Published in final edited form as:

Curr Opin Behav Sci. 2019 February ; 25: 57–65. doi:10.1016/j.cobeha.2018.07.004.

Chromatin Regulation in Complex Brain Disorders

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Abstract

Chromatin-related phenomena regulate gene expression by altering the compaction and accessibility of DNA to relevant transcription factors, thus allowing every cell in the body to attain distinct identities and to function properly within a given cellular context. These processes occur not only in the developing central nervous system, but continue throughout the lifetime of a neuron to constantly adapt to changes in the environment. Such changes can be positive or negative, thereby altering the chromatin landscape to influence cellular and synaptic plasticity within relevant neural circuits, and ultimately behavior. Given the importance of epigenetic mechanisms in guiding physiological adaptations, perturbations in these processes in brain have been linked to several neuropsychiatric and neurological disorders. In this review, we cover some of the recent advances linking chromatin dynamics to complex brain disorders and discuss new methodologies that may overcome current limitations in the field.

Introduction

Complex brain disorders impede an individual's ability to function optimally in daily life, and they exert an extreme financial and personal burden on society. Unfortunately, most current therapeutics are only mildly efficacious and produce unwanted side effects. This has led many researchers in the field to try and identify more specific mechanisms of disease origin. While some rare brain disorders are the result of very specific, heritable genetic defects (e.g., mutations), the majority of neurological diseases have low to moderate heritability suggesting that their etiology results from environmental effects on gene expression. At the intersection between environment and gene regulation is epigenetics, which results in gene expression changes without altering the DNA sequence. While identical DNA templates provide the building blocks for every cell in a given individual, only a very small subset of genomic regions within each cell are accessible for regulation, thereby creating unique cellular identities and the promulgation of proper cellular functions. Epigenetics powers these transitions by altering the compaction of chromatin (i.e., DNA wrapped around a core octamer of histone proteins) to either allow access to specific loci of

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Conflict of Interest
Nothing declared.

the DNA template for gene transcription (i.e. euchromatic regulation) or reduce access to inhibit gene expression (i.e., heterochromatic regulation). These dynamic conformational changes are driven by enzymes and chromatin-interacting proteins that “read,” add, or remove chemical modifications on/to DNA and histone proteins (Figure 1).

Neurons, like most cells, undergo epigenetic regulation during development, which tightly and temporally controls specific gene transcriptional programs leading to differentiation into mature neurons. However, unlike most other cells in the body, post-mitotic neurons remain in this mature state for the entirety of an organism’s life. This is not to imply that neurons are static; on the contrary, they are highly dynamic and undergo constant epigenetic gene regulation to aid in adapting and functioning properly in response to an ever-changing environment. Fittingly, dysfunction in these processes is thought to underlie several neurodevelopmental, neurodegenerative and adult neuropsychiatric disorders. In this review, we discuss how epigenetics fits into the etiology of complex brain disorders, how this may lead to novel therapeutics and how future technologies may overcome current limitations in the field.

Learning, memory and related pathologies

In order for mature neurons to respond and adapt to changes in the environment, coordinated firing of neural circuits must result in long-lasting functional changes. One of the most adaptive abilities of the brain is to form new memories, which is critical for driving future behavior and survival. Theories of memory suggest discrete cell populations across many brain regions form neuronal ensembles or “engrams” that maintain stable, long-lasting connections with each other through alterations in protein content within the neuron, particularly at the synapse [1]. Initial learning is established by activity-dependent gene expression, where both heightened extracellular stimulation and intracellular signaling reaches the nucleus to initiate *de novo* gene transcription [2]. Importantly, expression of these “early-response” or immediate early genes (e.g., *Fos*, *Egr1*, *NPas4*, *Arc*, etc.) is under the control of chromatin regulation. Several epigenetic processes have been shown to be critical in memory formation and recall including DNA methylation [3] and hydroxymethylation [4], histone acetylation/methylation [5], histone turnover [6], chromatin looping [7] and DNA damage [8]. While this regulation sets up initial increased responsiveness to stimulation, long-term memories are maintained across extended periods of time that far exceed the turnover rate of any individual protein or histone post-translational modification (e.g., histone acetylation/phosphorylation). Therefore, epigenetic “priming” of plasticity-related genes within engram memory cells may occur following exposure to a highly salient stimulus or repeated exposure to the same stimulus that produces stable chemical modifications (e.g., DNA methylation/demethylation), substitution of transcriptional co-factors (e.g., CRTC1-KAT5) and incorporation of specific histone variants (e.g., H2A.Z, H3.3, etc.) at given genomic loci to allow for efficient and responsive transcription of memory-stabilizing genes upon reactivation [3,6,9,10]. This theory fits well with recent data indicating that long-term memories can persist even when learning-induced synaptic modifications are erased [11,12] suggesting that other neuronal compartments (e.g., the nucleus) may be ‘tagged’ to help maintain specific memories.

While epigenetic priming of synaptic- and plasticity-related genes supports the maintenance of memories, these same processes are activated following both positive and negative experiences. For example, drug and stress-induced events can create memories that persist across the lifespan of an animal leading to related pathologies. Both drug addiction and post-traumatic stress disorder (PTSD) are brain disorders that involve rewiring of learning circuits to fuel either the motivational drive to seek drugs upon re-exposure to drug-associated cues or elicit intense fear responses to otherwise innocuous stimuli. Like most memory processes, several epigenetic mechanisms are required for fear and reward memory to be established and maintained in animals (for review, see [13–17]). In such studies, observing similar effects in human post-mortem brain tissues increases translational value. For example, Hurd and colleagues recently found that human heroin addicts and rats trained to self-administer heroin display increased H3K27ac in striatum, a modification that correlates with increased expression of glutamate receptor subunits (e.g., GRIA1), as well as increased activity of striatal bromodomain-containing proteins that normally “read” acetylated lysines [18]. Further translational value has been demonstrated by the use of pharmacological agents that target chromatin-interacting molecules, including bromodomain inhibitors (e.g., JQ1) that alter cocaine- and fear-associated memories [18–21] and BRG1 (a subunit of the SWI/SNF nucleosome remodeling complex) inhibition that blocks cue-induced motivation in rats following extended cocaine withdrawal, a model of relapse-like behavior [22]. One consideration that needs to be taken into account with all animal models of drug addiction and PTSD is the distinction between adaptive and maladaptive reward and fear memory. Some have used procedures to produce long-lasting phenotypes that resemble several psychiatric diagnostic characteristics, as well as classifying animals according to symptom severity [23,24]. Such approaches will aid future studies in determining whether adaptive vs. maladaptive memories produce distinct epigenetic signatures.

Stress produces epigenetic vulnerability

A history of stress is known to produce vulnerability to the precipitation of several psychiatric disorders later in life. Major depressive disorder (MDD), for example, is one of the most common mood disorders where only ~60% of individuals are responsive to current antidepressant medications, thus highlighting the need for better therapeutics and a greater understanding of the underlying molecular etiology of this illness. Many common animal models used for studying depressive-like behavior are stress-related [e.g., early life stress (ELS) and chronic social defeat stress (CSDS)]. These procedures produce a reliable constellation of depressive-like phenotypes that include social avoidance, anhedonia, anxiety and learned helplessness [25]. Furthermore, an additional strength of these models is their ability to discriminate between susceptible (i.e., exhibiting depressive-like symptoms) vs. resilient animals based on social interaction measures. Many epigenetic changes occur in susceptible but not in resilient mice following CSDS—or vice versa—for review see [26]), and targeting these processes has been shown to impact behavioral outcomes [27–33]. Furthermore, ELS during adolescence has been shown to create greater vulnerability to depression-like behavior following CSDS in adult mice and produces a distinct set of transcriptional changes in the ventral tegmental area (VTA), a major hub for reward processing, compared to non-stressed controls [34]. Similarly, another study revealed that individuals with PTSD with or without childhood maltreatment had strikingly non-

overlapping transcriptomic and DNA methylation profiles in blood [35]. Therefore, stress during specific developmental windows may establish important epigenetic signatures that contribute to adulthood psychopathologies.

Genetic and epigenetic risk interactions

With diseases such as schizophrenia (SZ) and Alzheimer's disease (AD) having relatively high heritability [36,37], researchers have been actively seeking genetic causes for these diseases. From genome-wide association studies (GWAS), several single-nucleotide polymorphisms (SNPs) have been identified in loci of SZ and AD patients. SNPs are often found in non-coding and regulatory regions, which prove challenging for the identification of functional effects on downstream protein-coding genes. Furthermore, mutations in regions that reside within heterochromatin may be less functionally relevant than ones within euchromatin, although many questions remain regarding the actual impact of heterochromatic unsilencing in neurological disease states. One recent approach to investigate genome-wide accessibility of functional loci involves the use of ATAC-seq (Assay for Transposase-accessible Chromatin with sequencing) [38]. This technique allows for the detection of open chromatin regions (OCR) that are accessible to transcriptional regulation (both permissive and repressive). Forrest and colleagues [39] recently used ATAC-seq in excitatory neurons from SZ patient-derived induced pluripotent stem cells (IPSCs) to validate and functionally characterize GWAS SZ candidate loci. They found that an OCR flanking the *MIR137* locus contains common GWAS SZ mutations. Upon CRISPR/Cas9-mediated gene editing in control neurons to drive these GWAS mutations, *MIR137* expression and neuronal arborization were found to be directly impacted suggesting a functional role for this SNP in SZ. This approach, coupled with neuronal fluorescence-activated nuclear sorting (FANS), has also been utilized in post-mortem human SZ brain to identify a functional, rare and neuronal-specific SNP upstream of a vesicular transport protein, *SNX19* [40]. With respect to AD, Kellis and colleagues [41] recently found that in CK-p25 mice (a murine model of AD), several enhancer regions associated with immune-response genes were significantly enriched with active histone modifications (e.g., H3K4me3, H3K27ac) late in the development of neurodegenerative-related phenotypes, and that this correlated with increased expression of respective immune-related genes. These same enhancer regions overlaid significantly with SNPs in aggregated GWAS studies from humans with AD. Furthermore, these enhancer-residing SNPs were found to increase transcriptional activity compared to control sequences suggesting a causal genetic link. Thus, in using chromatin accessibility readouts and downstream validations, researchers were able to gain a clearer picture as to how genetic variants under epigenetic control may contribute to brain disorders with high heritability.

Epigenetic regulation of neurodevelopmental disorders

One of the critical functions for chromatin regulation in brain involves tightly controlling gene expression patterns—in a cell-type specific manner—during central nervous system (CNS) development. Autism spectrum disorders (ASDs), and other neurodevelopmental illnesses, are characterized by abnormal neural growth, connectivity and synaptic plasticity that lead to behavioral dysfunctions, such as deficits in social interaction/communication and increased repetitive behaviors. While the causes of these changes have not yet been fully

delineated, research suggests that a combination of genetic vulnerability, coupled with environmental perturbations such as toxins and maternal immune activation during pregnancy, may contribute to these disorders [42]. Several mutations have been linked to ASDs, but the diversity of these syndromes limits prescribing one causal gene link. Recent genome-wide histone acetylation profiling (e.g., chromatin immunoprecipitation plus sequencing, ChIP-seq, for the enhancer modification H3K27ac) in post-mortem ASD *vs.* control brains found a significant ASD-related signature that spans across the heterogeneity of ASD-related clinical presentations [43]. These signatures were also enriched in regulatory elements controlling many genes with rare mutations highlighting potential genetic/epigenetic influences. In addition, some mutations in chromatin modifiers themselves have been found to be associated with ASDs and have been tested in relevant animal models. For example, haploinsufficiency (i.e., only having one copy of the gene) of *ARID1B*, a component of the human BRG1/BRM-associated factor (BAF) chromatin-remodeling complex, was found to be causally responsible for a subset of ASD cases (e.g., Coffin-Siris syndrome). Jung and colleagues recapitulated this finding using *Arid1b*^{+/-} heterozygous mice and identified severe deficits in social and cognitive behavior that resulted from reduced cortical inhibitory tone [44]. Another causally related ASD linked haploinsufficiency gene, *SHANK3*, controls post-synaptic actin and glutamatergic scaffolding. Heterozygous *Shank3*^{+/-} mice display pronounced social impairments and repetitive behavior. Interestingly, H3 acetylation was reduced in these mice, with concomitant increases in histone deacetylase (HDAC) expression/activity observed. Strikingly, a brief, 3-d administration of the HDAC inhibitor Romidepsin produced alleviation of these deficits for ~3 weeks [45]. Such findings may offer alternative pharmacotherapeutic avenues for alleviating intellectual and behavioral deficits present in certain individuals with ASD.

Candidate genes alter epigenetic landscapes to prime apoptotic genes in neurodegeneration

AD is a debilitating neurodegenerative disorder caused by neuronal atrophy and cell death, which leads to severe cognitive impairments, memory loss and dementia. Two key molecules identified histochemically have been at the forefront of AD research: 1) extracellular plaques containing Amyloid- β protein, and 2) intracellular hyperphosphorylated neurofibrillary-associated Tau protein. Recently, these molecules have been linked to epigenetic consequences that further perpetuate cellular dysregulation and pathology. Indeed, widespread changes in histone modifications and DNA methylation have been reported in AD [46]. For example, amyloid-induced neuroinflammation leads to increases in HDAC2 expression in mouse hippocampus resulting in enrichment at, and decreases in the expression of, synaptic plasticity-related genes [47,48]. This results in reduced spine density and memory impairment, which is rescued by HDAC2 knockdown. Graff and colleagues further demonstrated that HDAC2 is upregulated in human AD patients, and that blocking HDAC2 in mouse models of AD rescues many memory-associated deficits [48]. Additionally, tauopathies have been shown to reduce levels of repressive histone marks (e.g., H3K9me2) and relax heterochromatin, thereby allowing for the expression of normally silenced genes that can send post-mitotic neurons back into the cell cycle [49].

Along with AD, other neurodegenerative diseases share common cellular phenotypes that lead to cell death and autophagy, which include oxidative cellular stress, protein aggregation, neuroinflammation and DNA damage repair. These processes have been linked to changes in chromatin that can increase the expression of apoptotic genes over time [50]. For example, TDP-43 is an RNA- and DNA-binding protein that underlies the cellular toxicity of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 aggregates in the nucleus of motor and cortical neurons and produces cellular stress and apoptosis. TDP-43 was recently found to interact directly with CHD1, a chromatin remodeler, to inhibit its function, which is to activate the expression of neuroprotective stress-responsive genes [51]. Additionally, loss of gene repression at normally silenced loci in mature neurons lead to neurodegenerative-like phenotypes [52].

New technologies to assess and manipulate histone post-translational modifications

Great advances have been made over the last decade in uncovering potential roles for histone post-translational modifications (PTMs), along with their associated enzymatic machinery, in the regulation of gene expression in brain [53]. Most histone-related research *in vivo* is currently centered on the use of antibodies that recognize a single histone PTM (e.g., ChIP, western blotting, etc.). However, site-specific antibodies suffer from many critical drawbacks, such as cross-reactivity with similar epitopes on the same or different proteins, limited specificity, lack of combinatorial PTM recognition, epitope occlusion by adjacent PTMs and the fact that they cannot be used to discover new PTMs [54]. Therefore, recent work has developed high-throughput on-line mass spectrometry (MS) platforms for the specific identification of distinct, simultaneously present histone PTMs; MS is currently the only method available for detection of multiple occurring histone PTMs on the same protein [55,56]. In addition, MS sample size requirements needed to assess PTM states are low, which allows for the detection of PTMs from single cells. This technology, in conjunction with single-cell transcriptomic, metabolomic and phenotypic analyses, will begin to elucidate the contributions of cell-type specific histone PTMs in complex brain disorders (Figure 2).

Furthermore, much debate still exists as to whether histone PTMs themselves are causally responsible for differences in chromatin states, and thus gene expression, or whether such differences are simply the consequence of dynamic processes associated with transcription and/or nucleosome remodeling. This is due to the fact that inferences of causality with respect to histone biology—in all biological systems, not just in brain—are often based upon correlations, as opposed to direct manipulations of the PTMs themselves in the absence of needing to alter the activity/expression of their associated enzymes; manipulations of which may result in unintended or intractable biological effects [57]. To this effect, directly modifying chromatin at specific genomic loci can now be achieved by the use of zinc finger proteins (ZFPs) or CRISPR/dCas9 fused to DNA sequence recognition domains. By conjugating domains of known enzymatic transcriptional activators and repressors (e.g., VP64, p65, G9a), these loci can be modified to alter gene expression via endogenous chromatin modifications. ZFP approaches have been shown to be effective in rescuing AD-related deficits in mouse models [58], in altering depression-like phenotypes [59] and in promoting the attenuation of cocaine-associated memories [60]. Furthermore, recent work

has demonstrated the regulation of multiple genes simultaneously using CRISPR/dCas9 *in vivo* [61], which may better recapitulate endogenous epigenetic modes of regulation. While this approach allows for the establishment/removal of locus-specific histone PTMs, the ability to alter global levels of a single or combination of histone PTMs (without altering endogenous related enzymatic activities)—in a cell-type specific manner—is also on the horizon employing a new and exciting methodology utilizing protein-splicing approaches. David and colleagues [62] recently demonstrated the use of trans-splicing inteins to incorporate non-native histone PTMs into chromatin in live cells. Protein splicing involves the use of intervening polypeptides, referred to as inteins (aka “protein introns,” originally discovered in yeast), which cleave themselves from a host protein (extein) to form a novel spliced peptide product. Inteins exist either as contiguous polypeptides (cis) or in split form (trans). In protein *trans* splicing, the intein fragments (N-terminal, Int^N and C-terminal, Int^C) initially bind, followed by rearrangement, and then finally self-excision leaving their exteins ligated [63]. David and colleagues genetically fused Int^N to a truncated histone H2B protein, expressed it constitutively by transfection in 293T cells, and delivered an Int^C carrying synthetic cargo, which was complementary to the truncated H2B, using a cell-penetrating peptide (CPP); a process that can be temporally controlled (Figure 3). With this method, it was possible to generate H2B (and other histones, such as H3) with an array of modifications *in vivo*. Thus, these approaches have the exciting prospect of being able to causally link histone PTMs to neuronal patterns of gene expression and behavior, both normal and aberrant.

Conclusions

As discussed above, recent advances in molecular biology and bioengineering have given researchers unprecedented insights into the role of epigenetic gene regulation in the CNS. With the advent of novel approaches that employ tagging of “activated” neurons (e.g., FosTRAP mice) [64] and cell type-specific nuclear isolation procedures (e.g., INTACT) [65] coupled to genome-wide DNA/histone modification analyses (e.g., Bisulfite-seq, ChIP-seq), chromatin 3-dimensional conformational studies (e.g., Hi-C) [66] and chromatin accessibility assessments (e.g., ATAC-seq) [38], neuroepigeneticists are now armed with the toolkits necessary to begin truly achieving greater specificity in studies attempting to causally link epigenetic processes to behavior and the precipitation of neurological disease states (Figure 2). Furthermore, recent pioneering work demonstrating that critical transcriptional regulators during development form biophysical/physiochemical barriers—as phase-separated liquid-like droplets—around important regulatory DNA elements (e.g., super-enhancers in stem cells) highlights the ever growing complexity in our knowledge of chromatin structure and regulation [67], attributes that will most certainly play a role in the mediation of neuronal transcriptional plasticity. Therefore, using cutting-edge epigenomic approaches, along with sophisticated animal models and both pre-/post-mortem human sample analyses, promise to elucidate these diverse roles for chromatin state changes in the development, maintenance and potential treatment of complex brain disorders.

Acknowledgements

We thank the USA National Institutes of Drug Abuse for funding (DP1DA042078 to ISM and T32DA007135 to RMB).

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Highlights:

- Experience can alter chromatin to perpetuate maladaptive behaviors and psychiatric disorders.
- Chromatin accessibility can influence functional genetic variants in heritable brain disorders.
- Neurodegeneration is fueled by changes in chromatin.
- Advances in histone biology will establish causal links between histone PTMs and behavior.

Figure 1. Epigenetic regulation of gene expression.

Chromosomes are comprised of chromatin fibers that contain nucleosomes: DNA (~146 bp) wrapped around the histone octamer (four core histone proteins, with two copies each of H2A, H2B, H3 and H4). Stretches of chromatin can either be open (i.e., euchromatic) or closed (i.e., heterochromatic) to transcriptional regulation. Enzymes and chromatin-interacting proteins that deposit (i.e., “writers”), remove (i.e., “erasers”) and “read” chemical modifications on DNA and histone tails alter the compaction of chromatin. DNA methylation is typically associated with heterochromatin, while depending on the type of chemical modification and residue, histone PTMs can either be associated with eu- or hetero-chromatin formation.

Figure 2. Techniques for assessing cell-type specific chromatin states in brain.

Alterations in chromatin following environmental stimuli/experiences underlie the development of several complex brain disorders. Accurate assessments of these changes can be achieved by using neural cell-type specific reporter lines where neurons are fluorescently tagged (can even be achieved following “activation” by environmental stimuli). Cells/nuclei can be sorted using INTACT or FACS/FANS and used for several downstream analyses: **1.** RNA-seq can measure relative levels of transcripts. **2.** ChIP-seq utilizes antibodies specific for histone PTMs and/or transcription factors to examine alterations in their genomic enrichment in response to experience. **3.** Hi-C can be used to discover contacts between proximal and distal genomic loci that may affect gene expression and/or chromatin structure. **4.** ATAC-seq can be used to access chromatin accessibility and for transcription factor footprinting. **5.** Histone MS can be used to identify and quantify single and combinatorial histone PTMs simultaneously. **6.** Global MS can be used to characterize the entire proteome within a given set of cells. INTACT = isolation of nuclei tagged in specific cell types; FACS/FANS = fluorescent-activated cell/nuclear sorting; seq = high-throughput sequencing; ChIP = chromatin immunoprecipitation; TSS = transcriptional start site; PTM = post-translational modification; ATAC = assay for transposase-accessible chromatin; MS = mass spectrometry.

Figure 3. The potential use of trans-splicing inteins for synthesis of modified histone proteins in brain.

Specific populations of neurons *in vivo* could be transduced to express truncated versions of histone proteins lacking segments of their tails fused to an intein fragment (e.g., Int^N). Then, using temporal delivery of a synthetic cargo in brain—via a cell-penetrating peptide (CPP) conjugated to the other intein fragment (e.g., Int^C)—modified histone tails could theoretically be delivered and fused to the truncated histone protein in a traceless manner. On the cellular level, the synthetic peptide would be taken-up by the endocytic pathway, after which it would be released to the cytosol following endosomal rupture. The naturally reducing environment of the cytosol would promote the release of the intein peptide from the CPP. The free intein containing the modified histone tail would then diffuse into the nucleus where it would splice with its intein counterpart, thereby leaving a site-specific modified histone incorporated in neuronal chromatin.