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Epigenetic Mechanisms of Serotonin Signaling

Terrell Holloway[†] and **Javier González-Maeso**[†,‡,§,*]

[†]Department of Psychiatry, Icahn School of Medicine at Mount Sinai New York, New York 10029, United States

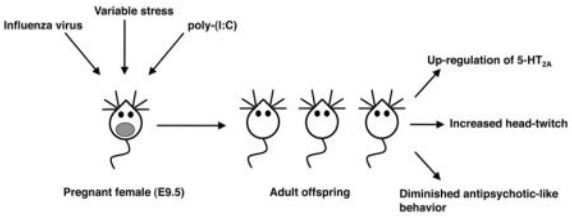
[‡]Department of Neurology, Icahn School of Medicine at Mount Sinai New York, New York 10029, United States

[§]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai New York, New York 10029, United States

Abstract

Histone modifications and DNA methylation represent central dynamic and reversible processes that regulate gene expression and contribute to cellular phenotypes. These epigenetic marks have been shown to play fundamental roles in a diverse set of signaling and behavioral outcomes. Serotonin is a monoamine that regulates numerous physiological responses including those in the central nervous system. The cardinal signal transduction mechanisms via serotonin and its receptors are well established, but fundamental questions regarding complex interactions between the serotonin system and heritable epigenetic modifications that exert control on gene function remain a topic of intense research and debate. This review focuses on recent advances and contributions to our understanding of epigenetic mechanisms of serotonin receptor-dependent signaling, with focus on psychiatric disorders such as schizophrenia and depression.

Graphical abstract



Keywords

Schizophrenia; psychosis; antipsychotics; serotonin 5-HT_{2A} receptor; hallucinogens; lysergic acid diethylamide (LSD); epigenetics; histone deacetylases (HDACs); G protein-coupled receptors (GPCRs)

[*]Corresponding Author: Javier.Maeso@mssm.edu.

Notes

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The term “epigenetics” was coined by Conrad H. Waddington in the 1940s and alluded to the fundamental question of how a particular genome generates a highly complex organism containing innumerable cell types derived from a single fertilized egg.[1] To that end, this field was mostly focused on processes related to embryonic development and cellular differentiation. Over the past decade, however, the definition of epigenetics has changed as the term now refers to the complete description of these potentially heritable changes across the genome that regulate gene transcription without modifying the underlying DNA sequence.[2–7] Because these changes, which include primarily many types of histone and nucleotide modifications such as DNA methylation and DNA hydroxymethylation, are mitotically or meiotically heritable but environmentally modifiable, epigenetics has emerged as an important area of molecular biological studies focused on how environmental factors could mediate stable changes in brain function. In this review, we discuss recent advances in our understanding of epigenetic contribution to serotonin-dependent signaling, in particular the role of histone and DNA modifications in psychiatric disorders and their treatment, including schizophrenia[8] and depression.[9]

OVERVIEW OF MECHANISMS OF EPIGENETIC REGULATION

The completion of the sequencing of the human genome is viewed as an important milestone.[10,11] However, the primary sequence represents only the beginning of our understanding of how the genetic information is stored and, most importantly, read. In eukaryotic cells, in contrast to prokaryotes, the DNA is packaged in the form of a nucleoprotein complex called chromatin. The nucleosome is the basic repeating structural unit of chromatin, which contains 147 base pairs of DNA wrapped twice around an octamer of two copies of each core histone protein (H2A, H2B, H3, and H4). Histones are deeply evolutionarily conserved proteins with a globular domain and a flexible amino-terminal tail.[5,12,13] By this design, biochemical modifications of these subunits can cause the histones to exist in one of two possible states: “open”, where the histone subunits are spaced apart and DNA that is wrapped around them is exposed to be transcribed, and “closed”, in which the histone subunits are packed tightly together and the DNA is not exposed and cannot be transcribed. Thus, the status of chromatin organization, and hence open or closed states of chromatin and DNA accessibility, depends on the so-called epigenetic modifications that fall into two main categories: DNA methylation and histone modifications.[5,12,14–17] This epigenetic information has been shown to be fundamental during embryonic development and tissue-specific cellular differentiation.[18,19] Recent studies also suggest that epigenetic modifications might constitute a new template for psychiatric interventions.[20]

DNA methylation is an epigenetic mark often associated with stable variations in gene expression that consists of the addition of a methyl group to the C5 position of cytosine at CpG dinucleotides. Most of the methylations occur within CpG islands, which are regions of DNA containing a high GC content (>55%).[21] These GC-rich sequences colocalize with approximately 60% of all promoters and are largely free of DNA methylation. Only a small proportion of these CpG islands become methylated during development, particularly within genomic regions that are cell-type specific. Methylation of DNA is catalyzed by a family of DNA methyltransferases (DNMTs): DNMT1, DNMT2, DNMT3A, and DNMT3B. The regulatory factor DNMT3L stimulates the DNA methylation activity of DNMT3A and

DNMT3B.[22] During embryonic development, DNMTs are differentially expressed, and their spatiotemporal distribution has been suggested to play an important role in neurogenesis, neuronal maturation and memory formation.[23–26] In mammals, patterns of DNA methylation are established during embryonic development by DNMT3A and DNMT3B, and maintained by a DNMT1-mediated copying mechanism when cells divide. DNA methylation is increased following learning[26] and maintained to support long-term information storage in the cortex.[27] In addition, demethylation of DNA appears to be involved in long-term changes of neuronal responsiveness.[28] These and other findings suggest that methylation of cytosines in CpG sites influences normal brain physiology and neuropsychiatric disorders.

Although dynamic variation in the accumulation of 5-methylcytosine (5-mC) has emerged as a key factor in the regulation of brain function, 5-mC is not the only covalent modification of DNA in eukaryotes. Thus, it has been demonstrated that CpG dinucleotides can be successively oxidized and converted to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC) by the TET family of DNA dioxygenases. An unusual DNA nucleotide, 5-hmC was detected while comparing the levels of 5-mC in cerebellar Purkinje neurons.[29] In parallel, another group identified the ten-eleven translocase (TET) proteins that hydroxylate 5-mC to form 5-hmC.[30] There is now considerable interest in this field because hydroxylation of 5-mC is likely the first step in the mechanism through which DNA methylation is reversed. These newly revealed DNA base modifications immediately drew broad attention from the research community and have been extensively reviewed. Understanding the dynamics of these modifications in living biological systems could lead to novel treatments for a number of psychiatric conditions.[31,32]

Covalent modifications at the N-terminal tail of histones correlate with open or closed states of chromatin depending on the type of modification. Thus, acetylation of histone H3 (H3ac) and acetylation of histone H4 (H4ac) are modifications that create a more open chromatin architecture.[33] Histone methylation, in contrast, correlates with either transcriptional activation, such as methylation of lysine 4 on histone H3 (H3K4me) and methylation of lysine 36 on histone H3 (H3K36me), or repression, such as methylation of lysine 9 on histone H3 (H3K9me) and methylation of lysine 27 on histone H3 (H3K27me), depending on the histone and amino acid residue being methylated. Histone acetylation is catalyzed by histone acetyltransferases (HATs), and this modification can be reversed by the enzymatic action of histone deacetylases (HDACs). According to neighbor joining methods and Bayesian analysis,[34] members of the HDAC family fall into four different phylogenetic classes: class I (HDAC1, 2, 3, and 8), class II (HDAC4, 5, 6, 7, 9, and 10), class III (SIR2 family of NAD^+-dependent HDACs), and class IV (HDAC11). Class I and II (Zn-dependent) and class III (NAD^+-dependent) show different distribution and are expressed among distinct cell types, including neurons, oligodendrocytes, and astrocytes.[35] Most HDACs were found to be expressed primarily in neurons, whereas HDAC2–5 and HDAC11 were present in oligodendrocytes (suggesting a potential role in myelination) and only HDAC1, 3, and 5 were expressed in choroid plexus.[35] The unusual expression of HDAC11 in hippocampus suggests a potential role in cognition and memory, whereas the high level of HDAC11 expression selectively in the Purkinje cell layer indicates a role in locomotor activity.[35] These and other recent findings discussed below suggest that HDAC inhibitors might emerge as a new target for drug discovery in molecular psychiatry.[36]

ROLE OF EPIGENETIC MECHANISMS IN SEROTONIN-DEPENDENT SIGNALING

Abnormalities in serotonin (5-hydroxytryptamine; 5-HT)-dependent signaling during critical periods of neurodevelopment have been implicated in psychiatric disorders such as schizophrenia, depression, and autism.[37,38] Atypical antipsychotic drugs, such as clozapine, olanzapine, and risperidone, present a high affinity as antagonists/inverse agonists for serotonin receptors, including 5-HT_{2A} and 5-HT_{1A}, and a much lower affinity for dopamine D_2 receptors.[39,40] It has also been demonstrated that clozapine is a partial agonist at cloned human 5-HT_{1A} receptors.[41] Based on their pharmacological properties, antidepressant drugs are classified into six major groups: tricyclic antidepressants (e.g., amitriptyline, imipramine, desipramine, and amoxapine), selective serotonin reuptake inhibitors (SSRIs; e.g., fluoxetine, paroxetine, and citalopram), selective serotonin and norepinephrine reuptake inhibitors (SSNRIs; e.g., venlafaxine), selective norepinephrine reuptake inhibitors (SNRIs; e.g., reboxetine), monoamine oxidase (MAO) inhibitors (e.g., isocarboxazid), and atypical antidepressants (e.g., mirtazapine and mianserin). Tricyclic antidepressants are classified into three subgroups based on their central ring of five links and are characterized by their inhibition of serotonin and norepinephrine uptake, as well as their properties to block the function of several monoaminergic receptors. Atypical antidepressants, such as mirtazapine and its structurally related mianserin, also block the function of 5-HT_{2A} receptors. Recent findings suggest epigenetic mechanisms that affect both the serotonin system and phenotypes induced by treatment with antipsychotic and antidepressant drugs.

Transient inhibition of serotonin transporter (5-HTT) during early stages in development with the SSRI fluoxetine produces abnormal emotional behaviors in mice, such as a reduction in the total distance traveled in an open field and a decrease in the total number of arm entries in the elevated plus-maze.[42] This behavioral phenotype was mimicked in mice genetically deficient in 5-HTT expression.[42] Additionally, blockade of 5-HTT during postnatal days P2–P11 in mice leads to dendritic hypotrophy and reduced excitability of infralimbic cortical pyramidal neurons, whereas prelimbic cortical pyramidal neurons, which normally inhibit fear extinction, show an increased excitability.[43] In contrast, it has also been demonstrated that administration of the SSRI escitalopram during postnatal days P5–P21 reduces anxiety-related behavior in adolescent mice,[44] whereas *5-HTT* knockout mice exhibited increased anxiety-related behaviors in both elevated plus maze and open field test.[44] Because exploratory behavior was reduced in *5-HTT* knockout mice, and early exposure to fluoxetine produced some but not all features associated with constitutive 5-HTT deficiency,[44] these findings suggest changes in adult behavior induced by developmental exposure to antidepressants that are not recapitulated in *5-HTT* knockout mice. Additional work will be necessary to identify the molecular and neurodevelopmental mechanisms that affect emotional behavior by early life blockade of the 5-HT transporter.

Using bisulfite sequencing to compare methylation of 20 CpG sites close to the transcription start site of the promoter region of the serotonin transporter gene (*SLC6A4*), recent findings in two cohorts (saliva-derived DNA from 80 young adults, and blood-derived DNA from 96 adolescents) suggest that methylation of the *SLC6A4* promoter predicts amygdala reactivity

using blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI).[45] Together, these results suggest that epigenetic modulation of *5-HTT* (*SLC6A4*) affects behaviorally and clinically relevant brain functions. Insights gained from these studies will be key to identifying novel targets for diagnosis and therapy.

The serotonin 5-HT_6 receptor, whose canonical signaling pathway consists of activation of heterotrimeric G_s proteins and the cyclic adenosine monophosphate (cAMP) pathway, is suspected to modulate neuronal development. Recent findings also demonstrate an alternative 5-HT_6 receptor-dependent signaling pathway that participates in the control of neuronal migration.[46] It has been reported that the carboxyl terminus of the 5-HT_6 receptor recruits a population of signaling proteins,[47] such as cyclin-dependent kinase 5 (Cdk5), which is implicated in various neuropsychiatric disorders including schizophrenia, major depression, and Alzheimer's disease.[48,49] This 5-HT_6/Cdk5-dependent signaling pathway affected neuronal migration, neurite growth, and dendritic structure through a mechanism that required phosphorylation of the Cdk5 substrate histone H1.[47] Additionally, these structural effects were independent of 5-HT_6 receptor coupling to G_s.[47] This effect on neurite growth of primary neurons was also reduced by mutating Ser350 into alanine in the carboxyl terminus of the 5-HT_6 receptor.[47] Although these findings suggest that Cdk5 and 5-HT_6 are both implicated in the epigenetic regulation of neurite growth and neuronal migration, events that may be perturbed in developmental disorders, further work will be necessary to determine the precise epigenetic role of histone H1 as Cdk5 substrate in neuronal differentiation.

Long-term memories last a lifetime, whereas the RNA or protein markers that may underlie these memory traces are replaced with new functional copies on the order of hours or days.[50] An attractive hypothesis to explain how memories remain stable despite the constant molecular turnover is related to epigenetic mechanisms that may affect the intrinsic properties of neurons in a long-term fashion. An example of this model is the epigenetic control of serotonin-dependent modulation of synaptic plasticity. Using the *Aplysia* central nervous system as a model, researchers found that serotonin induces methylation of a conserved CpG island in the promoter region of the *CREB2* gene, leading to enhanced long-term synaptic facilitation.[51] Interestingly, this epigenetic hypothesis is further supported by recent findings suggesting that induction of long-term memory (LTM) by serotonin in *Aplysia* requires epigenetic changes.[52] LTM has been linked with functional strengthening of existing synapses and other processes including de novo synaptogenesis.[50] A manipulation that can erase LTM permanently is inhibition of the constitutively active catalytic fragment of the atypical protein kinase Cζ (PKM).[53] It has been found that LTM can persist following reconsolidation blockade and inhibition of PKM,[52] indicating that consolidated memories may be far more refractory to modification or elimination than generally supposed. If these findings are confirmed in mammals, it would challenge the idea that the synapse is a cellular site for long-term memory storage.

Most antidepressants have a delayed onset of therapeutic efficacy.[54] Specifically, SSRIs and tricyclic antidepressants often require several weeks of administration to reach full clinical efficacy. Using chronic social defeat stress as a model of depression in mice, researchers demonstrated that defeat stress induces lasting down-regulation of *BDNF* transcripts

together with increased repressive histone methylation at their respective promoters in the hippocampus.[55] Importantly, chronic treatment with the antidepressant imipramine, which inhibits serotonin uptake, induces a selective down-regulation of HDAC5.[55] In addition, it was demonstrated that herpes simplex virus (HSV)-mediated overexpression of HDAC5 in the hippocampus prevented the antidepressant-like behavioral effects of chronic treatment with imipramine.[55] More recent findings further support this view based on the demonstration that chronic treatment with the antidepressant fluoxetine induced a transient increase in *BDNF* expression in the adult visual cortex, an effect that occurred in association with increased H3ac at *BDNF* promoter regions and down-regulation of *Hdac5*.[56] These experiments highlight an important role for the serotonergic system in processes of histone remodeling related to depression.

Most of the studies focused on the roles of HDACs in the CNS have been centered on the canonical function of these enzymes (i.e., deacetylation of histone tails). However, recent studies demonstrate that histones represent a small fraction of the total acetylome affected by HDAC-dependent function.[57] Almost any kind of threat to homeostasis or stress will cause plasma glucocorticoid levels to rise, which has traditionally been linked to the physiological function of enhancing the organism's resistance to stress. Using rodent models of post-traumatic stress disorder (PTSD), it has been suggested that cytoplasmic HDAC6 modulates acetylation of a key component of the glucocorticoid chaperone complex in the brain: Hsp90.[58] According to findings based on transgenic *Pet1-Cre* mice crossed to *HDAC6*$^{loxP/loxP}$ mice in which a portion of the *Hdac6* gene (exons 8–9) is floxed, it has been reported that deletion of HDAC6 exclusively in serotonin neurons reduces the anxiogenic-like effects of the glucocorticoid hormone cortico-sterone.[58] The role of HDAC6 in emotional behavior via deacetylation of non-histone proteins, including α-tubulin, Hsp90, and cortactin, has been supported by the behavioral changes observed in HDAC6-KO mice, such as hyperactivity, less anxiety, and antidepressant-like behavior.[59]

Psychoactive drugs such as dissociatives (phencyclidine [PCP] and ketamine) and hallucinogens (lysergic acid diethylamide [LSD], psilocybin, and mescaline) induce alterations in perception, cognition, sensorimotor gating, and social skills that exhibit certain similarities with the endogenous psychosis of schizophrenia patients.[60–64] These drugs, when injected in rodents, also elicit their own specific set of behaviors, such as deficits in working memory, deficits in sensorimotor gating, modulation of locomotion, and a particular side-to-side head movement known as the head-twitch response.[60,65,66] Due to the psychotomimetic action of these compounds, LSD and other hallucinogens have been used repeatedly in the preclinical search for more effective antipsychotic compounds.[65] In the 1990s, considerable interest was focused on the possible connection between the serotonergic and glutamatergic systems in schizophrenia.[66,67] Milestone findings revealed that the mGlu2/3 receptor agonist LY354740 reversed the effects of PCP using paradigms such as working memory, deficits in sensorimotor gating, hyper-locomotor activity, and cortical glutamate efflux in an animal model.[68]

Follow-up studies by other groups demonstrated that LY354740 antagonizes the induction of excitatory postsynaptic potential and currents (EPSPs and EPSCs) via activation of the serotonin 5-HT$_{2A}$ receptor.[69] It was also shown that LY354740 suppresses the head-twitch

behavior induced by the hallucinogen (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI).[70] Based on these findings, there was an enormous increase in the number of publications describing the potential use of mGlu2/3 agonists as antipsychotic-like drugs in rodents, as compounds such as LY354740, LY379268, and LY404039 were found to repress the cellular, electrophysiological, and behavioral effects induced by hallucinogenic 5-HT_{2A} agonists.[71] It has also been demonstrated that the antipsychotic-like effects of these mGlu2/3 receptor agonists are mediated via mGlu2-dependent and not mGlu3-dependent signaling.[72–74]

In light of these animal studies, it was shown that the mGlu2/3 receptor agonist LY404039 (active compound of LY2140023) improved both positive and negative symptoms of schizophrenia compared with placebo treatment.[75] Importantly, however, follow-up studies provided convincing evidence that LY2140023 does not separate from placebo, whereas atypical antipsychotics such as risperidone and olanzapine were efficacious in the treatment of positive symptoms.[76–79] Thus, improvement in PANSS total score (Positive and Negative Syndrome Scale) was significantly greater in the standard of care (SOC: olanzapine, risperidone, or aripiprazole) group.[76–79] These findings preceded the press release by Eli Lilly and Company with the decision to stop clinical trials with LY2140023 for the treatment of schizophrenia. Recent preclinical and clinical findings, however, may provide an epigenetic model of why some of the schizophrenia patients do not respond to treatment with the mGlu2/3 receptor agonist LY404039 (Figure 1).

In schizophrenia patients, many articles describe improvement hours or days immediately after intramuscular anti-psychotic drug administration.[80] However, psychiatric disorders in general, and schizophrenia in particular, are often characterized by chronic drug administration (weeks, months, or even years of sustained drug treatment). The prolonged use of these drugs in schizophrenia treatment emphasized the importance of understanding the consequences of the use of these compounds long-term. In fact, a recent 20-year follow-up study demonstrates that long-lasting treatment with anti-psychotic medications does not eliminate or reduce the frequency of psychosis in schizophrenia patients.[81] Thus, treated patients will usually enter a stabilization phase in which psychotic symptoms have been controlled, but patients remain at risk for relapse if treatment is interrupted.[81] This demonstrates that the antipsychotics currently available are designed only to treat the symptoms of schizophrenia and not the underlying causes of the disease. Additionally, whereas these findings suggest that the therapeutic effects of antipsychotic drugs are acute, they also validate the importance of understanding the consequences of long-term antipsychotic drug treatment. For chronic treatment with typical and atypical antipsychotic drugs in mouse models, it has been demonstrated that chronic treatment with atypical antipsychotics markedly decreases the density of 5-HT_{2A} receptors in frontal cortex.[82–84] Considering that the density of 5-HT_{2A} receptors is increased in post-mortem frontal cortex of antipsychotic-free schizophrenic subjects, and reduced to control levels in postmortem frontal cortex of antipsychotic-treated schizophrenic subjects,[84,85] these findings suggest that down-regulation of 5-HT_{2A} receptor density by chronic atypical antipsychotic treatment may be one of the molecular mechanisms involved in their therapeutic effects (see refs 86 and 87 for review articles discussing the level of expression of 5-HT_{2A} receptor in the schizophrenia brain). However, it was also demonstrated that this down-regulation of 5-

HT_{2A} receptor density in mouse frontal cortex by chronic treatment with atypical antipsychotic drugs leads to down-regulation of the transcription of *mGlu2*, an effect that was associated with repressive histone modifications at the promoter region of the *mGlu2* gene in mouse and human frontal cortex.[83] This epigenetic change occurs in association with a 5-HT_{2A} receptor-dependent up-regulation of HDAC2 and increased binding of HDAC2 to the *mGlu2* promoter.[83,88] Based on these findings (Figure 1), it was proposed that chronic treatment with atypical antipsychotic drugs induces a selective 5-HT_{2A} receptor-dependent up-regulation of HDAC2 in frontal cortex of individuals with schizophrenia, which consequently induces repressive epigenetic marks at the *mGlu2* promoter and thereby limits the therapeutic effects of mGlu2/3 agonists in these patients.[83,88,89]

Notably, this hypothesis has recently been substantiated by re-evaluation of previous clinical work. Thus, preliminary data suggest that previous exposure of schizophrenia patients to chronic treatment with atypical antipsychotic drugs, such as olanzapine and risperidone, prevents the therapeutic effects of LY404039, whereas this mGlu2/3 receptor agonist induces antipsychotic effects in patients previously treated with typical antipsychotics, such as haloperidol, that separate from placebo.[90] In addition, a genetic association between SNPs in the *5-HT_{2A}* (*Htr2a*) gene and the response to LY404039 treatment has been demonstrated.[91] Further preclinical and clinical investigation is definitely needed to validate the molecular mechanisms underlying these effects of typical versus atypical antipsychotic drug treatment on therapeutic responses to LY404039. However, based on previous findings,[83] a potential approach to reverse or prevent the repressive epigenetic changes induced at the *mGlu2* promoter in frontal cortex of schizophrenia patients chronically treated with atypical antipsychotics may be the use of adjunctive treatment with HDAC2 inhibitors. Thus, adjunctive treatment with suberoylanilide hydroxamic acid (SAHA), which is a selective inhibitor of class I and class II HDACs, prevented the repressive histone modifications induced at the *mGlu2* promoter by chronic treatment with atypical antipsychotic drugs and improved their therapeutic-like effects in mouse models.[83]

ROLE OF PRENATAL ENVIRONMENT IN SEROTONIN-DEPENDENT SIGNALING

There is extensive evidence to suggest that genetics plays a significant role in the etiology of schizophrenia.[92–94] The genetic determinants, however, are complex, as clearly elucidated from studies on twins. Monozygotic twins, whose DNA sequences are ~100% identical, have a concordance for schizophrenia of nearly 50%.[95–98] Such results favor a significant contribution of genetic factors to the etiology of schizophrenia. At the same time, they argue for a significant role of environmental events in the development of this complex psychiatric disease. Epidemiological studies have indicated that maternal infection during pregnancy with a wide variety of agents, including viruses (influenza[99,100] and rubella[101]), bacteria (bronchopheumonia[102]), and protozoa (*Toxoplasma gondii*[103]) increase the risk of schizophrenia in the adult offspring. Similarly, maternal adverse life events that occurred during pregnancy, such as war, famine, and death or illness in a first-degree relative, have been associated with schizophrenia in the adult offspring.[104–106] A conservative hypothesis

would be that the vulnerability to schizophrenia is multifactorial, caused by the interaction between genes and environmental factors. Nevertheless, recent genome-wide association studies focused on *de novo* mutations show that the *5-HT$_{2A}$* gene (*Htr2a*) might potentially be implicated in schizophrenia-related synaptic netwoks.[93] Additionally, alterations in density of 5-HT$_{2A}$ receptor binding have been reported in frontal cortex of schizophrenia subjects, using both radioligand binding assays in post-mortem samples[84,85,107] and positron emission tomography (PET) in schizophrenia patients.[108,109] (See also refs 86 and 87 for review articles). The potential link between alterations in 5-HT$_{2A}$ receptor-dependent signaling and schizophrenia-related behaviors has been suggested recently by findings based upon rodent models of prenatal adverse life events.

A relevant line of research has recently been focused on the importance of maternal infection during pregnancy as a risk factor for schizophrenia.[110] Using the human influenza virus A/NWS/33CHINI, it has been shown that maternal influenza infection induces behavioral changes in the adult offspring that model schizophrenia-related symptoms, such as deficits in PPI in the acoustic startle response and alterations in exploratory behavior and social interaction.[111] Notably, more recent findings demonstrate that maternal infection with the mouse-adapted influenza A/WSN/33 (H1N1) virus causes up-regulation of 5-HT$_{2A}$ binding and *5-HT$_{2A}$* mRNA expression in frontal cortex in the adult offspring.[112] Additionally, the behavioral response to the hallucinogenic 5-HT$_{2A}$ agonist DOI was shown to be affected by maternal influenza viral infection.[112] Thus, adult mice born to influenza virus-infected mothers show increased head-twitch behavior and dysregulated locomotor activity in response to DOI.[112] Additional findings obtained by independent groups testing the effects of several environmental factors, such as variable stress during pregnancy,[113] maternal immune activation during pregnancy,[114] Roman Low- and High-Avoidance (RLA-I versus RHA-I) rat strains,[115] transgenic mice with a knock-in of a *tryptophan hydroxylase 2* (*Tph2*) R439H mutation,[116] and repeated administration of methamphetamine,[117] validate that adverse environmental effects induce up-regulation of 5-HT$_{2A}$ receptor in frontal cortex and dysregulation of 5-HT$_{2A}$ receptor-dependent signaling and behavioral function (Figure 2). Although together with previous findings in post-mortem human brain of schizophrenic subjects and in schizophrenia patients (see above), these results suggest that up-regulation of 5-HT$_{2A}$ receptor density might be involved in schizophrenia-related phenotypes, further investigation will be necessary to understand the molecular and epigenetic mechanisms responsible for the effects of prenatal insults on 5-HT$_{2A}$ receptor density and its function. The fundamental role of gene–environment interactions in the function of the 5-HT$_{2A}$ receptor is further supported by an association between changes in placental methylation of the *5-HT$_{2A}$* gene and deficits in infant neurobehavioral outcomes.[118] Together with the schematic model presented in Figure 1 (see above), these data also suggest that whereas up-regulation of 5-HT$_{2A}$ receptor density in frontal cortex of untreated schizophrenic subjects might predispose to psychosis, down-regulation of frontal cortex 5-HT$_{2A}$ receptor density by chronic treatment with atypical antipsychotic drugs may be one of the mechanisms underlying their therapeutic effects.

Additional evidence suggests that environmental factors affect serotonin-dependent function in mouse and humans. For example, using immunohistochemical approaches, it was reported that maternal stress in rats induces a decrease 5-HT$_{1A}$-like immunoreactivity in the

hippocampus of the adult offspring.[119] These findings correlate with the increased DNA methylation in the promoter region of the *5-HT$_{1A}$* (*Htr1a*) gene observed in schizophrenia and bipolar disorder.[120] Combining the paradigms of maternal stress with heterozygous 5-HTT deficient mice (*5-HTT$^{+/-}$*), genome-wide hippocampal gene expression profiling suggests that numerous genes and related pathways are differentially affected by prenatal stress and the *5-HTT* genotype.[121] Among these, MAPK and neurotrophin signaling pathways were shown to be dysregulated by both the *5-HTT$^{+/-}$* genotype and prenatal stress exposure.[121] Additionally, exposure of *5-HTT$^{+/-}$* mice to prenatal stress induced behavioral deficits related to cognition.[121]

Post-mortem human brain studies have revealed decreased forebrain expression of glutamic acid decarboxylase (*GAD1*), which correlates with increased DNA methylation of the *GAD1* promoter.[122] It is known that maternal behavior affects epigenetic programming in the offspring.[123–125] When the effects of maternal care on *GAD1* promoter activity were examined in the hippocampus of rats, it was demonstrated that mice born to high licking/grooming mothers showed enhanced hippocampal *GAD1* expression, which was accompanied by decreased DNA methylation at the *GAD1* promoter, compared with mice born to low licking/grooming mothers.[126] Epigenetic assays revealed enhanced H3K9ac binding to the *GAD1* promoter in the hippocampus of mice born to high licking/grooming mothers.[126] Additionally, treatment of hippocampal neuronal cultures with 5-HT significantly increased *GAD1* mRNA levels.[126] Together, these data suggest that serotonin-dependent signaling may serve as a tool to epigenetically affect the function of GAD1 and consequently the GABAergic system, which is linked to the pathophysiology of schizophrenia.

FUTURE DIRECTIONS

This review has summarized the increasing array of findings that support a role of serotonin-dependent epigenetic regulation of physiological and behavioral responses. We have focused our attention on two main aspects related to epigenetic mechanisms of serotonin signaling in whole animal models of psychiatric disorders such as schizophrenia and depression: the role of epigenetic mechanisms in serotonin receptor-dependent signaling and how environmental events during pregnancy induce stable adaptations and maladaptations of the serotonin system in the brain. The mechanisms of basic transcriptional and epigenetic mechanisms are varied and highly complex, and further studies are needed to understand what controls the epigenetic state of specific subsets of genes. An important question is related to how epigenetic changes are translated into transcriptional modulation. For example, no single epigenetic modification examined to date has been shown to be deterministic for a change in gene promoter activity. This suggests that numerous modifications that work in concert are required for transcriptional changes. Although epigenetic enzymes such as HDACs have emerged recently as attractive therapeutic targets, a major limitation is that manipulation of such targets will affect chromatin structure at hundreds or thousands of genes throughout the brain. Attractive approaches to succeed with these limitations are the use of synthetic zinc finger proteins (ZFPs) or sequence-specific transcription activator-like effectors (TALEs) coupled to an enzymatic moiety. These approaches would be designed to target a particular

chromatin modification to a given gene within a region of the CNS. These goals represent perhaps one of the greatest challenges in serotonin research.

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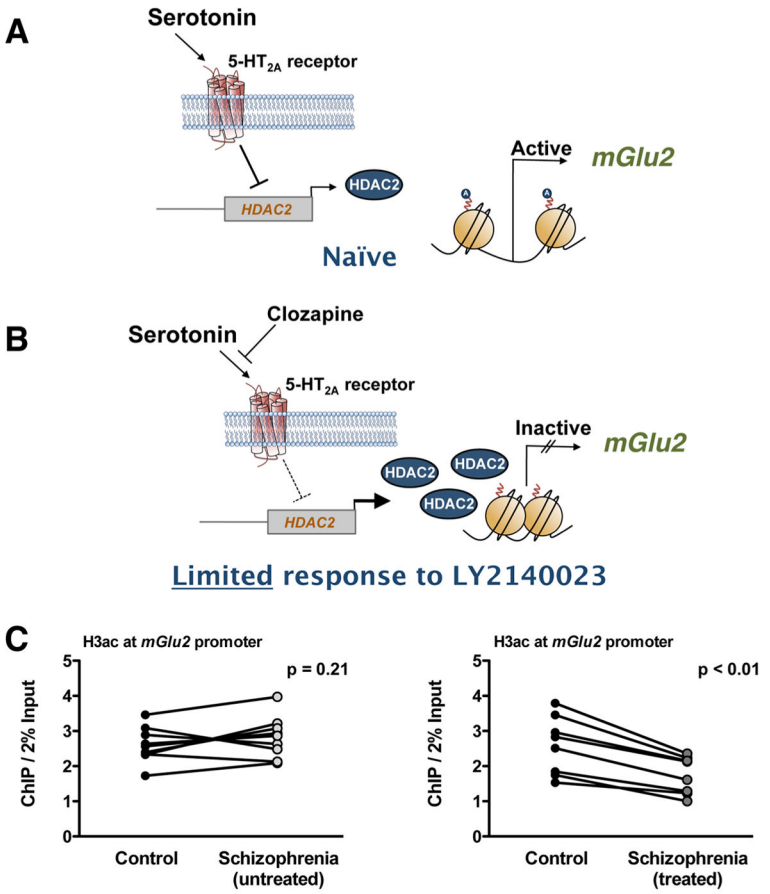

Figure 1.

(A, B) Schematic model of the effect of chronic atypical antipsychotic treatment on the epigenetic status of the *mGlu2* (*Grm2*) gene. Activation of the 5-HT_{2A} receptor by the endogenous neurotransmitter serotonin represses the promoter activity of the *HDAC2* gene in mouse and in human frontal cortex (see also ref 83). Atypical antipsychotic drugs, such as clozapine and risperidone, reverse the 5-HT_{2A} receptor-dependent repression of *HDAC2*, an effect that is associated with increased *HDAC2* promoter activity and repressive histone modifications at the *mGlu2* promoter. This epigenetic effect of chronic atypical antipsychotic treatment may limit the therapeutic effects of mGlu2/3 agonists such as LY2140023 (pomaglumetad, pro-drug of LY404039). (C) Decreased acetylation of histone H3 (H3ac) at the *mGlu2* promoter in prefrontal cortex of schizophrenia patients treated with atypical antipsychotic drugs (see also ref 83).

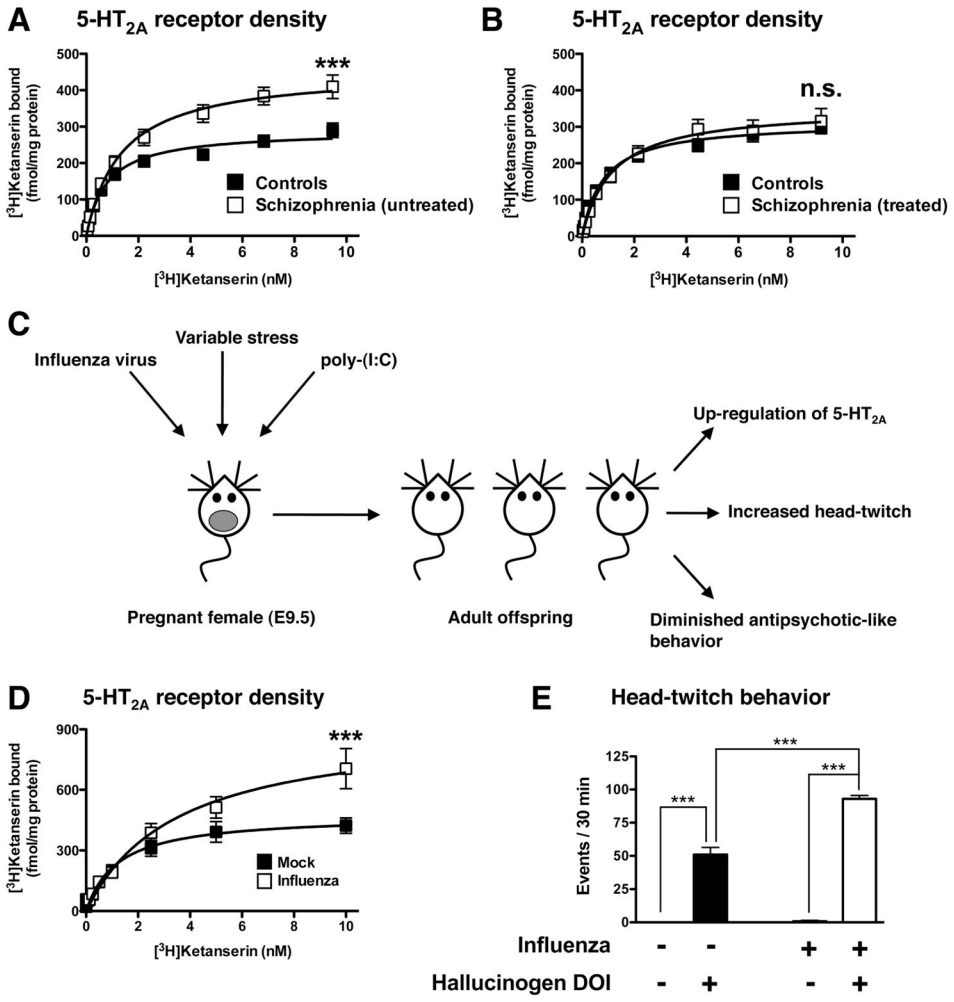

Figure 2.

(A, B) Increased 5-HT_{2A} receptor density as defined by [^{3}H]ketanserin binding saturation curves in post-mortem frontal cortex of schizophrenic subjects and individually matched controls. Note that 5-HT_{2A} receptor density is increased in untreated schizophrenic subjects (A) and unaffected in antipsychotic-treated schizophrenic subjects (B) compared with controls (see also refs 84 and 85). (C) In mouse models, prenatal insults during pregnancy such as maternal influenza viral infection, maternal variable and unpredictable stress, and maternal immune activation by injection of poly(I:C) induce alterations in expression and behavior function of the 5-HT_{2A} receptor in the adult offspring. These prenatal insults also decrease mGlu2-dependent antipsychotic-like behavioral effects of the mGlu2/3 agonist LY379268. (D) Increased 5-HT_{2A} receptor binding in frontal cortex of adult mice born to influenza virus-infected mothers (see also refs 112 and 113). (E) Increased head-twitch behavior induced by the hallucinogenic 5-HT_{2A} receptor agonist DOI in adult mice born to influenza virus-infected mothers (see also refs 112 and 113).