A Review on the Role of Epigenetics in Psychiatry: Etiology, Contemporary Medicine and Future Perspectives

By

Sadia Afrin Oshin
15236003

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
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Student's Full Name & Signature:

Sadia Afrin Oshin
15236003

Approval

The thesis/project titled “A Literature Review on the Role of Epigenetics in Psychiatry: Etiology, Contemporary Medicine and Future Perspectives” submitted by

Sadia Afrin Oshin (15236003)

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Examining Committee:

Supervisor:
(Member)

_____

Iftekhar Bin Naser
Assistant Professor, Mathematics and Natural Sciences
Brac University

Program Coordinator:
(Member)

_____

Iftekhar Bin Naser
Assistant Professor, Mathematics and Natural Sciences
Brac University

Departmental Head:
(Chair)

_____

Prof. A. F. M. Yusuf Haider
Chairperson, Mathematics and Natural Sciences
Brac University

Abstract

Psychiatric disorders are complex phenomena that have neurological, genetic and epigenetic bases. The epigenetic underpinnings of such disorders have been discovered and rediscovered in past research and provide a promising ground for drug development involving fewer side effects and faster recovery. This paper illustrates the epigenetics underlying the development of depression, anxiety disorders, and schizophrenia. In addition, it elaborates on epigenetic pharmacotherapy, focusing on HDAC and DNMT inhibitory activities of contemporary medicine and prospective epigenetic drugs.

Keywords: psychiatric epigenetics; pharmacology; drug development; HDAC inhibitor; DNMT inhibitor

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List of Acronyms

5-mc	5-methyl cytosine
5-hmc	5-hydroxymethyl cytosine
5-LOX	5-Lipoxygenase
ac-H3	acetylated histone H3
ac-H4	acetylated histone H4
AMP	Adenosine Monophosphate
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid
BAD	BCL-2 Associated Agonist of cell Death
Bax	Bcl-2 associated x
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra Large
BP	Bipolar Disorder
CDK	Cyclin Dependent Kinase
Chip	Chromatin Immunoprecipitation
Cip1	CDK interacting protein 1
CRH	Corticotropin-Releasing Hormone
COMT	Catechol-O-Methyltransferase
DNAM	DNA Methylation
DNMT	DNA Methyltransferase
DIV	Days In Vitro
DRD2	Dopamine Receptor D2
EPM	Elevated Plus Maze
GABA	Gamma-Aminobutryric Acid

GAD67	Glutamate Decarboxylase 67
GSK-3	Glycogen Synthase Kinase-3
HAT	Histone Acetyltransferase
HDAC	Histone Deacetylase
HES-1	Hairy and Enhancer of Split-1
HMT	Histone Methyltransferase
HSP70	Heat Shock Protein 70kDa
IC50	Half Maximal Inhibitory Concentration
IL-6	Interleukin-6
LPS	Lipopolysaccharide
LTG	Lamotrigine
MCP-1	Monocyte Chemoattractant Protein-1
MIP-1	Macrophage Inflammatory Protein-1
MMP-9	Matrix Metallopeptidase 9
NeuroD	Neuronal Differentiation
NIH	Novelty-Induced Hypophagia
NMDA	N-Methyl-D-Aspartate
pCREB	cAMP Response Element-Binding protein
pERK	Endoplasmic Reticulum Kinase protein
RELN	Reelin
SB	Sodium Butyrate
SZ	Schizophrenia
tPA	tissue Plasminogen Activator
TSA	Trichostatin A

Chapter 1

Introduction

1.1 Background

The idea of environmental epigenetics has been around since French naturalist Lamarck proposed in 1802 that the environment can alter the phenotype of an organism in a heritable manner. After new knowledge of genes and genetics in embryology was uncovered, Conrad Waddington coined the term Epigenetics, which etymologically means “above genetics” and refers to the branch of genetics that studies the molecular pathways that regulate gene expression. The environment shapes organisms in ways that have not missed the human eye before, epigenetic studies portray the different ways in which this takes place, along with the role environmental factors play in disease pathogenesis by way of gene regulation. As of 2017, it was estimated by Ritchie and Roser [1] that 792 million people worldwide has or had a mental illness in their lifetimes, all the while mental health disorders being widely under-reported. There have been numerous genetic mapping studies focused on identifying a genetic basis for psychiatric disorders in the past few decades, two main methods performed repeatedly were linkage and association studies. While some of the neuropsychiatric disorders, like Alzheimer’s disease, Rett syndrome, Huntington’s disease and fragile X syndrome showed mutations and polymorphisms as causes, others, called idiopathic psychiatric disorders, like schizophrenia, bipolar disorder and depression led researchers to resort to alternative theories where the DNA sequence was not a contributing factor to the illnesses. Many of who theorized the causes to be aberrations in gene expression. There is sufficient evidence corroborating that claim, which are presented in section 1.3. Some researchers think that epigenetic mechanisms may be one factor explaining the missing heritability in major depression, because it is a mechanism for environmental exposure to have long-lasting and sometimes even transgenerational marks on gene transcription. Epigenetics has not only garnered attention for explaining neuropsychiatric disease pathogenesis but also that of other complex diseases like cancer, cardiovascular diseases, type 2 diabetes and metabolic disorders. [2] The contemporary methods of treatment that are predominantly used are psychotherapy, psychoactive drugs, and lifestyle changes.

On the other hand, current methods of social interventions in mental health, for example efforts in alleviating poverty, reducing discrimination etc. as a measure of tackling public mental health are comparatively negligible in use and efficacy. More work needs to be done in the social sector to prevent the devastating effects of mental illnesses.

While the social work on changing environments that blight mental health worldwide is of utmost importance, it is also necessary to have treatment options that reverse the changes caused by such environments. This is where epigenetic therapy can play a vital role. Current treatment options, however helpful, has numerous and debilitating side effects and offer little guarantee for a recovery without relapse. A large number of patients treated with these methods go through multiple relapses, while there are many who are resistant to medications. Additionally, psychotherapy, while being deemed the safer treatment option, takes a considerably long time to bring about significant improvements. Epigenetic therapy, with drugs or nutrition, may provide patients with better treatment and pathways to recovery.

1.2 Epigenetic Mechanisms

Epigenetic mechanisms are mitotically and/or meiotically heritable, potentially reversible mostly covalent modifications of DNA, RNA, and DNA associated proteins that do not alter the DNA or RNA sequence of organisms. They also involve nucleosome remodeling/repositioning, and higher order chromatin organization. [3] The covalent modifications express themselves as methylation at 5’ position of cytosine residues in CpG sites, methylation of RNA in various sites, and acetylation of DNA associated histone proteins. The study of RNA epigenetic modification, often called epitranscriptomics, is indeed limited in its capacity, but the remaining epigenetic modifications have been widely studied and the knowledge has been applied to diagnostics, treatment and measure of treatment progress for a wide range of ailments such as cardiovascular diseases, cancer, diabetes and mental illnesses. The major mechanisms of epigenetic modifications are as follows.

DNA methylation

Chemical alterations of DNA molecules were first detected in 1948, and a function of DNA methylation, in particular 5-methylcytosine (5mC), in gene regulation was proposed in the mid-1970s. In the 1980s, a functional association of DNA methylation with gene repression was established, alongside the existence of CpG islands. [4] Methylated cytosines are targets

for DNA-binding proteins (e.g., MBD1, MBD3, MBD4 and MeCP2) leading to chromatin condensing and gene silencing. Other funtions of DNA methylation include genomic imprinting, X chromosome inactivation in cells of female origin and containment of transposable elements. [5] The first 'epigenetic drug', 5-azacytidine (also known as 2′-deoxy-5-azacytidine and later called decitabine), which blocks DNA methylation, was used to alter gene expression and phenotypes in fibroblast cell lines. [6] DNA methylation is catalyzed by a family of DNA methyltransferases that shift a methyl group from *S*-adenyl methionine (SAM) to the fifth carbon of a cytosine residue to form 5mC. All Dnmts in this family of enzymes are extensively involved in the development of an embryo. "By the time cells reach terminal differentiation, Dnmt expression is much reduced. This would seem to suggest that the DNA methylation pattern in postmitotic cells is stable. However, postmitotic neurons in the mature mammalian brain still express substantial levels of Dnmts, raising the possibility that Dnmts and DNA methylation may play a novel role in the brain." [4]

DNA hydroxymethylation

"Recent studies of genomic DNA on human and mouse brain, neurons and from mouse embryonic stem cells have shown that 2-oxoglutarate and Fe(II)-dependent oxygenases of the ten-eleven translocation (Tet) proteins can catalyze the oxidation of 5mC at CpG dinucleotides to form 5-hydroxymethylcytosine (5-hmC)." Provoking evidence shows the role of the Tet protein family and 5-hmC in normal development as well as in many diseases. [7]

Nucleosomal organization

The chromatin subunit model, was put forth in 1974 after substantial research suggesting the nucleosomal organization of chromatin. [6] The essence of this model is the control this organization has (alongside other factors such as higher order packaging of chromatin) over the accessibility of genes. Eukaryotic chromatin is made responsive to environmental, metabolic, and developmental cues by the action of a family of proteins called the "nucleosome remodeling" ATPases. These enzymes undergo conformational changes when they simultaneously bind to target nucleosomes and bind and hydrolyze ATP, causing assembly/disassembly of nucleosome, movement of the histone octamers on DNA or exchange of histone proteins for variants. All of these affect the accessibility of genes to interacting proteins. [8]

Histone modification

Since 1960's it has been known that histone proteins are post-translationally modified. This happens via acetylation, methylation, phosphorylation and ubiquitination, leading to changes in chromatin structure, which in turn influence gene expression. Acetylation of the ε-amino group of lysine residues in the aminoterminal tails of histones H3 and H4 is the best-denoted histone modification and have consistently been demonstrated to promote transcription. Histone acetylation is catalyzed by histone acetyl transferases, which are brought to the site of acetylation by transcriptional cofactors AMP response element-binding protein and p300. Histone deacetylation, on the other hand, is correlated with CpG methylation and inactive chromatin states. There are four classes of HDACs that catalyze deacetylation, which are themselves regulated by posttranslational modifications. Histone Lysine methylation has complex results regarding promotion or repression of genes, depending on where the lysine residue resides and the extent of the methylation. Like histone acetylation, histone methylation is also reversible, with histone lysine methyltransferases that target specific lysines and specific mono-, di- or trimethylation states. [9]

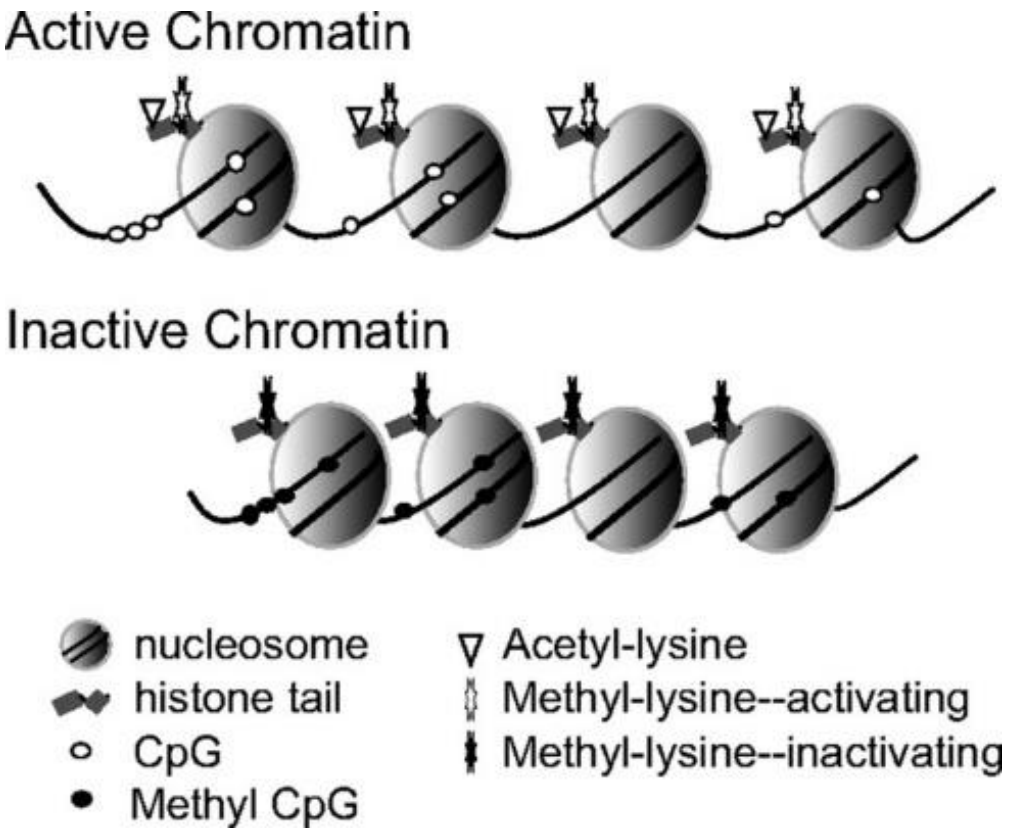

Figure 1. Chromatin structure and epigenetic tags. Nucleosomes are indicated as spheres, with sites of methylation and acetylation on DNA and histone proteins listed in the key. [9]

Non coding RNA

Long non-coding RNA sequences can silence genes by recruiting remodeling complexes that in turn facilitates histone methylation. They can also recruit RNA-binding proteins that disrupt histone deacetylation and prevent transcription factor from binding to the promoter region. “Small inhibitory RNAs and dicer-dependent microRNAs, as short noncoding RNAs, have also been shown to play a role in transcriptional suppression through several mechanisms, including the recruitment of specific argonaute proteins to form epigenetic remodeling complexes that promote histone deacetylation, histone methylation, and DNA methylation.”

Transgenerational inheritance of RNA-induced epigenetic silencing is partly supported by a single stranded subclass of these small noncoding RNAs called protein interaction world-interacting RNAs (21–30 nt). [9]

RNA epigenetics

Posttranscriptional RNA modification make up another type of epigenetics, RNA epigenetics.

MRNA, tRNA, and rRNA can be methylated at various positions in the nucleotide base and at the 2 position of ribose. The RNA methyltransferases are categorized into four families, which use S-adenosylmethionine as a universal methyl donor. RNA methylation has multiple functions including stabilization, enhanced function and quality control.

1.3 The Role of Epigenetics in Psychiatric Disease Pathogenesis

Besides the unique roles of epigenetics in developmental programming, the epigenetic landscape interacts with the micro and macro environmental dynamics (e.g., hormones, neurotransmitters, neuronal activation/inhibition, nutrition, drugs, metabolic states, seasonal/climate changes, microbiota and contaminants) to help adaptation to the everchanging conditions in an age and generation specific manner. [10] These interactions may or may not lead to detrimental health effects.

1.3.1 Determinants of Altered DNA Methylation in Psychopathogenesis

Methionine, folate and vitamin B12 play key roles in methylation reactions. Additionally, a 2013 study found that pregnant mice with a folate and vitamin B12 imbalance birthed offspring with global hypomethylation of their epigenome. [10]

“Dietary levels of methionine, the metabolic precursor of S-adenosylmethionine during prenatal and early postnatal life, influences susceptibility to chronic adult diseases because dietary methionine influences DNA methylation. Environmental factors also modulate proteins like enzymes associated with DNA methylation and histone modification. Other proteins like polycomb proteins (proteins that maintain many genes important for development in a repressed state) also mediate the epigenetic effects of the environment on gene expression.” [2]

In addition to nutritional factors, maternal distress also influences epigenetic portrait of the brain during fetal period which is carried into adulthood. In rats, stress during pregnancy cause methylation of the BDNF exon IV gene by DNMT1 and DNMT3a, causing down-regulation of the BDNF gene in the amygdala and hippocampus of offspring both at weaning and adulthood. Prenatal stress also increases promoter DNAM of corticotrophin-releasing hormone gene, resulting in increased responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to acute stress and anxiety-like behaviors in adolescent rats. Prenatal restraint

stress in mice was found to increase the 5-mC and 5-hmc levels in CpG regions of GAD67 and RELN promoters in offspring, leading to schizophrenia-like phenotypes in adults, which was attenuated by administration of anti-psychotic drugs. [10] Glutamic acid decarboxylase 67 (GAD67) is crucial for gamma-aminobutyric acid (GABA) neurotransmission in the brain, it catalyzes the decarboxylation of glutamate to form GABA. [11] GABA plays critical roles in the transmission of sensory messages through the thalamus and also bringing in large groups of thalamic cells under collective oscillation that underlie changes in conscious state. [12] “Work from animal experiments strongly suggests that deficiency in either GAD65 or GAD67 is detrimental to the animal's ability to resist stress-induced pathology.” [13] RELN (Reelin) on the other hand, is involved with neuronal migration and cortical lamination during embryonic development, and with synaptic strength, and connectivity in the adult brain, among other functions. [14]

Drug abuse also meddles with the epigenetic profile. For example, long-term exposure of pregnant mice to methamphetamine alters DNAM in hippocampal cells, modifying behavior in offspring. Moreover, genome-wide DNA methylome profiling revealed methylation alteration in more than two dozen CpG sites in ten genes in the offspring as a result of maternal smoking during pregnancy. In addition, depression, blood nutritional elements and vitamins, body mass index, the infants gestational age, birth order and birth weight have been associated with alterations in DNAM. Other human studies have found that seasonal availability of nutrients during preconceptional period could have life-long alterations of DNA methylome in offspring. [10]

Although the fetal period is linked to the most influential changes in DNA methylome, notable modifications can occur in childhood and adulthood. For example, insufficient maternal care during childhood alters the promoter DNAM pattern of glucocorticoid receptor gene in the rat hippocampus causing maladaptive behavior in adulthood. [10] “These differences emerged over the first week of life, and were reversed with cross-fostering.” [2] In mice, early life stress was correlated with reduced neurogenesis in the hippocampus in adulthood cause by increased methylation of the BDNF (Brain Derived Neurotrophic Factor) gene. BDNF plays an important role in the synthesis of new synapses. Thus, down-regulation of the gene that produces BDNF led to decreased number of new synapses and reduced symbolisation of experiences, curtailing the ability to memorise and learn. This methylation marker remains stable throughout life and is inherited by the next generation. [15-18] Early life maternal deprivation in monkeys was correlated with widespread DNAM changes in the

prefrontal cortex and T cells, and altered 5-hmc in genes linked with neuronal functions and psychological diseases in the cortex that could remain in adulthood. [10] Noteworthy in these studies is the fact that parental care only constitutes maternal care in case of most non-human animals, whereas for humans, parental care can be provided by both or all parents.

Even the type of milk fed to infants poses a risk of altering the DNA methylome; milk derived opioid peptides (bovine milk is rich in opioid peptides) induces genome-wide DNAM alterations similar to that caused by morphine, interfering with the function of key genes involved in redox and methylation homeostasis. [10] Increased methylation of the BDNF gene is also observed

A comparative whole-genome DNAM analysis using the Illumina 450K Bead Chips discovered methylation changes in >2800 CpG sites in 96 maltreated children who were separated from their parents because of abuse or neglect compared with matched control subjects. Long-lasting DNAM of this kind was also found in genome-wide DNAM analysis of individuals with a history of childhood and adolescent traumatic life events (25 cases versus 16 controls). The latter study presented 362 alternatively methylated promoters (248 hyper- and 114 hypomethylated), genes related to cellular and neuronal plasticity, particularly ASL2 with reduced expression, exhibited the most significant deviation in DNAM. Despite blood and saliva samples not being appropriate representations of brain cells affected in psychiatric diseases, several studies show that some of the epigenetic modifications are reflected in peripheral tissues. [10] “Epigenetic alterations in peripheral tissues are useful for development of potential biomarkers for monitoring disease status.” [19]

Chapter 2

The Epigenetic Etiology of Psychiatric Disorders

There is accumulating evidence that the root cause of mental illnesses are different kinds of trauma – psychological, nutritional, transgenerational, prenatal, childhood trauma are some categories, and the categories may and do converge to create, according to many experts, the basis of all mental illnesses.

2.1 Depression

Chronic exposure to various kinds of stress alters chromatin structure in the brain of rodents. One type of modification is global (acetylation and methylation of histones in specific limbic regions of the brain, presumably causing a genome-wide skew towards a more permissive or restrictive chromatin activity), and the other is locus specific, that is histone methylation or acetylation or DNA methylation at a particular gene that regulates its modified expression. [20]

These epigenetic regulation of stress responses is demonstrated in the chronic social stress defeat model created by Vialou, Feng, Robision, and Nestler in 2013. Under chronic social stress, *susceptible* mice show depression-like symptoms, these deleterious symptoms are mostly avoided in *resilient* mice in spite of undergoing the same level of stress. The aforementioned study illustrates how this susceptibility and resilience is party controlled by changes in histone acetylation and a repressive form of histone methylation - dimethylation of Lys9 of histone H3 (H3K9me2) – in nucleus accumbens respectively. Global level of increased histone acetylation was seen, which encourages resiliency due to HDAC inhibition in this specific brain region being antagonistic to susceptibility and conducive to antidepressant-like reactions. On the other hand, global increases in H3K9me2 are pathological, increasing susceptibility to depression. In addition, genomewide measure were used in this study to pinpoint numerous genes that were affected by alterations in these histone modifications, further elucidating the ways in which susceptibility is developed or avoided. [20]

There is also strong evidence supporting that early life events give way to lifelong vulnerability to stress. The previously mentioned study showing a correlation between lack of maternal care in rats and DNAM of glucocorticoid receptor gene in the rat hippocampus exemplifies how early life adversity can mediate vulnerability to stress.

Moreover, this hyperresponsiveness to stress was seen to be passed on to next generations as anxious mother rats were less capable of providing sufficient maternal care to their offspring. [20]

Some studies show reduced hippocampal volume and circulating BDNF in blood in human patients suffering from major depression. The minimized BDNF concentration in blood normalizes after chronic antidepressant treatment. Post-mortem evaluations have shown decreased BDNF levels in the brains of suicide victims and depressed patients. Reduced

BDNF expression has been shown to undermine neuronal differentiation in the dentate gyrus and depression-like behavior in rodent models. In comparison, infusion of BDNF in hippocampus of these rodents had antidepressant effects. [15] “BDNF levels reduction in traumatic childhood may also have a teleological meaning, i.e., like a natural protection, so as to reduce accurate symbolisation of painful memories and support emotional survival. On the other hand, traumatic childhood increases stress in order to serve the need for hypervigilance, since the world can be perceived as a potential enemy, or even as a minefield.” Studies have also found hypermethylation in the 5’ region of ribosomal RNA in the hippocampal area of suicidal children which controls the HPA axis. The result is atypical regulation of the response to stress and suicidal tendencies. [21]

It is difficult to prove that the environmental epigenetic changes mentioned above contribute to suicidality, since the epigenetic state before exposure to adverse environment cannot be monitored in cases where brain tissue needs to be analyzed, and since it is known that diverse stochastic epigenetic changes already take place in genetically and environmentally stable organisms. [20] This is unless, a saliva or blood test is used as proof of correlation.

2.2 Schizophrenia

In 2003, a study utilized two pairs of monozygotic twins, one pair being concordant with schizophrenia, the other being discordant. The authors examined the methylation patterns of lymphocytes at the 5’ regulatory region of the dopamine D2 receptor gene in all of the subjects. Many DNA methylation differences within the analysed region were found between and within the twin pairs. The affected twin in the discordant pair was epigenetically more similar to the concordant twins than to his unaffected monozygotic co-twin [2], portraying the epigenetic basis for schizophrenia (SZ).

In another study, promoter of the RELN gene was hypermethylated in frontal lobe samples of 10 schizophrenia patients. Further consolidating the previously mentioned study done on mice that underwent prenatal stress and showed hypermethylated RELN promoters with schizophrenia-like symptoms in adulthood. Moreover, an additional study investigated the methylation of CpG sites on RELN promoters in genomic DNA of 15 patients with schizophrenia and 10 non-psychiatric subjects, discovering hypermethylation. In studies of post mortem temporal cortex of patients with schizophrenia showed significantly altered expression of several genes including that encoding histone deacetylase 3. “A recent

observation has suggested a link between the BDNF Val66Met polymorphism and abnormal DNA methylation levels at CpG islands at the BDNF locus in the frontal cortex of schizophrenic patients. More specifically, it was shown that there was a decrease in DNA methylation at exonic CpG islands of the BDNF gene in the frontal cortex of individuals carrying the Met allele. All in all, these data support the notion that genetic variations in BDNF are associated with a distinct epigenetic regulation of BDNF gene expression." [15]

Although the methylation state of this gene in schizophrenia is controversial, COMT has been recognized as a candidate gen, and encodes catechol-O-methyltransferase, which is associated with dopamine metabolism. Dopamine is considered to underlie symptoms such as hallucinations and delusions. In addition, SOX10 gene encoding oligodendrocyte specific transcription factor, is observed to be downregulated in the prefrontal cortices of SZ patients, which corresponds with reduced expression of oligodendrocyte-related and key myelination genes, causing oligodendrocyte dysfunction. [19] Oligodendrocytes are highly specialized neural cells whose function is to myelinate central nervous system axons. [22] "DNA methylation alterations of other genes, such as FOXP2 (which encodes a transcription factor required for language development) and HTR2A (which en- codes serotonin receptor 2A, one of the main target molecules of antipsychotic drugs), have also been reported in postmortem brain samples from schizophrenia patients." Other genes affected epigenetically in schizophrenia are 5HTR1A (which encodes serotonin receptor 1A), ST6GALNAC1 (encodes α-N-acetylgalactosaminide α- 2,6-sialyl transferase 1, an enzyme that transfers sialic acid to O-linked N-acetylgalactosamine residues, but its function has not been fully elucidated), S-COMT (which encodes the soluble form of COMT), GABRB2 (which encodes the β2 subunit of GABA(A) receptor), etc. [19]

2.3 Anxiety Disorders

Environmental factors that have been known to influence the epigenetic landscape of an organism to create anxiety-like behaviors can be grouped into three categories: 1. Prenatal and preconception factors, 2. Early-life factors, and 3. Juvenile and adult life factors. Here, prenatal and preconception factors are explained in detail.

Prenatal and preconception factors

Most animal studies examining effect of environmental factors during prenatal and preconception periods manipulated maternal and/or offspring exposure to nutritional deficiencies, stress or drugs of abuse. Maternal stress during pregnancy were indicated to disrupt neuroendocrine levels and negatively impact offspring development. Dams exposed to restraint stress during gestational days 8-21 have adolescent male and female offspring with modified CRH DNAM in hypothalamus and elevated anxiety-like behaviors. "Specifically, offspring show decreased entries into the center zone of the open field and decreased entries into the open arm of the elevated plus maze (EPM)."

The CRH gene codes for corticotropin-releasing hormone, which is responsible for stimulating the release of ACTH (Adrenocorticotropic hormone), which in turn binds to its receptor on the adrenal cortex and triggers the release of stress hormones such as cortisol. Prenatally stressed offspring have DNAM of three CpG sites in the CRH promoter region within the hypothalamus and have higher basal and post-acute-stress serum corticosterone levels. "Interestingly, prenatal stress exposure has sex-dependent effects in anxiety-like behaviors but not in Crh DNA methylation in hypothalamus" Anxiety behaviors were witnessed to a greater extent in male mice offspring rather than the female ones in the EPM test. [23]

The quality of nutrition during brain development can convert epigenetic machinery, dams were fed a diet high or low on omega-3 fatty acids and the consequential events on offspring were recorded. After weaning, the offspring were either continued on the same diet or were transitioned to a western diet until adulthood. Offspring that shifted from a low omega-3 diet to a western one showed more anxiety-like behaviors as indicated by decreased time spent in the open arms of the EPM compared to offspring raised and continued on the low omega-3 diet. Animals raised on a high omega-3 diet did not exhibit changes in their level of anxiety, suggesting that early-life exposure to a high omega-3 diet had protective effects on western diet-induced anxiety. "Animals raised and maintained on the low omega-3 diet had increased DNA methylation of the BDNF exon IV promoter within the frontal cortices that was further elevated in low omega-3 animals that transitioned to a western diet." [23] "BDNF has been shown to mediate synaptic plasticity, known to be critically important in learning and memory processes, in particular for the consolidation and extinction of fear

memory.” Altered expression may affect memory and extinction learning, which have its importance in anxiety-related behavior. [24]

Prenatal stress on the last 10 days of pregnancy elevates anxiety-like effects on the EPM in adult offspring coupled with decreased RLN mRNA expression and increased DNA methylation of the RLN distal promoter region. Reelin is an important protein that regulates brain development via cytoskeleton modifications. The correlation between decreased Reelin expression and anxiety-like behavior is accordant with previous human post-mortem studies that found decreased RLN mRNA in patients with schizophrenia and bipolar disorder with psychosis. Thus, increased DNAM of RLN distal promoter region may contribute to anxiety disorders. [24]

Paternal exposure to drugs of abuse led to increased basal anxiety levels in animal offspring in a sex dependent manner. To exemplify, adult sires exposed to cocaine prior to mating with a cocaine-naïve female produce male offspring with increased anxiety-like behavior suggested by greater latency to feed in a novelty-induced hypophagia task. Novelty-induced hypophagia (NIH) tasks are those which evaluates stress-induced anxiety in mouse models by measuring the latency of the mouse to approach food in a novel environment. This test uses food that is palatable and familiar which the mouse would approach without hesitation in a familiar environment. The experiment showed no behavioral effects on female offspring. Male offspring of cocaine exposed sires have increased expression of BDNF mRNA and greater association of histone 3 acetylation with BDNF promoters in the medial prefrontal cortex. This phenomenon is also observed in the sperm of cocaine sires. “In contrast, paternal alcohol exposure results in increased anxiety-like behaviors in female, but not male, offspring. However, paternal alcohol exposure increases DNA methylation of the paternally expressed 3 (PEG3) gene promoter region in offspring of both sexes, a methylation pattern also found in the sperm of sires. Deficiencies in PEG3 expression cause fetal growth restriction as well as anxiety-like and abnormal maternal behaviors.” [24]

Chapter 3

Epigenetic Therapy

In light of recent advances, it is evident that epigenetics plays a key role in mediating human behavior, and in the pathogenesis of virtually every type of mental illness. Consequently,

epigenetic biomarkers can be developed to diagnose diseases with an epigenetic background. Additionally, epigenetic therapy can be used to prevent those diseases using a diverse array of modalities, and measure progress of the respective treatment plans. Epigenetic therapy is the use of drugs or other epigenome-influencing modalities to treat medical conditions. Other diseases that are influenced by epigenetic mechanisms are cancer, heart disease and diabetes. Although epigenetic therapy for mental illnesses is often said to be limited to drugs, it can also include nutrition, psychotherapy, electroconvulsive therapy (ECT), and physical exercise, since all of these therapeutic methods impact the epigenome. The progress of these treatments can also be monitored by evaluating changes in the specific epigenetic profile. Some epigenetic mechanisms in the brain have also been identified which can be used as a guideline to create novel drugs targeting the related pathways.

In this section, I focus on HDAC and DNMT inhibition activity of currently available psychoactive drugs, and the prospects for other HDAC and DNMT inhibitors available in psychiatry. Various other epigenetic effects of the mentioned drugs have been discovered, but here I describe only the effects that have been proven to be related to HDAC and DNMT inhibitor activity. The methods and materials are explained in detail for Valproate and Lithium.

3 Epigenetic Effects of Contemporary Medicine

3.1. Histone Deacetylase Inhibitors

As outlined in this paper, chromatin remodeling events are involved with the development of psychiatric disorders. The nucleosome, being the fundamental unit of chromatin, allows open and closed states of transcription units to form based on histone tail modifications, including acetylation. “Altered HDAC expression affects learning, memory and mood related behaviors while different HDAC expression patterns have been associated with bipolar disorder, major depressive disorder, schizophrenia, and neurodegeneration.” [25] Class I and II HDACs are the main target for drugs since they are modified in psychiatric diseases.

Tricyclic antidepressants, monoamine oxidase inhibitors or selective serotonin-reuptake inhibitors are the main drug classes prescribed for treating mood disorders. Generally, the therapeutic effects of antidepressants are brought about only after chronic treatment and are not effective in all patients.

3.1.2 Antidepressants

Current pharmacological interventions using SSRIs and SNRIs for depression have significant limitations. For example, there is a substantial delay between the onset of pharmacological action and achievement of a therapeutic response. Additionally, even with appropriate pharmacological treatments, less than 50% of patients with depression achieve full remission (Little, 2009). It is therefore necessary to develop a new epigenetic drug with a faster onset and greater efficacy in the treatment of major depression. [26]

Imipramine

Imipramine belongs to a class of medications called tricyclic antidepressants (TCAs). It is mainly used in the treatment of depression and also anxiety and panic disorders. It works by restoring the balance of neurotransmitters such as norepinephrine in the brain. Imipramine and the other TCAs have decreased in use in recent decades, due to the introduction of the selective serotonin reuptake inhibitors (SSRIs), which have fewer side effects and are safer in overdose.

HDAC inhibition activity of Imipramine

Tsankova et al. administered chronic social defeat stress followed by chronic imipramine (a tricyclic antidepressant) to mice and studied adaptations at the levels of gene expression and chromatin remodeling of five brain-derived BDNF splice variant mRNAs (I-V) and their unique promoters in the hippocampus. Defeat stress induced lasting downregulation of BDNF transcripts III and IV and strongly increased repressive histone methylation at their corresponding promoters. Chronic imipramine reversed this downregulation and enhanced histone acetylation at these promoters. This hyperacetylation by chronic imipramine was associated with a selective downregulation of HDAC5. Moreover, viral-mediated HDAC5 overexpression in the hippocampus blocked imipramine's ability to reverse depression-like behavior. [27]

3.1.3 Antipsychotics

Valproate

Valproate, or valproic acid (VPA) is an anticonvulsant used in the treatment of epilepsy schizophrenia and bipolar disorder often in conjunction with atypical antipsychotics, in

patients resistant to lithium therapy. Valproic acid (N-dipropylacetic acid) is a simple, short and branched fatty acid chain, structurally unique compared to other antiepileptic drugs.

The acetylation of histone octamers is controlled by activities of histone acetylases (HATs) and HDACs. This remodeling of chromatin structure in promoter regions of genes associated with mood control is suggestive of the development of mood disorders. Various antidepressants and mood stabilizers have a direct or indirect effect on these epigenetic factors. Therefore, HDAC inhibitors have gained paramount attention in treating such disorders. Chronic VPA treatment in bipolar patients often diminishes mood swings to either the manic or depressive state. [28]

HDAC inhibition activity of VPA

"VPA is the prototypical HDAC inhibitor in major psychiatric disorders research." [29] Other antiepileptic drugs that are known to inhibit HDACs are topiramate, levetiracetam (its major carboxylic acid metabolite in humans: 2-pyrrolidinone-n-butyric acid), phenobarbital, phenytoin, "Considering that about 10 days of oral valproate administration are required before its mood-stabilizing effect becomes significant, and that this effect persists well after valproate cessation, it has been assumed that the mechanism of action of valproate involves not only acute and short-term biochemical effects but also changes at the genomic level." Studies found that valproate directly inhibits various HDACs at therapeutically relevant IC50 values, but its metabolites seem to be weaker HDAC inhibitors. [30]

Par-4 has been proposed to be a positive modulator of intracellular DRD2 (Dopamine Receptor D2) signaling, which is considered to be related to mood-associated behavior in experimental animals. When cultured mouse primary neurons at DIV 7 where treated with VPA, levels of Par-4 protein increased in a treatment time and concentration dependent fashion. A notable increase was observed after 6 hours of treatment, demonstrating the need for prolonged VPA treatment to boost Par-4 levels significantly. To examine transcription of Par-4, primary cultured hippocampal neurons at DIV 7 were treated with VPA for different durations, and Par-4 mRNA levels were measured using a quantitative real time PCR. A significant increase in Par-4 mRNA was recorded after 6 hours of treatment, which correlated with the time of protein production, signaling that the increase in Par-4 protein was caused by increased transcription of the Par-4 gene upon VPA treatment. To test whether the transcriptional changes were brought about by HDAC inhibition, the question of whether Par-

4 gene is affected by known HDAC inhibitors, like sodium butyrate (SB) and trichostatin A (TSA), was brought up. Cultured hippocampal and striatal neurons (DIV 7) were incubated with 1mM SB and 100 nM TSA for 30 hours, and Par-4 protein levels were inspected. The HDAC inhibitors effectively upregulated Par-4 production. It was also tested if other mood stabilizers, namely carbamazepine (CZM), lamotrigine (LTG) and LiCl, resemble these effects on Par-4. After 48 hours of treatment CZM, LTG and LiCl did not vitally increase Par-4 levels in cultured hippocampal neurons. Furthermore, to evaluate whether HDAC inhibition is the causal factor behind the upregulation of Par-4, shRNAs were constructed for specific HDACs and effective knock-down of HDACs were confirmed with semi-quantitative RT-PCR in Cath.-a-differentiated (CAD) cells, which showed weighty VPA-dependent induction of Par-4. After 5 days of differentiation and concurrent knockdown of HDACs, persistent induction of Par-4 was observed by the knockdown of HDAC4 and HDAC5, indicating that HDAC4 and HDAC5 is involved in the transcriptional activation of Par-4 upon chronic VPA treatment. [28]

In order to further investigate the effect of VPA on HDAC inhibition, the levels of acetylated histone H3 (ac-H3) and H4 (ac-H4) in the Par-4 promoter region were measured using cultured hippocampal neurons at DIV 7. After 24 hours of VPA treatment, an increase in ac-H3 and ac-H4 was measured by using chromatin immunoprecipitation (ChIP) with anti ac-H3 and ac-H4 antibodies. The recorded levels of increase were time and dose dependent, indicating that VPA exerts its Par-4 upregulation effects by way of increased H3 and H4 acetylation in the Par-4 promoter region. [28]

To evaluate whether these histone modifications occur in physiologic conditions, adult mice were injected with 100 mg/kg VPA intraperitoneally. Experimental groups were split into three groups, including control (CTL; saline for 7 days), acute (saline for 6 days+VPA on the last day), and chronic (VPA for 7 days). Mice were sacrificed at fixed time points after final injection and their hippocampal regions were collected for ChIP assay using anti ac-H3 and ac-H4 antibodies. A notable increase in ac-H4 and a less significant increase in ac-H4 were observed. However, the results were not as robust as seen in cultured neurons, which might be because of the complex experimental condition. Factors such as faster VPA clearance in the brain could play a role in the differing results. Additionally, the increase in ac-H3 and ac-H4 were seen after acute VPA treatment, at which point protein expression was mostly unaffected. This reveals more complex intermediary processes between chromatin remodeling and transcription upregulation. [28]

VPA changes chromatin structure through downregulation of chromatin associated proteins such as several members of Structural Maintenance of chromatin (SMC) proteins, SMC-associated proteins, DNA methyltransferase and heterochromatin proteins. This is related to decondensation of chromatin and increased sensitivity of DNA to nucleases. Downregulation of heterochromatin proteins is brought about by downstream effects of histone acetylation. VPA likely inhibits HDAC by binding to the catalytic center of the enzyme. Alongside inhibition of catalytic activity of class I HDAC, VPA triggers proteasomal degradation of HDAC2 in contrast to other HDAC inhibitors such as trichostatin A (TSA). VPA activity has often been compared to other HDAC inhibitors, like TSA, to confirm the HDAC inhibitory effect of VPA. In multiple cell lines, TSA and VPA induce expression of the endogenous reelin promoter, TSA being more efficient. In addition, VPA reverses down-regulation of RELN and GAD67 in wild type and heterozygous reeler mice. This phenomenon demystifies VPA's effect on mood disorders, since down-regulation of RELN and GAD67 in psychiatric patients is well documented. Moreover, VPA enhances the binding of ac-H3 to RELN and GLAD67 promoters, indicating that histone H3 covalent modifications moderate DNA demethylation in terminally differentiated neurons. VPA also induces replication-independent DNA demethylation in non-dividing cells. Together, this solidifies the concept that chromatin acetylation and DNA methylation is interrelated and that changes in histone acetylation bring about changes in DNA state. [28]

Both *in vitro* and *in vivo* models have showed that VPA's HDAC inhibitory activity regulates genes involved in neuronal survival or death. For instance, several HDAC inhibitors including VPA acts on class I HDACs, HDAC 1 and 3 which exceptionally increased the protein levels of Hypoxia Inducing Factor (HIF)-1 alpha and its transcriptional capacity in cell lines. VPA has also been suspected to increase neurotrophins expression, particularly BDNF and GDNF, providing an explanation for the neuroprotective and memory-enhancing properties of this drug. [28] "VPA modifies upregulation of BDNF protein by increasing levels of exon IV-containing BDNF mRNA in rat cortical neurons." [25] Furthermore, VPA increases the expression of 5-lipoxygenase (5-LOX) in neuronal cultures and, *in vivo*, VPA treatment enhances the acetylation of histone H3 and the 5-LOX in mouse hippocampus. [30] "VPA has been shown to induce differentiation and cell death of neuroblastoma cells, possibly associated with its HDACs inhibiting activity and with an activated Notch signaling cascade, as shown by increased levels of intracellular Notch-1 and Hes-1. In human neuroblastoma cells, p21(Cip1) is a target of induction of transcription and protein expression

for both lithium and VPA, while only VPA induces a concomitant increase of p27(Kip1) gene expression. Similarly, in medulloblastoma cells, VPA determines induction of histone (H3 and H4) hyperacetylation, activation of p21, and suppression of TP53, CDK4, and CMYC expression, with a strong correlation between induction of histone hyper-acetylation and regulation of pathways critical for maintaining growth inhibition and cell cycle arrest. Taken together, all these developmental effects of VPA lead to associate VPA-induced teratogenesis with histone deacetylase inhibition in vivo, possibly explaining why VPA causes spina bifida if taken during pregnancy." VPA's effect on HDACs upregulates neuron-specific genes via a neuron-located basic helix-loop-helix transcription factor, NeuroD, and it raises expression of cyclin D2 as well as survival genes, such as Bad and Bcl-2, leading to an increase in GABAergic neuron number, neurite outgrowth and neuronal differentiation. Neuroprotection from malonate toxicity in rat striatum *in vivo* is associated with an increase in histone acetylation and a decrease in Heat Shock Protein 70 (HSP70) and fos expression, whereas levels of Bcl-2 were unaffected. Further, VPA prevents the acetylation of histone H3 in the ischemic brain, generates HSP70 and halts ischemia-induced downregulation of phospho-Akt, in addition to blocking ischemia-induced upregulation of p53, inducible nitric oxide synthase and cyclooxygenase-2. Two studies provided insights on the influence of VPA on neuroprotective and transcription related genes. Activation of FGF-1B gene promoter, member of the fibroblast growth factor family, by VPA-related inhibition of HDACs and in glioblastoma cells was investigated. VPA treated cells showed activation of FGF-1B promoter through acetylation of histone H3 secondary to HDAC inhibition. (29) VPA also pacifies cerebral inflammation and perihematomal cell death after intracerebral hemorrhage by way of several mechanisms mediated by transcriptional activation following HDAC inhibition. In fact, by increasing histone H3 acetylation along with the translation and post-translational modification of pro-survival proteins pERK, pAKT, pCREB and HSP70, VPA upregulates the expression of anti-apoptotic genes such as bcl-2 and bcl-xL, while downregulating pro-apoptotic genes, such as bax. Equally important, VPA decreases the expression of inflammatory genes, mainly Fas-L, IL-6, MMP-9, MIP-1, MCP-1 and tPA. VPA effectively blocked seizure-induced neurogenesis, seemingly by HDAC inhibition and normalization of HDAC-dependent expression of genes in the epileptic dentate area, and it protected animal models from seizure-induced cognitive impairment in a hippocampus-dependent learning task. VPA's influence on histone acetylation has also been seen in a clinical setting, involving schizophrenic and bipolar patients, in which ac-H3 and ac-H4 levels from lymphocyte nuclear protein extracts significantly increased by VPA treatment.

[28] “The probable mechanisms of actions of VPA in depression are related with its effects on NMDA and AMPA glutamate receptors, induction of acetylation of brain H3, or modification of DNA methylation.” [25]

HDAC inhibition cannot explain all of the biochemical effects of VPA, however it can explain some of them: increase in Akt activation via activation of GSK-3, and an increased expression of β-catenin. “Additionally, while no data are available to associate HDAC inhibition with valproate-induced downregulation of brain PKCs, it should be noted that in epithelial cells, HDAC inhibitors have been shown to reduce the expression of PKC-a and PKC-e.” Besides increasing BDNF production, the neuroprotective effects of valproate have been attributed to the activation of Akt following inhibition of HDAC by valproate. HDAC inhibition by valproate has also been implicated in the *in vitro* neuroprotection from glutamatergic excitotoxicity: in cerebellar granule cell culture, valproate treatment causes a time-dependent elevation in acetylated H3, jointly with a decrease in the levels of the pro-apoptotic enzyme glyceraldehyde-3- phosphate dehydrogenase, whose gene is associated with H3. [30]

Lithium

Lithium (Li) is an element. Its name originates from "lithos," the Greek word for stone, because it is present in trace amounts in virtually all rocks. Lithium compounds, also known as lithium salts are the most widely prescribed mood stabilizers. Lithium is also the drug of choice when antidepressants are unable to treat major depressive disorder and bipolar disorder. In these disorders, lithium reduces the risk of suicide.

HDAC inhibition activity of Lithium

Similarly, Hobara et al. examined the effect of Li in class I and II HDACs (HDAC-2, -4, -5, -6, and -8) in lymphocytes from male C57BL/6 and BALB/c mice that underwent a forced swim test, a highly reliable model of depressive-like behavior. After 21 days of Li administration, expression of HDAC-2, -4, -5, -6, and -8 mRNA levels were comparable to those of stress-free control mice, suggesting a non-selective nature of Li as HDAC inhibitors. The region-specific changes in histone deacetylase expression and histone H3 acetylation

(AcH3) induced by Li in C57BL/6 mice was observed in another study. Treatment with Li notably increased AcH3 expression in nucleus accumbens and cingulate cortex. [29]

Evidence suggests that HDAC inhibitors can employ antimanic effects in different treatment regimens and animal models. Accordingly, results of a 2013 study by Stertz et al. show that treatment with Li was able to partly reverse AMPH-induced hyperactivity after repeated AMPH administration. Furthermore, AMPH increased HDAC activity in the PFC, which was partially reversed by Li. [31] In a study measuring neuroprotective effects of Li, HDAC1 siRNA and other HDAC inhibitors (phenylbutyrate, sodium butyrate or trichostatin A), the latter caused synergistic neuroprotection together with lithium in cerebellar granule cells of . Cotreatment of HDAC inhibitors with lithium seemed to have only slight effects on HDAC inhibitor-induced histone hyperacetylation. However, given that Western blotting measures genome-wide levels of histone acetylation in bulk chromatin and that HDAC inhibitors are likely to influence a small fraction of nucleosomes to regulate gene transcription, additional experiments are necessary to address the issue regarding the roles of HDAC-regulated genes in mediating the neuroprotective synergy. [32]

Mice with a mutation in the Clock gene (ClockDelta19) have been recognized as a model of mania that respond to lithium treatment. Arey found that Cholecystokinin (Cck) is a direct transcriptional target of CLOCK and levels of Cck are reduced in the ventral tegmental area (VTA) of ClockDelta19 mice. Selective knock-down of Cck expression via RNA interference (RNAi) in the VTA of wild type mice is enough to produce a manic-like phenotype. Moreover, chronic treatment with lithium rehabilitates Cck expression to near wild type and this increase is necessary for the therapeutic actions of lithium. This effect is partly achieved by an increase in histone acetylation at the Cck promoter by Li. [33]

In vitro studies have illustrated that lithium can induce autophagy, which is a major intracellular degradation system that utilizes lysosomes to degrade long-lived proteins and damaged organelles. As a crucial recycling system, autophagy is important for intracellular quality control, cell death and differentiation, tumor suppression, organism development, and other processes. Defects in autophagy have been frequently correlated with diseases as well as tumorigenesis, immune deficiency, and neurodegeneration. Lithium-induced autophagy promotes the disposal of toxic, long-lived, aggregate-prone proteins, such as mutant huntingtin, α-synuclein, and even pathological prions. Wu et al. discovered that Lithium significantly down-regulated HDAC1 at the translational level by targeting HDAC1 mRNA.

The authors of the study also showed that depletion of HDAC1 is essential for the neuroprotective effects of lithium and for the lithium-mediated degradation of mutant huntingtin through the autophagic pathway. [34]

3.1.4 Anxiolytic Drugs

Currently no anti-anxiety drugs are known to have HDAC inhibitor activity.

3.2 DNA Methyltransferases Inhibitors

3.2.1 Antidepressants

Amitriptyline, Imipramine and Paroxetine

Amitriptyline is part of a group of medicines called tricyclic antidepressants. They are thought to work by increasing the neurotransmitter serotonin in the brain. This can improve mood and can also change the way that nerves receive pain signals. Paroxetine is a selective serotonin reuptake inhibitor (SSRI) that increases the presence of serotonin in the brain and implements similar mood enhancing effects.

DNMT inhibition activity of Amitriptyline, Imipramine and Paroxetine

Zimmerman, Zschocke, Perisic, Yu, Holsboer, and Rein explored the specificity and the mode of action of the reported inhibitory effect of the tricyclic antidepressant amitriptyline on DNMT activity in primary astrocytes from the rat cortex. They found that the impact on DNMT was shared by another tricyclic antidepressant, imipramine, and by paroxetine, but not by venlafaxine or the mood stabilizers carbamazepine and valproic acid. DNMT activity in subventricular neural stem cells was refractory to the influence of antidepressants. Among the recognized DNMTs, antidepressants primarily targeted DNMT1. The attenuation of enzymatic DNMT1 activity was neither due to reduced DNMT1 expression nor due to direct drug interference. We tested putative DNMT1-inhibitory mechanisms and discovered that a known stimulator of DNMT1, the histone methyltransferase G9a, exhibited reduced protein levels and interactions with DNMT1 upon antidepressant exposure. Adding recombinant G9a completely reversed the antidepressant repressive effect on DNMT1 function.

3.2.2 Antipsychotics

Olanzapine

Olanzapine is a second-generation antipsychotic that is used to treat certain mood conditions such as schizophrenia and bipolar disorder. It can also be used in combination with other medication to treat depression. This medication can help to decrease hallucinations and help patients think more clearly and positively about themselves, feel less agitated, and take a more active part in everyday life. Olanzapine is linked to a class of drugs called atypical antipsychotics. It exerts its action primarily on dopamine and serotonin receptors. It acts as a receptor antagonist and restores normal dopamine and serotonin neurotransmission.

DNMT inhibition activity of Olanzapine

In a 2018 study, 21-day restraint stress was performed on rats and two different drugs were administered to detect epigenetic effects of the restraint stress and subsequently the drugs. The restraint stress procedure affected BDNF mRNA expression levels and the epigenetic regulation of BDNF promoter exon IV, HDAC5, DNMT1, and DNMT3a in the rat hippocampus. Chronic restraint stress also decreased the levels of total and exon IV BDNF mRNA, reduced acetylated histone H3 and increased MeCP2 at promoter IV of the BDNF gene, and increased the levels of HDAC5, DNMT1, and DNMT3a mRNA expression. Changes induced by 21-day restraint stress were significantly reversed by olanzapine administration. [35]

DNA methylation is reliant on the enzymatic function of several DNA methyltransferases, including DNMT1, DNMT3a, and DNMT3. Specifically, DNMT1 and DNMT3a are required for maintaining DNA methylation in central nervous system neurons in adult mice. Therefore, the impact of antipsychotic drugs on DNMT1 mRNA levels in the stress-free and stress conditions was investigated in the study by qRT-PCR. [35]

DNA methylation is involved with common and critical processes in mammals, including transposon silencing and genomic imprinting. The DNMT3a, DNMT3b, and DNMTl proteins are principally responsible for the establishment of genomic DNA methylation patterns and play important roles in human development, reproduction, and mental health. Boersma et al. [36] found that Prenatal Stress (PNS) decreased total BDNF expression and increased DNMT1 and DNMT-3a expression in the amygdala and hippocampus of PNS-exposed

offspring. Another study showed that clozapine reduced stress-induced elevations in DNMT1 binding to BDNF promoters in the frontal cortex of PNS-exposed offspring [37]. Although the method of stress induction used in the 2018 study differed from those used in these previous studies, 21 days restraint stress also elevated the levels of DNMT1 and DNMT3a mRNA expression in the rat hippocampus; however, these increases were subsequently reversed by olanzapine.

Clozapine

Clozapine, sold under the brand name Clozaril among others, is an atypical antipsychotic drug. It is primarily used for schizophrenia that does not improve following the use of other antipsychotic medications. In those with schizophrenia and schizoaffective disorder it may reduce the rate of suicidal behavior and hallucinations. It is more effective than typical antipsychotics, especially in those who are treatment-resistant. It is used via mouth, or by injection into a muscle. It works by blocking receptors in the brain for several neurotransmitters including dopamine type 4 receptors, serotonin type 2 receptors, norepinephrine receptors, acetylcholine receptors, and histamine receptors.

DNMT inhibition activity of Clozapine

Dong, Tueting, Matrisciano, Grayson and Guidotti have reported that mice born from dams stressed during pregnancy (PRS mice), in adulthood, have behavioral deficits reminiscent of behaviors observed in SZ and bipolar (BP) disorder patients. Furthermore, they have shown that the frontal cortex and hippocampus of adult PRS mice, like that of postmortem chronic SZ patients, are characterized by elevations in DNMT1, ten-eleven methylcytosine dioxygenase 1 (TET1) and exhibit an enrichment of 5-methylcytosine (5MC) and 5-hydroxymethylcytosine (5HMC) at neocortical GABAergic and glutamatergic gene promoters. In a 2016 study, they showed that the behavioral deficits and the increased 5MC and 5HMC at GAD1, RELN and BDNF promoters and the reduced expression of the mRNAs and proteins corresponding to these genes in frontal cortex of adult PRS mice is reversed by treatment with clozapine. Interestingly, clozapine had no effect on either the behavior, promoter methylation or the expression of these mRNAs and proteins when given to offspring of non-stressed pregnant mice. Behavioral tests were used to measure locomotor activity and social interaction in the mice. [37]

3.2.3 Anxiolytic drugs

No anxiolytic drugs have been known to have DNMT inhibitor activity.

3.2 Future Perspectives

3.2.1 HDAC Inhibitors with Psychoactive Properties

MS-275

Chronic social defeat stress in mice causes a temporary decrease, followed by a persistent increase, in levels of acetylated histone H3 in the nucleus accumbens, an important limbic brain region that plays a central role in the brain's reward circuit. This persistent increase in H3 acetylation is related to decreased levels of histone deacetylase 2 (HDAC2) in the nucleus accumbens. Similar effects were seen in the nucleus accumbens of depressed humans studied postmortem. These changes in H3 acetylation and HDAC2 expression create long-lasting positive neuronal adaptations, since infusion of HDAC inhibitors into the nucleus accumbens, which increases histone acetylation, exerts potent antidepressant-like effects in the social defeat paradigm and other behavioral assays. In addition, HDAC inhibitor [N-(2-aminophenyl)-4-[N-(pyridine-3-ylmethoxy-carbonyl)aminomethyl]benzamide (MS-275)] infusion reverses the effects of chronic defeat stress on global patterns of gene expression in the nucleus accumbens, as determined by microarray analysis, with notable similarities to the effects of the standard antidepressant fluoxetine. [38] MS-275 can modulate RELN and GAD67 gene expression. Simonini et al. reported the hyperacetylation potential of MS-275 treatment on lysine residues in histone H3 tails. The inhibitory effect of MS-275 on Ac-H3 is greater than that of VPA and is brain-region specific. Thus, MS- 275 had greater efficacy in mouse frontal cortex while fourfold to tenfold higher doses were needed to increase Ac-H3 in hippocampus and striatum, respectively. Moreover, MS-275 moderated activation of nucleosomal H3 acetylation can regulate the interaction of GAD67 and RELN promoters with histone 3. [25]

Vorinostat

Vorinostat also known as suberanilohydroxamic acid (SAHA) is a member of a larger class of compounds that inhibit histone deacetylases (HDAC). Around 30% of schizophrenics are resistant to antipsychotic therapy. The HDAC inhibitors, vorinostat and MS-275 increased

mGlu2 and mGlu3 expression in mouse frontal cortex by increasing H3 acetylation in the promoter regions of the Glu2 and Glu3 genes. [39] Schizophrenia is linked with diminished glutamatergic transmission and compounds that activate the mGlu 2 receptor are of interest as antipsychotics. Furthermore, up-regulation of mGlu2 expression might overcome the resistance to antipsychotic drug treatment in patients with schizophrenia. [25]

IN14

A recent study evaluated the toxicity, the capacity to inhibit HDAC activity and antidepressant-like activity of three recently described class I HDAC inhibitors IN01, IN04 and IN14, using A. salina toxicity test, in vitro fluorometric HDAC activity assay and forced-swimming test, respectively. The data showed that IN14 possesses a better profile than the other two. Therefore, the pro-cognitive and antidepressant effects of IN14 were investigated. In the forced-swimming test model of depression, intraperitoneal administration of IN14 (100 mg/Kg/day) for five days decreased immobility, a putative marker of behavioral despair, significantly more than tricyclic antidepressant desipramine, while also enhancing climbing behavior, a putative marker of motivational behavior. These results suggest that novel HDAC class I inhibitor IN14 may be a promising new antidepressant with low toxicity and encourages further studies on this compound. [40]

Sodium Butyrate

BDNF gene is involved with the etiology of depression. Chronic stress causes down-regulation of BDNF expression while antidepressant therapy activates BDNF expression. The expression of the BDNF promoter of exon 4 (BDNF P4) influences the expression of total brain BDNF and is regulated by DNA methylation and histone modification mechanisms. [41] Sodium Butyrate modifies upregulation of BDNF protein by increasing levels of exon IV-containing BDNF mRNA in rat cortical neurons. By activating promoter IV, NaBu indicates that it is inhibiting HDAC1. [42]

The HDAC inhibitor sodium butyrate exerted antidepressant effects in a depression mouse model, and it could enhance memory function in an Alzheimer's disease mouse model. [43]

If chromatin condensation occurs due to the exposure of stress by the aberrant expression and activity of HDACs, then the binding of transcription factors, such as phospho-CREB, to their binding site may be impeded, even if the levels of transcription factors are increased by

antidepressant treatment. If the accessibility of the transcription factor to their binding site is decreased via chromatin condensation in major depression, the efficacy of antidepressants may fail to be realized. In this situation, a combined administration of an antidepressant and an HDAC inhibitor could be helpful in treatment of antidepressant-resistant patients with major depression. [26]

3.2.2 Development of Selective HDAC Inhibitors

HDAC inhibitors have been significantly researched on as psychiatric drugs. However, it has been observed that these broad HDAC inhibitors have very common side effects and toxicities such as thrombocytopenia, diarrhea, and fatigue, which considerably limit their therapeutic applications in patients.

These side effects occur due to influences on many cellular mechanisms including cytotoxicity, cell cycle control and immune modulation. [43] The development of isozyme-selective HDAC inhibitors might be an important gateway to achieving enhanced therapeutic efficacy and reduced toxicity. [26]

In an effort to discover existing scaffolds with HDAC inhibitory activity, a drug library approved by the US Food and Drug Administration and a National Institutes of Health Clinical Collection compound library in HDAC enzymatic assays were screened. Ebselen, a clinically safe compound, was identified as a weak inhibitor of several HDACs, including HDAC1, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, and HDAC9 with half maximal inhibitory concentrations approximately single digit of μM. Two ebselen analogs, ebselen oxide and ebsulfur (a diselenide analog of ebselen), also inhibited these HDACs, but with improved potencies on HDAC8. Benzisothiazol, the core structure of ebsulfur, specifically inhibited HDAC6 at a single digit of μM but had no inhibition on other HDACs. Further efforts on structure–activity relationship based on the core structure of ebsulfur led to the discovery of a novel class of effective and selective HDAC6 inhibitors with RBC-2008 as the lead compound with single-digit nM potency. This class of HDAC inhibitor features a novel pharmacophore with an ebsulfur scaffold selectively targeting HDAC6. [44]

In a 2013 study the impact of chronic, systemic treatment with Compound 60 (Cpd-60), a slow-binding, benzamide-based inhibitor of the class I histone deacetylase (HDAC) family members, HDAC1 and HDAC2, in mood-related behavioral assays responsive to clinically effective drugs were examined in wild type mice. Cpd-60 treatment for one week was linked with attenuated locomotor activity following acute amphetamine challenge.

Furthermore, treated mice demonstrated decreased immobility in the forced swim test. These changes are in alignment with established effects of clinical mood stabilizers and antidepressants, respectively. Whole-genome expression profiling of specific brain regions (prefrontal cortex, nucleus accumbens, hippocampus) from mice treated with Cpd-60 discovered gene expression changes, including a small subset of transcripts that significantly overlapped those previously reported in lithium-treated mice. HDAC inhibition in brain was confirmed by increased histone acetylation both globally and, using chromatin immunoprecipitation, at the promoter regions of upregulated transcripts, an observation consistent with in vivo engagement of HDAC targets. [45]

It has been observed in a study that two compounds, ACY-738 and ACY-775, inhibit HDAC6 with low nanomolar potency and a selectivity of 60- to 1500-fold over class I HDACs. In contrast to tubastatin A, a reference HDAC6 inhibitor with similar effectiveness and peripheral activity, but more limited brain bioavailability, ACY-738 and ACY-775 induce dramatic increases in α-tubulin acetylation in brain and stimulate mouse exploratory behaviors in novel, but not familiar environments. Further, it has been shown that ACY-738 and ACY-775 share the antidepressant-like properties of other HDAC inhibitors, such as SAHA and MS-275, in the tail suspension test and social defeat paradigm. These features of ACY-738 and ACY-775 are directly attributable to the inhibition of HDAC6 expressed centrally, as they are fully abrogated in mice with a neural-specific loss of function of HDAC6. Furthermore, administered in combination, a behaviorally inactive dose of ACY-738 markedly improves the anti-immobility activity of a sub-active dose of the selective serotonin reuptake inhibitor citalopram. The results validate new isoform-selective probes for in vivo pharmacological studies of HDAC6 in the central nervous system and reinforce the viability of this HDAC isoform as a potential target for antidepressant development. [46]

3.2.3 DNMT Inhibitors with Psychoactive Properties

5-azaD

we have investigated the effects induced by systemic or intra-hippocampal administration of inhibitors of DNA methyltransferase (DNMT) in rats submitted to a range of behavioural tests. Systemic administration of DNMT inhibitors induced a dose-dependent antidepressant-like effect, which was followed by decreased DNA methylation and increased brain-derived neurotrophic factor (BDNF) levels in the hippocampus. Hippocampal inhibition of DNA

methylation induced similar behavioral effects. No treatment induced any locomotor effects in the OFT. Antidepressant-like effects of 5-azaD were confirmed in mice submitted to the FST or the tail suspension test. [47]

Zebularine

CNS Inflammation has been linked to depression and fatigue. [48]

A study was designed to investigate a link between DNA methylation and neuroinflammation. The authors hypothesized that central administration of lipopolysaccharide (LPS) and the DNMT inhibitor zebularine in adult mice would cause an exacerbated neuroinflammatory response, in that there would be exaggerated sickness behavior and a pro-inflammatory gene expression profile that would be associated with DNA demethylation of Il-1β. Surprisingly, rather than intensifying the LPS-induced sickness response and increasing inflammation in the brain, zebularine led to a quicker recovery of LPS-induced sickness behavior and decreased cytokine gene expression. Results of the Fluidigm analysis indicated that DNMT inhibition formulated an anti-inflammatory profile in microglia at 4 h, as many inflammatory genes were decreased by zebularine alone or in combination with LPS (Il-1β, Tnf, etc.). [49]

The findings using the Fluidigm analysis indicated differential expression of epigenetic regulators and sensome genes in microglia with central zebularine and LPS. [49] Nearly all Hdacs analyzed were affected by LPS, something that has been demonstrated elsewhere [50]. Further, Hdac1's notable downregulation with zebularine supports the dependency of HDAC regulation on DNA methylation [51].

3.2.4 Development of Selective DNMT Inhibitors

Similar to broad (pan) HDAC inhibitors, pan DNMT inhibitors also elicit a number of disturbing side effects.

According to Pan Xu, Guang Hu, Cheng Luo, and Zhongjie Liang, the resolved autoinhibitory structures of DNMTs afford a novel strategy for targeting the protein-protein interface involved in the autoinhibitory interactions. The molecular mechanism underlying the conformational transitions may also shed new light on the design of allosteric inhibitors.

Both strategies would likely produce inhibitors with more selectivity compared to nucleotide derivatives. [52] More selective DNMT inhibitors are the ultimate goal for providing patients with epigenetic drug therapy.

Chapter 4

Discussion

Mental Health impacts virtually every area of people's lives, including physical health, productivity, learning, socializing, parenting, and even survivability. This extensive review reconsolidates the accumulating evidence showing the contribution of epigenetic pathways to psychiatric disorders, portrays how traditional psychoactive drugs have the ability to change the epigenetic landscape, and develops a comprehensive list of prospective epigenetic drugs based on their HDAC and DNMT inhibitory activities. Awareness and resource building regarding mental illnesses should improve upon the realization of how environmental factors can act as a foundation for these disorders. The plethora of knowledge illustrating the importance of stressors like poverty, financial problems, lack of neighborhood facilities/safety, parenting, intergenerational trauma, prenatal, postnatal and adult nutrition, etc. open up wider political, economic and social discourse. Understanding the mechanisms behind epigenetic development of mental illnesses as well as other diseases provides the most robust evidence of socioeconomic causes of ill health. As such, this knowledge might steer our societies and thus governments towards systemic changes like spending more in social benefits, working towards a society free of factors that contribute to mental illness, making the workplace more inclusive to diversity to beat stress and thus improving the overall wellbeing of humankind.

Meanwhile, this decade-long effort in delineating the role of epigenetics in mental health have unlocked opportunities for alternative, potentially better therapeutics. The findings presented in this review allow an insight into epigenetic effects of contemporary psychiatric drugs, which may aid in developing novel drugs targeting the respective epigenetic reactions. As previously mentioned, the resistance to contemporary psychiatric medicine, their side effects and a high relapse rate pose as significant challenges in the field of psychiatry. Thus, the development of drugs that reverse the root molecular aberrations is a promising step.

Prospective HDAC and DNMT inhibitors covered in this review provides a starter for efforts in epigenetic drug development, and examples of ways in which the drugs can be made more selective and thus less toxic and side-effect laden. Epigenetic effects of current and prospective drugs that are not proved to be mediated by HDAC and DNMT inhibition are left out of this review, studying the exact mechanism behind these gene expression states altered by way of medication is a necessary endeavour that future research can focus on. Further potential areas of research are the development of more isozyme-selective HDAC and DNMT inhibitors, as well as other epigenetic drugs that are specific in nature.

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Appendix

In this paper, the terms “mental illness” and “psychiatric disorder” have been used interchangeably since the term “mental illness” was initially formulated to destigmatize psychiatric disorders.