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Proteomic Investigation of Epigenetics in Neuropsychiatric Disorders: A Missing Link between Genetics and Behavior?

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Neuropsychiatric disorders affect a large segment of the human population and result in large costs to society. The majority of such disorders have unknown underlying causes. Recent evidence suggests an important role for epigenetic regulation in the emergence of neuropsychiatric disease. Epigenetics may provide a link between genetic and environmental factors and behavior. Epigenetic signaling involves changes on the structure of chromatin; such changes are often triggered and maintained by the post-translational modification of chromatin proteins and/or DNA. Recent proteomic technologies have enabled the study of epigenetic mechanisms in a high-throughput manner. This review will provide an overview of the major epigenetic pathways and modern techniques for their study, before focusing on experimental evidence supporting a strong role for epigenetics in selected psychiatric disorders such as depression, schizophrenia, and drug addiction. These results highlight a great need for the inclusion of the proteomic characterization of epigenetic mechanisms in the study of gene/disease associations in psychiatric disorders.

Keywords: epigenetics • chromatin • histone modification • DNA methylation • neuropsychiatric disorders

Introduction

Neuropsychiatric disorders generally have unknown underlying causes. Several hypotheses seeking to explain the neurobiology of psychiatric disease have been postulated: alterations in monominergic neurotransmission, neurogenesis, and glial development and transport are only a few of the proposed mechanisms of disease.[1] Changes in gene expression have been proposed as one unifying molecular mechanism that could provoke and maintain the stable brain modifications and changes in behavior correlated with psychiatric ailments. However, virtually all studied transcription factors revert to normal very quickly after chronic perturbation.[2] Recent evidence points to a role for epigenetic regulation in the emergence of neuropsychiatric disorders; epigenetics may represent a mechanism behind the occurrence of disease, serving as a largely uncharacterized link between genetic and environmental factors.[1,3] Furthermore, epigenetic mechanisms are accessible targets for pharmaceutical treatments and thus they can open the door to new, alternative strategies in the treatment of psychiatric disease.[4] Our knowledge of the dynamic interplay between genetic and environmental factors in the occurrence of neuropsychiatric disease—via epigenetic signaling—can be significantly enhanced by exploiting available proteomic technologies (Figure 1). This review will provide an overview of two major epigenetic mechanisms (histone modification and DNA methylation) and the current proteomic approaches for their study, before focusing in findings supporting a strong role for epigenetics on selected psychiatric disorders such as depression, schizophrenia and drug addiction. The experimental results reviewed make a strong argument for the inclusion of epigenetic markers in the study of gene/disease associations in psychiatric disorders.

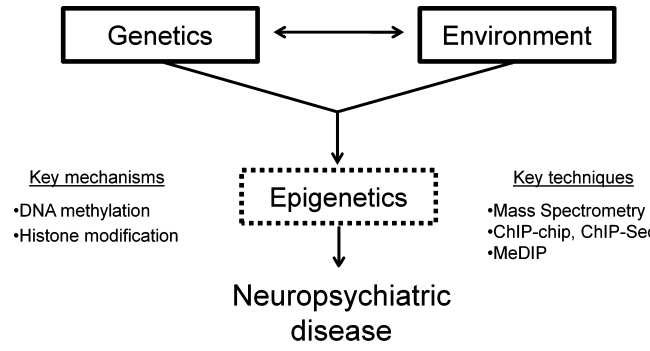

Figure 1. Interplay between genetics and environment by way of epigenetics gives rise to neuropsychiatric disorders.

Short Introduction into Chromatin, Epigenetics and Its Study

Eukaryotic DNA is packaged into chromatin, a highly organized protein−DNA complex. Once thought to be a mere DNA compaction scheme, the structure of chromatin has now been found to regulate gene accessibility and transcription. Disruption of chromatin architecture is intimately associated with

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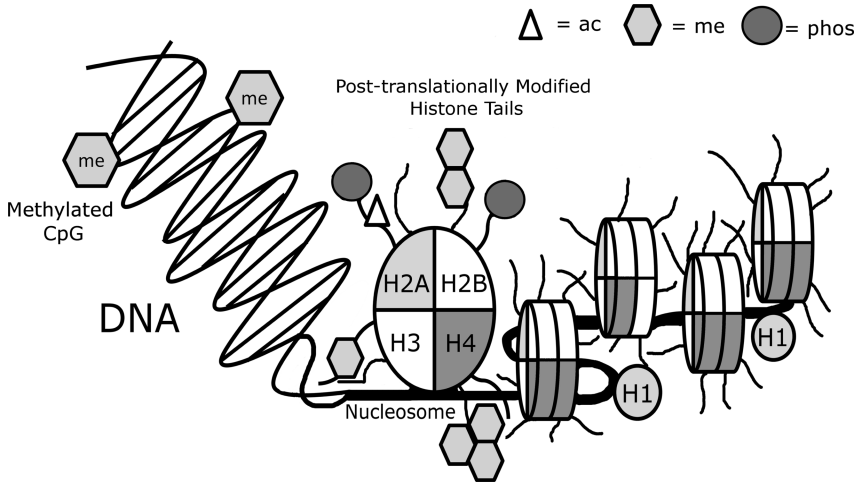

Figure 2. Simplified view of key epigenetic mechanisms. Changes in chromatin structure are triggered and maintained through the methylation of DNA and the post-translational modification of histone proteins. White triangles represent acetylation, while dark gray stars and light gray hexagons represent phosphorylation and methylation, respectively. Multiple hexagons reflect the occurrence of histone di- and trimethylation.

various human diseases, such as cancer,[5] and several neurological syndromes including α-thalassemia/mental retardation, Rett, Coffin-Lowry, and Rubinstein-Taybi syndromes.[6,7] Chromatin domains differing in structure and transcriptional activity are formed and maintained by the interaction and post-translational modification of chromatin proteins,[8] which can alter gene expression in both dynamic and stable fashions.[9,10] Actively transcribed chromatin domains are termed euchromatin, while transcriptionally inert genes constitute heterochromatin.[11,12] Euchromatin is less condensed, more accessible, and easily transcribed, whereas heterochromatin is highly condensed, ordered, and thus inaccessible to the transcriptional machinery.[11]

The basic unit of chromatin is the nucleosome which consists of approximately 146bp of DNA wrapped around an octameric histone core formed by one H3−H4 tetramer and two H2A-H2B dimers. The N-terminal "tails" of histone proteins project out of the nucleosome core.[13] The linker histone H1 stabilizes DNA in between nucleosomes and has an important role in the higher-order folding of chromatin (Figure 2).[14,15] Despite the fact that the histone core binds the DNA backbone in 14 distinct locations (resulting in over 120 atomic interactions),[16] nucleosomes are highly dynamic and can slide along DNA with the aid of ATP-dependent nucleosome-remodeling complexes.[17−19] Chromatin/nucleosome structure is known to change locally in response to processes such as gene transcription, DNA damage, replication and recombination.[20] Changes in the composition and structure of chromatin are sufficient to cause heritable phenotypic changes. These changes—occurring without alterations in DNA sequence—are termed epigenetic. Epigenetics determines whether, when, and how particular genes will be transcribed.[21] Epigenetic mechanisms involve mainly the post-translational modification of histone proteins and the methylation of DNA.

Post-Translational Modification of Histones

The protruding histone N-terminal tails are decorated with myriad post-translational modifications (PTMs) including phosphorylation, methylation and acetylation (Figure 2).[22,23] Though all histones—including H1[15]—have been found to be modified, PTMs are higher in number and abundance on histones H3 and H4 and thus this review will focus on these. PTMs occur on multiple, but specific, amino acids—particularly on lysine and arginine residues.[24] Certain modifications, such as acetylation and phosphorylation, are dynamic and easily reversible and thus are associated with dynamic changes in gene expression.[25] On the other hand, more stable modifications such as histone methylation are thought to participate in the long term maintenance of genomic regions.[25]

Histone PTMs have been linked to diverse cellular events including apoptosis,[26] cell differentiation,[27] cancer,[28] and the cell cycle.[22] Unlike DNA methylation (see below), particular histone PTMs can either activate or repress gene transcription.[29,30] For example, histone H3 trimethylation at lysine 4 (H3K4me3) and histone acetylation are linked to transcribed genomic regions (euchromatin),[31] while trimethylation of histone H3 on lysine 9 (H3K9me3) is associated with gene silencing (heterochromatin).[32] Distinct "writers" and "erasers" of the histone code have been found to be responsible for adding or removing a particular PTM.[30] For instance, histone acetyl transferases (HATs) are responsible for acetylating histones, while histone deacetylases (HDACs) remove acetyl groups. In general, histone modifying enzymes are very specific; the modifying enzyme depends on the particular histone variant and the specific site modified.[30] For instance, different enzymes are responsible for methylating histones with varying pre-existing degrees of methylation.[33] There are seven lysine methyltransferases (HMTases) responsible for the methylation of lysine 9 on histone H3 alone;[30] among these, HMTase G9a is responsible for adding two methyl groups to unmodified histone H3 on K9 (resulting in H3K9me2), while SUV39H1 adds two methyl groups to monomethylated K9 (resulting in H3K9me3).[34]

Although histone PTMs were initially thought to affect cellular processes through modifying the interaction between the histone core and DNA, the remarkable diversity and biological specificity associated with histone modification patterns has led to the "histone code" hypothesis.[25] This theory proposes that individual histone PTMs and their combinations are "codes" that function as binding locations for other "effector" proteins that "interpret" these codes[35] and dynamically regulate DNA-templated processes.[36] Supporting this hypothesis, several histone PTM "read-

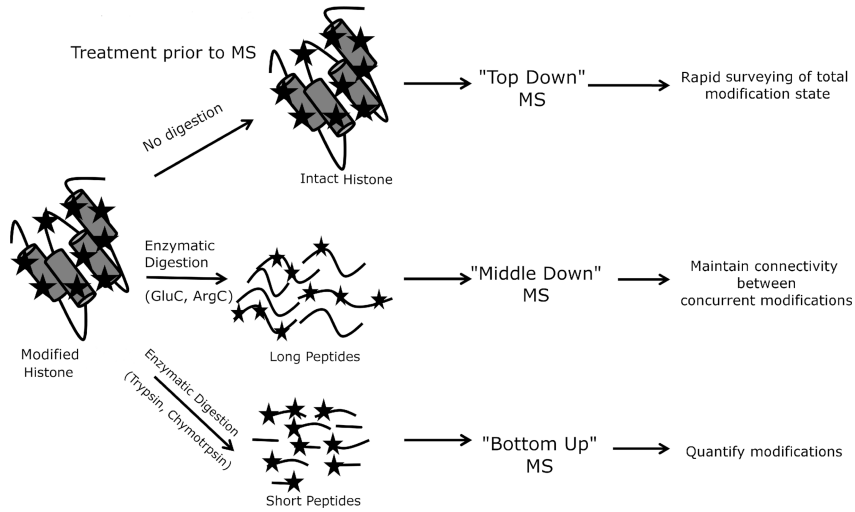

Figure 3. MS-based approaches to study histone modifications. "Top Down" and "Middle Down" MS methods analyze the concurrent modifications on intact proteins or large histone polypeptides, while the "Bottom Up" approach enzymatically digests histones into short peptides prior to MS and allows for the relative quantification of PTMs.

ers" have been discovered.[37,38] For example, histone H3 methylation at lysine 4 (H3K4me3) and lysine 36 (H3K36me3) are linked to transcribed regions and are correspondingly recognized by proteins containing Tudor and PHD domains.[31] Conversely, methylation of histone H3 on lysine 9 (H3K9me3) and lysine 27 (H3K27me3) are associated with heterochromatin as these marks recruit the gene silencing proteins Heterochromatin Protein 1 (HP1) and Polycomb group (PcG), respectively.[11,39]

Binding of protein readers to particular histone PTMs might be influenced by other histone PTMs. For instance, regulation of HP1 binding to H3K9me3 is provided by phosphorylation of serine 10 (the adjacent residue to lysine 9); phosphorylation of this residue releases HP1 from chromatin.[40,41] This effect is known as the "Binary Switch."[5,42] Other Binary Switches may be present on histone H3 and other histones, but have yet to be experimentally confirmed. Adding to the complexity of the code, some histone modifications have been shown to be cross-regulated by other modifications—or lack thereof—on a different histone (usually within the same nucleosome).[43] For instance, H3 K36 di- and trimethylation by the HMTase Set2 is trans-regulated by a critical lysine residue on histone H4; the N-terminus of Set2 needs to interact with H4K44 in order to be able to bind the nucleosome and methylate H3K36.[44]

Analysis of histone modifications has conventionally relied on modification-specific antibodies used in labor-intensive immunoassay methods (such as Western blotting, immunofluorescence, etc).[29,35] Although these methods have provided considerable insight into the significance of histone PTMs, problems such as cross-reactivity or epitope occlusion impair the analysis.[35] Furthermore, the use of site-specific antibodies predetermines which modifications can be detected. Circumventing these issues, mass spectrometry (MS) has emerged as an alternative and unbiased approach to characterize histone modifications.[35] Analysis of histones by MS can be performed in several ways: Top Down,[45,46] Middle Down[24] and Bottom Up (Figure 3).[47] These approaches will be briefly visited here. For a detailed discussion of MS methods to characterize histone PTMs, readers are directed to reviews by Garcia et al.[35] and Trelle and Jensen.[48] Top Down and Middle Down MS methods analyze modifications occurring simultaneously on intact proteins or large histone polypeptides (generated by digestion by enzymes such as ArgC and GluC), respectively. Top Down proteomics allows for rapidly surveying modified histone forms in a single experiment. For instance, Top Down MS has been used to quantitatively analyze combinatorial modifications on histone H4.[45] Likewise, Middle Down proteomics has been used in combination with hydrophilic interaction chromatography (HILIC) to identify modified forms on the N-terminal tail peptide of the histone H3 variant H3.2.[24,49] On the other hand, the Bottom Up approach enzymatically digests histones into short peptides prior to MS analysis[35] allowing for both the characterization and quantification of histone modified forms.[15,50] Digestion with trypsin is usually utilized to generate such histone peptides. Bottom Up MS was used to identify the aforementioned "binary switch" on histone H3, involving K9 and S10.[41]

The MS-based methods mentioned so far are suited to provide a global view of the histone modification landscape in a given cellular state. However, very often the characterization of histone PTMs within specific genes or promoters is desired. To this end, methods targeting particular histone modifications or histone binding proteins and their associated DNA have been developed. These methods—termed Chromatin Immunoprecipitation or ChIP—take advantage of antibodies designed to bind and enrich nucleosomes containing the feature of interest from fragmented chromatin.[29] The DNA associated with the immunoprecipitated nucleosomes can then be detected on a microarray (ChIP-chip).[51,52] This technique has been used to profile histone PTMs on *Saccharomyces cerevisiae*, *Drosophila melanogaster* and mammalian genomes.[52−55] More recently, DNA resulting from ChIP has been sequenced using next generation sequencing technology (ChIP-seq).[29,51,56] This method is capable of higher resolution, with less noise and better genome coverage than ChIP−chip.[29,56] ChIP-seq has been used to profile histone modifications in human T cells.[57] Histone monomethylation on H3K9, H3K27, H3K79, and H4K20, for instance, was found to be linked to gene activation, whereas trimethylations on H3K27, H3K9, and H3K79 were linked to transcriptional repression.

DNA Methylation

Another major mechanism of epigenetic regulation involves the methylation of DNA. In mammals, DNA methylation refers to the addition of a methyl group to a cytosine base in a CpG dinucleotide (Figure 2).[51,58] These nucleotides particularly occur in regions of DNA known as CpG islands. Methylated CpG islands are usually associated with promoter regions, and can act as methylation marks for imprinted genes.[29] DNA methylation has been strongly correlated to transcriptional repression, though the exact silencing mechanism remains unclear.[29] DNA methylation is regulated by DNA methyltransferases (DNMT); DNMT3a and DNMT3b "*de novo*" methylate, while DNMT1 maintains the pre-existing methylation state of DNA.[58,59] DNA methylation can be bound by methyl binding domain (MBD) proteins.[29,60] Not surprisingly, DNA methylation is closely coupled to histone PTMs. For instance, H3K9 recruits DNMTs that methylate DNA and methylated DNA in turn recruits other proteins that interact with HDACs to mediate gene repression.[61]

The most widely used techniques for the characterization of DNA methylation sites are not entirely proteomic and include: (1) mapping of cleavage sites by restriction enzymes that are capable of discriminating between methylated and unmethylated CpG DNA sequences, (2) sequencing of DNA treated with sodium bisulfite, which converts all methylated cytosines to uracil, and (3) immunoprecipitation of DNA with an antibody that binds methylated cytosine (termed MeDIP, mDIP or mCIP).[51] In the first method, restriction enzymes that only recognize unmethylated DNA (e.g., *Hpa*II[62,63] and XhoI)[64] are used to identify a specific region as unmethylated (cleaved) or methylated (intact). Alternatively, restriction enzymes that recognize methylated DNA sequences (e.g., McrBC and Mrr)[65] can be used. In this case, methylated DNA would be cleaved while unmethylated DNA would remain intact.[51] These methods have been combined with microarrays and direct sequencing to profile DNA methylation on a genome-wide scale.[66−68] In the second approach, reaction with sodium bisulfite converts unmethylated cytosines to uracil.[69] PCR on the resulting DNA causes uracil to be replaced with a thymine base; thus, methylated cytosines are found by preservation of cytosine at a particular nucleotide position when searching the amplified DNA against a reference genome. Conversely, unmodified cytosines appear as substitutions from cytosine to thymine.[29] Microarray[70,71] and next-generation sequencing[72−74] have been used to analyze bisulfite-treated DNA, recently achieving single base resolution on a genome-wide level.[29,51,75] Lastly, ChIP with an antibody recognizing methylcytosine, followed by DNA detection on a microarray has been successfully used to locate methylation sites across entire genomes.[76−79]

Epigenetic Alterations in Major Psychiatric Disorders

Through the study of epigenetics marks, researchers can determine whether or not a gene is actively transcribed or silenced during a particular disease state. In this way, epigenetics may provide a basis for determining transcriptional states and identifying genes and protein products implicated in neuropsychiatric illness. However, the potential impact of epigenetics in this field is far greater; epigenetics could provide a unifying theory behind the occurrence of mental disease, linking genetic and various environmental factors and illuminating an underlying etiology for selected neuropsychiatric illnesses.[3,80] Epigenetics may explain several aspects of mental disorders, including discordant rates between monozygotic twins and the chronic relapsing nature of these diseases.[81] Numerous recent experimental data support a critical role for epigenetics in several psychiatric pathologies.

Depression. Depression is a serious psychological condition that presents with wide-ranging symptoms that include depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, low energy and poor concentration.[81,82] Over 121 million people worldwide are affected by it;[82] approximately one-sixth of Americans will suffer from depression during their lifetime.[81] In the year 2000, the economic burden of depression was estimated to be over $80 billion in the United States alone.[83] Recent studies have reported alterations in epigenetic markers in suicide victims, suggesting a link between epigenetics and depression.[1] In the frontopolar cortex, DNMT3b expression is increased in suicide completers (compared to controls that died suddenly from causes not involving any diseases of the central nervous system).[84] DNMT3b upregulation may contribute to the hypermethylation of the gamma-aminobutyric acid type A (GABA-A) receptor promoter; this result would explain decreased levels of GABA-A expression in subjects that committed suicide.[84]

The brain-derived neurotrophic factor BDNF and its receptor tropomyosin-related kinase B (TrkB) are also decreased in suicide completers[85] and these two proteins are decreased in the serum of depressed patients.[86] mRNA for the TrkB splice variant TrkB.T1 was found to be decreased in the brains of suicide victims. Moreover, promoter regions for this gene were found to be hypermethylated, suggesting altered DNA methylation may be responsible for the decrease in TrkB gene expression.[87] In rats, acute immobilization stress correlated with a decrease in histone acetylation at BDNF exons, correspondingly decreasing BDNF transcript levels.[88] In mice, chronic social defeat stress (an animal model for depression) caused decreases in BDNF mRNA as well as increases in repressive H3K27 dimethylation at the BDNF promoter.[89] Stress-related gene repression and decreased BDNF levels were found to be reversed by chronic (not acute) antidepressant treatment.[89] However, H3K27me2 was present weeks after the cessation of stress and was not reversed by antidepressant treatment. This epigenetic mark may therefore represent a long-lasting sign of repression on BDNF promoters resulting from chronic defeat stress. Rather than removing this methylation mark, chronic antidepressant treatment seems to reverse repression of the BDNF gene by inducing H3 acetylation, as well as methylation on H3K4 (activating mark) at the same promoters. Therefore, drugs capable of reversing - or overcoming - the epigenetic changes associated with depression have great potential as a new and improved family of antidepressant treatments.

Schizophrenia. Schizophrenia (SZ) is a common mental disorder (1% prevalence in US adults)[90] characterized by psychotic symptoms including delusions, hallucinations and disordered thoughts, as well as social withdrawal, lack of motivation and general apathy.[91] Although the underlying causes of SZ are unclear, experimental evidence suggests a role for DNA and histone methylation in the pathogenenesis of the disease. The mRNA level of reelin, an extracellular matrix protein previously implicated in neuronal migration and SZ, was found to be significantly decreased in brain tissue from patients diagnosed with SZ; this decrease correlated with an increased level of DNMT1.[92−94] This suggests that DNA hypermethylation in the promoter region of the reelin gene may be responsible for lower reelin expression in SZ patients. Treatment with HDAC inhibitors

increased methylation at the reelin gene promoter (in both cell culture and *in vivo*). This suggests that an interplay between DNA demethylation and histone acetylation is responsible for controlling reelin expression and may also contribute to the regulation of other genes involved in SZ.[92]

Epigenetic dysregulation of other proteins such as membrane-bound catechol-*O*-methyltransferase (MB-COMT) and glutamate decarboxylase 67 (GAD_{67}) have been implicated in SZ. MB-COMT promoter DNA has been found to be frequently hypomethylated in post-mortem brain samples from the frontal lobe of SZ patients. This change is accompanied by an expected increase in transcript levels of MB-COMT.[95] These findings suggest that MB-COMT overexpression due to promoter hypomethylation may increase dopamine degradation in the frontal lobe.[96] Conversely, post-mortem brain samples from SZ patients show reduced GAD_{67} mRNA and protein. In addition to DNA hypermethylation,[97] this decrease was correlated with hypermethylation on H3K27 (heterochromatic mark), and hypomethylation of H3K4 (euchromatic mark) in the promoter region of the gene encoding GAD_{67}.[98] Recently, it was shown that HDAC inhibitors activate reelin and GAD_{67} with dose and time dependence comparable with that of DNMT inhibitors; both classes of drugs attenuate, directly or indirectly, the enzymatic and transcriptional repressor activities of DNMTs and HDACs.[99] This result points at the possibility of using epigenetic drugs, individually or in combination, as potential novel therapeutics to alleviate protein deficits and clinical symptoms associated with SZ.[99]

Drug Addiction. Drug addiction is a chronic relapsing disorder where motivation to seek and consume drugs of abuse becomes compulsive despite its negative consequences.[100,101] Drugs of abuse, such as cocaine, heroin, and methamphetamines, usurp the brain's natural reward pathways, including the mesolimbic dopamine system.[2] Addiction does not occur immediately upon exposure to drugs of abuse, but rather involves neural changes that develop over time.[102] Nevertheless, acute administration of cocaine has been found to rapidly increase histone H4 acetylation (an activating mark) in genes known to be involved in cocaine-related behaviors. Although acute cocaine consumption does not affect every control gene promoter, it results in a global increase on histone H4 acetylation accompanied by concurrent phosphorylation and acetylation of histone H3 in striatum.[103] Furthermore, alterations in H3 acetylation in response to cocaine self- administration have been found to persist long after cessation of drug administration.[104] These results may implicate histone acetylation in drug addiction withdrawal and relapse. Interestingly, cocaine exposure also results in a global increase in H3K9 trimethylation (a silencing mark). Inhibition of the aforementioned histone methyltransferase (HMTase) G9a—which results in a decrease in the levels of H3K9me3—potentiates behavioral response to cocaine.[105] This suggests that the H9K9me3 increase results from a compensatory mechanism to offset the negative effects of histone hyperacetylation. Increased histone acetylation through decreased HDAC5 function, a class II HDAC, has also been reported to occur in response to chronic cocaine consumption.[106]

Learning and Memory. The formation of long-term memories is thought to require lasting changes in gene expression.[2] As for depression and drug addiction, there is experimental evidence supporting a role for epigenetic mechanisms in this process. Contextual fear conditioning is a learning model by which an animal associates a new context with a negative stimulus.[107] Acetylation of histone H3, but not H4, has been found to be significantly increased after contextual fear conditioning.[108] Both contextual fear conditioning and/or the activation of the ERK pathway, which is thought to contribute to memory formation, result in increased levels of histone H3 phosphorylation and acetylation in the hippocampus.[109,110] Mutant mice lacking CREB-binding protein (CBP), a transcriptional coactivator with intrinsic HAT activity, have deficits in several hippocampus-dependent memory tests; an HDAC inhibitor can restore normal long-term memory formation in the mutants,[111] and even enhance it in normal animals.[108,109] Recent findings have also implicated changes in DNA methylation in learning and memory. Contextual fear conditioning induced DNMT3a and DNMT3b expression in the hippocampus and administration of DNMT inhibitors blocked the induction of this type of learning process. These results demonstrate that DNA methylation is dynamically regulated and that this cellular mechanism may be crucial for memory formation.[112]

This consideration of epigenetics in learning and memory takes on added importance on light of recent evidence that natural brain aging is also associated with alterations in histone acetylation. HDACs inhibitors have been reported to enhance learning behavior and access to long-term memories in a mouse model of Alzheimer's disease through an increase in histone acetylation.[113] More recently, the acetylation of histone H4 at lysine 12 (H4K12) has been shown to be dysregulated in aged mice.[114] This aberrant regulation results in the loss of most memory-associated transcription in the hippocampus. The administration of an HDAC inhibitor increased H4K12 acetylation and rescued the expression of learning-induced genes leading to the recovery of cognitive abilities.[114,115] This raises the possibility of epigenetic approaches to the treatment of cognitive decline associated with aging.

Future Directions: Including Epigenetics in the Study and Treatment of Psychiatric Disorders

The evidence reviewed above emphasizes the need to include the study of epigenetic marks in the study of gene-disease associations in neuropsychiatric disorders. Epigenetics offers a mechanism through which environmental factors can modify gene function (with no changes in the DNA sequence) and account for the lack of direct relationship between genotype and phenotype in major psychiatric disorders and the observed variability in the symptoms of disease in individuals with similar genetic makeup.[95] Epigenetic alterations in gene activity could either exacerbate or counteract and obscure genetic predisposition to psychiatric disease. For example, decreases in elements of the serotonin signaling pathway have been long associated with several psychiatric diseases.[116] However, the mechanism by which these alterations may be related to mental disorders remains elusive, with the exception of a few known polymorphisms.[1,117−119] Analysis of epigenetic marks in the promoter regions of serotonergic target genes may reveal new insights into how this system may be susceptible to these mechanisms. This example highlights the value of exploring genome-wide chromatin modifications—by way of the high throughput proteomic and genomic techniques reviewed here—to explore other genes in which changes at the chromatin level contribute to neuropsychiatric disorders. Experimental workflows integrating the study of mRNA, DNA sequence and epigenetic marks are needed to achieve a comprehensive view of the origins of mental disorders. Genome-wide epigenetic studies have produced groundbreaking results in the areas of developmental and cancer biology,[2,120,121] and similar potential is available in the nascent neuroepige-

netics field. In addition to revealing new ways to predict the occurrence and outcome of neuropsychiatric diseases, the study of epigenetic marks can also offer exciting new avenues for their treatment. For instance, DNMT inhibitors and HDACs have been tested for the treatment of amyotrophic lateral sclerosis, Alzheimer's disease, Rubinstein-Taybi syndrome, spinal muscular atrophy, multiple sclerosis, epilepsy, Rett syndrome, stroke, Fragile X syndrome, and Huntington's disease.[4,122] These drugs also hold promise for therapy relevant to the psychiatric disorders discussed in this review, including schizophrenia, depression, and drug addiction.[122,123]

Conclusions

The abundant experimental evidence reviewed here highlights a need to include the study of epigenetics and chromatin in the analysis of human polymorphisms associated with psychiatric diseases. Epigenetics allows environmental factors to modify gene function and explain the lack of direct relationships between genotype and phenotype in major psychiatric disorders. Recently developed high-throughput technologies to characterize histone modifications and DNA methylation will greatly facilitate this task. The integration of the epigenetic code with genetic information will hopefully provide a unifying hypothesis about the origins of neuropsychiatric disorders. However, exactly how environmental factors relay information onto epigenetic marks to control gene transcription is still unknown; understanding these connections will certainly be key to uncovering new therapeutic targets for the prevention, treatment and eventual cure of mental illnesses.

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References

(1) Autry, A. E.; Monteggia, L. M. Epigenetics in suicide and depression. *Biol. Psychiatry* **2009**, *66* (9), 812–3.
(2) Tsankova, N.; Renthal, W.; Kumar, A.; Nestler, E. J. Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* **2007**, *8* (5), 355–67.
(3) Rutten, B. P.; Mill, J. Epigenetic mediation of environmental influences in major psychotic disorders. *Schizophr. Bull.* **2009**, *35* (6), 1045–56.
(4) Urdinguio, R. G.; Sanchez-Mut, J. V.; Esteller, M. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *Lancet Neurol.* **2009**, *8* (11), 1056–72.
(5) Wang, G. G.; Allis, C. D.; Chi, P. Chromatin remodeling and cancer, Part I: Covalent histone modifications. *Trends Mol. Med.* **2007**, *13* (9), 363–72.
(6) Hendrich, B.; Bickmore, W. Human diseases with underlying defects in chromatin structure and modification. *Hum. Mol. Genet.* **2001**, *10* (20), 2233–42.
(7) Renthal, W.; Nestler, E. J. Chromatin regulation in drug addiction and depression. *Dialogues Clin. Neurosci.* **2009**, *11* (3), 257–68.
(8) Polo, S. E.; Almouzni, G. Chromatin assembly: a basic recipe with various flavours. *Curr. Opin. Genet. Dev.* **2006**, *16* (2), 104–11.
(9) Waddington, C. H. Gene regulation in higher cells. *Science* **1969**, *166* (905), 639–40.
(10) Bird, A. Perceptions of epigenetics. *Nature* **2007**, *447* (7143), 396–8.
(11) Grewal, S. I.; Jia, S. Heterochromatin revisited. *Nat. Rev. Genet.* **2007**, *8* (1), 35–46.
(12) Craig, J. M. Heterochromatin--many flavours, common themes. *Bioessays* **2005**, *27* (1), 17–28.
(13) Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **1997**, *389* (6648), 251–60.
(14) Catez, F.; Yang, H.; Tracey, K. J.; Reeves, R.; Misteli, T.; Bustin, M. Network of dynamic interactions between histone H1 and high-mobility-group proteins in chromatin. *Mol. Cell. Biol.* **2004**, *24* (10), 4321–8.
(15) Garcia, B. A.; Joshi, S.; Thomas, C. E.; Chitta, R. K.; Diaz, R. L.; Busby, S. A.; Andrews, P. C.; Ogorzalek Loo, R. R.; Shabanowitz, J.; Kelleher, N. L.; Mizzen, C. A.; Allis, C. D.; Hunt, D. F. Comprehensive phosphoprotein analysis of linker histone H1 from Tetrahymena thermophila. *Mol. Cell. Proteomics* **2006**, *5* (9), 1593–609.
(16) Luger, K. Structure and dynamic behavior of nucleosomes. *Curr. Opin. Genet. Dev.* **2003**, *13* (2), 127–35.
(17) Becker, P. B. Nucleosome sliding: facts and fiction. *EMBO J.* **2002**, *21* (18), 4749–53.
(18) Becker, P. B.; Horz, W. ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **2002**, *71*, 247–73.
(19) Cosgrove, M. S.; Boeke, J. D.; Wolberger, C. Regulated nucleosome mobility and the histone code. *Nat. Struct. Mol. Biol.* **2004**, *11* (11), 1037–43.
(20) Jin, J.; Cai, Y.; Li, B.; Conaway, R. C.; Workman, J. L.; Conaway, J. W.; Kusch, T. In and out: histone variant exchange in chromatin. *Trends Biochem. Sci.* **2005**, *30* (12), 680–7.
(21) Probst, A. V.; Dunleavy, E.; Almouzni, G. Epigenetic inheritance during the cell cycle. *Nat. Rev. Mol. Cell. Biol.* **2009**, *10* (3), 192–206.
(22) Cheung, P.; Allis, C. D.; Sassone-Corsi, P. Signaling to chromatin through histone modifications. *Cell* **2000**, *103* (2), 263–71.
(23) Peterson, C. L.; Laniel, M. A. Histones and histone modifications. *Curr. Biol.* **2004**, *14* (14), R546–51.
(24) Garcia, B. A.; Pesavento, J. J.; Mizzen, C. A.; Kelleher, N. L. Pervasive combinatorial modification of histone H3 in human cells. *Nat. Methods* **2007**, *4* (6), 487–9.
(25) Strahl, B. D.; Allis, C. D. The language of covalent histone modifications. *Nature* **2000**, *403* (6765), 41–5.
(26) Cheung, W. L.; Ajiro, K.; Samejima, K.; Kloc, M.; Cheung, P.; Mizzen, C. A.; Beeser, A.; Etkin, L. D.; Chernoff, J.; Earnshaw, W. C.; Allis, C. D. Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. *Cell* **2003**, *113* (4), 507–17.
(27) Meshorer, E.; Misteli, T. Chromatin in pluripotent embryonic stem cells and differentiation. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7* (7), 540–6.
(28) Fraga, M. F.; Ballestar, E.; Villar-Garea, A.; Boix-Chornet, M.; Espada, J.; Schotta, G.; Bonaldi, T.; Haydon, C.; Ropero, S.; Petrie, K.; Iyer, N. G.; Perez-Rosado, A.; Calvo, E.; Lopez, J. A.; Cano, A.; Calasanz, M. J.; Colomer, D.; Piris, M. A.; Ahn, N.; Imhof, A.; Caldas, C.; Jenuwein, T.; Esteller, M. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat. Genet.* **2005**, *37* (4), 391–400.
(29) Evertts, A. G.; Zee, B. M.; Garcia, B. A. Modern Approaches for Investigating Epigenetic Signaling Pathways. *J. Appl. Physiol.* 2010.
(30) Kouzarides, T. Chromatin modifications and their function. *Cell* **2007**, *128* (4), 693–705.
(31) Ruthenburg, A. J.; Li, H.; Patel, D. J.; Allis, C. D. Multivalent engagement of chromatin modifications by linked binding modules. *Nat. Rev. Mol. Cell. Biol.* **2007**, *8* (12), 983–94.
(32) Grewal, S. I.; Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **2003**, *301* (5634), 798–802.
(33) Steward, M. M.; Lee, J. S.; O'Donovan, A.; Wyatt, M.; Bernstein, B. E.; Shilatifard, A. Molecular regulation of H3K4 trimethylation by ASH2L, a shared subunit of MLL complexes. *Nat. Struct. Mol. Biol.* **2006**, *13* (9), 852–4.
(34) Shi, Y.; Whetstine, J. R. Dynamic regulation of histone lysine methylation by demethylases. *Mol. Cell* **2007**, *25* (1), 1–14.
(35) Garcia, B. A.; Shabanowitz, J.; Hunt, D. F. Characterization of histones and their post-translational modifications by mass spectrometry. *Curr. Opin. Chem. Biol.* **2007**, *11* (1), 66–73.
(36) Cosgrove, M. S. Histone proteomics and the epigenetic regulation of nucleosome mobility. *Expert Rev. Proteomics* **2007**, *4* (4), 465–78.
(37) Wang, Y.; Fischle, W.; Cheung, W.; Jacobs, S.; Khorasanizadeh, S.; Allis, C. D. Beyond the double helix: writing and reading the histone code. *Novartis Found. Symp.* **2004**, *259*, 3–17; discussion 17−21, 163−9.
(38) Daniel, J. A.; Pray-Grant, M. G.; Grant, P. A. Effector proteins for methylated histones: an expanding family. *Cell Cycle* **2005**, *4* (7), 919–26.
(39) Bernstein, B. E.; Meissner, A.; Lander, E. S. The mammalian epigenome. *Cell* **2007**, *128* (4), 669–81.

(40) Hirota, T.; Lipp, J. J.; Toh, B. H.; Peters, J. M. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature* **2005**, *438* (7071), 1176–80.
(41) Fischle, W.; Tseng, B. S.; Dormann, H. L.; Ueberheide, B. M.; Garcia, B. A.; Shabanowitz, J.; Hunt, D. F.; Funabiki, H.; Allis, C. D. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature* **2005**, *438* (7071), 1116–22.
(42) Fischle, W.; Wang, Y.; Allis, C. D. Binary switches and modification cassettes in histone biology and beyond. *Nature* **2003**, *425* (6957), 475–9.
(43) Latham, J. A.; Dent, S. Y. Cross-regulation of histone modifications. *Nat. Struct. Mol. Biol.* **2007**, *14* (11), 1017–24.
(44) Du, H. N.; Fingerman, I. M.; Briggs, S. D. Histone H3 K36 methylation is mediated by a trans-histone methylation pathway involving an interaction between Set2 and histone H4. *Genes Dev.* **2008**, *22* (20), 2786–98.
(45) Pesavento, J. J.; Mizzen, C. A.; Kelleher, N. L. Quantitative analysis of modified proteins and their positional isomers by tandem mass spectrometry: human histone H4. *Anal. Chem.* **2006**, *78* (13), 4271–80.
(46) Boyne, M. T., 2nd; Pesavento, J. J.; Mizzen, C. A.; Kelleher, N. L. Precise characterization of human histones in the H2A gene family by top down mass spectrometry. *J. Proteome Res.* **2006**, *5* (2), 248–53.
(47) Plazas-Mayorca, M. D.; Zee, B. M.; Young, N. L.; Fingerman, I. M.; LeRoy, G.; Briggs, S. D.; Garcia, B. A. One-pot shotgun quantitative mass spectrometry characterization of histones. *J. Proteome Res.* **2009**, *8* (11), 5367–74.
(48) Trelle, M. B.; Jensen, O. N. Functional proteomics in histone research and epigenetics. *Expert Rev. Proteomics* **2007**, *4* (4), 491–503.
(49) Young, N. L.; DiMaggio, P. A.; Plazas-Mayorca, M. D.; Baliban, R. C.; Floudas, C. A.; Garcia, B. A. High throughput characterization of combinatorial histone codes. *Mol. Cell. Proteomics* **2009**, *8* (10), 2266–84.
(50) Garcia, B. A.; Busby, S. A.; Shabanowitz, J.; Hunt, D. F.; Mishra, N. Resetting the epigenetic histone code in the MRL-lpr/lpr mouse model of lupus by histone deacetylase inhibition. *J. Proteome Res.* **2005**, *4* (6), 2032–42.
(51) Schones, D. E.; Zhao, K. Genome-wide approaches to studying chromatin modifications. *Nat. Rev. Genet.* **2008**, *9* (3), 179–91.
(52) Bernstein, B. E.; Kamal, M.; Lindblad-Toh, K.; Bekiranov, S.; Bailey, D. K.; Huebert, D. J.; McMahon, S.; Karlsson, E. K.; Kulbokas, E. J., 3rd; Gingeras, T. R.; Schreiber, S. L.; Lander, E. S. Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* **2005**, *120* (2), 169–81.
(53) Bernstein, B. E.; Humphrey, E. L.; Erlich, R. L.; Schneider, R.; Bouman, P.; Liu, J. S.; Kouzarides, T.; Schreiber, S. L. Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (13), 8695–700.
(54) Schubeler, D.; MacAlpine, D. M.; Scalzo, D.; Wirbelauer, C.; Kooperberg, C.; van Leeuwen, F.; Gottschling, D. E.; O'Neill, L. P.; Turner, B. M.; Delrow, J.; Bell, S. P.; Groudine, M. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* **2004**, *18* (11), 1263–71.
(55) Heintzman, N. D.; Stuart, R. K.; Hon, G.; Fu, Y.; Ching, C. W.; Hawkins, R. D.; Barrera, L. O.; Van Calcar, S.; Qu, C.; Ching, K. A.; Wang, W.; Weng, Z.; Green, R. D.; Crawford, G. E.; Ren, B. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* **2007**, *39* (3), 311–8.
(56) Park, P. J. ChIP-seq: advantages and challenges of a maturing technology. *Nat. Rev. Genet.* **2009**, *10* (10), 669–80.
(57) Barski, A.; Cuddapah, S.; Cui, K.; Roh, T. Y.; Schones, D. E.; Wang, Z.; Wei, G.; Chepelev, I.; Zhao, K. High-resolution profiling of histone methylations in the human genome. *Cell* **2007**, *129* (4), 823–37.
(58) Sananbenesi, F.; Fischer, A. The epigenetic bottleneck of neurodegenerative and psychiatric diseases. *Biol. Chem.* **2009**, *390* (11), 1145–53.
(59) Feng, J.; Fan, G. The role of DNA methylation in the central nervous system and neuropsychiatric disorders. *Int. Rev. Neurobiol.* **2009**, *89*, 67–84.
(60) Nan, X.; Meehan, R. R.; Bird, A. Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. *Nucleic Acids Res.* **1993**, *21* (21), 4886–92.
(61) Klose, R. J.; Bird, A. P. Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* **2006**, *31* (2), 89–97.
(62) Singer-Sam, J.; Grant, M.; LeBon, J. M.; Okuyama, K.; Chapman, V.; Monk, M.; Riggs, A. D. Use of a HpaII-polymerase chain reaction assay to study DNA methylation in the Pgk-1 CpG island of mouse embryos at the time of X-chromosome inactivation. *Mol. Cell. Biol.* **1990**, *10* (9), 4987–9.
(63) Singer-Sam, J.; LeBon, J. M.; Tanguay, R. L.; Riggs, A. D. A quantitative HpaII-PCR assay to measure methylation of DNA from a small number of cells. *Nucleic Acids Res.* **1990**, *18* (3), 687.
(64) Guha, S. Determination of DNA sequences containing methylcytosine in Bacillus subtilis Marburg. *J. Bacteriol.* **1985**, *163* (2), 573–9.
(65) New England Biolabs. Restriction Endonucleases Overview. http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/overview.asp (Accessed May 10th, 2010).
(66) Yan, P. S.; Chen, C. M.; Shi, H.; Rahmatpanah, F.; Wei, S. H.; Caldwell, C. W.; Huang, T. H. Dissecting complex epigenetic alterations in breast cancer using CpG island microarrays. *Cancer Res.* **2001**, *61* (23), 8375–80.
(67) Hatada, I.; Kato, A.; Morita, S.; Obata, Y.; Nagaoka, K.; Sakurada, A.; Sato, M.; Horii, A.; Tsujimoto, A.; Matsubara, K. A microarray-based method for detecting methylated loci. *J. Hum. Genet.* **2002**, *47* (8), 448–51.
(68) Rollins, R. A.; Haghighi, F.; Edwards, J. R.; Das, R.; Zhang, M. Q.; Ju, J.; Bestor, T. H. Large-scale structure of genomic methylation patterns. *Genome Res.* **2006**, *16* (2), 157–63.
(69) Clark, S. J.; Statham, A.; Stirzaker, C.; Molloy, P. L.; Frommer, M. DNA methylation: bisulphite modification and analysis. *Nat. Protoc.* **2006**, *1* (5), 2353–64.
(70) Adorjan, P.; Distler, J.; Lipscher, E.; Model, F.; Muller, J.; Pelet, C.; Braun, A.; Florl, A. R.; Gutig, D.; Grabs, G.; Howe, A.; Kursar, M.; Lesche, R.; Leu, E.; Lewin, A.; Maier, S.; Muller, V.; Otto, T.; Scholz, C.; Schulz, W. A.; Seifert, H. H.; Schwope, I.; Ziebarth, H.; Berlin, K.; Piepenbrock, C.; Olek, A. Tumour class prediction and discovery by microarray-based DNA methylation analysis. *Nucleic Acids Res.* **2002**, *30* (5), e21.
(71) Gitan, R. S.; Shi, H.; Chen, C. M.; Yan, P. S.; Huang, T. H. Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis. *Genome Res.* **2002**, *12* (1), 158–64.
(72) Bibikova, M.; Lin, Z.; Zhou, L.; Chudin, E.; Garcia, E. W.; Wu, B.; Doucet, D.; Thomas, N. J.; Wang, Y.; Vollmer, E.; Goldmann, T.; Seifart, C.; Jiang, W.; Barker, D. L.; Chee, M. S.; Floros, J.; Fan, J. B. High-throughput DNA methylation profiling using universal bead arrays. *Genome Res.* **2006**, *16* (3), 383–93.
(73) Cokus, S. J.; Feng, S.; Zhang, X.; Chen, Z.; Merriman, B.; Haudenschild, C. D.; Pradhan, S.; Nelson, S. F.; Pellegrini, M.; Jacobsen, S. E. Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature* **2008**, *452* (7184), 215–9.
(74) Lister, R.; O'Malley, R. C.; Tonti-Filippini, J.; Gregory, B. D.; Berry, C. C.; Millar, A. H.; Ecker, J. R. Highly integrated single-base resolution maps of the epigenome in Arabidopsis. *Cell* **2008**, *133* (3), 523–36.
(75) Lister, R.; Ecker, J. R. Finding the fifth base: genome-wide sequencing of cytosine methylation. *Genome Res.* **2009**, *19* (6), 959–66.
(76) Zhang, X.; Yazaki, J.; Sundaresan, A.; Cokus, S.; Chan, S. W.; Chen, H.; Henderson, I. R.; Shinn, P.; Pellegrini, M.; Jacobsen, S. E.; Ecker, J. R. Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. *Cell* **2006**, *126* (6), 1189–201.
(77) Weber, M.; Davies, J. J.; Wittig, D.; Oakeley, E. J.; Haase, M.; Lam, W. L.; Schubeler, D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat. Genet.* **2005**, *37* (8), 853–62.
(78) Zilberman, D.; Gehring, M.; Tran, R. K.; Ballinger, T.; Henikoff, S. Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. *Nat. Genet.* **2007**, *39* (1), 61–9.
(79) Shen, L.; Kondo, Y.; Guo, Y.; Zhang, J.; Zhang, L.; Ahmed, S.; Shu, J.; Chen, X.; Waterland, R. A.; Issa, J. P. Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet.* **2007**, *3* (10), 2023–36.
(80) Nestler, E. J. Epigenetic mechanisms in psychiatry. *Biol. Psychiatry* **2009**, *65* (3), 189–90.
(81) Krishnan, V.; Nestler, E. J. The molecular neurobiology of depression. *Nature* **2008**, *455* (7215), 894–902.
(82) World Health Organization. Depression. http://www.who.int/mental_health/management/depression/definition/en/ (Last accessed on Dec. 3, 2009).
(83) Greenberg, P. E.; Kessler, R. C.; Birnbaum, H. G.; Leong, S. A.; Lowe, S. W.; Berglund, P. A.; Corey-Lisle, P. K. The economic burden of

depression in the United States: how did it change between 1990 and 2000. *J. Clin. Psychiatry* **2003**, *64* (12), 1465–75.
(84) Poulter, M. O.; Du, L.; Weaver, I. C.; Palkovits, M.; Faludi, G.; Merali, Z.; Szyf, M.; Anisman, H. GABAA receptor promoter hypermethylation in suicide brain: implications for the involvement of epigenetic processes. *Biol. Psychiatry* **2008**, *64* (8), 645–52.
(85) Dwivedi, Y.; Rizavi, H. S.; Conley, R. R.; Roberts, R. C.; Tamminga, C. A.; Pandey, G. N. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch. Gen. Psychiatry* **2003**, *60* (8), 804–15.
(86) Karege, F.; Perret, G.; Bondolfi, G.; Schwald, M.; Bertschy, G.; Aubry, J. M. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res.* **2002**, *109* (2), 143–8.
(87) Ernst, C.; Deleva, V.; Deng, X.; Sequeira, A.; Pomarenski, A.; Klempan, T.; Ernst, N.; Quirion, R.; Gratton, A.; Szyf, M.; Turecki, G. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. *Arch. Gen. Psychiatry* **2009**, *66* (1), 22–32.
(88) Fuchikami, M.; Morinobu, S.; Kurata, A.; Yamamoto, S.; Yamawaki, S. Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus. *Int. J. Neuropsychopharmacol.* **2009**, *12* (1), 73–82.
(89) Tsankova, N. M.; Berton, O.; Renthal, W.; Kumar, A.; Neve, R. L.; Nestler, E. J. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat. Neurosci.* **2006**, *9* (4), 519–25.
(90) Snyder, E. M.; Murphy, M. R. Schizophrenia therapy: beyond atypical antipsychotics. *Nat. Rev. Drug Discovery* **2008**, *7* (6), 471–2.
(91) Graff, J.; Mansuy, I. M. Epigenetic dysregulation in cognitive disorders. *Eur. J. Neurosci.* **2009**, *30* (1), 1–8.
(92) Chen, Y.; Sharma, R. P.; Costa, R. H.; Costa, E.; Grayson, D. R. On the epigenetic regulation of the human reelin promoter. *Nucleic Acids Res.* **2002**, *30* (13), 2930–9.
(93) Costa, E.; Chen, Y.; Davis, J.; Dong, E.; Noh, J. S.; Tremolizzo, L.; Veldic, M.; Grayson, D. R.; Guidotti, A. REELIN and schizophrenia: a disease at the interface of the genome and the epigenome. *Mol. Interv.* **2002**, *2* (1), 47–57.
(94) Akbarian, S. The molecular pathology of schizophrenia-Focus on histone and DNA modifications. *Brain Res. Bull.* 2009.
(95) Abdolmaleky, H. M.; Smith, C. L.; Zhou, J. R.; Thiagalingam, S. Epigenetic alterations of the dopaminergic system in major psychiatric disorders. *Methods Mol. Biol.* **2008**, *448*, 187–212.
(96) Abdolmaleky, H. M.; Cheng, K. H.; Faraone, S. V.; Wilcox, M.; Glatt, S. J.; Gao, F.; Smith, C. L.; Shafa, R.; Aeali, B.; Carnevale, J.; Pan, H.; Papageorgis, P.; Ponte, J. F.; Sivaraman, V.; Tsuang, M. T.; Thiagalingam, S. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Hum. Mol. Genet.* **2006**, *15* (21), 3132–45.
(97) Kundakovic, M.; Chen, Y.; Costa, E.; Grayson, D. R. DNA methyltransferase inhibitors coordinately induce expression of the human reelin and glutamic acid decarboxylase 67 genes. *Mol. Pharmacol.* **2007**, *71* (3), 644–53.
(98) Huang, H. S.; Matevossian, A.; Whittle, C.; Kim, S. Y.; Schumacher, A.; Baker, S. P.; Akbarian, S. Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1-regulated histone methylation at GABAergic gene promoters. *J. Neurosci.* **2007**, *27* (42), 11254–62.
(99) Kundakovic, M.; Chen, Y.; Guidotti, A.; Grayson, D. R. The reelin and GAD67 promoters are activated by epigenetic drugs that facilitate the disruption of local repressor complexes. *Mol. Pharmacol.* **2009**, *75* (2), 342–54.
(100) Koob, G. F.; Le Moal, M. Drug abuse: hedonic homeostatic dysregulation. *Science* **1997**, *278* (5335), 52–8.
(101) Koob, G. F.; Kenneth Lloyd, G.; Mason, B. J. Development of pharmacotherapies for drug addiction: a Rosetta stone approach. *Nat. Rev. Drug Discovery* **2009**, *8* (6), 500–15.
(102) Kauer, J. A.; Malenka, R. C. Synaptic plasticity and addiction. *Nat. Rev. Neurosci.* **2007**, *8* (11), 844–58.
(103) Kumar, A.; Choi, K. H.; Renthal, W.; Tsankova, N. M.; Theobald, D. E.; Truong, H. T.; Russo, S. J.; Laplant, Q.; Sasaki, T. S.; Whistler, K. N.; Neve, R. L.; Self, D. W.; Nestler, E. J. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **2005**, *48* (2), 303–14.
(104) Freeman, W. M.; Patel, K. M.; Brucklacher, R. M.; Lull, M. E.; Erwin, M.; Morgan, D.; Roberts, D. C.; Vrana, K. E. Persistent alterations in mesolimbic gene expression with abstinence from cocaine self-administration. *Neuropsychopharmacology* **2008**, *33* (8), 1807–17.
(105) Maze, I.; Covington, H. E., 3rd; Dietz, D. M.; LaPlant, Q.; Renthal, W.; Russo, S. J.; Mechanic, M.; Mouzon, E.; Neve, R. L.; Haggarty, S. J.; Ren, Y.; Sampath, S. C.; Hurd, Y. L.; Greengard, P.; Tarakhovsky, A.; Schaefer, A.; Nestler, E. J. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* **2010**, *327* (5962), 213–6.
(106) Renthal, W.; Maze, I.; Krishnan, V.; Covington, H. E., 3rd; Xiao, G.; Kumar, A.; Russo, S. J.; Graham, A.; Tsankova, N.; Kippin, T. E.; Kerstetter, K. A.; Neve, R. L.; Haggarty, S. J.; McKinsey, T. A.; Bassel-Duby, R.; Olson, E. N.; Nestler, E. J. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* **2007**, *56* (3), 517–29.
(107) Phillips, R. G.; LeDoux, J. E. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* **1992**, *106* (2), 274–85.
(108) Levenson, J. M.; O'Riordan, K. J.; Brown, K. D.; Trinh, M. A.; Molfese, D. L.; Sweatt, J. D. Regulation of histone acetylation during memory formation in the hippocampus. *J. Biol. Chem.* **2004**, *279* (39), 40545–59.
(109) Levenson, J. M.; Sweatt, J. D. Epigenetic mechanisms in memory formation. *Nat. Rev. Neurosci.* **2005**, *6* (2), 108–18.
(110) Chwang, W. B.; O'Riordan, K. J.; Levenson, J. M.; Sweatt, J. D. ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. *Learn Mem.* **2006**, *13* (3), 322–8.
(111) Korzus, E.; Rosenfeld, M. G.; Mayford, M. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron* **2004**, *42* (6), 961–72.
(112) Miller, C. A.; Sweatt, J. D. Covalent modification of DNA regulates memory formation. *Neuron* **2007**, *53* (6), 857–69.
(113) Fischer, A.; Sananbenesi, F.; Wang, X.; Dobbin, M.; Tsai, L. H. Recovery of learning and memory is associated with chromatin remodelling. *Nature* **2007**, *447* (7141), 178–82.
(114) Peleg, S.; Sananbenesi, F.; Zovoilis, A.; Burkhardt, S.; Bahari-Javan, S.; Agis-Balboa, R. C.; Cota, P.; Wittnam, J. L.; Gogol-Doering, A.; Opitz, L.; Salinas-Riester, G.; Dettenhofer, M.; Kang, H.; Farinelli, L.; Chen, W.; Fischer, A. Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science* **2010**, *328* (5979), 753–6.
(115) Sweatt, J. D. Neuroscience. Epigenetics and cognitive aging. *Science* **2010**, *328* (5979), 701–2.
(116) Mockus, S. M.; Vrana, K. E. Advances in the molecular characterization of tryptophan hydroxylase. *J. Mol. Neurosci.* **1998**, *10* (3), 163–79.
(117) Zhang, X.; Gainetdinov, R. R.; Beaulieu, J. M.; Sotnikova, T. D.; Burch, L. H.; Williams, R. B.; Schwartz, D. A.; Krishnan, K. R.; Caron, M. G. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **2005**, *45* (1), 11–6.
(118) Cichon, S.; Winge, I.; Mattheisen, M.; Georgi, A.; Karpushova, A.; Freudenberg, J.; Freudenberg-Hua, Y.; Babadjanova, G.; Van Den Bogaert, A.; Abramova, L. I.; Kapiletti, S.; Knappskog, P. M.; McKinney, J.; Maier, W.; Jamra, R. A.; Schulze, T. G.; Schumacher, J.; Propping, P.; Rietschel, M.; Haavik, J.; Nothen, M. M. Brain-specific tryptophan hydroxylase 2 (TPH2): a functional Pro206Ser substitution and variation in the 5′-region are associated with bipolar affective disorder. *Hum. Mol. Genet.* **2008**, *17* (1), 87–97.
(119) Haavik, J.; Blau, N.; Thony, B. Mutations in human monoamine-related neurotransmitter pathway genes. *Hum. Mutat.* **2008**, *29* (7), 891–902.
(120) Lee, M. P. Genome-wide analysis of epigenetics in cancer. *Ann. N.Y. Acad. Sci.* **2003**, *983*, 101–9.
(121) Lee, T. I.; Jenner, R. G.; Boyer, L. A.; Guenther, M. G.; Levine, S. S.; Kumar, R. M.; Chevalier, B.; Johnstone, S. E.; Cole, M. F.; Isono, K.; Koseki, H.; Fuchikami, T.; Abe, K.; Murray, H. L.; Zucker, J. P.; Yuan, B.; Bell, G. W.; Herbolsheimer, E.; Hannett, N. M.; Sun, K.; Odom, D. T.; Otte, A. P.; Volkert, T. L.; Bartel, D. P.; Melton, D. A.; Gifford, D. K.; Jaenisch, R.; Young, R. A. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* **2006**, *125* (2), 301–13.
(122) Grayson, D. R.; Kundakovic, M.; Sharma, R. P. Is there a future for histone deacetylase inhibitors in the pharmacotherapy of psychiatric disorders. *Mol. Pharmacol.* **2010**, *77* (2), 126–35.
(123) Abel, T.; Zukin, R. S. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Curr. Opin. Pharmacol.* **2008**, *8* (1), 57–64.

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