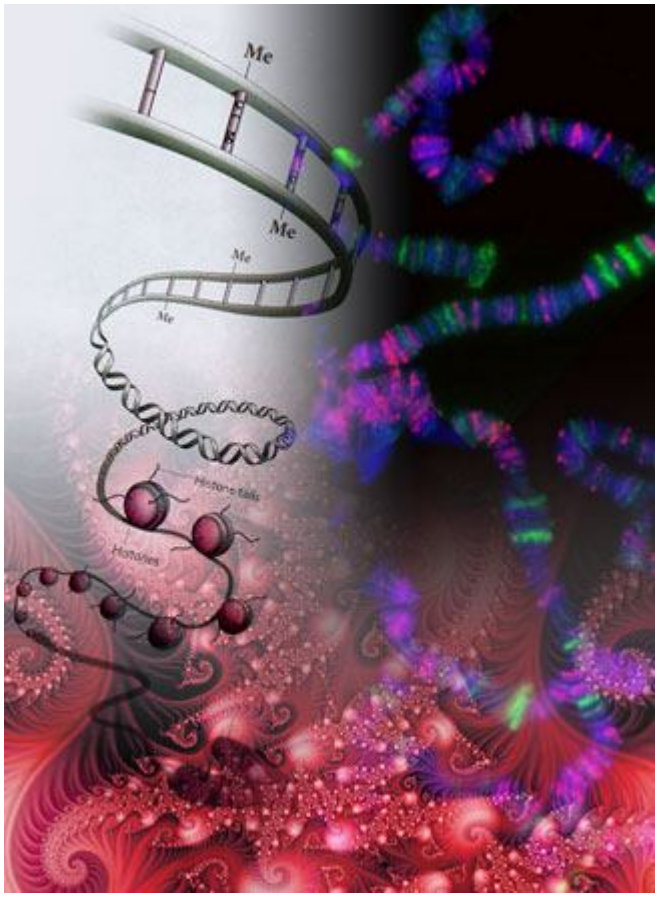
Epigenetic involvement in psychiatric disorders and psychiatric drugs



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1. Introduction

1.1 General introduction

For a long time scientists have sought the origin of psychiatric diseases in genetic patterns. Genome-wide association studies delivered a wide range of candidate genes for depression, schizophrenia and bipolar disorder, such as BDNF, GR, COMT, DAOA, 5HTT and DRD2. However, as increasingly more association studies attempted to pinpoint the etiology of such disorders to genetic polymorphisms, it became clear that the make-up of our genes does not comprehend the full story. Findings are often inconsistent and in some cases, genotype per se does not affect disease-risk, but modifies the deleterious influence of environmental stressors on the risk of developing disease. From epidemiological research it has long been known that environmental factors and significant life events increase the likelihood of developing psychiatric symptoms. The incidence of traumatic events and the inconsistent correlation with candidate genes, indicate that genes and environment interact, and synergistically cause psychiatric behavior. In the field of epigenetics, genes and environment converge, providing better insight into mental disorders.

In 1975, C.H. Waddington revolutionized the field of developmental biology by merging the theories of genetics and epigenesis. While previously developmental mechanisms and genetics were studied separately, Waddington focused on the interplay between genetic factors and embryological growth and differentiation, to produce the full-grown features of an organism. He coined the term "epigenetics" to describe this novel area, and soon it was widely in use (Van 2002). After many years of research "epigenetics" is now used to describe "the study of stable alterations in gene expression potential that arise during development and cell proliferation". Epigenetic processes underlie cellular development and differentiation, but they can also occur in mature mammals, either at random or under influence of the environment (Jaenisch and Bird 2003). An increasing amount of research is dedicated to understanding environmental influences on genetic expression, to unravel the etiology of disease. One area in which it is of special importance is the field of psychiatry.

In this paper, research on the role of epigenetic mechanisms in psychiatry will be reviewed. In addition, a chapter is dedicated to the current status of epigenetic medication for psychiatric disorders. Several traditional drugs have been found to alter epigenomal structure, and in a variety of animal studies, experimental compounds with epigenetic targets are investigated. The aim of this review is twofold; to get better insight into the involvement of epigenetic mechanisms in psychiatric disorders, and to establish the future prospects for epigenetic psychiatric treatment. Understanding the role of epigenetic mechanisms in psychiatry is of key importance for psychiatric treatment, since it may be reversible under the influence of social factors, drugs or behavioral interventions. In addition, a deep understanding can lead to development of better medication, and better comprehension of the medication currently in use.

1.2 The relevance of epigenetics in depression and anxiety disorders

Major depressive disorder is a chronic disease that severely affects quality of life. It has a high prevalence; 15% of US citizens are afflicted with depression (Marcotte, Wilcox-Gok et al. 1999). It is primarily characterized by low mood and self-esteem, loss of energy and interest, and an inability to experience pleasure. Major depressive disorder is frequently comorbid with anxiety disorders, such as panic-, generalized anxiety- and posttraumatic-stress disorder. Depression and anxiety disorders have shared etiology, since both often originate in response to stressful life events, and adverse

childhood experiences such as abuse and neglect increase the likelihood of developing symptoms (Widom, DuMont et al. 2007). Importantly, the genotype of patients can modulate the impact of such an event, influencing the development of these disorders. For example, serotonin transporter (5HTT) genotype affects the influence of childhood adversity and life events on developing post-traumatic stress disorder, and a polymorphism of the brain-derived neurotrophic factor (BDNF) gene alters the effect of childhood sexual abuse on severity of adult depressive symptoms (Aguilera, Arias et al. 2009;Beach, Brody et al. 2010). 5HTT and BDNF are, among many others, candidate genes for major depressive and anxiety disorders. Furthermore, prenatal factors may predispose to these disorders, as maternal stress during pregnancy increases risk of adult depression (Allen, Lewinson et al. 1998) and has been associated with low birth weight, which in turn correlates with female adolescent depression (Costello, Worthman et al. 2007). This is thought to be mediated by prenatal overexposure to glucocorticoids, coinciding with hypotheses of an overly sensitive HPA-axis in depression and anxiety (Cameron 2006). Thus the intrauterine environment may modulate epigenetic mechanisms, introducing vulnerability to a psychiatric phenotype later in life.

1.3 The relevance of epigenetics in psychotic disorders

Schizophrenia and bipolar disorder severely disrupt quality of life and are widely investigated in psychiatric research. Despite many attempts to unravel a molecular cause, it remains unclear how these disorders commence. Schizophrenia affects approximately 1% of the population and is characterized by psychotic episodes with hallucinations and delusions, and by depression-like symptoms such as deficits in memory and concentration and lack of emotion. Bipolar disorder has a similar prevalence, and is defined by periods of mania, with an extremely elevated mood and excessive risk-taking, alternated with episodes of depression. In manic periods psychotic symptoms can occur, similar to those that are experienced in schizophrenia. To some extent the clinical characteristics of schizophrenia and bipolar disorder overlap, as do the molecular mechanisms that underlie them. Many of the same chromosomal loci are found in genetic linkage studies, and in association research candidate genes are found to overlap, such as COMT, DAOA, DISC1 and BDNF (Ivleva, Thaker et al. 2008). Several lines of research support the involvement of epigenetic mechanisms in the etiology of schizophrenia and bipolar disorder. The development of psychotic symptoms is generally thought to correlate with adverse life experiences, suggesting a gene-environment interaction. For example, people with bipolar disorder frequently experienced childhood trauma, affecting the age of onset and severity of the disease. This is mediated by the BDNF genotype of bipolar patients, which moderates the harmful effects of stressful life events on their depressive episodes, further indicating a role for epigenetic mechanisms (Etain, Henry et al. 2008). A large body of epidemiological findings in schizophrenia patients may also be explained by epigenetic modulation. Maternal stress during gestation elevates disease-risk and it is associated with emotional and cognitive deficits early in life. In addition, monozygotic twin discordance has been consistently reported in psychotic disorders, and differences in epigenetic structure may explain why the same genotype can lead to opposing phenotypes. Other environmental factors that correlate with schizophrenia include migration, urban rearing environment, paternal age, and fetal hypoxia and folate deficiency. Hypoxia is thought to have long-lasting effects on dopamine neurotransmission, possibly mediated by epigenetic mechanisms. Paternal age may affect the DNA methylation pattern of sex cells and introduce altered genetic expression in offspring of older fathers. DNA methylation is also influenced by folate deficiency, since folate is a precursor to S-

Adenosyl-Methionine, a methyl-donor important in DNA methylation (Van, Rutten et al. 2008). Taken together these lines of evidence provide a solid basis for epigenetic research in psychotic disorders.

1.4 Search strategy

To achieve this review a literature search was conducted in PubMed, with a publication date between January 1st 2003 and July 20th 2010. The following search strategy was run for the chapter Epigenetic psychiatric mechanisms: *(depressive disorder OR anxiety OR psychosis OR schizophrenia OR bipolar disorder) AND (epigenetic OR DNA methylation OR histone OR chromatin),* and for the chapter Epigenetic psychiatric drugs: *(antidepressant OR antipsychotic) AND (epigenetic OR DNA methylation OR histone OR chromatin) ; (HDAC inhibitor OR DNMT inhibitor) AND (depressive disorder OR anxiety OR psychosis OR schizophrenia OR bipolar disorder) ; lithium AND (depressive disorder OR anxiety OR psychosis OR schizophrenia OR bipolar disorder) AND (epigenetic OR methylation OR histone OR chromatin).* Also cross references were included. The resulting papers were each judged for relevance to the research questions based on the journal, title and abstract. Search results of different entry terms displayed overlap, as many studies investigate both a psychiatric disorder and the effects of a psychiatric drug or multiple drugs. Consequently these are cited repeatedly in this review. A final selection of 123 papers was included; 35 on epigenetic mechanisms in depression and anxiety, 23 on psychotic disorders, 10 on antidepressants, 7 on antipsychotics, 41 on HDAC inhibitors and 7 on other drugs.

2. Epigenetics

2.1 The epigenome

Within the nucleus of a cell, the DNA sequence lies wrapped around histone proteins. The complex of DNA, histones and non-histone proteins, such as scaffold and polycomb proteins, forms a highly condensed structure called chromatin. The basic unit of chromatin is the nucleosome, which consists of a histone octamer with a standard length of 147 base pairs of DNA wound around it. This results in a five- to tenfold volume reduction (Kornberg 1974). A histone octamer exists of two copies of each of the core histone proteins H2A, H2B, H3 and H4. Histones are small proteins consisting of a globular domain and a charged NH^2-terminus, or "histone tail", that protrudes from the nucleosome (Jenuwein and Allis 2001). Each nucleosome is connected to the next by a short segment of linker DNA of 10-80 base pairs long, to which a histone H1 protein binds. The string of nucleosomes is folded into a strongly condensed fiber of 30 nanometers in diameter, bringing about a compaction of roughly 50-fold. Further details on the folding of the nucleosomestring are still elusive, and in particular the higher order structure of chromatin is not yet fully understood.

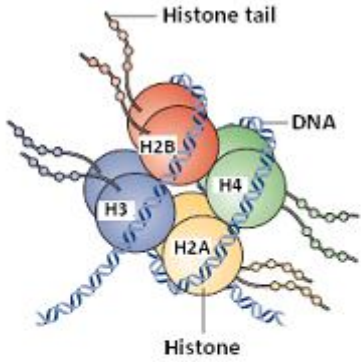

Figure 1: The nucleosome
Schematic representation of the nucleosome with DNA wrapped around histone proteins H2A, H2B, H3 and H4. Adapted from Tsankova, 2007.

Within the chromatin structure, the DNA sequence is methylated at certain nucleotides. The methylation pattern of DNA together with the chromatin structure is referred to as the epigenome, and regulates the availability of genes to transcription factors.

2.2 Gene expression

Every cell in an organism contains identical DNA, even though many different cell types exist. Without altering the genetic code, diversifications in the patterns of gene expression give rise to differentiating tissues. Epigenetic mechanisms drive this cellular development and differentiation, and are therefore at the heart of genetic expression. At the start of embryological development, cells are pluripotent, after which they can specialize into many cell types. Epigenetic processes activate some genes and inhibit others and genetic expression becomes increasingly more defined, restricting the properties of the cell. When cells are fully differentiated, they have accumulated epigenetic marks that set them apart from cells of other types. Their epigenetic profile is maintained during mitosis, aiming to assure the continuity of cellular traits. In neurons, mitosis does not occur, so any modifications are sustained within individual cells. As in other cells, epigenetic mechanisms are essential to the development of the nervous system. The epigenetic machinery drives both embryonic and postnatal neural development. It is involved in neurogenesis (Kuwabara, Hsieh et al. 2004;Zhao, Ueba, Christie et al. 2003), neuronal differentiation, cell fate specification (Fan, Beard et al. 2001) and development of dendrites (Wu, Lessard et al. 2007).

The course of development of the epigenetic profile is influenced by environmental factors in utero. In different species environmental factors such as temperature or the presence of predators, have been shown to affect the phenotype of the offspring. In humans and mice, the physiology of the baby is affected by the nutritional state of the mother. Maternal stress in rats also alters the phenotype of their offspring (Dolinoy, Weidman et al. 2007). According to Bateson and colleagues,

such epigenetic developmental plasticity may involve preparing the offspring for the type of environment they are likely to live in (Bateson, Barker et al. 2004).

It was previously thought that epigenetic properties that arise during development, will always remain the same. However, it is now clear that these mechanisms are dynamically regulated. Epigenetic remodeling takes place throughout adult life, under the influence of environmental factors such as nutrition, drugs, and chemical, physical and psychosocial factors (Dolinoy, Weidman et al. 2007;Sutherland and Costa 2003). When epigenetic regulation is aberrant, it can modify susceptibility to disease. It has been implicated in imprinting disorders such as Prader-Willi syndrome and Angelman syndrome, Alzheimer's disease, asthma and autism (Dolinoy, Weidman et al. 2007).
Epigenetic modifications are also a large contributor to cancer, as they serve as a surrogate for genetic mutations and are often found in cancerous cells (Feinberg and Tycko 2004;Iacobuzio-Donahue 2009) In addition, psychiatric disorders such as depression and schizophrenia appear to be modulated by epigenetic alterations, as is the focus of this review (Rutten and Mill 2009). Since environmental factors are known to contribute to these diseases, epigenetic regulation may be the field where genes and environment interact, to produce a psychiatric phenotype.

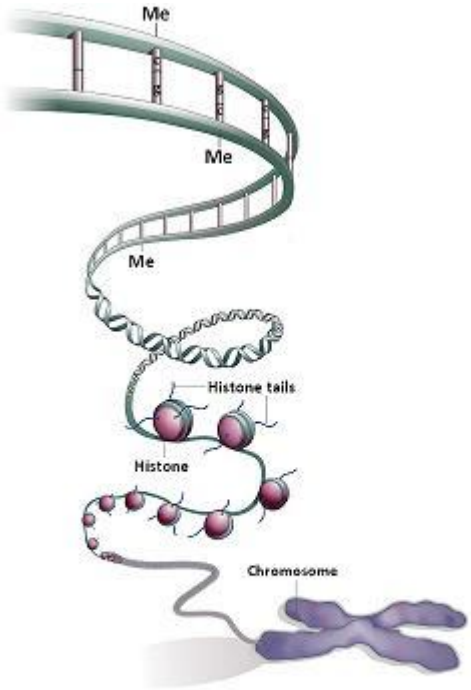

Figure 1: The epigenome
Unfolded chromosome with histones, histone tails and DNA methylation sites (Me). Adapted from Qiu, 2006.

Since the epigenetic pattern of a cell is mitotically heritable, it is possible that environmental factors in certain stages of life produce long-term phenotypic changes. Epigenetic mechanisms are particularly prone to deregulation during embryological growth, neonatal development, puberty and old age. It is most vulnerable to modification during embryogenesis, when epigenetic signalling is highly active in cell growth and differentiation. When environmental factors influence the developing epigenome in the prenatal period, it may cause vulnerability to adult chronic diseases (Dolinoy, Weidman et al. 2007;Rutten and Mill 2009). Interestingly, several studies suggest that the epigenetic profile is also meiotically heritable (Anway, Cupp et al. 2005;Rakyan, Chong et al. 2003). If this is the case, epigenetic effects of environmental and psychosocial influences can have an impact on the next generation, even before conception.

2.3 Epigenetic mechanisms

There are two major types of epigenetic mechanisms that regulate gene expression in the nervous system. The first is posttranslational modification of histones and the second is DNA methylation. Other types of epigenetic mechanisms exist, such as RNA-based mechanisms and polycomb protein-mediated chromatin remodeling (Gibney and Nolan 2010;Goodrich and Kugel 200). However, these are yet to be characterized in the brain and thus of less relevance to this review.

Histone modifications and DNA methylation interact to modify the structure of the epigenome, determining the accessibility of DNA to transcription. When chromatin is in an activated and accessible state, it is called euchromatin. In contrast, heterochromatin is condensed and

inactivated, and does not allow transcription. In reality, not only these two states exist, but also a continuum in-between (Tsankova, Renthal et al. 2007).

Posttranslational modification of histones

Posttranslational histone modifications take place at the histone tail of the nucleosome. The most common are the small covalent modifications acetylation, methylation and phosphorylation. Less frequent modifications include ubiquitination, sumoylation, ADP ribosylation and deimination. Their effects on transcriptional activity are not yet well understood (Gibney and Nolan 2010;Tsankova, Renthal et al. 2007).

Histone acetylation

Histone acetylation occurs most frequently on the lysine residues at H3 and H4 of the NH^2-terminal, though it can also take place at other histones and in the globular domains. This dynamic process is controlled by specific enzymes that either add or remove the acetyl group. Histone acetyl transferases (HATs) catalyze the addition of acetyl groups. Over a dozen HATs have been identified, of which some can also acetylate non-histone proteins such as transcription factors Several transcription factors even contain intrinsic HAT activity to activate gene activity. Histone deacetylases (HDACs) catalyze the removal of acetyl groups from histone proteins, as well as non-histone proteins among which p53, Sp1 and CREB (Sleiman, Basso et al. 2009). There are four classes of HDACs, of which class I and II are the most relevant to this review, as the first is expressed throughout brain and body, and the second primarily in the brain, heart and muscle. Histone hyperacetylation is associated with decondensation of chromatin and an increase in gene activity, whereas hypoacetylation correlates with repression of chromatin and a decrease in gene activity. The balance between the opposing activity of HATs and HDACs on histone tails, is an important factor in regulating transcription. Defects in this interplay can lead to neurodegenerative diseases as well as many types of cancer (Hahnen, Hauke et al. 2008;Hassa, Haenni et al. 2006).

Histone methylation

Histone methylation occurs on lysine residues of the histone tail. This modification can exist in a mono-, di-, or trimethylated state, each with a different effect on transcriptional activity through distinct coregulators (Kouzarides 2007). Interestingly, methylation of different lysine residues can achieve opposite effects on gene activity; it can cause both repression and activation depending on which lysine residue of the histone tail is methylated. In psychiatric epigenetics, focus lies on methylation of histone H3. Trimethylation of histone H3 at lysine 4 (H3K4) is important for transcriptional activation, while the same methylation state is repressive at H3K9 and H3K27, and dimethylation of H3K9 and H3K27 are repressive as well (Ebert, Lein et al. 2006;Kouzarides 2007;Mosammaparast and Shi 2010).

As with acetylation, methylation is regulated by specific enzymes that can exert effects on histone as well as on non-histone proteins. Histone methyltransferases (HMTs) add methyl groups to lysine residues and histone demethylases (HMDs) remove these methyl groups. There are distinct HMTs and HDMs for various lysine residues, each with specific abilities to catalyze mono-, di-, or trimethylated states (Mosammaparast and Shi 2010). The methylation process gives rise to many unique possibilities in influencing transcriptional activity, by combining a variety of enzymes with different lysine residues in different states.

Histone phosphorylation

Although histone phosphorylation is somewhat less well understood, several nuclear protein kinases and protein phosphatases are known that add or remove phosphate groups from the histone tail. The protein kinase MSK 1 and the protein phosphatase inhibitor DARRP-32 have been shown to regulate phosphorylation in the brain. Phosphorylation of histones is associated with the promotion of transcriptional activity. It is the best characterized at serine 10 on histone H3, where it recruits a HAT to halt repressive methylation on lysine 9. This HAT in turn acetylates the lysine residue in a process called phosphoacetylation, which further increases transcriptional activity (Renthal and Nestler 2009).

DNA methylation

In cytosine or DNA methylation, a cytosine nucleotide is methylated by transfer of a methyl group from S-adenosyl methionine (SAM), resulting in 5-methyl-cytosine. It is catalyzed by DNA methyltransferases (DNMT) and occurs in approximately 3% of cytosines in human DNA. In mammals, this only takes place at the dinucleotide sequence CpG. The CpG sequence occurs in low frequency throughout the genome and in high frequency in so-called CpG islands (Gibney and Nolan 2010). These were first thought to be concentrated in the promoter regions of genes, but recently evidence was found that only half of the CpG islands are localized in these areas. It is suggested that the rest lies within or in-between genes, in transcription start-sites of non-coding RNA's (Illingworth, Kerr et al. 2008) and island shores (5). DNA methylation is associated with transcriptional repression, and this process is enhanced by methyl-binding proteins. These proteins bind specifically to methylated DNA, and further repress genetic transcription by recruiting chromatin-remodeling complexes. For example, methyl-CpG-binding protein 2 (Mecp2) recruits the HDAC Sin3 complex and the histone H3K9 trimethyl-transferase Suv39H1, and methyl-binding-domain protein 1 (MD1) recruits the histone H3K9 dimethyltransferase Setdb1. The cooperation between methyl-binding proteins and chromatin-remodeling enzymes illustrates that different epigenetic mechanisms act synchronically to influence genetic transcription.

3. Epigenetic psychiatric mechanisms

3.1 Depression and anxiety disorders

A variety of neurobiological hypotheses of depression have been investigated for epigenetic modulation. The majority of research has been carried out in rodents, but there are also a substantial number of human post mortem studies. Studies can be roughly categorized into two paragraphs: 1) the epigenetic correlates of depressive behavior and 2) the epigenetic mark of childhood adversity. The first contains research in animal models for depression and anxiety, and in the brains of depressed individuals and suicide victims. In the second paragraph the focus lies on the lasting epigenetic alterations induced by adverse childhood experiences in rodents and humans, associated with adult depressive behavior. The aim of this chapter is to provide insight into the epigenetic mediation of depression and anxiety.

The epigenetic correlates of depressive behavior

Restraint stress is often used as a model to investigate the neurobiological characteristics of stress and depression in rodents. It induces alterations in genetic expression, at least partly regulated by epigenetic mechanisms, that are also found in the brains of depressed humans. One of these is a decrease in brain-derived-neurotrophic factor (BDNF) functioning, a growth factor that is involved in neural development, adult neurogenesis and neural plasticity. It is thought to mediate cognitive dysfunction and altered neural networks in psychiatric disorders, and the gene for BDNF is extensively studied for its regulation by chromatin remodeling (Angelucci, Brene et al. 2005). The BDNF gene has different splice variants that result in at least nine different mRNA transcripts with distinct promoter regions, that each code for an isoform of the same BDNF protein, allowing specific expression in different brain regions (Aid, Kazantseva et al. 2007;Liu, Lu et al. 2006). Restraint stress induces decreased levels of total BDNF mRNA in the hippocampus and decreased acetylation at histone H3 at the BDNF gene, associated with reduced levels of BDNF protein. Interestingly, after 24 hours BDNF mRNA and protein levels were back to normal, showing that environmental cues can induce plastic chromatin regulation (Fuchikami, Morinobu et al. 2009). Findings in two other rodent models for depression, namely novelty stress and forced swimming, indicate that stress also induces phosphoacetylation at the BDNF gene in the hippocampus. This is regulated by the GABAA and the NMDA receptor, as drugs that target these strongly affect levels of phosphoacetylation, suggesting that GABA and glutamate can set intracellular mechanisms into action to alter BDNF-associated chromatin (Chandramohan, Droste et al. 2008;Papadopoulos, Chandramohan et al. 2010). Furthermore, restraint stress induces histone methylation in the hippocampus in a complex and regionally specific pattern. Hunter and colleagues found that levels of H3K9 trimethylation in the dentate gyrus (DG) and CA1 region were increased, while levels of H3K9 mono-methylation and H3K27 trimethylation in the same regions were reduced and H3K4 trimethylation remained unchanged (Hunter, McCarthy et al. 2009). However, increased trimethylation of H3K4 in the dentate gyrus has also been reported (Cassel, Carouge et al. 2006).

Similar results were found with a different model for depression, social defeat stress. Rodents are repeatedly confronted with a dominant peer to induce defeat, and this leads to exaggerated stress-related behavior up to six weeks afterwards. This is accompanied by repressive histone dimethylation of H3K27 at the BDNF exon 3 and 4 promoters in the hippocampus, lasting for a month after defeat (Tsankova, Berton et al. 2006). In addition, it is associated with a transient

increase in histone H3 acetylation of the glucocorticoid receptor (GR) gene in the hippocampus, peaking 30 minutes after defeat stress (Hollis, Wang et al. 2010). As histone acetylation is indicative of genetic activity, this finding concurs with theories of exaggerated stress responsiveness and HPA-axis reactivity in depression and anxiety disorders. However, in a study on altered GR expression in major depressive disorder, no correlation with DNA methylation was found (Alt, Turner et al. 2010).

Each of these studies suggests that stress-related changes in the hippocampus are under epigenetic influence, supporting the role of the hippocampus in depressive behavior. In addition, social defeat stress affects epigenetic regulation in the nucleus accumbens, a brain region that is implicated in depression through its role in reward sensitivity. It increases global dimethylation of H3K9 and H3K27, both repressive modifications, and phospho-CREB binding, which is a marker of transcriptional activity. Furthermore social defeat induces dynamically regulated acetylation of H3K14 in the nucleus accumbens. This was decreased directly after defeat, but strongly increased 1 to 10 days later, accompanied by a decrease in HDAC2 mRNA. In human post-mortem brain tissue similar findings were reported, with elevated H3K14 acetylation in the nucleus accumbens of depressed individuals, associated with decreased levels of HDAC2 mRNA (Covington, Maze et al. 2009). Alterations in expression of HDAC genes are indirect markers of the involvement of epigenetic mechanisms, as the enzymatic activity of HDACs influences the structure of the epigenome. Moreover, according to recent work by Hobara and colleagues, the genetic expression of HDAC genes in mood disorder patients depends on whether they are currently experiencing a depressive episode. During depression HDAC2 and HDAC5 mRNA is increased in leukocytes suggesting decreased acetylation, compared to major depressive disorder patients who are not in a depressive episode. (Hobara, Uchida et al. 2010). Changes in DNMT genes are also indicative of a role of epigenetic mechanisms. In the brains of suicide completers who were diagnosed with major depression, mRNA levels of DNMT1 and DNMT3B, but not DNMT3A were altered in the frontopolar cortex, amygdala and the paraventricular nucleus of the hypothalamus. Interestingly, transcript levels of the DNMTs were highly intercorrelated in control subjects, while only low correlation was present in the suicide brain. In addition, in the frontopolar cortex the promoter region of the GABAA alpha 1 receptor subunit gene was hypermethylated in suicide victims, accompanied by diminished expression (Poulter, Du et al. 2008).

A number of studies investigated the role of histone modifications through disruption of HMT and HAT genes. Mice with increased expression of the histone H3K9 methyltransferase Setdb1 in forebrain neurons have antidepressant-like phenotypes, as they are more resistant to anhedonia, despair and learned helplessness in behavioral paradigms. This appears to be mediated by glutamate receptor subunit genes, which are highly methylated in these mice (Jiang, Jakovcevski et al. 2010). Disruption of the methyltransferasecomplex GLP/G9a, which is also specific for H3K9 dimethylation, leads to activation of neural progenitor genes. These mice display severe mental retardation accompanied by cognitive impairment and behavioral abnormalities, such as attenuated interest in the environment and impaired emotional responsiveness, comparable to symptoms of human depression. (Schaefer, Sampath et al. 2009). Furthermore, knockdown of the p300/CBP-associated factor (PCAF) gene which encodes a HAT, causes heightened behavioral responses to acute stress, forced swimming and conditioned fear paradigms, accompanied by increased plasma corticosterone levels. This indicates that histone acetylase by PCAF is of great importance to stress responsivity (Maurice, Duclot et al. 2008). Taken together, these studies in transgenic mice further support the involvement of chromatin remodeling in depression and anxiety.

While most animal models are meant to induce depression, the paradigm of environmental enrichment protects against depressive behavior. When rodents are exposed to a stimulating living environment, their behavioral sensitivity to stress decreases and concomitantly hippocampal BDNF gene expression increases. Similar to the repression of BDNF activity, this is mediated by epigenetic mechanisms. Environmental enrichment significantly increases H3K4 trimethylation at the BDNF exon 1 and exon 6 promoter regions, a decrease in H3K9 trimethylation at the exon 4 promoter and in H3K27 trimethylation at the exon 3 and 4 promoters. This was not associated with changes in expression of histone methylases and demethylases, suggesting that other mechanisms were at play to regulate histone methylation (Kuzumaki, Ikegami et al. 2010). The expression of the BDNF exon 1 variant in the hippocampus is regulated by NMDA activity, controlled by chromatin remodeling mechanisms. Activation of the NMDA receptor leads to increased hippocampal BDNF mRNA, and this correlates with increased H3K4 dimethylation, decreased H3K9 dimethylation and increased H3K9 and K14 acetylation. In addition, HDAC1 and the methyl-binding proteins MBD1 and MeCP2 are released following H3K9 dimethylation (Tian, Hu et al. 2009). These findings are a good illustration of how different epigenetic mechanisms act in concert to bring genetic expression to the desired level. In a similar manner, BDNF expression is repressed in chemically induced depression in mice. Histone H3K27 is trimethylated, while H3 is hypoacetylated and DNA is methylated at promoter IV, all reducing genetic activity (Onishchenko, Karpova et al. 2008). In humans, BDNF mediates the beneficial effects of electroconvulsive therapy (ECT), which is commonly used to treat people with medication-resistant depression (Baghai and Möller 2008). Tsankova and colleagues found that ECT increased BDNF transcription through chromatin modifications, which differ for acute and chronic administration. Whereas in both conditions an increase in BDNF expression is mediated by histone H4 acetylation at promoters 3 and 4, chronic ECT also increased acetylation at H3. The authors suggest that this finding may shed light onto the fact that ECT is only effective in treating depression after repeated administration, as the 'true' mechanism of action may lie at the more resistant H3 acetylation (Tsankova, Kumar et al. 2004).

The research described above dealt with theories on BDNF and GR malfunction in depression, and both appear to be mediated by histone modifications and DNA methylation. Another important hypothesis of major depressive disorder is decreased serotonin neurotransmission. The serotonin transporter gene 5HTT is associated with depression, and it contains a short allelic variant that predisposes to mood symptoms, and a long allelic variant that is considered protective. Ollson et al. recently found evidence that the influence of 5HTT genotype is modulated by levels of DNA methylation. In human buccal cells hypermethylation of the promoter of 5HTT only associates with depressive symptoms, if one carries the 5HTTLPR short allele (Olsson, Foley et al. 2010).

The epigenetic mark of childhood adversity

A widely established contributor to the development of depression and anxiety is childhood adversity (Weich, Patterson et al. 2009). Stress early in life likely interacts with genetic factors to produce altered behavior later in life. In a groundbreaking study, Weaver and co-workers examined how poor maternal care can affect epigenetic regulation of the GR gene. Adult rats that received poor maternal care had hypermethylated GR promoter regions in the hippocampus, associated with lower expression levels. In addition, methylation of the NGFI-A binding site on the GR promoter was investigated in the first postnatal week of rats with high versus low maternal care. The NGFI-A binding site was highly methylated directly after birth. However, on the sixth day after birth, methylation had ceased only in the group with good maternal care. Methylation remained in the rats

that received poor mothering and this effect persisted into adulthood (Weaver, Cervoni et al. 2004). In later work, Weaver and colleagues suggested that DNA methylation of the NGFI-A binding site lowers GR expression, as it interferes with NGFI-A binding to the GR promoter (Weaver, D'Alessio et al. 2007). Administration of methionine, a precursor to the methyl-donor SAM, increased DNA methylation within the NGFI-A binding site, thereby inhibiting NGFI-A binding and reducing GR expression. In addition, methionine reversed the effects of maternal care on NGFI-A binding to the GR promoter, and on hippocampal GR expression and HPA responses to stress, thus demethylating the binding site. As one would expect methionine to increase DNA methylation, the authors suggest that methionine may affect DNMT or demethylase activity through an unknown mechanism. Nonetheless, these findings provide evidence that influences genetic expression through epigenetic modulation (Weaver, Champagne et al. 2005). This is further supported by research in humans, investigating the effects of childhood abuse on epigenetic regulation of the GR promoter in suicide victims. When suicide completers had experienced abuse during childhood, levels of GR mRNA were reduced and DNA methylation of the promoter was elevated. In addition, rRNA genes that code for ribosomal RNA and are vital to protein synthesis, were hypermethylated in the hippocampus throughout the promoter region, and expression was reduced. Childhood abuse is associated with decreased hippocampal volume and cognitive impairments, and this may be mediated by an epigenetic mark left by adverse experiences (McGowan, Sasaki et al. 2009). When infant rats are maltreated by a caregiver, BDNF mRNA levels in the adult prefrontal cortex are decreased, regulated by an increase in DNA methylation at exon 9 of the BDNF gene. These rats also displayed abusive behavior towards their offspring, which in turn displayed increased DNA methylation in the prefrontal cortex at BDNF exon 4 (Roth, Lubin et al. 2009). It thus seems that methylation of the BDNF gene forms a molecular mark of early life stress. This is further supported by the finding that regulation of the BDNF gene is involved in the consolidation of fear memory. Contextual fear learning generates changes in DNA methylation leading to altered regulation of several BDNF mRNA's (Gupta, Kim et al. 2010;Lubin, Roth et al. 2008).

In rhesus macaques early life stress interacts with 5HTT methylation status. The 5HTT short allele generally has higher methylation levels than the long allele, leading to low 5HTT expression in both macaques and humans (Philibert, Madan et al. 2007). High 5HTT methylation levels are associated with increased stress reactivity when macaques have experienced early life stress, while 5HTT genotype does not influence stress reactivity associated with early stress (Kinnally, Capitanio et al. 2010). Similar results were found in humans, where methylation levels of the 5HTT gene correlated with abuse during childhood, independent of genotype (Beach, Brody et al. 2010). Another recent study also found 5HTT genotype to interact with levels of DNA methylation. In carriers of the long allele, higher methylation of the 5HTT promoter area was associated with increased risk of unresolved responses to trauma. The short allele variant only predicted unresolved trauma when methylation levels were low (van Ijzendoorn, Caspers et al. 2010). These findings strongly indicate that epigenetic regulation mediates the interaction between genes and environment to produce an individual's behavior.

To elucidate the effects of prenatal stress mice were exposed to stress early in gestation. In adulthood, these mice displayed maladaptive behavioral responses to stress, anhedonia and an increased sensitivity to selective serotonin reuptake inhibitor (SSRI) treatment. Long-term alterations in central corticotropin-releasing factor (CRF) and GR expression, as well as increased responsivity of the hypothalamic-pituitary-adrenal (HPA) axis, were present in these mice and likely contributed to their heightened sensitivity to stress. Changes in CRF and GR gene methylation correlated with

altered gene expression, providing evidence of epigenetic programming during early prenatal stress (Mueller and Bale 2008). Interestingly, maternal mood during pregnancy also affects epigenetic regulation. Children of mothers who were depressed or anxious during the third trimester have increased methylation at the GR gene, and an increased cortisol stress response at 3 months of age. This indicates that epigenetic regulation mediates increased HPA axis activity induced by maternal mood before birth (Oberlander, Weinberg et al. 2008).

3.2 Psychotic disorders

In recent years a variety of studies have aimed to shed light on epigenetic factors in the development of psychotic disorders. Global changes in DNA methylation levels were found, indicating that an altered methylation process may contribute to developing such disorders. In cortical neurons of schizophrenia patients global DNA hypermethylation is observed (Grayson, Chen et al. 2006). In leukocytes contradictory results were found, as global methylation was decreased in male schizophrenia patients. DNA methylation was similar in female patients and controls, suggesting that methylation is differentially involved between the sexes (Shimabukuro, Sasaki et al. 2007).

One of the major hypotheses on the etiology of psychosis is dysfunctional neural development and plasticity, mediated by deficits in GABAergic neurotransmission. GABAergic interneurons release the reelin protein, which is important for regulating synaptic plasticity and neural migration. Decreased expression of reelin and glutamic acid decarboxylase 67 (GAD67), an enzyme that is involved in the synthesis of GABA, are consistently observed in schizophrenia and bipolar patients (Akbarian and Huang 2006). Additionally, the reelin and GAD67 genes show genetic association with psychotic disorders and this is likely mediated by epigenetic remodeling, as several studies report that the promoter area of these genes is hypermethylated in the frontal cortex of schizophrenia patients (Kundakovic, Chen et al. 2009). In such patients, Huang measured DNA methylation of the GAD67 gene in GABAergic neurons that were separated for open and repressed chromatin, based on the presence of repressive versus activating histone trimethylation. Contradictory to expectations in repressive chomatin a decrease in DNA methylation was found at the GAD67 promoter. Though only a limited number of CpG sites was investigated, these results raise the possibility that histone methylation at the GAD67 gene may function independently from DNA methylation (Akbarian and Huang 2006;Huang and Akbarian 2007).

To mimic the downregulation of reelin and GAD67 that is observed in psychotic patients, methionine can be administered. Methionine decreases reelin and GAD67 expression in the frontal cortex and induces hypermethylation of the reelin and GAD67 promoters. Mice that are treated with this compound also demonstrate behavioral aspects of schizophrenia, and so methionine is used as an animal model for this disorder. Tremolizzo and colleagues showed that mice treated with methionine display social withdrawal and impaired attention and cognition (Tremolizzo, Doueiri et al. 2005). The methionine-induced hypermethylation of reelin and GAD67 is associated with increased binding of MeCP2 and MBD2 to the promoters, likely mediating DNA hypermethylation (Dong, gis-Balboa et al. 2005). Like methionine, folate is a precursor of SAM, and some evidence exists that folate-deficiency is involved in schizophrenia (Brown and Susser 2008). In addition, a gene that influences folate metabolism, MTHFR, displays genetic association with schizophrenia, suggesting that this gene may affect disease-risk through altering DNA methylation (Lewis, Zammit et al. 2005).

Each of these studies indicates that the downregulation of GABAergic neurotransmission in the frontal cortices of schizophrenia patients is mediated by promoter hypermethylation. DNMTs are

of great importance in this process. Genetic expression of reelin and GAD67 is associated with reduced DNMT activity and DNMT1 protein levels, and downregulation of reelin and GAD67 expression correlates with increased DNMT activity (Kundakovic, Chen et al. 2007;Noh, Sharma et al. 2005). In the frontal cortex of schizophrenia and bipolar patients with psychosis, DNMT1 mRNA and protein expression are increased, accompanied by a decrease in GAD67 mRNA-positive neurons (Guidotti, Dong et al. 2009). DNMT3a is overexpressed in GABAergic neurons of the frontal cortex of schizophrenia subjects, while DNMT3b occurs in very low concentrations (Veldic, Kadriu et al. 2007;Zhubi, Veldic et al. 2009). In medium spiny neurons of the striatum of schizophrenia but not bipolar patients, DNMT1 expression is also increased paralleled by decreases in reelin and GAD67 expression (Veldic, Kadriu et al. 2007).

Another important hypothesis of the etiology of schizophrenia is hyperfunction of dopamine, one of the catecholamines. The synthesis of catecholamines is mediated by the Catechol-O-methyltransferase (COMT) enzyme, and the COMT gene has often been associated with schizophrenia. In recent years epigenetic regulation of this gene has also been investigated. COMT has two isoforms encoded by the same gene; Membrane Bound (MB)-COMT, mainly expressed in the brain, and Soluble (S)-COMT, which is predominantly expressed in peripheral cells. MB-COMT promoter DNA is frequently hypomethylated in schizophrenia and bipolar disorder patients, particularly in the left frontal lobe. This corresponds with an increase in transcript levels of MB-COMT in both schizophrenia and bipolar disorder patients, and a decrease in expression of the dopamine receptor gene DRD1 (Abdolmaleky, Cheng et al. 2006). More evidence for epigenetic mediation of COMT is delivered by Pedrosa, who screened several schizophrenia and bipolar disorder candidate genes in fetal brain matter for peaks in H3K4 methylation and H3K9 acetylation, because this indicates genetic expression. Many peaks were found, among others at the COMT gene, signalling regulatory regions under epigenetic control (Pedrosa, Locker et al. 2009). In contrast, methylation levels at the COMT gene were similar in leukocytes of schizophrenia patients and controls, and also in monozygotic twins discordant for schizophrenia (Murphy, O'Reilly et al. 2005). But as leukocytes express S-COMT which has lower affinity for catecholamines, the relevance of these findings is hard to judge. However, in the cerebellum of schizophrenia patients COMT methylation does not differ either, nor in a genome-wide methylation analysis (Dempster, Mill et al. 2006). These findings are inconclusive as to whether COMT is involved in psychotic disorders, and if so, whether it is epigenetically regulated. In another genome-wide analysis in brain matter of schizophrenia and bipolar patients, methylation changes in a wide variety of genes were revealed. Among these were genes that are involved in glutamatergic and GABAergic neurotransmission and in neural development. Methylation status of these genes did not correlate between the male and female sex in bipolar patients, suggesting that there are hormonal influences in the etiology of bipolar disorder. In contrast, in schizophrenia patients there was a significant overlap in methylation profile between males and females (Mill, Tang et al. 2008).

Another candidate gene for schizophrenia is the SOX10 gene, that encodes a transcription factor that is involved in embryonic development and cell fate. It is highly methylated in brains of schizophrenia patients correlating with reduced mRNA levels of SOX10. SOX10 protein is specific voor oligodendrocytes, and DNA methylation status of the SOX10 gene also associates with other oligodendrocyte-related gene expressions. Hence, the authors suggest that the extent of methylation of the SOX10 gene may be an epigenetic sign of oligodendrocyte dysfunction in schizophrenia (Iwamoto, Bundo et al. 2005). Moser and colleagues posed that the SLC12A6 gene may have a role in schizophrenia through epigenetic mediation as well. SLC12A6 is involved in cellular proliferation and

has been associated with schizophrenia and bipolar disorder, and the allelic variance at this gene leads to an additional methylation site (Moser, Ekawardhani et al. 2009).

The majority of research focuses on DNA methylation, but there is also some evidence that histone modifications contribute to altered genetic expression in schizophrenia. Elevated HDAC1 expression levels are detected in the prefrontal cortices of schizophrenia subjects. The mRNA levels of GAD67 show a strong and negative correlation with the mRNA levels of HDAC1, HDAC3 and HDAC4 (Sharma, Grayson et al. 2008). In Korean patients HDAC3 and 4 genes were associated with schizophrenia, though this may not be generalizable to other populations (Kim, Rincon Castro et al. 2008). Furthermore, schizophrenia patients have higher lymphocyte baseline levels of histone H3K9 dimethylation. Interestingly, lymphocyte cultures of schizophrenia patients responded differently to a HDAC inhibitor than cultures of healthy people; dimethylation of histone H3K9 was less responsive in lymphocytes of schizophrenia patients than of healthy people (Gavin, Rosen et al. 2009). In addition, schizophrenia patients have lower lymphocyte baseline levels of H3K9 and H3K14 acetylation levels, but these levels increased significantly more in lymphocyte cultures from healthy controls than from patients with schizophrenia after incubation with an HDAC inhibitor (Gavin, Kartan et al. 2008). These findings could imply that the chromatin of schizophrenia patients is more restrictive and more rigid than of people without this diagnosis.

4. Epigenetic psychiatric drugs

The recent interest for the role of epigenetics in the brain has led researchers to explore the possibility of epigenetic psychiatric drugs. Several psychiatric medications were discovered to influence epigenetic mechanisms, suggesting that this is part of their mechanism of action. In addition, the therapeutic potential of experimental compounds that specifically target epigenetic mechanisms is extensively investigated. This chapter aims to give a broad overview of the current knowledge of epigenetic therapy in psychiatric disorders. First, antidepressants are described, second antipsychotics, third HDAC inhibitors and fourth drugs that do not fit into one of the previous categories. To our knowledge, this chapter covers every psychiatric drug and experimental compound that has been investigated for its epigenetic influence in depression, anxiety or psychotic disorders.

4.1 Antidepressants

Various antidepressants have been investigated for their epigenetic action. In theory, it is likely that these drugs affect epigenetic mechanisms, as it could provide an explanation as to why antidepressants are generally only effective after several weeks of treatment. Since neurotransmitter levels increase immediately after use, the real mechanism of action may lie in epigenetic mechanisms, taking some time for these neurotransmitters to induce epigenetic adaptations. Upon binding to receptors intracellular cascades may be set into action that ultimately intrude the cell nucleus and influence enzymes that regulate the pattern of histone modifications and DNA methylation. However, for a detailed analysis of these processes more research is needed.

Imipramine

Imipramine is a tricyclic antidepressant that has been used to treat depression since the 1950s. Its primary mechanism of action is inhibiting the reuptake of serotonin and norepinephrine, thus elevating the levels of these neurotransmitters in the brain. Recently, the epigenetic effects of imipramine have been uncovered, providing a deeper understanding into the therapeutic potential of this drug. Since BDNF dysfunction is often implicated in the etiology of depression, Tsankova and colleagues investigated the effectiveness of imipramine on epigenetic regulation of the BDNF gene in the hippocampi of mice. Chronic social defeat led to repressive histone dimethylation of H3K27 at the BDNF exon 3 and 4 promoters, continuing for a month after social defeat. Chronic treatment with imipramine could not reverse this, even though depressive symptoms disappeared. However, after defeat stress chronic imipramine did lead to hyperacetylation of H3 at the BDNF promoters, mediated by downregulation of HDAC5. Moreover, the efficacy of imipramine was blocked by overexpression of HDAC5, suggesting that downregulation of HDAC5 may be essential to the efficacy of imipramine. The authors suggest that these findings might have strong implications for antidepressant treatment in humans. Antidepressant medication often seems only effective in curing symptoms and not the underlying disease, since many patients relapse once they are off medication. Since histone H3 hypermethylation was not affected by imipramine this could be a robust mechanism that lies at the heart of depression, providing a possible new target for antidepressant therapy. (Tsankova, Berton et al. 2006)

Imipramine influences epigenetic regulation in the nucleus accumbens, by largely reversing repressive dimethylation of H3K9 and H3K27 in the nucleus accumbens and increased phospho-CREB

binding induced by social defeat stress. An interesting characteristic of the social defeat model is its ability to differentiate between animals that are vulnerable versus resilient to depression, thereby mimicking human differences. The global pattern of H3 dimethylation in the nucleus accumbens of these resilient mice strongly resembled the dimethylation profile of mice that received chronic imipramine treatment after social defeat. This may indicate that resilient animals somehow naturally overcome the effects of stress on gene expression in the accumbens. Of course the genes that show dimethylation in resilient mice, but not in mice treated with imipramine, could also be investigated as novel targets for antidepressant medication. (Wilkinson, Xiao et al. 2009)

Amitriptylin

Amitriptylin is a tricyclic antidepressant, and Perisic et al. employed this medication in an epigenetic study in rat astrocytes. It induced global DNA hypomethylation without affecting histone acetylation, and this was partially reversible after withdrawal. In addition, amitriptylin strongly reduced enzymatic activity of DNMT1 without altering DNMT1 protein levels, and this reduction in activity may mediate the reduced global methylation levels (Perisic, Zimmermann et al. 2010).

Fluoxetine

Fluoxetine is a widely used antidepressant, belonging to the class of selective serotonin reuptake inhibitors. In addition, fluoxetine was recently investigated for its epigenetic effects in the hippocampus. Restraint stress induced trimethylation of histone H3K9 and H3K4 in the dentate gyrus in the hippocampus. Treatment with fluoxetine reversed H3K9 but not H3K4 trimethylation, selectively enhancing genetic activity, since only H3K9 trimethylation is repressive (Hunter, McCarthy et al. 2009). In healthy rats, fluoxetine also induces epigenetic modulation. Chronic fluoxetine treatment decreases acetylation of H3 in three serotonin projection areas; the caudate putamen, the frontal cortex and the dentate gyrus of the hippocampus. In addition, expression of the methyl-binding proteins MeCP2 and MBD1 was increased, accompanied by increased HDAC2 expression, further inhibiting transcriptional activity in these brain regions (Cassel, Carouge et al 2006).

Karpova and colleagues investigated prenatal exposure to fluoxetine in mice. Expression of BDNF in the hippocampus showed longterm upregulation into adulthood, but this was not accompanied by changes in acetylation or trimethylation of H3 in the BDNF gene, though these modifications are known for stimulating genetic activity. This suggests that the BDNF-upregulation may be mediated by another, possibly less well characterized epigenetic modulation (Karpova, Lindholm et al. 2009). In mice that have been subjected to social defeat stress, a global characteristic pattern of gene transcription is induced in the nucleus accumbens. Treatment with fluoxetine reverses this, further indicating that fluoxetine acts by epigenetic regulation (Covington, Maze et al. 2009).

MAO inhibitors

Monoamine oxidase (MAO) inhibitors form a group of highly effective antidepressant medications. They act by inhibiting the MAO enzymes, thereby preventing the breakdown of the monoaminergic neurotransmitters serotonin, dopamine, epinephrine and norepinephrine. MAO inhibitors are capable of enhancing histone methylation by breaking down lysine-specific demethylase 1 (LSD1), a histone demethylase that is structurally similar to MAO A and B (Culhane, Wang et al. 2010). LSD1 specifically demethylates mono- and dimethylated H3K4 and H3K9, which are repressive modifications (Binda, Valente et al. 2010). The MAO inhibitors phenelzine and tranylcypramine both

inhibit demethylation of histone H3K4, resulting in a global increase in H3K4 methylation. Interestingly, tranylcypromine was ten times more effective in inhibiting LSD1 than MAO A or B, suggesting that selective LSD1 inhibitors might be more efficient in treating depression. Other experimental MAO inhibitors also increase H3K4 methylation, but the effects of such drugs on H3K9 methylation have not yet been investigated, as the role of LSD1 in histone demethylation has only recently been uncovered (Lee, Wynder et al. 2006).

4.2 Antipsychotics

Haloperidol is a widely used typical antipsychotic that preferentially antagonizes dopamine D2 receptor activity. Some evidence now indicates that haloperidol also induces histone modifications to achieve its therapeutic effects. In mice histone phosphoacetylation is observed in the striatum after striatal infusion of haloperidol. Specifically, histone H3 at serine 10 is phosphorylated and H3K14 is acetylated, and this is mediated by the NMDA receptor, since administration of an NMDA receptor antagonist blocks phosphoacetylation. Administration of the D2 receptor antagonist raclopride had the same results (Li, Guo et al. 2004b). Another recent study also observed increased H3 phosphorylation in the striatum, but no changes in acetylation were found. Two more studies also reported an increase in mouse striatal H3 phosphorylation in response to haloperidol, though no changes in acetylation levels remained unchanged (Bertran-Gonzalez, Bosch et al. 2008;Bertran-Gonzalez, Hakansson et al. 2009). Furthermore, one study reported that haloperidol decreases global DNA methylation levels in the brain of female rats. However, haloperidol did not influence methylation of the reelin and GAD67 gene promoters in the frontal cortex and striatum (Shimabukuro, Jinno et al. 2006).

Treatment with the atypical antipsychotic clozapine, which blocks dopamine D1, D2, and 5HT2 receptors, increases GAD67-associated trimethylation of H3K4 by threefold in the rat cerebral cortex. In addition, mRNA expression of the H3K4-specific histone methyltransferase gene Mll1 was increased and Mll1 occupancy at the GAD67 promoter had doubled. In the prefrontal cortex of humans who had been treated with clozapine, H3K4 trimethylation at the GAD67 gene had increased by twofold. As this modification is associated with transcriptional activation, these findings indicate that the therapeutic action of chromatin relies at least in part on increased GABAergic activity mediated by histone methylation (Huang, Matevossian et al. 2007). Further supporting this hypothesis, methionine-induced reelin promoter hypermethylation was strongly decreased after administration of clozapine, together with an increase in promoter-associated H3K9 and H3K14 acetylation (Dong, Nelson et al. 2008).

Two structurally similar antipsychotics, sulpiride and amisulpiride, act by antagonizing the dopamine D2 receptor as well. Moreover, they are highly effective in increasing acetylation of H3 associated with reelin and GAD67 gene promoters in the frontal cortices of mice. These drugs have the same effect in the hippocampus and the striatum, but a much higher dose is needed, indicating selectivity for brain regions (Simonini, Camargo et al. 2006). In the frontal cortex and striatum of mice that displayed methionine-induced hypermethylation of the reelin promoter, methylation was strongly decreased after administration of sulpiride, together with an increase in promoter-associated H3K9 and H3K14 acetylation (Dong, Nelson et al. 2008). Risperidone is another atypical antipsychotic that antagonizes D2 receptors, but it has highest affinity for serotonin 5HT2 receptors. It induces global phospho-acetylation of H3 in the striatum, mediated by the NMDA receptor,

showing that dopamine, serotonin and glutamate act synchronically to influence chromatin regulation (Li, Guo et al. 2004a).

4.3 HDAC inhibitors

A well-balanced regulation of HDACs and HATs is essential to well-functioning genetic transcription. As alterations in histone acetylation are implicated in many cancers as well as neurodegenerative disorders, compounds that inhibit HDACs are extensively studied in models of such diseases. These HDAC inhibitors are now also the focus of psychiatric research, since some psychiatric medications have been found to possess HDAC-inhibiting properties. Consequently also experimental HDAC inhibitors were applied in models for brain and behavior.
HDAC inhibitors differ in their specificity for the separate classes of HDACs, though most target class I and II. HDAC inhibitors can be classified into categories based on their chemical structure, and the most important are the hydroxamates, the short fatty-chain acids and the benzamides. Unfortunately, the epigenetic actions of psychiatric medications have not yet been thoroughly investigated, and their specific HDAC targets have not yet been defined. In this paragraph, research on HDAC inhibitors is described that underlines a role of histone acetylation in psychiatric disorders. Moreover, HDAC inhibitors were selected based on their prevalence in psychiatric epigenetic literature, indicating that they may be promising in treating psychiatric disorders. Many more HDAC inhibitors exist that have been primarily investigated in the treatment of cancer, such as SAHA, trapoxin and LBH589 (Sleiman, Basso et al. 2009).

Valproate

The drug valproic acid (VPA) is commonly used in the treatment of epilepsy and bipolar disorder. It increases GABAergic activity by inhibiting GABA transaminase, an enzyme that is involved in the synthesis and degradation of GABA. Moreover, VPA is a potent HDAC inhibitor of class I and II HDACs and the most extensively investigated inhibitor in psychiatric epigenetics. It belongs to the short fatty-chain acids and it increases levels of acetylated histone H3 and H4, facilitating genetic activity (Gottlicher 2004). Many efforts have been dedicated to elucidating the effects of VPA on neuronal processes in the brain. According to a study of D'Souza, the therapeutic actions of VPA are mediated through regulation of tyrosine hydroxylase (TH) expression, as VPA increases mRNA and protein levels (D'Souza, Onem et al. 2009). TH is involved in the biosynthesis of the catecholamines dopamine, norepinephrine and epinephrine, and these neurotransmitters are thought to be involved in psychiatric disorders.

When VPA is administered to rat cortical neurons, mRNA levels and protein levels of exon 1-9-containing BDNF are elevated, and in astrocytes also glial-derived neurotrophic factor is increased (Fukuchi, Nii et al. 2009;Wu, Chen et al. 2008;Yasuda, Liang et al. 2009a). In a study in rat glioma cells, VPA failed to induce any change in levels of BDNF mRNA per se, but it enhanced the stimulatory effect of serotonin on BDNF gene expression (Morita, Gotohda et al. 2009). VPA also affects expression of glutamic acid decarboxylases (GAD), which are responsible for GABA synthesis. Fukuchi et al. found VPA to dose-dependently decrease expression of GAD65, GAD67 and the major GABAA receptor subunit γ2 in rat cortical neurons (Fukuchi, Nii et al. 2009). However, increases in GAD in response to VPA are also reported (Kundakovic, Chen et al. 2009). In the lymphocytes of healthy humans, VPA increases GAD67 mRNA almost fourfold and nearly doubled acetylation of histone H3K9 and H3K14. The effects of VPA were also examined in lymphocytes of bipolar and schizophrenia

patients who had received VPA for four weeks. VPA significantly increased GAD67 mRNA expression and H3K9, H3K14 and H4 acetylation levels (Gavin, Kartan et al. 2009;Sharma, Rosen et al. 2006). In the frontal cortex of schizophrenia and bipolar disorder patients with psychosis, DNMT1 mRNA and protein expression preferentially increased in layer I, II, and IV interneurons. Interestingly, DNMT1 expression only increased when patients received antipsychotic monotherapy, and not when they were treated with a combination of VPA and antipsychotics treatment. This strongly suggests that the mechanism of action of VPA is at least partly epigenetic, and that the symptoms VPA treats may be of epigenetic nature (Guidotti, Dong et al. 2009;Veldic, Kadriu et al. 2007).

In rat forebrain stem cells, VPA induced neurogenesis of mainly GABAergic neurons, indicated by high concentrations of GABA and GAD65 and GAD67. In addition, neurite outgrowth was stimulated (Laeng, Pitts et al. 2004). In a similar study, Yu and colleagues also found that VPA increased neuronal differentiation both in vitro and in vivo, together with increased levels of acetylated H3 and H4. However, proliferation of hippocampal progenitor cells was decreased (Yu, Park et al. 2009). The reelin gene is also involved in neural development and is similarly targeted by VPA. In neural progenitor cells VPA increased acetylation of H3 and H4 and induced expression of reelin (Chen, Sharma et al. 2002;Kundakovic, Chen et al. 2009;Mitchell, Chen et al. 2005). This is most likely mediated by demethylation of the reelin promoter area, as treatment with VPA stimulates promoter demethylation at the reelin and GAD67 genes in the cortex and hippocampus of mice (Dong, Guidotti et al. 2007;Mitchell, Chen et al. 2005). Additionally, DNMT1 and 3A and B protein levels are downregulated in response to VPA in frontal GABAergic interneurons, likely mediating increased genetic activity (Dong, Guidotti et al. 2007;Kundakovic, Chen et al. 2009). When VPA is administered together with methionine, it blocks the hypermethylation that is normally induced by methionine alone (Dong, gis-Balboa et al. 2005;Tremolizzo, Doueiri et al. 2005). In addition, VPA attenuates behavioral schizophrenia-like characteristics that are induced by methionine. It is suggested that this is mediated by VPA induced hyperacetylation of histone H3 at the reelin and GAD67 promoters, which facilitates a state of euchromatin and enhances DNA demethylase activity. In mice that exhibit methionine-induced hypermethylation, VPA induces demethylation of the reelin promoter in the frontal cortex, and increased acetylation of H3K9 and H3K14 associated with the promoter. When VPA was administered in conjunction with clozapine or sulpiride, both demethylation and acetylation were further enhanced (Dong, Nelson et al. 2008).

In an extensive study Perisic and coworkers examined epigenetic effects of a variety of psychoactive compounds in hippocampal and cortical astrocytes, among which VPA. This was the only drug to exert effects on histone acetylation, inducing dose-dependent global hyperacetylation of histones H3 and H4 and decreased levels of repressive H3K9 dimethylation. Furthermore, the increased acetylation by VPA caused a four-fold increase in glutamate transporter GLT-1 mRNA and reduced DNA methylation of the GLT-1 gene, which concurs with hypotheses of glutamate hypofunction in psychotic disorders (Farber 2003). In addition, VPA induced global demethylation of CpG sites in astrocytes of the cortex and hippocampus. The effects on acetylation were transient, since they peaked during the first hour of treatment and then declined. Fourty-eight hours after VPA treatment acetylation levels had returned to baseline, showing that the effects of VPA are fully reversible (Perisic, Zimmermann et al. 2010). Furthermore, VPA increases melatonin MT1 receptor expression in glioma cells. Altered function of melatonin has been implicated in the etiology of depression, providing a possible mechanism of action of VPA in bipolar disorder. In the same study, VPA increased expression of HDAC 1, 2 and 3, and of MeCP2, indicating that VPA affects both histone acetylation and DNA methylation (Kim, Rincon Castro et al. 2008), and the upregulation of HDACs

and MeCP2 may constitute a compensatory mechanism to VPA-induced hyperacetylaton. A final clue to an epigenetic mechanism of action is the finding that in rats, VPA heightened levels of histone H4 acetylation in the striatum, associated with the fosB promoter gene. Striatal fosB protein levels were also elevated, and since fosB is an ubiquitous transcription factor this likely indicates that VPA enhances genetic expression in the striatum (Shen, Kalda et al. 2008).

Sodium butyrate

Sodium butyrate is a short fatty-chain acid that is used in many experimental paradigms to induce inhibition of histone deacetylase. It selectively inhibits HDAC class I and II and it is applied in a range of studies to elucidate the involvement of HDACs in development and disease (Davie 2003). In recent years SB has been used to investigate epigenetic regulation in neural processes. Similar to VPA, SB upregulates BDNF and GDNF mRNA levels in astrocytes, with marked increases in GDNF promoter activity and promoter-associated histone H3 acetylation (Wu, Chen et al. 2008). As BDNF is involved in memory formation, SB might have beneficial effects on this process, as was shown by Stefanko and colleagues. Treatment with SB enhances long term memory for object recognition in mice and it improves memory formation in fear conditioning. Following fear conditioning, mice had elevated trimethylation levels of H3K4 and dimethylation of H3K9. Treatment with SB elevated trimethylation while it diminished dimethylation, enhancing genetic activity. This shows that while SB primarily is an HDAC inhibitor it also affects histone methylation (Gupta, Kim et al. 2010). In a contextual fear model, fear extinction is highly accelerated by SB therapy with fear decreasing eight times as fast as in the control condition, again suggesting that SB enhances fear-related learning and memory (Lattal, Barrett et al. 2007). In hippocampal neurons, TSA induced transcription of promoter 1 of the BDNF gene, associated with hyperacetylation at H3K9 and H3K14 and increased BDNF protein levels. Interestingly, also an increase in HDAC mRNA and protein levels was observed, suggesting a compensatory mechanism in response to HDAC inhibition (Tian, Marini et al. 2010).

Some inconsistent results have been reported on the effectiveness of SB on depressive behavior in animals. Schroeder found that while chronic and acute administration of SB improves depressive behavior in the tail suspension test, no consistent results were found in three other models for depression. Nevertheless, acute SB treatment increased histone H3 and H4 acetylation in the hippocampus and the frontal cortex, with a peak 30 minutes after administration (Schroeder, Lin et al. 2007). Gundersen et al. found similar results, with acute SB administration having an antidepressant effect in only two out of five tests, accompanied by increased hippocampal H4 acetylation. Chronic treatment did not alter depressive behavior and it decreased H4 acetylation, while neither treatment affected H3 acetylation in the hippocampus (Gundersen and Blendy 2009).

In astrocytes of the hippocampus, SB led to an increase in glutamate transporter GLT-1 mRNA after 72 hours, but not after 24 hours of treatment, indicating that SB is more effective after chronic treatment. Further, SB induced a dose-dependent global increase in acetylation of histone H3 and H4 (Perisic, Zimmermann et al. 2010). In the central nucleus of the amygdala, SB induces acetylation and phosphoacetylation of histone H3K14 accompanied by increased c-Fos expression (Kwon and Houpt 2010b). Similar to VPA, SB increases TH mRNA and protein levels. As mentioned above, the fact that a HDAC inhibitor acts on catecholaminergic pathways is promising for a therapeutic potential in psychiatric disease.

Trichostatin A

Originally an antibiotic, two decades ago Trichostatin A (TSA) was discovered to possess HDAC-inhibiting properties. Among the HDAC inhibitors it belongs to the hydroxymates, targeting both class I and class II HDACs. In the brain, inconsistent effects have been reported of TSA on BDNF expression. In the hippocampus TSA enhances expression of BDNF exon 1 but not of exon 4 (Tian, Hu et al. 2009) and in astrocytes TSA upregulates both BDNF and GDNF mRNA levels, with marked increases in GDNF promoter activity and promoter-associated histone H3 acetylation (Wu, Chen et al. 2008). However, in glioma cells TSA does not induce any change in the basal levels of BDNF mRNA. TSA did strengthen the positive influence of serotonin on BDNF transcription in glioma cells (Morita, Gotohda et al. 2009), suggesting that TSA may enhance BDNF expression in glia through serotonin-mediated pathways. TSA is also beneficial in fear extinction, which is accelerated by eight-fold in a contextual fear paradigm, pointing to a major role of epigenetic modulation in this process (Lattal, Barrett et al. 2007).

TSA induces transcription of the melatonin MT1 receptor gene in glioma cells. As mentioned earlier, melatonin is implicated in depression (Kim, Rincon Castro et al. 2008). Another depression-related gene is activated by TSA; the GR gene. In a study on the influence of maternal care on GR expression, Weaver and colleagues suggested that poor mothering lowers GR mRNA levels through methylation of the NGFI-A binding site. This interferes with NGFI-A binding to the GR exon 1 promoter, inhibiting expression (Weaver, D'Alessio et al. 2007). In adulthood, TSA can reverse the epigenetic effects of poor maternal care. It increased hippocampal GR expression by increasing H3K9 acetylation, DNA demethylation and binding of NGFI-A to the GR exon 1 promoter, to comparable levels with rats that received good mothering. (Weaver, Cervoni et al. 2004). In addition, Weaver and co (2006) found that behavioral effects of poor mothering are reversible by treatment with TSA. In a different line of research they found that over 900 genes are regulated by maternal care. Of these, less than 2% is affected by TSA treatment, among which genes that are involved in learning and memory, X-linked mental retardation and neurodegenerative disorders.

The epigenetic effects of TSA have also been investigated in psychotic disorders. As mentioned above, glutamate hypofunction is implicated in psychosis and therefore glutamate-related genes are of interest in epigenetic medication. In astrocytes, TSA decreased DNA methylation of the GLT-1 gene, and this correlated with a four-fold increase in GLT-1 mRNA (Perisic, Zimmermann et al. 2010). Similar to VPA, TSA increases mRNA levels of the reelin and GAD67 genes in neural precursor and neural blastoma cells. By increasing acetylation of H3 and H4 and decreasing expression and enzymatic activity of DNMT1 and 3A and B, the reelin promoter is demethylated and more available for transcription (Chen, Sharma et al. 2002;Kundakovic, Chen et al. 2009;Mitchell, Chen et al. 2005). The influence of TSA was also investigated in human lymphocytes. It induced an increase of nearly four-fold in GAD67 mRNA levels, an almost two-fold increase in acetylation of H3K9 and K14, and a five-fold rise in attachment of acetylated H3K9 and K14 to the GAD67 promoter (Gavin, Kartan et al. 2009).

MS-275

MS-275 is a benzamide-based HDAC inhibitor that selectively targets class I HDACs, but it is not as popular in scientific research as the previous compounds. In mouse models for depression, infusion of MS-275 into the nucleus accumbens delivered strong antidepressant-like effects. In these mice, chronic defeat stress led to a unique global pattern of gene transcription in the nucleus accumbens, and this could be reversed by MS-275 treatment. Almost a third of all genes that were induced by

this compound were regulated similarly in stressed and control animals, suggesting that in the majority of genes MS-275 has differential effects in case of stress. The effects of MS-275 on genetic expression were compared with the effects of fluoxetine, in search for novel antidepressant targets. A number of genes were only regulated by MS-275, among which genes involved in gap junction formation and in adrenergic receptor function (Covington, Maze et al. 2009). In addition, MS-275 increases mRNA levels of reelin and GAD67 in neural precursor and neural blastoma cells. By decreasing expression and enzymatic activity of DNMTs, the reelin promoter is demethylated and more available for transcription (Kundakovic, Chen et al. 2009). Simonini and colleagues report that MS-275 increases acetylation of histone H3 in histones associated with reelin and GAD67 gene promoters preferentially in the frontal cortices of mice. To have an effect in the hippocampus and the striatum a much higher dose is needed, indicating that MS-275 is selective for brain regions (Simonini, Camargo et al. 2006).
Treatment with MS-275 stimulates promoter demethylation and histone H3 acetylation at the reelin and GAD67 genes in the cortex and hippocampus of mice (Dong, Guidotti et al. 2007). In neural progenitor cells MS-275 dose-dependently decreases methylation at the reelin and GAD67 promoters as well, and it upregulates expression of reelin and GAD67, correlating with the extent of inhibition of HDAC activity. Further it downregulates DNMT1, DNMT3A and DNMT3B protein levels. These DNMTs, together with MeCP2 and HDAC1 appear to constitute gene-specific repressor complexes at the reelin and GAD67 promoters, as activation of these genes by MS-275 is associated with dissociation of these proteins from the promoter regions. In addition, MS-275 decreased expression of MeCP2 and HDAC1. The synchronic involvement of DNMTs, MeCP2 and HDAC1 again show that DNA methylation and histone acetylation are intertwined mechanisms, synchronically controlling genetic activity (Kundakovic, Chen et al. 2009).

4.4 Other

DNMT inhibitors

In scientific research DNMTs inhibitors are used to investigate the functional characteristics of DNMTs. As with HDAC inhibitors, they are extensively investigated in cancer therapy. DNMT inhibitors are employed in neural research as well, albeit less then the compounds previously described. Nevertheless, some evidence indicates that DNMT inhibitors may be useful in psychiatric therapy. The compounds 5-aza-dC (AZA), zebularine and doxorubicin inhibit DNMT1 and DNMT3 and decrease DNA methylation of the reelin promoter in neural progenitor cells. This dramatically increases reelin and GAD67 mRNA levels, showing that the expression of the reelin and GAD67 genes is mediated by DNMTs. In addition, these DNMT inhibitors reduced DNMT enzymatic activity, decreased DNMT1 protein levels and increased H3 acetylation in the promoter area (Chen, Sharma et al. 2002;Kundakovic, Chen et al. 2007;Mitchell, Chen et al. 2005). Treating hippocampal neurons with AZA leads to increased BDNF exon 4 mRNA, indicating that this splice variant is methylated by DNMT1 or DNMT3 (Tian, Hu et al. 2009).

Lithium

Lithium has been the foremost treatment for bipolar disorder since the 1970's. Its molecular therapeutic actions are not completely understood, though it has been clear for some time that it inhibits glycogen synthase kinase 3. This enzyme decreases protein activity by phosphorylation and promotes cellular proliferation in tumors, hence lithium is investigated for applications in cancer

therapy (Adler, Hottinger et al. 2009). In rat cortical neurons lithium enhances the expression of BDNF promoter IV, through inhibiting GSK-3 (Yasuda, Liang et al. 2009b). When injected into the central nucleus of the amygdala expression of the transcription factor c-Fos is increased, and this is accompanied by highly increased acetylation and phosphoacetylation of histone H3K14, with a time course slightly preceding and corresponding to c-Fos induction. Acetylation and phosphoacetylation also colocalized with c-Fos expression in the central amygdala. However, no changes in acetylation were induced in other nuclei of the amygdala, indicating that lithium acts region-specific (Kwon and Houpt 2010a).

5. Discussion

5.1 Involvement of epigenetic mechanisms in psychiatry

Depression and anxiety disorders

The current evidence indicates that epigenetic modifications mediate the development of depression and anxiety disorders. Studies are varied in character, and together they show that epigenetic mechanisms likely mediate several important hypotheses of the etiology of depression. First, it has long been known that stressful life events contribute to developing depression and anxiety, and a variety of animal studies have shown that stressful experiences alter epigenetic regulation in the hippocampus and nucleus accumbens. Consequently, it seems likely that stress influences genetic expression through altered epigenetic mechanisms thereby inducing sensitivity to depression. Second, from epidemiological research it is known that adverse experiences during childhood severely influence risk on depressive symptoms. Research in animals and humans indicates that childhood adversity leaves a lasting epigenetic mark, influencing genetic expression long after these experiences have ceased. Third, one of the earliest hypotheses of depression is serotonin hypofunction, and this is likely mediated by DNA methylation. Methylation levels at the serotonin transporter gene correlate with sensitivity to depression and are associated with childhood abuse. Fourth, in animals models of depression and in human patients deficits in BDNF-functioning are consistently found. From a variety of animal studies it now seems clear that stress decreases BDNF levels through altered epigenetic mechanisms both in the hippocampus and the nucleus accumbens, and stress early in life has similar effects. Fifth, depression and especially anxiety disorders are characterized by exaggerated sensitivity to stress. This is mediated by the HPA-axis in which the GR receptor takes part. Research now shows that this hypothesis is also supported by epigenetic mechanisms, as both acute and early life stress lead to hypermethylation and decreased expression of the GR gene, correlating with heightened responsivity to stress in adulthood. Even during gestation maternal stress influences methylation and expression of the GR gene and the CRF gene in offspring. This is associated with increased physiological responses to stress and depression-like behavior, further strengthening the involvement of GR methylation in depression.

Adding to these lines of evidence are medication studies. Several commonly used antidepressants influence chromatin structure and DNA methylation, suggesting that this may be part of their therapeutic action and consequently that epigenetic mechanisms are altered in depressive disorder. In addition, the finding that HDAC inhibitors affect stress-related behavior and stress-induced genetic expression shows that histone acetylation mediates depression and anxiety.

Psychotic disorders

Epigenetic studies in schizophrenia and bipolar disorder are less varied, probably because these disorders are less prevalent and more challenging to model in animals than depressive disorders. Nevertheless, two important hypotheses on the etiology of psychotic disorders can to some extent be confirmed by epigenetic research. First, the majority of studies deals with deficits in GABAergic neurotransmission. Reduced frontal functioning of GABA is thought to underlie psychosis, characterized by reduced expression of reelin and GAD67. Converging evidence suggests that this is mediated by epigenetic modifications, as the expression of reelin and GAD67 in the frontal cortex is mediated by DNA hypermethylation. This process is regulated by DNMTs and has a negative correlation to HDAC expression, providing further evidence for epigenetic involvement. Second, an

important theory on the development of psychosis is hyperdopaminergic neurotransmission. Elevated dopamine levels are found in patients and inhibiting this neurotransmitter is the primary treatment for psychosis. Being involved in dopamine synthesis, the COMT gene is relevant to this process. Some studies have reported that epigenetic regulation of this gene is altered in psychotic disorders, but negative results are also reported, and the evidence remains inconclusive. Two more candidate genes for psychotic disorders are linked to epigenetic regulation. The SOX10 gene is hypermethylated in the schizophrenia brain and a polymorphism of the SLC12A6 gene leads to an additional methylation site, enhancing methylation of this gene.

Another line of evidence for epigenetic mediation of psychosis is delivered by research on antipsychotics. Several widely used antipsychotic drugs have been shown to influence chromatin structure in the frontal cortex and striatum. This likely contributes to their mechanisms of action, which suggests that histones and DNA methylation may be modified in psychotic disorders. Furthermore, HDAC inhibitors enhance schizophrenia-like GABAergic deficits in the frontal cortex and ameliorate behavioral symptoms in a mouse model for this disease, supporting a role of histone acetylation in psychosis.

In addition, epigenetic remodeling is implicated in bipolar disorder through the epigenetic effects of two mood-stabilizers; VPA and lithium. VPA is a HDAC inhibitor, and a single study reports that lithium induces phosphoacetylation in the central amygdala.

From the current evidence it is tempting to conclude that epigenetic mechanisms are involved in psychotic disorders. However, due to the limited number of studies conclusions are premature.

5.2 Current status and future prospects for epigenetic drugs

Antidepressants

Several antidepressant drugs have been shown to influence epigenetic mechanisms. Amitriptylin reduces DNA methylation in the nucleus accumbens, mediated by reduced activity of DNMTs. Fluoxetine decreases histone acetylation in the caudate putamen, frontal cortex and the hippocampus, and increases expression of methyl binding proteins and HDAC2. MAO inhibitors enhance repressive histone methylation by breaking down LSD1, an enzyme that specifically inhibits repressive histone methylation. These studies were carried out in healthy animals and clearly indicate that antidepressants have an epigenetic mechanism of action. However, it is hard to infer epigenetic effectivity during depression. This has only been confirmed for fluoxetine and imipramine, as these drugs are able to reverse epigenetic modifications induced by stress. Fluoxetine can reverse global stress-induced histone methylation in the dentate gyrus of the hippocampus, and imipramine increases stress-induced BDNF acetylation in the hippocampus and global acetylation in the nucleus accumbens. Correlating with this process is a decrease in depressive symptoms.

Based on the limited number of studies it is difficult to draw solid conclusions on the epigenetic effects of each antidepressant. However, current evidence seems promising that antidepressant action is mediated by epigenetic mechanisms, as every drug under investigation shows epigenetic action, despite their different mechanisms of action. Of course these antidepressants have in common that they enhance activity of serotonin, thus serotonergic neurotransmission may influence chromatin remodeling. This likely occurs at the BDNF gene, as TCA's, SSRI's and MAO inhibitors have all been associated with increased BDNF expression in the hippocampus.

Antipsychotics

A number of antipsychotics have been investigated in epigenetic research, delivering promising results. The most prominent finding is that antipsychotics can reverse the epigenetic repression of GABAergic neurotransmission in the frontal cortex that occurs in psychotic disorders. Clozapine, sulpiride and amisulpiride strongly increase expression of the GAD67 and reelin genes, by altering histone methylation, increasing histone acetylation and decreasing DNA methylation associated with these genes. Furthermore, haloperidol, risperidone and raclopride induce global histone phosphorylation and phosphoacetylation in the striatum, mediated by the NMDA receptor. Haloperidol also decreases global DNA methylation levels, but only in female rats. However, though this supports the epigenetic action of this drug, it is difficult to infer how such global modifications may contribute to the therapeutic action of these drugs. As each of the drugs under investigation primarily targets the dopamine D2 receptor, their epigenetic effects are likely mediated by this receptor. Based on the current literature it is probable that antipsychotics influence epigenetic mechanisms, though one should be conservative in drawing conclusions because of the limited number of studies. More research is needed to acquire full insight into the epigenetic action of antipsychotic medication.

HDAC and DNMT inhibitors

HDAC inhibitors inherently have epigenetic action and many studies suggest that they could be beneficial in the treatment of psychiatric disorders. First, they likely have potential in treating depression. By remodeling chromatin and DNA methylation, HDAC inhibitors are able to induce BDNF activity, stimulate neural plasticity and enhance melatonin function, which are all implicated in the development of depression. In addition, HDAC inhibition reverses the effects of fear conditioning on histone methylation, accelerates fear extinction, and reverses a global pattern of transcription induced by stress. Moreover, it is able to reverse the effects of early-life stress on GR expression, an important risk factor to developing depression and anxiety. Second, HDAC inhibition may be therapeutic in psychotic disorders. Deficits in GABAergic neurotransmission contribute to schizophrenia and bipolar disorder, and HDAC inhibitors promote GABAergic neurotransmission by demethylating related genes and inducing expression. Furthermore, HDAC inhibitors could possibly relieve reduced glutamatergic activity that is observed in psychosis. They reduce methylation of a glutamate transporter gene and increase expression, enhancing glutamate neurotransmission. Also, HDAC inhibitors promote the synthesis of catecholamines and these are implicated in both depression and psychosis. The dual potential of ameliorating both depressive and psychotic characteristics is not surprising considering that a potent HDAC inhibitor, VPA, is commonly used to treat bipolar disorder, which entails both depressive and psychotic symptoms. This supports the possibility that other HDAC inhibitors may have clinical actions in humans as well. However, as of yet we can only speculate about the potential of these drugs. The number of studies is small and most findings have not been replicated. This holds even more for DNMT inhibitors. As of yet the evidence on the efficacy of such compounds is too limited to support possibilities for treatment. Nevertheless, current results are promising as different DNMT inhibitors decrease DNA methylation of the reelin promoter and increase reelin, GAD67 and BDNF expression in neural progenitor cells, accompanied by reduced DNMT activity and increased acetylation.

5.3 Limitations and recommendations

Psychiatric disorders originate in the brain and therefore ideally, epigenetic modifications would always be investigated in brain material of patients. This limits scientific research to the post mortem brain, severely restricting possibilities for studies in humans. Instead, a variety of cell and animal models is used, but one cannot be certain that observations fully generalize to the human brain. For example, genetic expression in leukocytes may be different from expression patterns in the brain and in animal research the full spectrum of a psychiatric disorder is hard to grasp. Especially psychotic disorders are challenging to model because symptoms are complex and heterogeneous.

Importantly, the use of different paradigms makes it more difficult to draw hard conclusions on epigenetic involvement. This heterogeneity in research methods could possibly explain some inconsistencies in findings. Furthermore, many studies investigate different epigenetic mechanisms separately, though it has become clear that none of the histone modifications nor DNA methylation acts independently. It would be advisable to measure each modification simultaneously, to get better insight into the epigenetic interactions relevant to psychiatry. In line with this, another limitation to current epigenetic psychiatry is the focus on a subset of mechanisms. In each study, histone acetylation, methylation, phosphorylation or DNA methylation is investigated, as these mechanisms are the best characterized in other fields of expertise and are easily measured with a chromatin immunoprecipitation (ChIP) assay (Zecchini and Mills 2009). However, this does not preclude other epigenetic mechanisms as modulator of genetic expression and behavior. The effects of less frequent histone modifications on genetic activity, such as ubiquitination and sumoylation, are not yet fully understood. In addition, RNA- and polycomb-based mechanisms are likely important in epigenetic regulation, but as of yet they have not been well characterized in the brain. Future research should be directed at understanding the effect of these mechanisms on genetic activity, providing a more solid base for epigenetic psychiatric research.

In research on the therapeutics of HDAC and DNMT inhibitors, a drawback is that these compounds have very general mechanisms of action. This could pose difficulties for their clinical potential. First, HDAC and DNMT inhibitors are not specific to a class of HDACs or DNMTs. Second, different epigenetic mechanisms are intertwined so influencing one will concomitantly influence another, as has been shown for several HDAC inhibitors. Third, HDACs and DNMTs are ubiquitous in the human body and drugs that inhibit these enzymes most likely affect epigenetic regulation outside the brain. This is supported by the vast amount of research with HDAC inhibitors in many types of cancer. Therefore, the effects of HDAC and DNMT inhibitors on histone modifications and DNA methylation should be extensively investigated in peripheral tissues and at non-psychiatric genes, before therapeutic use is considered. Furthermore, the specific properties of each HDAC and DNMT have not yet been firmly established. Once this has been achieved more specific inhibitors may be developed.

5.4 Conclusion

The field of psychiatric epigenetics increases our understanding of the neurobiology of psychiatric disorders and of the therapeutic action of psychiatric drugs. The current research strongly suggests that epigenetic mechanisms mediate the development of psychiatric symptoms, and the epigenetic changes that are associated with these disorders fit into existing theories of the etiology. Stress and major life events have long been known to increase the risk of developing psychiatric symptoms, and

epigenetic mechanisms aid in explaining how this occurs. Epigenetic research also supports and possibly explains several neurobiological hypotheses on psychiatric behavior. This is sustained by a variety of studies in cellular and animal models and in humans, providing a broad base for epigenetic involvement. The precise processes by which epigenetic alterations arise, such as the interplay between neurotransmission and different epigenetic mechanisms, need to be further addressed. Furthermore, the great variety in methodologies can be a disadvantage, as it may lead to inconsistent results and brings about problems of generalization. Moreover, the number of studies is still limited, making it difficult to draw hard conclusions. In future research the solidity of current knowledge can be enhanced by replicating previous studies, and by directly correlating epigenetic alterations in the various experimental paradigms that are in use.

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