTP), which could be reversed by treatment with the non-selective HDAC inhibitor trichostatin A. Additional pioneering work in this area by Sweatt and colleagues demonstrated that the acetylation of histone H3 in area CA1 of the hippocampus was increased upon contextual fear conditioning in a manner dependent upon activation of the NMDA receptor and Erk1/2 kinase activity (Levenson et al., 2004). Furthermore, they showed that elevating levels of histone acetylation through the use of the non-selective HDAC inhibitor sodium butyrate enhanced the induction of hippocampal LTP at Schaffer-collateral synapses in area CA1, and that administration of the non-selective HDAC inhibitor sodium butyrate prior to contextual fear conditioning enhanced hippocampus-dependent memory (Levenson et al., 2004). Building on these studies using HDAC inhibitors to pharmacologically modulate memory, the enhancement of hippocampus-dependent memory and hippocampal synaptic plasticity by HDAC inhibitors is mediated by CREB and recruitment of CBP (Vecsey et al., 2007).

Complementing these studies on the facilitatory role of HAT activity in synaptic plasticity and memory formation, and inspired by the remarkable ability of sodium butyrate to provide access to memories even in the face of neurodegeneration (Fischer et al., 2007), studies that have used gain-of-function and loss-of-function mouse models have identified a key role of Hdac2, but not Hdac1, as a suppressor of chromatin-mediated neuroplasticity (Guan et al., 2009). As part of these studies, it was demonstrated that Hdac2 overexpression causes a loss of synapse number and decreased memory formation, whereas conversely Hdac2 deficiency caused an increase in synapse number and enhanced memory formation using a fear conditioning paradigm (Guan et al., 2009). Consistent with these findings, Hdac2 was shown to bind to the promoters of multiple genes critical to synaptic plasticity and genes known to be regulated in an activity-dependent manner (Guan et al., 2009, Graff et al.,

2012). Additionally, these studies demonstrated that the detrimental effects of Hdac2 overexpression could be reversed by treatment with SAHA, whereas in the *Hdac2* knockout mice, SAHA was unable to enhance memory further (Guan et al., 2009). Taken together, these findings suggest that Hdac2 is a key target of non-selective HDAC inhibitors that enhance memory and that selective HDAC2 inhibitors may enhance synaptogenesis and memory formation. In further support of this notion, virus-mediated delivery of a short-hairpin-RNA (shRNA) that selectively targets *Hdac2* into the hippocampus of adult mice was sufficient to restore synaptic plasticity deficits, enhance synaptogenesis, and ameliorate behavioral deficits in a mouse model of neurodegeneration (Graff et al., 2012). Along similar lines, but focusing on a different class I HDAC family member and different behavioral paradigms, a role for Hdac3 as a memory suppressor has also been demonstrated (McQuown et al., 2011). These studies took advantage of virus-mediated delivery of Cre recombinase to causes focal deletions of *Hdac3* in subregions of the hippocampus due to the conditional nature of the transgenic line. Taken together with the findings on Hdac2, these data provide clear evidence for a role for HDAC-containing co-repressor complexes in the epigenetic regulation of synaptic plasticity and memory formation.

2.2.3 Existing psychiatric drugs affecting mood have direct effects on epigenetic mechanisms

At least two clinically-applied classes of neuropsychiatric medications have direct effects on enzymes involved in epigenetic regulation, though whether this effects is necessary for their therapeutic effects has not been established. First, the anticonvulsant and antimanic/mood stabilizing valproic acid inhibits Class I HDACs but not Class II-IV HDACs (Phiel et al., 2001). Second, the antidepressant, and monoamine oxidase inhibitor, tranylcypromine, which also inhibits the amine oxidase activity of the histone lysine demethylase (KDM) lysine-specific demethylase 1 (LSD1) (Lee et al., 2006). Most recently, the tricyclic antidepressants (TCA) amitriptyline and imipramine, along with the selective serotonin reuptake inhibitor (SSRI) paroxetine, were shown to inhibit the activity of DNA methyltransferase Dnmt1 in primary rat cortical astrocytes (Zimmermann et al., 2012). Instead, of a direct effect on the methyltransferase catalytic activity or expression levels of Dnmt1, the authors identified euchromatic histone-lysine N-methyltransferase 2 (EHMT2), a positive regulator of Dnmt1 activity, to be decreased at the level of protein expression (Zimmermann et al., 2012). Although the precise mechanism through which this downregulation of Dnmt1 and Ehmt2 occurs remains elusive, nonetheless, given the demonstration that multiple structurally distinct antidepressants from both the TCA and SSRI classes similarly impact a major regulator of the initiation and maintenance of DNA methylation marks and histone methylation, may have important therapeutic implications. Determining whether changes in specific histone acetylation, histone methylation, and DNA methylation sites known to be regulated by Class I HDACs, LSD1/EHMT2, and DNMT1, respectively, occur in post-mortem brains of mood disorder subjects, would be of great interest for future studies.

As described in more detail in section 2.2.6 below, since the initial discoveries of the epigenetic effects of valproic acid and tranylcypromine, efforts have begun to determine whether more selective HDAC and KDM inhibitors have effects on mood in animal models. Examples of these efforts include the observations that the selective Class I HDAC inhibitor, MS-275, which matches the pharmacological profile of valproic acid in terms of HDAC selectivity but is 500-1000 times more potent (Bradner et al., 2010, Fass et al., 2010), has antidepressant-like effects in the mouse social defeat stress model (Covington et al., 2009), and the hydroxamic acid HDAC inhibitor SAHA, which is even more potent than MS-275 but targets additional HDAC isoforms (Bradner et al., 2010), reverses several depression-

like behaviors in stressed mice (Uchida et al., 2011). While valproic acid attenuates mania-like behavior in the rodent amphetamine-induced hyperlocomotion assay (Kim et al., 2008), effects of more selective HDAC inhibitors in this assay have not yet been reported. Lastly, progress has been made in developing selective LSD1 inhibitors (Neelamegam et al., 2012), which do not inhibit monoamine oxidases, such that future experiments may reveal the on-target effects of these inhibitors in rodent behavioral model.

2.2.4 Epigenetic alterations as biological markers of mood disorder pathophysiology and treatment

A growing body of work investigating blood-derived lymphocytes and post-mortem brain tissue from mood disorder patients have begun to reveal alterations in chromatin modification profiles that are distinctly different from healthy control subjects, suggesting a potential role for epigenetic mechanisms in the pathophysiology and/or treatment of these disorders. In one study, Gavin and colleagues (Gavin et al., 2009) reported that acetylation of histone H3 was altered in BPD patients' lymphocytes. Moreover, valproic acid therapy in BPD patients results in an increase in H3 acetylation (Sharma et al., 2006). Also, methylation of lysine 9 on histone H3 is reduced in postmortem brain samples from human depressed patients (Covington et al., 2011). Finally, the expression of several chromatin-modifying enzymes (HDACs or KDMs) is altered in MDD and BD in peripheral blood lymphocytes or post-mortem brain tissue (Iga et al., 2007, Hobara et al., 2010, Abe et al., 2011, Covington et al., 2011). Although many of these findings await replication in larger cohorts and a more comprehensive investigation of potential confounding effects and role of genetic variation, taken together these data suggest that alterations in epigenetic marks and chromatin modifying enzyme expression levels may provide promising biomarkers of mood disorders.

Following the observation of differential epigenetic state of chromatin in mood disorder subjects, profiling of the mRNA levels of 11 HDACs (HDAC1-11) in peripheral white blood cells of MDD and BPD subjects during depressive and periods of symptomatic remission has revealed that *HDAC2* and *HDAC5* mRNA levels are decreased in a depressed but not remitted state of MDD (Hobara et al., 2010). In contrast, in BPD subjects *HDAC6* and *HDAC8* mRNAs levels were decreased in both the depressed and remitted state, and *HDAC4* mRNA levels were increased only in the depressive state (Hobara et al., 2010). Interestingly, no significant changes in *HDAC6* or *HDAC8* mRNA levels were observed in the first-degree unaffected relatives of BPD subjects. Since there was no correlation between mRNA expression of any of the HDACs and type of medication (tricyclics, SSRI, SNRI, sulpiride, lithium, valproate, and carbamazepine), it was concluded that the alteration of HDAC mRNA expression was unlikely to be due to effects of medication alone. To test this further, analysis of leukocytes from mice treated with behaviorally effective doses of antidepressants and mood stabilizers did not reveal any changes in the expression of the HDACs whose expression changed in the MDD or BPD subjects (Hobara et al., 2010). Beyond zinc-dependent HDACs, similar studies performed on the mRNA expression levels of *SIRT1-7* in peripheral white blood cells of patients with MDD or BPD during depressive and remissive states compared to normal healthy subjects has revealed the state-dependent, selective decrease of *SIRT1*, *SIRT2* and *SIRT6* mRNA levels in both MDD and BPD patients only during a depressive episode (Abe et al., 2011). Taken together, these results provide evidence of both state-dependent regulation of expression of HDACs (*HDAC2*, *HDAC5*, *SIRT1*, *SIRT2*, *SIRT6* in MDD; *HDAC4*, *SIRT1*, *SIRT2*, *SIRT6* in BPD) and trait-dependent regulation of expression of HDACs (*HDAC6* and *HDAC8* in BPD) suggesting that dysregulated expression of HDACs may be an importance factor in mood disorder pathophysiology.

An important next step is simply replication of the findings of differential regulation of HDACs in mood disorder subjects in additional larger cohorts in a manner that takes into consideration possible confounding genetic and medication factors. In addition, it would be helpful if future studies can determine whether the altered mRNA expression levels observed in these studies cross over to the level of altered protein expression levels and if this impacts the chromatin state and expression level of specific genes. It would also be important to understand the mechanism through which such changes in HDAC mRNA expression are occurring be it epigenetic itself or due to post-transcriptional mechanisms affecting mRNA processing or stability. Furthermore, in the case of BPD additional studies of individuals the manic phase of illness would be revealing. Such knowledge would help interpret existing and new whole-genome transcriptome profiling data from mood disorder patients and may help focus efforts towards specific HDACs for investigating in animal models and with specific pharmacological probes.

2.2.5 Role of epigenetic mechanisms in animal models of stress and depression

The idea that adverse environmental influences – stress - can result in meaningful, lasting changes in an organism's behavioral response is a central theme underlying basic survival as well as higher-order brain functions such as learning and memory. Epigenetic mechanisms provide insight about how external stress can influence changes in DNA methylation and posttranslational modification of histone proteins resulting in altered gene expression and ultimately, impact behavioral response. In a groundbreaking study demonstrating the epigenetic impact of early life stress, the Meaney laboratory showed for the first time that maternal behavior produces stable alterations of DNA methylation and histone acetylation in offspring (Weaver et al., 2004). Comparing offspring of rat dams that exhibited natural differences in pup licking and grooming behavior, the study found that deficient maternal care resulted in elevated levels of transcriptionally repressive DNA methylation at a glucocorticoid receptor (GR) promoter exon, evident one week after birth. These changes could be reversed by cross-fostering pups to dams exhibiting enhanced maternal care. Resulting patterns of DNA methylation were still present 90 days later when the pups reach adulthood where histone H3 acetylation and presence of a GR-transcription factor were concomitantly reduced – along with hippocampal GR protein levels - in rats raised in conditions with deficient maternal care. Further, when these now-adult rats were exposed to acute restraint stress, elevated corticosterone levels indicated that deficient maternal care resulted in hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, an effect not seen in rats raised under or cross-fostered, to conditions of enhanced maternal care. Proposing that increases in promoter-region histone acetylation may increase deficient GR expression, the authors further showed that central infusion of the Class I/II HDAC inhibitor, trichostatin A, elevated hippocampal GR protein levels and normalized the corticosterone response to acute restraint stress. Overall, this work demonstrated the impact that acute and chronic stress can have on epigenetic mechanisms that can yield gene expression differences that affect the neural circuitry underlying stress-induced neurochemical and behavioral changes. As discussed more below in section 2.2.6, these early efforts also provided an initial hint of the promise of using chromatin-modifying agents– particularly HDAC inhibitors– to reverse pathological changes that may underlie diverse stress-associated psychiatric diseases.

Another early example in which an external stress was linked to epigenetic changes came from the first study of chromatin following electroconvulsive seizures (ECS) in rats (Tsankova et al., 2004). Here, the idea that effective treatment of depression may involve changes in chromatin was supported by the novel result that both acute and repeated ECS treatment induced transient increases in hippocampal histone acetylation and

phosphoacetylation. Such short-lasting increases in open-chromatin-associated histone modification were coupled, using chromatin immunoprecipitation, to the promoter regions of plasticity-associated transcripts including a variant of brain derived neurotrophic factor (*Bdnf*) whose expression was increased by ECS (Tsankova et al., 2004). Chronic ECS also revealed more robust decreases in promoter region histone acetylation compared to acute treatment, indicating a shift in predominant chromatin modification over the duration of stress exposure (Tsankova et al., 2004).

Additional studies have also begun to further examine the role and specificity of different types of stressor on histone modifications, chromatin state, and gene transcription (Hunter et al., 2009). Utilizing a paradigm of restraint stress in rats, acute restraint resulted in decreased monomethylation and increased trimethylation of hippocampal histone H3 lysine (K) 9 (H3K9me3) compared to unstressed animals. H3K9me3 is associated with heterochromatin and, thus, is consistent with the observed absence of H3K4me3, which is associated with active transcription. That the levels of hippocampal histone H3K27me3 were decreased after acute stress suggests that cellular resources to restore homeostasis likely involve maintaining transcriptional activity, at least at some level. These patterns changed after seven or twenty one days of restraint stress and appear to involve a relative increase in open chromatin histone marks over time. Specifically, although H3K4me3 and H3K27me3 were decreased after seven days restraint stress, H3K9me3 was increased. Further, chronic restraint stress revealed subtly increased hippocampal H3K4me3 and reduced H3K9me3 levels. These findings highlight the dynamic response of chromatin to stress and demonstrate important differences that emerge over time that can provide insight into the histone modifying enzymes regulating acute or prolonged stress responses.

Exposure to the acute stress of forced swimming is another well-established rodent behavioral test with predictive validity for currently used antidepressant drugs. Importantly, exposure to two days of forced swimming results in the acquisition of behavioral immobility in the test and has been shown to result in transient increases in the active chromatin marks, histone H3 phosphorylation and phosphoacetylation as well as immediate early gene expression in the dentate gyrus (Chandramohan et al., 2008). These behavioral and epigenetic effects were disrupted by blockade of the neural signaling via N-methyl-D-aspartate (NMDA) receptors and the extracellular signal-regulated kinases (Erk) 1/2 signaling pathways and were similarly prevented by genetic ablation of the mitogen and stress-activated kinases 1 and 2 (*Msk1/2*), which are kinases predominantly responsible for phosphorylation of histone H3, that are downstream of the NMDA receptor and Erk1/2 (Chandramohan et al., 2008).

Another important development in identifying the epigenetic mechanisms resulting from chronic stress is the rodent model of chronic social defeat stress. In this model, subordinate behavior is induced in a test mouse by daily, ten minute physical exposure to an aggressive male. Following each session of social defeat exposure, the test mouse is housed with, but physically separated from, the aggressive male. This paradigm is continued with a novel aggressive male each day for ten days and results in marked depressive-like behaviors in a subset of test mice including reduced social interaction and decreased sucrose consumption (a putative model of anhedonia). This model has reasonable face validity for inducing lasting depressive-like behaviors in rodents and is unique from other depression-related rodent behavioral test in that, similar to the lag in efficacy associated with clinical antidepressant treatment, chronic, rather than acute administration of antidepressant drugs is required to alleviate social-defeat induced behavioral deficits.

Importantly, work from the Nestler laboratory has demonstrated that chronic treatment with the antidepressant imipramine reverses social-defeat associated decreases in hippocampal

Bdnf mRNA levels (Tsankova et al., 2006). Changes in promoter region chromatin revealed that repressive histone methylation (H3K27me2) was evident in defeated mice, independent of treatment, one month after social defeat. This indicates the lasting presence of a repressive histone mark resulting from long-term stress exposure. However, that the monoaminergic antidepressant imipramine restored *Bdnf* transcript levels was most likely the result of observed increases in H3K9,14 diacetylation and H3K4 methylation; two marks associated with active chromatin. A parallel and unique effect in defeated mice treated with imipramine was decreased expression of the Class II histone deacetylase, Hdac5 (Tsankova et al., 2006). Consistent with this result, pro-depressant effects and reduced histone H3 acetylation were observed in mice with hippocampal overexpression of Hdac5 (Tsankova et al., 2006). Further, Hdac5 overexpression prevented the antidepressant-like effects of imipramine in socially-defeated mice (Tsankova et al., 2006). A follow-up study using chromatin immunoprecipitation and promoter microarray chip (ChIP-chip) revealed that social defeat resulted in increased promoter region histone methylation (H3K9me2 and H3K27me2) on number of genes in the nucleus accumbens and that chromatin changes were similar between mice treated with imipramine compared to those animals resilient to the social defeat model (Wilkinson et al., 2009). Along similar lines, rats with a natural propensity to explore novel environment were recently shown to have higher hippocampal histone H2B and H3K14 acetylation compared to low-exploring counterparts (Hollis et al., 2011). Following exposure to social defeat, these high-responding rats had decreased histone acetylation, whereas low-responding rats revealed increased acetylation of H3K14, although in this study no differences were found for the expression of *Hdac3*, *Hdac4* or *Hdac5* in the hippocampus. Taken together, these reports highlight the potential utility of describing dysregulated histone modification in differentially responding subpopulations of mice and provide further support that aberrant histone acetylation can shed light on the mechanism of stress-induced behavioral deficiencies.

One of the most important directions for future studies of the role of epigenetic mechanisms in regulating stress responses and resiliency is to identify specific genes or transcriptional programs affected by stressful events that in turn can be targeted pharmacologically and exhibit antidepressant-like activity. While our understanding of this repertoire of genes involved in mood regulation that is under epigenetic regulatory control remains far from complete, Uchida and colleagues have recently described a prototypical example of such a gene that governs the behavioral response to chronic stress (Uchida et al., 2011). In this work, the DNA methylation level and histone acetylation level of the promoter region of the glial cell-derived neurotrophic factor (*Gdnf*) gene in the striatum was shown to be correlated with the response of stress-resilient C57BL/6 mice compared to stress-vulnerable BALB mice using chronic ultra-mild stress (CUMS) exposure. The authors were able to specifically implicate Hdac2 as the key driver of these epigenetic changes involved in *Gdnf* expression as while CUMS was found to increase the binding of MeCP2 to the *Gdnf* promoter in both strains, the binding and formation of an Hdac2-MeCP2 complex uniquely occurred on the *Gdnf* promoter in stress-vulnerable BALB mice (Uchida et al., 2011). Implicating a causal role for Hdac2 in these behavioral effects, viral-mediated delivery of a constitutively active form of Hdac2 that could not be subject to inhibitory S-nitrosylation into the striatum of normally stress-resilient C57BL/6 mice enhanced the detrimental effects of stress as measured in the social interaction test (Uchida et al., 2011). Conversely, a catalytically inactive Hdac2 mutant (H141A) that was interpreted as a dominant-negative form of Hdac2 caused mice to show increased social interaction and increased sucrose preference tests indicative of an antidepressant-like effect (Uchida et al., 2011). Paralleling these genetic manipulations, the systemic administration of the non-selective HDAC inhibitor SAHA for the last 5 days of each 6-week CUMS sessions and during behavioral testing had antidepressant-like activity in the social interaction, sucrose preference, FST, and novelty-suppressed feeding tests (Uchida et al., 2011). Interestingly, administration of standard

antidepressant such as the TCA imipramine and SSRI fluoxetine under similar conditions were ineffective, providing evidence of a more robust and faster acting antidepressant-like effect of HDAC inhibition.

In addition to a role for Hdac2 in the regulation of *Gdnf* mRNA expression and susceptibility to stress, consistent with their evidence for alteration of DNA methylation in the *Gdnf* promoter, the authors in this study also demonstrated, quite remarkably, that the delivery of zebularine, a nucleoside-based DNA methyltransferase (DNMT) inhibitor, into the striatum of stress-vulnerable BALB mice attenuated the detrimental effects of stress in multiple behavioral paradigms, including social interaction, sucrose preference, novelty suppressed feeding, and FST with similar results obtained with RG108, a nonnucleoside-based DNMT inhibitor that does not require integration into DNA to affect DNA methylation (Uchida et al., 2011). Taken together, these studies point again to an important role for Hdac2-mediated chromatin modification acting in concert with DNMTs to regulate the expression of key genes for determining susceptibility and resistance to stressful events.

As part of studies aim to understand the role that specific genes or transcriptional programs have in determining mood behavior and response to antidepressants, another important direction for future studies is to identify the specific DNA binding elements that confer specificity to the chromatin-modifying and remodeling machinery. One example of these efforts has been the identification by Trono and colleagues of KAP1, an essential cofactor of Kruppel-associated box zinc finger proteins (KRAB-ZFPs) that bind to DNA at defined loci (Jakobsson et al., 2008). Deletion of KAP1 in the adult forebrain of mice caused elevated anxiety-like behavior and stress-induced deficits in spatial learning and memory, which were associated with the dysregulation of a small subset of genes (Jakobsson et al., 2008). Given the association of KAP1 with chromatin-modifying complexes, this provides an example of how deficits in the recruitment of epigenetic machinery to chromosomal loci can impact resiliency and susceptibility to stress leading to behavioral deficits that manifest at the level of cognitive deficits as can be seen in patients with mood disorders. Understanding the role of similar co-repressor and DNA binding components in the context of other behavioral models of stress-induced epigenetic regulation and pathophysiology will further shed light on possible targets for therapeutic intervention.

Besides nuclear localized HDACs and transcription factors that function to regulate transcription, a key role for the predominantly cytoplasmic Class IIb HDAC6 has emerged in the regulation of affective behaviors (Fukada et al., 2012). While Hdac6-deficient mice exhibited normal basal locomotor activity in their home cage, normal circadian rhythms, and normal phenotypes in a battery of neurophysiological functions, exposure of these mice to a novel environment led to hyperactivity (Fukada et al., 2012). Hdac6 expression was shown to predominantly localize to the dorsal and median raphe nuclei, which are key areas of serotonergic neuron activity involved in controlling emotional behaviors (Fukada et al., 2012). Most exciting from a therapeutic perspective, administration of an Hdac6-specific inhibitor, NCT-14b, had antidepressant-like behavioral effects in the TST in mice in a manner that was correlated with changes in cytoplasmic tubulin acetylation and could be phenocopied by the complete knockout of *Hdac6* (Fukada et al., 2012). In addition to these studies, studies from Berton and colleagues have provided additional genetic and pharmacological data further outlining a key role of Hdac6 in regulating stress response and resilience (Espallergues et al., 2012). Here, the investigators have shown the Hdac6 deacetylates the chaperone protein Hsp90 that is known to play a key role in GR function. Consistent with a functional relevance of this acetylation of Hsp90 and disrupted interaction with the GR, knockout of Hdac6 selectively within serotonergic cells attenuated the anxiogenic effects of corticosterone administration as assessed in the open-field, elevated plus maze and social interactions tests, as well as prevented social avoidance in mice subject to

chronic social defeat (Espallergues et al., 2012). Taken together, these findings with Hdac6 provide an important example of an effect of HDAC family members on stress response and depression-like behavior through mechanisms that at least in part are mediated wholly, or at least in part, through the regulation of the acetylation of non-histone substrates. Given the abundance of nonhistone substrates in the acetylome (Choudhary et al., 2009), the observation of effects of genetically or pharmacologically altering HDACs, and likely also other enzymes with chromatin-modifying capacity, such as EZH2 which regulates actin cytoskeleton dynamics (Su et al., 2005), should not immediately be ascribed to effects on epigenetic mechanisms. In other words, they demonstrate the importance of HDACs but not necessarily histone *per se* in at least some models of antidepressant-like effect.

In summary, the findings illustrated in Figure 1 demonstrate the growing understanding of the widespread impact of chromatin-modifying complexes, particularly those associated with HDAC activity, on stress-related behavioral responses and pathophysiology. In total, these findings indicate that the relationship of stress to epigenetic changes and transcriptional regulation is highly regulated and that changes are nuanced depending on the type and duration of stress as well as cell type. Future studies in this area should seek to define the precise role and temporal dependency of different chromatin-modifying complexes in mediating these effects. Additionally, it will be important to elucidate the nature of the transcriptome response to these changes in histone modifications and whether changes in gene expression occur coordinately or divergently in different cell types within the underlying neurocircuitry that are affected. Fortunately, as described below in section 2.2.6 these findings also suggest new avenues for therapeutics intervention as treatment with chromatin-modifying drugs can alleviate stress-induced behavioral deficits. Thus, it is likely that continuing to understand the role of HDACs, KDMs and histone-targeting kinases will lead to the development of improved clinical treatments that can be used as monotherapies or in combination with existing therapeutics.

2.2.6 Antidepressant-like effect of prototypical HDAC inhibitors

Largely driven by interests in targets outside of the nervous system, there has been significant interest and recent progress in developing pharmacological probes of HDACs with four major classes of HDAC inhibitors either currently in clinical trials or already approved by the F.D.A.: i) carboxylic acids (e.g., sodium butyrate, valproate), ii) hydroxamic acids (e.g., trichostatin A and suberoylanilide hydroxamic acid (SAHA); iii) *o*-aminoanilides (e.g., MS-275); and iv) depsipeptides (e.g. Romidepsin/FK228)(Haggarty and Tsai, 2011). Most HDAC inhibitors function through chelating the active site zinc ion, and on the basis of the structures of these inhibitors, a general model for HDAC inhibitors has been put forth consisting of “cap-linker-chelators” moieties (Bieliauskas and Pflum, 2008). Differences in HDAC isoform inhibitor selectivity, particularly between Class I and Class II HDAC isoforms, have been partially obtained by varying the chelator moiety as well as capping elements that extend outside of the enzyme active site, although truly isoform-selective inhibitors that can be used to reliably probe the function of HDACs *in vivo* in the nervous system remains an area of intense activity with numerous inherent challenges (e.g. (Beconi et al., 2012)). Despite the current limitations in the available pharmacological tool kit, given the recognition of the importance of epigenetic mechanisms in the response to stress and in depressive-like behaviors (Tsankova et al., 2007, Covington et al., 2010, Sun et al., 2012), there has been significant efforts put forth toward the use of these prototypical pharmacological probes to target HDAC-mediated regulation of epigenetic mechanisms (Figure 2).

Paralleling similar studies investigating the memory enhancing effects of HDAC inhibitors, sodium butyrate treatment was one of the first compounds reported to improve defeat-

induced social interaction deficits in mice — an antidepressant-like effect (Tsankova et al., 2006). Similar results have also been shown in normal mice when sodium butyrate was administered in combination with fluoxetine in the tail suspension test (TST) (Schroeder et al., 2007). Importantly, the antidepressant-like effects of chronic sodium butyrate treatment were likely influenced by stress-effects arising from a battery of behavioral tests, as discussed (Schroeder et al., 2007). While one recent study indicates that sodium butyrate, at a dose sufficient to induce histone acetylation in mouse brain, did not alter FST behavioral response after a 21 day treatment paradigm (Gundersen and Blendy, 2009), another recapitulates antidepressant-like effects chronic butyrate treatment in the FST and TST and further shows transcriptional regulation of genes, including serotonin 2A receptor (*Htr2a*) downregulation (Yamawaki et al., 2012). Despite the utility of sodium butyrate as a tool compound, the extremely high dose of compound (~0.1- 1 g/kg) needed and potential for off-target effects highlight the importance of the goal of identifying and characterizing the behavioral impact of HDAC inhibitors with improved potency, brain penetrance and HDAC subtype selectivity.

Progress toward this direction has come from Covington and colleagues who have showed that extended brain infusion of the potent HDAC inhibitor, SAHA, or the Class I HDAC selective inhibitor MS-275, resulted in significant antidepressant like effects in socially-defeated mice in the FST (Covington et al., 2009). Similar antidepressant-like effects were observed via increased social interaction score (Covington et al., 2009) and provides strong evidence that further resolving the HDAC subtypes responsible for regulating depressive-like behaviors and effective treatment response is an important aim for the development of improved therapeutics. Importantly, the demonstration for the first time that both the hydroxamic acid class of Class I/IIa HDAC inhibitors represented by SAHA, and the ortho-aminoanilide type of selective Class I HDAC inhibitors both exhibited antidepressant-like activity provides, along with the carboxylic acids (e.g. butyrate), and the previously mentioned thiolate-based HDAC6 inhibitor NCT-14b, along with DNMT inhibitors, provide a number of distinct chemical scaffolds for pursuing optimized pharmacological agents with antidepressant-like activity (Figure 2).

While additional work is needed to refine the mechanism of HDAC inhibitors as mood-stabilizing drugs, these initial studies provide a promising view that novel leads for the development of novel therapeutics may exist in highly selective HDAC inhibitors. It is interesting to note that the original work from the Nestler laboratory demonstrating a role of HDACs in social defeat model of depression was using *Hdac5* and the work from the Berton laboratory was on *Hdac6*— both of which are Class II HDACs that has been shown consistently by multiple groups to not be inhibited by butyrate, valproate, or MS-275 (Bradner et al., 2010, Fass et al., 2010), pointing to a clear role for multiple different types of HDAC containing complexes in regulating emotional behavior. With respect to the function of Class IIa HDACs (composed of HDAC5/7/9), recent studies have shown that their deacetylase activity toward histone substrates is mediated through a Class I HDAC3, which interacts with Class IIa HDACs via the nuclear corepressor NCoR, and that Class IIa HDACs may naturally target substrates other than histones despite their original namesake (Fischle et al., 2002, Verdin et al., 2003). Consistent with this notion, purified Class IIa HDACs lack the ability to deacetylate the same synthetic histone-based synthetic peptide substrates as Class I HDACs and are refractory to carboxylic acids (e.g. sodium butyrate), ortho-aminoanilides (e.g. MS-275), and most hydroxamate-containing HDAC inhibitors (e.g. SAHA), but instead can deacetylate trifluoroacetyl-modified substrates (Bradner et al., 2010). Thus, genetic alterations involving the deletion or overexpression of these Class IIa HDACs may not directly validate the deacetylase activity of these HDACs per se, but rather their co-repressor and scaffolding functions. Further resolving these issues in the context of animal behavioral models will require the development of pharmacological probes that can

selectively target the deacetylase activity or specific protein-protein interactions of Class IIa HDACs.

Along the same lines of needing improved pharmacological probes to better understand the role of specific HDACs in the regulation of chromatin-mediated neuroplasticity, given that MS-275 still targets three of the major Class I HDAC isoforms (HDAC1/2/3), but not HDAC8 or the Class II HDAC isoforms, it remains to be demonstrated if inhibition of a single HDAC isoform, or multiple HDAC isoforms, mediates the antidepressant-like properties of these pharmacological agents. Furthermore, since each Class I HDAC isoform is known to be part of multisubunit, chromatin-modifying complexes (e.g. mSIN3A, NURD, LSD1/CoREST), it remains an open, but critical, question from the perspective of selectivity and minimizing undesired effects, of whether one more specific chromatin-modifying complexes are more critically involved in regulating mood-related neuroplasticity. The identification of such a multisubunit complex would guide future efforts to develop more selective inhibitors. Finally, carrying this one step further, it would be important to understand if, and how, the function of such 'mood-relevant' chromatin machinery is regulated both spatially in different cell types in the nervous system that comprise different aspects of the neurocircuits that mediate affective behaviors, and temporally in response to stress and other experiences that impact emotional responses and mood. Lastly, it might be expected that certain types of genetic variation affecting susceptibility to mood disorders will be found to affect the regulatory regions of the genome that are under epigenetic control of mood-relevant chromatin machinery—a notion that can be tested experimentally through mapping of the chromosomal loci to which the complexes that regulate chromatin-mediated neuroplasticity bind using

2.2.7 Role of epigenetic mechanisms in mouse models of mania and psychotic behavior

Beyond stress and depression models, emerging reports have begun to reveal that HDAC inhibitor treatment may function more broadly as a mood-stabilizing drug with effects on behaviors resembling mania and psychosis. For example, psychostimulant-induced hyperactivity, has been shown to be attenuated by treatment with valproate and sodium butyrate (Arent et al., 2011) (Kim et al., 2008), although to date there have not been reports of testing more selective and potent HDAC inhibitors in this model. In the case of antipsychotic drug treatments and treatment with the mood stabilizer valproic acid, a number of studies have shown that the response of cortical neurons leads to alteration of epigenetic mechanisms and gene expression (Tremolizzo et al., 2002, Costa et al., 2003, Dong et al., 2005, Tremolizzo et al., 2005, Costa et al., 2006). Emerging from these studies is the notion that chromatin within particular cell types and promoters may be more responsive to epigenetic modulation than others, with strong evidence for the effects of multiple drugs on the epigenetic state and expression levels of genes involved in inhibitory GABAergic neuron function (Tremolizzo et al., 2005, Ruzicka et al., 2007, Kundakovic et al., 2009, Guidotti et al., 2011).

Extending these studies on the importance of epigenetic mechanisms to the behavioral effects of antipsychotics and additional neurotransmitter systems, González-Maeso and colleagues have recently demonstrated that chronic administration of atypical antipsychotic drugs, such as clozapine, causes an upregulation of the expression of *Hdac2* in the frontal cortex of mouse in a manner dependent upon the serotonin receptor 2A (Htr2a) activity, with similar results observed in post-mortem tissues from schizophrenics treated chronically with antipsychotics (Kurita et al., 2012). Correlated with this upregulation of *Hdac2* upon chronic clozapine treatment, the investigators observed a downregulation of the mRNA expression and function of the metabotropic glutamate receptor 2 (mGlu2). This loss of mGlu2 receptor

activity was correlated with the increased occupancy of the *mGlu2* promoter by Hdac2 and loss of histone H3 and histone H4 acetylation along with an increase in H3K27me3 without an effect on either histone H3K4 methylation or DNA methylation. In heterologous cells, the investigators demonstrate that Hdac2 is a critical regulator of transcription from the *mGlu2* promoter suggesting a direct causal link. They also demonstrate that chronic atypical antipsychotic drugs alter epigenetic mechanisms leading to the upregulation of *Hdac2* promoter activity in a manner dependent upon Htr2a receptors, which are also known to be antagonized by atypical antipsychotics including clozapine.

Most remarkably, in this same study, viral-mediated HDAC2, but not HDAC1 or HDAC4, overexpression in the frontal cortex diminished the ability of an mGlu2/3 agonist to attenuate behavioral responses in mice induced by the psychotomimetic, NMDA receptor antagonist MK801 in mice including alterations in locomotion, pre-pulse inhibition (PPI), and working memory in a T-maze (Kurita et al., 2012). Consistent with these findings, and complementary in the directionality of the behavioral effects, stereotactic injection of either MS-275 or SAHA into the frontal cortex led to an upregulation of *mGlu2* mRNA expression. While single agent dosing was ineffective, the combination of SAHA and an mGlu2/3 agonist attenuated MK801-dependent locomotor response. On its own, chronic SAHA treatment also prevented the PPI deficits induced by MK801 and diminished head-twitch response induced by the hallucinogenic drug DOI similar to that of the effects of chronic clozapine.

Taken together, the results summarized above provide compelling evidence of a causal role for HDAC-mediated epigenetic regulation in the neurochemical mechanisms through which atypical antipsychotic drugs are efficacious at least in rodent behavioral models resembling schizophrenia, and by inference also likely in humans. Through the link to post-mortem brain studies in humans, they also draw attention to the important role that compensatory epigenetic events at promoters of genes, such as *mGlu2* (Kurita et al., 2012), may play in the response to psychoactive drugs particularly over a chronic versus acute time course. Given the often poor response rate to many clinically used mood stabilizers and antipsychotics, understudying the long-term impact of these treatments on epigenetic mechanisms may lead to a better understanding of the underlying changes in neuroplasticity that are necessary and sufficient for onset of clinical benefit and factors that otherwise prevent or sensitive patients to such responses (Guidotti et al., 2011).

2.2.8 Mutations in the histone acetyltransferases *Clock* as a mouse model of bipolar disorder

While HDACs have seen significant attention due to the availability of a growing number of small-molecule probes and genetic models, other chromatin-modifying enzymes are emerging as potentially critical regulators of mood-relevant neurocircuitry and physiology (Borrelli et al., 2008). Most notably, a dominant-negative mutation in *CLOCK* (Doi et al., 2006), a histone acetyltransferase involved in generating circadian rhythms, causes mania-like behavior in mice (Roybal et al., 2007). *CLOCK* interacts physically or functionally with HDACs (Nakahata et al., 2008), histone methyltransferases (Etchegaray et al., 2006) (Katada and Sassone-Corsi, 2010), and histone demethylases (DiTacchio et al., 2011), strongly implicating a role for epigenetic regulation in circadian rhythms and mood. Dysregulated circadian rhythms (e.g. in sleep, energy, appetite, etc) have long been associated with BD. Indeed, impairments in the molecular mechanisms controlling circadian rhythms may in fact be part of the pathophysiology of BPD (Milhiet et al., 2011).

CLOCK is a DNA-binding transcription factor that takes part in one of the core transcription-translation-feedback mechanisms that generate circadian rhythms in mammals

(Brown et al., 2012). In this oscillatory mechanism, a heterodimer of CLOCK and BMAL1 activate transcription of the genes PER1-3 and CRY1,2; these in turn inhibit CLOCK-BMAL1 to repress their own transcription. CLOCK has intrinsic histone acetyltransferase activity that is essential for circadian rhythmicity (Doi et al., 2006). CLOCK also acetylates BMAL1, which has the effect of repressing CLOCK-BMAL1-mediated transcription (Hirayama et al., 2007). The HDAC SIRT1 regulates circadian rhythms, likely in part by interacting with CLOCK-BMAL1 and deacetylating BMAL1 (Nakahata et al., 2008). Also, class I HDACs in the SIN3A HDAC complex interact with PER-associated factors to repress CLOCK-BMAL1-mediated transcription (Duong et al., 2011). In addition, the histone methyltransferase MLL1 interacts with CLOCK-BMAL1, and this interaction is required for circadian transcriptional regulation of CLOCK-controlled genes (Katada and Sassone-Corsi, 2010). Another histone methyltransferase, EZH2, interacts with CLOCK-BMAL1, and is required for CRY-mediated repression of CLOCK-BMAL1-dependent transcription (Etchegaray et al., 2006). Lastly, the histone demethylase JARID1A regulates circadian transcription in a demethylase-independent mechanism involving interaction with CLOCK-BMAL1, and inhibition of HDAC1 repression of CLOCK-BMAL1-mediated transcription (DiTacchio et al., 2011). Thus, multiple epigenetic regulatory mechanisms critically modulate the circadian rhythmicity of CLOCK-BMAL1-mediated transcription.

Mice expressing a deletion mutant of CLOCK lacking exon 19 (CLOCK Δ 19) (King et al., 1997) exhibit multiple behavioral abnormalities with similarity to human mania, including decreased sleep, hyperactivity, lowered anxiety, and reduced depressive-like behavior (Roybal et al., 2007). CLOCK Δ 19 fails to activate transcription, despite being able to heterodimerize with BMAL1 and bind to DNA (Gekakis et al., 1998). However, CLOCK Δ 19 can not interact with MLL1, suggesting a key role for histone methylation in CLOCK-mediated transcriptional activation (Katada and Sassone-Corsi, 2010). CLOCK Δ 19 mutant mice have also proved to be a useful model for testing pharmacological means of alleviating dysregulated circadian rhythmicity-induced manic-like behavioral abnormalities. Indeed, chronic treatment with the mood stabilizer lithium restores near-normal responses in tests of anxiety and depressive-like behavior (Roybal et al., 2007). In addition, an inhibitor of the lithium target GSK3 β reduces novelty-induced hyperactivity in CLOCK Δ 19 mice (Kozikowski et al., 2011). Lastly, an inhibitor of the kinase CK1 also reverses some of the behavioral abnormalities in CLOCK Δ 19 mice (Arey and McClung, 2012).

In future studies, it will be important to test whether small molecule epigenetic modulators, such as the mood stabilizer and HDAC inhibitor valproic acid, can reverse behavioral abnormalities in CLOCK Δ 19 mice. Indeed, valproic acid does regulate circadian rhythmicity in mouse embryonic fibroblast reporter gene cells (Johansson et al., 2011), suggesting that it might alter the circadian rhythm deregulation in CLOCK Δ 19 mice that underlies their manic-like behavior. In addition, given the defect in MLL1 recruitment by CLOCK Δ 19, logical future drug candidates for testing in these mice would include inhibitors of histone demethylases. Such inhibitors might increase the level of histone methylation in the vicinity of CLOCK-activated gene promoters, thus overcoming the deficit caused by loss of MLL1 recruitment.

2.2.9 Future Directions & Open Questions

Although the data summarized above is provocative in providing evidence for both alteration of the expression in mood disorder subjects of key chromatin-modifying enzymes and in alteration of epigenetic, several critical unanswered questions remain about epigenetic alterations as biological markers of mood disorders pathophysiology. First, do the specific epigenetic alterations in mood disorder patients' blood cells represent pathological states, or beneficial compensatory changes in response to the underlying pathology and/or

medication? Second, if altered epigenetic markers in mood disorders represent pathological changes, are they caused by underlying genetic liability (e.g. (Gertz et al., 2011)) or environmental disease triggers, such as stress? Third, do epigenetic mechanisms interact with genetic variation to exacerbate, or dampen, risk for mood disorders (e.g. (Gamazon et al., 2012))? Finally, while these initial observations of epigenetic biomarkers in mood disorders, generally measured in whole-tissue or whole-cell lysates, are extremely intriguing, the opportunity now exists to greatly extend this analysis to specific genomic loci, using high resolution, whole-genome methodologies in human tissues or cells, such as ChIP-Seq (Uchida et al., 2011, Houston et al., 2012). It is quite possible that altered chromatin states occur in mood disorders at specific loci regulating the expression of genes that have functional roles in the pathophysiology of these disorders. In particular, an analysis of the epigenetic status of chromatin in the vicinity of risk genes implicated in genome-wide association studies in mood disorders (Sklar et al., 2011), would potentially be extremely informative. Answering these questions and improving our understanding of the role of epigenetics in mood disorders will require focus on experimental and translational studies aiming to link together the emerging understanding of the molecular and cellular mechanisms of epigenetic regulations both in model systems and ultimately in humans.

Besides these powerful new technologies of ChIP-Seq and RNA-Seq that will allow deep interrogation of the transcriptome in rodents and humans at an unprecedented level of resolution, another exciting new technological advance that is beginning to be brought to bear to attempt to address questions on the role of specific molecular and cellular mechanisms of epigenetic regulation in the nervous system is that provided by the ability to use reprogramming technologies to derive human induced pluripotent stem cells (iPSCs) or induced neurons (iNs) (Grskovic et al., 2011, Okita and Yamanaka, 2011, Yang et al., 2011). While in the context of neuropsychiatric disorders efforts to date have focused largely on Mendelian disorders with a single gene with a large effect size, such as Rett syndrome due to mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene (Cheung et al., 2012, Farra et al., 2012), and Fragile $\times$ syndrome (Sheridan et al., 2011), nascent efforts are underway to create populations of iPSC models from patients with bipolar disorder, major depressive disorder and schizophrenia (Brennand and Gage, 2011, Pedrosa et al., 2011). The ability to differentiate these patient-specific, stem cell models into defined neuronal lineages *en masse* on a scale compatible with the use of ChIP-Seq and RNA-Seq will enable testing whether observations made about epigenetic dysregulation in peripheral blood-derived cells and post-mortem tissues can be replicated *in vitro* with potentially more physiologically relevant neuronal cells (Lin et al., 2011, Houston et al., 2012). Future efforts using human iPSC-derived neurons will also be aimed towards probing the underlying molecular mechanisms of chromatin-mediated neuroplasticity and to screen for experimental therapeutics targeting these mechanisms. The fact that such studies can be performed in live human neurons capable of forming neural networks *in vitro* that can respond to electrophysiological and pharmacological stimuli provides new avenues for investigating activity-dependent regulation of the human neuronal transcriptome and much promise for helping elucidate fundamental aspects of the epigenetic regulation of human neurobiology. Additionally, given the plethora of human genetic studies of disorders such as bipolar disorder, major depression, and schizophrenia that have identified genetic variation in non-coding regions of the genome, these studies should also provide a new avenue for investigation of the impact of patient-specific genetic variation on the epigenetic regulation of neuronal gene expression in response to diverse pharmacological and other perturbations.

While the field of neuroepigenetics has proceeded using a rather limited number of small-molecule probes, efforts are underway to more systematically develop small molecules that can selectively and conditionally target epigenetic mechanisms in the context of the nervous system. For example, Marcaurelle and colleagues have described efforts to synthesize a

large collection of novel small-molecule probes using diversity-oriented synthesis that led to the discovery of a novel class of macrocyclic HDAC inhibitors, which display mixed enzyme inhibition kinetics, that were capable of increasing histone acetylation levels in cultured mouse neurons (Marcaurelle et al., 2010). Using a high-throughput, microfluidics-based, synapse microarray technology, Shi and colleagues recently described the identification of novel inducers of synaptogenesis, such as the potent HDAC inhibitor named 'synapsinostat' (Shi et al., 2011). Additionally, aiming to selectively target epigenetic mechanisms involved in CREB-mediated transcription, Fass and colleagues reported on the results of a high-throughput screen that led to the identification of another highly potent, and brain penetrant HDAC inhibitor named 'crebinostat' that regulated CREB-mediated transcription, enhanced synaptogenesis, and enhanced cognition in mice as assessed using contextual fear conditioning (Fass et al., 2013).

Outside of the area of HDAC inhibitors, one of the greatest opportunities and challenges for the field of neuroepigenetics moving forward will be to determine the merits of systematically developing selective, brain-penetrant, small-molecule probes that can be used to selectively and conditionally target other key enzymatic activities and protein-protein interactions integral to chromatin-modifying and remodeling complexes. An area of particular interest is the development of small-molecule probes that could allow more precise control of epigenetic mechanisms than currently feasible. For example, one application could be cell-type control of being able to affect chromatin-modification in defined neuronal cell types (e.g. excitatory glutamatergic neurons versus inhibitory GABAergic neurons). To date, only a few selected examples are known of cell-type specific components of chromatin-remodeling complexes. One example is the chromodomain, helicase DNA-binding protein 5 (CHD5), which is a neuron-specific subunit of the nucleosome remodeling and deacetylation (NuRD) complex. Thus, selectively targeting CHD5 may provide a mechanism for altering the function of specific NuRD complexes in the nervous system. Another opportunity for specificity comes through the potential existence of neuron specific expression of alternative splice forms of genes. For example, four alternative splice forms of LSD1 generated from a combination of inclusion or exclusion of two alternatively spliced exons, E2a and E8a, the latter of which encodes a 4 amino acid insert that is exclusively expressed in the nervous system were recently reported (Zibetti et al., 2010). Through an integrative approach of targeted proteomic analysis of purified chromatin-modifying complexes from the nervous system in combination with analysis of recently completed deep analysis of the human and mouse transcriptome (Higuchi et al., 2011), such efforts are likely to reveal numerous opportunities for affording cell-type specific control of epigenetic mechanisms. Extending this line of investigation for other possible sources of cell-type specific epigenetic regulation, recent studies on the existence of non-coding RNAs (ncRNA) associated with chromatin-modifying complexes (Rinn and Chang, 2012), such as such as the ncRNA HOTAIR that functions as a scaffold for both the polycomb repressive complex 2 (PRC2) and LSD1/CoREST/REST complex (Tsai et al., 2010), and the discovery of the overall abundance of transcription from non-coding regions of the genome (Derrien et al., 2012), suggests that cell-type specific components should not be limited to proteins but also include these molecules. Understanding the role of such cranes may also hold the key to being able to selectively target chromatin-modifying complexes in a locus specific manner in the nervous system since in principle the interaction of such cranes and protein components could be targeted with small molecules or other selective nucleic acid based probes.

Besides cell type-specific control of epigenetic mechanisms, another type of conditional control of chromatin-modifying complexes that would be particularly useful to have is that of precise temporal control ranging from rapid to prolonged time scales. While small molecules with different pharmacokinetic profiles in brain (e.g. long half-lives versus short

half-lives) that provide varying levels of exposure provide an example of such temporal control, it also would be highly desirable to identify probes whose properties could be selectively activated or inhibited much the same way that, for example, optogenetic methods provide control of neuronal firing (Fenno et al., 2011). Such developments will require creative engineering of proteins as has been done for GPCRs (Dong et al., 2010), and, or alternatively, advances in synthetic chemistry and screening to yield new generations of small-molecule probes.

Summary

The overall studies summarized here underscore a number of major topics in the emerging field of neuroepigenetics that will continue to be the focus of research in the area of mood disorders: namely: i) the impact of acute and chronic stress on the activity of chromatin-modifying enzymes; ii) the gene expression differences and neural circuitry underlying stress-induced neurochemical and behavioral changes; and iii) the promise of chromatin-modifying drugs—particularly HDAC inhibitors—in reversing changes that may underlie diverse stress-induced psychiatric diseases and in the regulation of mania and psychosis. Overall, through integrated efforts using animal models and the development of novel small-molecule probes that can then be used for conditional and combinatorial manipulation of neuroplasticity at the level of individual neurons and with intact brain circuits, it is promised to shed new light on the molecular mechanisms of neuroplasticity and will help advance our understanding if specific epigenetic mechanisms can be selectively targeted for the treatment or prevention of mood disorders. If dysregulation of epigenetic mechanisms are truly causally involved in the pathophysiology of mood disorders, one can speculate, and work towards testing experimentally, on the hypothesis that the benefits of therapeutic agents that more directly modulate epigenetic mechanisms (as opposed to indirect modulation by receptor-mediated signaling) could have a faster onset of clinical benefit, a more persistent effect, and the ability to treat otherwise treatment-resistant forms of mood disorders.

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Highlights

- Molecular mechanisms of mood disorders involving altered neuroplasticity.
- Epigenetic mechanisms play a critical role in the regulation of neuroplasticity.
- Histone-modifying enzymes reversibly alter neuronal gene transcription.
- Epigenetic mechanisms may provide new treatment targets for mood disorders.

Figure 1. Convergence of pathways involved in the epigenetic regulation of learning and memory with stress responses that may mediate the long-term pathophysiological effects of stress in chromatin-mediated neuroplasticity

Stimuli (I) such as glutamate and glucocorticoids released as response of the physiological response to stress and other experiences lead to the activation of both synaptic receptors (II) and intracellular signaling pathways (III) that include the MEK-ERK-MSK cascade involved in phosphorylation of histone H3, ERK-mediated activation of the histone acetyltransferase CBP as part of the chromatin-modifying complexes (IV) that lead to recruitment of additional chromatin-remodeling complexes (v) that alter chromatin structure (V) and lead in turn to altered gene expression states, as well as activation of nuclear hormone receptors, such as the glucocorticoid receptor (GR). Besides HATs and phosphorylation, the structure of chromatin is altered through deacetylation by HDACs, methylation by lysine methyltransferase (KMTs), demethylation by lysine demethylases (KDMs), which depending on the amino acid side chain and context can lead to activation or repression of transcription due to the alteration of charge of the histone tail but also interactions with various protein domains that bind to modified histone residues. Reversible DNA methylation by DNMTs further interacts with chromatin-modification complexes and transcription factors. HDAC6, a cytoplasmic deacetylase that deacetylates the chaperone Hsp90 facilitates the ligand binding, nuclear translocation, and transcriptional activation of GR. Both the inhibition of Class I HDACs (HDAC1/2/3) and the Class IIb HDAC6 with prototypical HDAC inhibitors, such as MS-275 and NCT-14b, respectively, and have been shown to have antidepressant-like behavioral effects providing evidence that these enzymes normally function as ‘mood suppressors’ that govern susceptibility and resilience to stress induced pathophysiology.

Figure 2. Prototypical small-molecule probes targeting epigenetic mechanisms with effects on moodrelated behavior

Lysine deacetylase inhibitors affect nuclear and non-nuclear members of the zinc-dependent family of histone deacetylases (HDACs). DNMT inhibitors affect DNA methylation through integration into DNA (zebularine) or direct inhibition of DNMTs (RG108).