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Epigenetic regulation of cognition: A circumscribed review of the field

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Abstract

The last decade has been marked by an increased interest in relating epigenetic mechanisms to complex human behaviors, although this interest has not been balanced, accentuating various types of affective and primarily ignoring cognitive functioning. Recent animal model data support the view that epigenetic processes play a role in learning and memory consolidation and help transmit acquired memories even across generations. In this review, we provide an overview of various types of epigenetic mechanisms in the brain (DNA methylation, histone modification, and noncoding RNA action) and discuss their impact proximally on gene transcription, protein synthesis, and synaptic plasticity and distally on learning, memory, and other cognitive functions. Of particular importance are observations that neuronal activation regulates the dynamics of the epigenome's functioning under precise timing, with subsequent alterations in the gene expression profile. In turn, epigenetic regulation impacts neuronal action, closing the circle and substantiating the signaling pathways that underlie, at least partially, learning, memory, and other cognitive processes.

The human brain encompasses approximately 86 billion neurons (Azevedo et al., 2009) organized in a set of large- and small-scale synaptic networks. The functional and structural properties of these networks are what substantiate learning in particular (i.e., the capacity to encode, process, consolidate/integrate, and retrieve information) and cognitive processes in general. Numerous cellular and molecular mechanisms have been recognized as important players in learning and cognition, but among these mechanisms, a crucial role is thought to be played by de novo protein synthesis (Flexner, Flexner, & Stellar, 1963). Protein synthesis is firmly controlled by gene expression, which is in turn orchestrated by a number of mechanisms, including epigenetic mechanisms (Allis et al., 2007), which act via modifications to the DNA and histone components of nucleosomes and noncoding RNA molecules. Broadly defined, epigenetic processes are biochemical processes that regulate gene expression without altering the corresponding primary DNA sequence (Nikolova & Hariri, 2015).

Metaphorically speaking, epigenetics (from the Greek *epi-*, meaning over, outside of, or around) can be viewed as the Cinderella of the genomic sciences: initially out of focus in the field studying the etiology of complex human traits, yet now one of the most rapidly expanding fields in genetics/genomics. Such a shift in attention did not happen overnight, and the distance between Conrad Waddington's pioneering work on epigenetics (1942) and today's burgeoning field is mind-boggling. Of note is that this distance has been overcome in strides, marking the accelerating progression of the field. This acceleration is also notable now. If at the beginning of the 21st century there were only whispers of "an emerging view that epigenetics . . . plays a role in complex diseases" (Hatchwell & Greally, 2007, p. 588), the growing conviction that "the ability of epigenetic marks to persist during development and potentially be transmitted to offspring may be necessary for generating the large range of different phenotypes that arise from the same genotype" (Portela & Esteller, 2010, p. 1057) can be heard as a shout! Today epigenetic/epigenomic mechanisms are understood to exert both indirect and direct effects on phenotypes (Kiser, Rivero, & Lesch, 2015). The former are generally considered to be exerted at the interface between the genes and phenotypes of any individual, moderating and contextualizing the effects of the genome on complex behavior traits, embedded in a particular developmental context. The latter assumes that epigenetic mechanisms act as independent factors (Czyz, Morahan, Ebers, & Ramagopalan, 2012), whose impact generates what is known as Gene × Environment interaction.

However, it is important to note that the rapid emergence of epigenetics as a distinct and growing field of study, as reflected in the literature on the etiology of complex human behavior traits, does not question or diminish the role of the structural variation in the genome in the development of

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complex traits. Multiple studies have indicated that the vast majority of complex human traits display substantial heritability estimates (Polderman et al., 2015; Yang et al., 2013). Yet, structural variation, however important, cannot explain how a stem cell (i.e., an omnipotent cell that contains the baseline genome configuration for a particular individual) may develop into any kind of cell, how cloned animals derived from the same donor DNA may turn out to be neither identical to their donor nor to each other (Esteller, 2008; Rideout, Eggan, & Jaenisch, 2001), and how monozygotic twins are identical at the DNA sequence level, but have different patterns of DNA methylation (Feinberg, 2011; Fraga et al., 2005; Kaminsky et al., 2009) and histone modification (Fraga et al., 2005). Moreover, as epigenetics is generally defined as the study of mechanisms that spatially (i.e., where in the genome) and temporally (i.e., when in the genome) control gene expression in a potentially heritable way (Portela & Esteller, 2010), there is a differentiation between one's genetic (i.e., DNA sequence based) inheritance, the magnitude of which is captured by genetic heritability, and one's epigenetic (i.e., non-DNA sequence based) inheritance, the magnitude of which is captured by epigenetic heritability (Chuang & Jones, 2007; Li, Beard, & Jaenisch, 1993). Genetic and epigenetic inheritance both involve the transmission of information from cell to daughter cell or from generation to generation.

The spectrum of phenomena that are covered by the field of epigenetics is vast. There are only two illustrations, but they provide a metric of the heterogeneity of what is encountered in the field (Bird, 2007). The first illustration comes from one of the best studied and most straightforward examples of coat color in mice (*Mus musculus*). It is controlled by the agouti (also known as nonagouti) locus, whose function is affected by the extent of DNA methylation at an upstream transposon. Offspring with different coat colors (i.e., brown vs. yellow) may be generated by genetically identical parents whose agouti genes are in different epigenetic states. The second illustration comes from research on the effects of one of the most essential and influential behaviors: that of early maternal care. Although it has long been known that the quality of early maternal care has lasting effects on the development of an offspring, only recently a potential mechanism for these effects has been delineated from a study that established that maternal nurturing in rats (*Rattus norvegicus*) alters DNA methylation at the gene encoding the glucocorticoid receptor (Weaver et al., 2004). Inadequate maternal care is associated with hypermethylation of this receptor in young offspring, which is consequently associated with the downregulation of the receptor's expression in adults and with functioning and behavior (e.g., elevated anxiety). It was further demonstrated that natural variation in maternal care in a rat not only affects the epigenetic states and expression of the gene in question but also is noticeable in a larger group of functionally related genes in the studied locus (McGowan et al., 2011). This finding suggests that natural variation in maternal behavior results in a detectable and coordinated epigenomic response in the brain that affects networks of genes. Given the heterogeneity of the phenomena that are labeled as epigenetic/epigenomic, it is not surprising that the corresponding fields of inquiry are vast.

Two dominant usages of the word *epigenetic* have been differentiated (Bird, 2007). One of these usages goes back to Conrad Waddington (1957), who was interested in the study of epigenesis, or the differentiation of phenotypes from genotypes during development, that is, how gene function causes the emergence of a phenotype. Following Waddington's meaning of developmental biology, Robin Holliday defined epigenetics as "the mechanisms that impart temporal and spatial control on the activities of all those genes required for the development of a complex organism" (Holliday, 1990, p. 329). The other is attributed to Arthur Riggs and his colleagues (Russo, Martienssen, & Riggs, 1996), interested in the study of mitotic and meiotic non-DNA sequence-based mechanisms involved in the inheritance of phenotypic characteristics, that is, inheritance outside of mutational changes. This multiplicity of the meaning of the term *epigenetic* has been recognized as problematic and led to extensive discussion in the literature (e.g., Jablonka & Lamb, 2002; Ptashne, 2007; Van Speybroeck, 2002; Wu & Morris, 2001). To clarify the current uses of the term, Bird (2007) proposed a definition that captures today's field of studies and the connotation of the concepts of *epigenetic* and *epigenomic*. According to his definition, epigenetic events are those that result in ". . . the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" (Bird, 2007, p. 398). Bird stresses four features of this definition: (a) it is inclusive of chromosomal marks so that both transient and stable DNA modifications are included; (b) it focuses on chromosomes and genes and excludes, for example, prions, except when they directly encroach on chromosomal function; (c) it views epigenetic processes as interpreters of genetic variation, so that an identical combination of genes might produce different developmental outcomes; and (d) it conceptualizes epigenetic marks as reactive, not proactive. It is intriguing that this definition meets the expectation of a mnemonic, which is a memory-assisting device (Gräff & Tsai, 2013), or a mnemogenic reaction, which is a biochemical process that achieves cellular information storage (Day & Sweatt, 2010; Roberson & Sweatt, 2001), as epigenetic mechanisms defined as such "react to learning (that is, neuronal activity triggered by new information); and they can convey such information into specific gene expression programmes, which are a prerequisite for long-lasting memories" (Gräff & Tsai, 2013, p. 97).

The essential role of epigenetic mechanisms has long been recognized in such cellular processes as cell division, growth, and fate determination. The recent accumulation of evidence has illuminated the importance of epigenetic mechanisms in a number of other processes, including DNA damage control, genome stability, cell-cell interaction, and neuronal gene expression. With regard to the latter, the research indicates that the relevance of epigenetic mechanisms lies not only in the

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control of a single neuronal cell but also in the control of neuronal circuits and networks (Kinde, Gabel, Gilbert, Griffith, & Greenberg, 2015). The long-range impact of epigenetic mechanisms has been particularly striking in the analyses of higher level behaviors, such as stress regulation, social behavior, learning, and other cognitive processes. Animal models have demonstrated the wide range of this impact, from causing severe deficiencies to substantiating considerable improvement.

This review is focused on the insights into the molecular mediators of epigenomic regulation that have revealed how they mediate phenotypic variability in complex behaviors in general and cognitive processes in particular. Such insights have contributed enormously to the field's understanding of the (a)typical development of these processes. As this issue presents numerous excellent articles on DNA methylation, this article is focused primarily on other epigenetic mechanisms (not to understate the importance of DNA methylation, but simply to minimize redundancy). Moreover, whereas making a reference to, drawing from, and commenting on the fields of epigenetics and epigenomics in general, this review is particularly fueled by a strengthening idea that long-lasting (i.e., a substantial portion of the life span) functional states of neurons involve epigenetic phenomena (Hong, West, & Greenberg, 2005). The unique feature of these types of cells compared to virtually all other types of cells is that their states are not transmittable to daughter cells because almost all neurons never divide. Finally, the majority of the field of behavior epigenetics/epigenomics focuses on phenotypes that pertain to social–emotional development, whether typical or atypical, whereas this review is concerned particularly with phenotypes capturing various aspects of learning and cognitive processing, however defined.

This overview is structured in such a way that, first, it introduces some key concepts and definitions. Second, it covers major methods used in the field of genetics and genomics. Third, it provides a brief overview of the status of the field's appreciation, both data and theory driven, of the role of epigenetic mechanisms in cognitive processes. Fourth, it offers a number of comments on the overall status of and potential future developments in the field of behavioral epigenetics/epigenomics, with a particular emphasis on cognitive processes.

Concepts and Definitions: A Brief Overview

Epigenetic processes include posttranslational modifications of histones (Imhof, 2006), DNA methylation (Klose & Bird, 2006) and hydroxymethylation, actions of noncoding RNAs (Weinberg et al., 2006), prions and prionlike phenomena, chromosomal position effects, polycomb mechanisms (Schwartz & Pirrotta, 2007), and numerous other processes. These processes can be grouped into three main didactic categories: histone modifications and nucleosome positioning (Portela & Esteller, 2010), DNA (hydroxyl)methylation, and noncoding RNA actions. These mechanisms and transcription factors and other specialized proteins (Leader, Wang, Popov, Fu, & Pestell, 2006) collectively instruct the genome as to which subsets of genes should be expressed in each cell type, when, and to what extent. When considered on a genome-wide scale, epigenetic mechanisms are quite loosely, but still informatively, referred to as the epigenome (Fazzari & Greally, 2004).

As mentioned above, the original definition of epigenetic mechanisms (Waddington, 1942) denoted the mismatch between one genotype and multiple phenotypes as they arose from that single genotype; that is, it addressed the question of how differentiated cells emerge from omnipotent (i.e., stem) cells due to the interaction with their environmental context (Kiser et al., 2015). This differentiation is assumed to be associated with the concept of an *epigenetic mark*, which is both a marker and a mechanism of cell differentiation (Nikolova & Hariri, 2015). In this section, some of these markers and related concepts are described.

The methyl group (Me or CH_3) consists of an alkyl derived from methane, containing one carbon atom bonded to three hydrogen atoms. When attached to the DNA molecule, it juts out from the cytosine nucleotide into the major groove of the DNA. Its effect is twofold. First, its presence alters the binding patterns of transcription factors, that is, proteins that normally bind to specific DNA sequences to regulate the rate of transcription (Kim, Kollhoff, Bergmann, & Stubbs, 2003). Second, it attracts specific proteins that bind to it, resulting in gene silencing and chromatin compaction (Bird & Wolffe, 1999).

Cytosine–guanine (cytosine nucleotide–phosphate–guanine nucleotide [CpG]) rich regions of the genome (CpG islands) were initially identified through the analyses of the strikingly discordant patterns in the digestion of genomic DNA by the restriction enzyme isoschizomers, *Moraxella* species 3416 (*Msp* I) and *Haemophilus parainfluenzae* series II (*Hpa*II), which differ from each other in only one aspect: their sensitivity to cytosine methylation (Singer, Roberts-Ems, & Riggs, 1979). *Msp* I, the methylation insensitive enzyme, digests the genome to completion, whereas *Hpa* II, the methylation-sensitive enzyme, digests the genome such that the DNA maintains a high molecular weight, as in mammalian genomes, where 55%–70% (Bird, 1980) of all *Hpa* II sites are methylated. According to a different source, of the roughly 28 million CpGs in the human genome, 60%–80% are generally methylated (Smith & Meissner, 2013). *Hpa* II digests the hypomethylated minority of the genome into fragments that are known as *Hpa* II tiny fragments. The analyses of *Hpa* II tiny fragments (Bird, 1986) revealed that they predominantly consist of G+C dinucleotides, and are CpG rich. This observation in turn led to the development of the definition of a CpG island. A sequence is designated as such if (a) its content is 55% or more (G+C); (b) the observed to expected CpG dinucleotide ratio is 60% or greater; and (c) both occur within a sequence of 500 base pairs or more (Takai & Jones, 2002). Compared to the largely methylated CpGs, CpG islands are usually unmethylated (apart from imprinted loci, X-inactivated loci, and transposable elements) or are

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largely resistant to DNA methylation; they are observed in ~20% of the mammalian genome, which is substantially rarer than chance. CpG islands are frequent in gene promoters in general, and at transcription start sites of housekeeping and developmental regulator genes in particular (Deaton & Bird, 2011). This underrepresentation of CpG islands is biochemically driven. Specifically, the deamination of cytosine gives rise to uracil; uracil is easily identified as foreign by the DNA repair machinery and replaced. Conversely, the deamination of methylcytosine (mC) gives rise to thymine, which is not recognized and replaced as easily and can consequently result in mutations and deletions in the genome (Duncan & Miller, 1980). Cytosine methylation differs among tissues in both quality and quantity and is therefore physiologically variable (Fazzari & Greally, 2004).

Nucleosomes are basic chromatin units in which large eukaryotic genomes are packed into the cell nucleus. Nucleosomes include an octamer of histone proteins containing two pairs of core histones (designated by the letter H followed by a number, H2A, H2B, H3, and H4) around which 147 base pairs of DNA are wrapped in about two turns of a left-handed superhelix. Nucleosomes are arranged into higher order chromatin formations (Harshman, Young, Parthun, & Freiras, 2013). Histone proteins have protuberant N-terminal tails that are able to interact with nucleosomal DNA; these tails undergo posttranslational modifications (i.e., an addition of one or more chemical groups), including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, or ADP-ribosylation, mediated by the counteracting activities of enzymes, the so-called writers and erasers (Jiang et al., 2008). There are more than 100 distinct histone modifications (Margueron, Trojer, & Reinberg, 2005), which can dynamically affect the interactions between the histones themselves, between histones and DNA, and between histones and additional nuclear proteins (Kouzarides, 2007). For example, such modifications affect the direct interaction of histone tails with the nucleosomal DNA, thereby altering the chromatin state (Bannister & Kouzarides, 2011) and thus determining how accessible a stretch of DNA is to molecules regulating transcription. In this way, they may determine how likely a particular gene is to be transcribed at any moment. Acetylation is mediated by histone acetyltransferases (HATs) and usually enhances transcription (i.e., compacted DNA is transformed into a more "relaxed" structure accessible to transcriptional machinery as a result of the placement of an acetyl group on lysine), facilitating transcription initiation and elongation (Shahbazian & Grunstein, 2007). Deacetylation is catalyzed by histone deacetylases (HDACs). Phosphorylation is mediated by nuclear kinase; dephosphorylation is catalyzed by protein phosphatase. Methylation is mediated by the SET domain of histone methyltransferases (HMTs); demethylation is catalyzed by demethylases (HDMs). The activity of these enzymes is thought to be coordinated so that patterns of their combinatorial interactions result in specific chromatin modifications referred to as the *histone code* (i.e., a code for gene regulatory information). These codes in turn are recognized by protein complexes whose action is binary: to activate or to repress gene expression (Strahl & Allis, 2000). However, some histone modifications are inherited epigenetically (Grewal & Moazed, 2003; Ringrose & Paro, 2004).

Noncoding RNA molecules (ncRNAs) are those whose structure is encoded in DNA, but not translated into proteins. They form a large class of molecules, such as micro-RNA (miRNA), small interfering RNA (siRNA), PIWI-interacting RNA (piRNA), and long noncoding RNA (lncRNA), with the capacity to participate in the epigenetic machinery directly, that is, by modifying other RNA molecules or RNA–protein complexes, or indirectly, by binding to DNA- or chromatin-modifying enzymes and deploying their activity to specific genomic sites, thereby modulating chromatin states (Castel & Martienssen, 2013; He & Hannon, 2004; Mercer & Mattick, 2013).

A Brief Overview of the Methods Used to Elucidate Epigenetic Mechanisms

The main goal of epigenomics has been stated as the identification of the features of the DNA sequence that direct epigenomic processes. This identification is complex, involves multiple steps, engages both molecular experiments and bioinformatics analyses, and generates large-scale databases. These databases are not easy to process and interpret for a number of reasons: first and foremost, because of the related issues of correlation and causality; that is, a particular DNA sequence feature might be a result of the epigenetic process or may be a player in this process itself. Second, epigenomic mechanisms are time specific and, if not sampled at the "right" time, might not be traceable by the available analytical methods.

Cell-specific patterns of cytosine methylation and histone modification (including histone methylation) are thought to be major players in the differentiation of cell types. The system represented by these patterns is referred to as the epigenome (Novik et al., 2002), in which all epigenetic processes are included; it is called the methylome (Feinberg, 2001) when only DNA methylation-related processes are included. Thus, the identification and characterization of this system as a whole or its different patterns is the task of epigenetics (for specific genes) and epigenomics (for the genome as a whole). There are different laboratories, multilaboratory teams, and international groups that are engaged in this awe-inspiring task.

The International Human Epigenome Consortium is aimed in particular at the identification of the sites in which the cytosine methylation component varies between cell types, that is, differentiates various cells and tissues. It promises to provide insights into CpG methylation in 30,000 human genes using 200 cell types (Bradbury, 2003). Although still in progress, this project provides public access to epigenomic data (http://epigenomesportal.ca/ihec/index.html). In addition, a number of other public databases are available for open access. Thus, the Human Roadmap Epigenomics project has recently compiled and analyzed a total of 127 reference human epigenomes (Roadmap Epigenomics, 2015)

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and raw data, including basic DNA sequences, DNA methylation sites, and messenger RNA (mRNA) levels, assayed in peripheral blood and brain, among other tissue types (http://www.roadmapepigenomics.org and http://compbio.mit.edu/roadmap). Yet another resource is Braincloud (http://braincloud.jhmi.edu), a database containing genomic, epigenomic, and transcriptomic data sampled across stages of development (Schultz, 2002; Wang et al., 2012). The data are currently available only for the prefrontal cortex, but there is a plan to add additional brain regions.

An important issue in studying epigenetic mechanisms pertains to the question of the selection of cell types and tissues to study. As epigenetic marks are expected to vary between cell types and tissues, it is logical to assume that in studying epigenetic marks it is important to sample from the tissue whose function is being studied; that would be, of course, the brain for the purposes of understanding the role of epigenetic mechanisms in cognitive processes. Yet to do this working with human participants is near impossible with rare exceptions (i.e., working with postmortem or surgical tissue). Thus, it has been investigated whether peripheral cell types (i.e., blood or saliva) can be used as substitutions or veritable proxies for studying epigenetic patterns in the brain. These studies report blood–brain correlations in DNA methylation of up to 0.90 (Horvath et al., 2012; Tylee, Kawaguchi, & Glatt, 2013). Thus, although it is known that there are numerous regions of the methylome in particular (and likely the epigenome in general) that are variable among tissues, and that some differ substantially (the largest methylation differences have been observed within or near genes involved in tissue differentiation, including neurogenesis and hematopoiesis; Davies et al., 2012), it has been deemed informative to use peripheral cell types for understanding how epigenetic mechanisms operate in the formation and regulation of complex traits such as cognitive processes. First, significant cross-tissue concordance in methylation patterns has been observed for CG-rich promoters across genes (Davies et al., 2012). Second, it is reassuring that there are now multiple studies that have compared patterns of methylation between blood and saliva and found them to be consistently converging (Thompson et al., 2013). It is interesting that it has been observed that the methylation patterns of DNA extracted from saliva compared to those extracted from blood resemble more closely the methylation patterns of DNA from the brain tissue (Smith et al., 2015).

Yet, whatever population of cells from whatever tissue is used in the analyses, epigenetics and epigenomics employ a number of different methods: both generic, suitable for a "broad-net" (or genome-wide) association analyses, and highly targeted, suitable for elucidating specific regulatory mechanisms. Here, some brief accounts of methods of studying DNA methylation and histone modification are provided.

Since the original postulation of *genome methylation* as an epigenetic regulator (Holliday & Pugh, 1975; Riggs, 1975), numerous techniques have been developed for its analyses, the goals of which are to detect methylated sequences throughout the genome (Fazzari & Greally, 2004). Restriction landmark genomic scanning (RLGS) was one of the early techniques utilized for genome-wide epigenetic patterning analyses (Shibata et al., 1994). RLGS uses a combination of restriction enzymes, some of which are specific to DNA modifications. Specifically, RLGS cuts DNA and then labels it directly with a radioactive isotope (usually phosphorus-32) to detect differences in methylation at restriction sites (e.g., *Nocardia argentinensis* I [*Nar* I]) using two-dimensional gel electrophoresis. The resulting radioactive second-dimension gel is then transferred onto a film. The radiation produced by the radioactive labeling will result in the film being exposed wherever the restriction fragments have migrated during electrophoresis. The film is then developed so that a visual representation of the results in the form of an autoradiograph is generated. Repeated analyses of the same DNA samples subjected to an interrogation with the same combination of restriction enzymes will produce the same pattern of "dots" (exposures). However, if methylated and unmethylated DNA regions are compared, different patterns will emerge. That is, the autoradiographs generated from methylated and unmethylated DNA can be compared to reveal any changes that have led to visual differences in the film. Each autoradiograph contains thousands of spots, each corresponding to a labeled DNA restriction landmark. It readily detects alterations deviating from normal, and has been exceptionally effective in identifying hyper/hypomethylation in tumors, as well as changes in the methylome throughout the development of an organism. This technique, although perhaps not the easiest to use, is known for its high sensitivity. For example, its utilization has resulted in the identification of several imprinted genes (Plass et al., 1996; Shibata et al., 1994).

Subsequent to RLGS, additional techniques have been developed. These techniques share several features: (a) the methylated fraction of the genome is enriched in a specific manner that depends on the restricted digestion of unmethylated sequences using a methylation-sensitive enzyme; and then (b) polymerase chain reaction (PCR) is carried out, so that the digested fragments fail to amplify. Specifically, in differential methylation hybridization (DMH; Yan et al., 2002), two restriction enzymes are used simultaneously: *Mse* I (*Micrococcus* species) to reduce the average size of the DNA while preserving CpG-rich sequences and a 5′ methylcytosine-sensitive restriction enzyme (e.g., *Bacillus stearothermophilus* 458 series I [*BstU* I] or *Hpa* II). The amplification of intermethylated sites (Frigola, Ribas, Risques, & Peinado, 2002) uses the methylation-sensitive *Sma* I (*Serratia marcescens*) restriction enzyme. A methylation target array (MTA; Chen, Chen, et al., 2003) uses a similar approach to DMH, cutting initially with an enzyme that spares CpG-rich sequences followed by the use of a methylation-sensitive enzyme such as *BstU* I or *Hpa* II. Both DMH and MTA require a subsequent hybridization to genomic microarrays, whereas amplification of intermethylated sites, given the limited number of *Sma*I sites in the genome, utilizes a fingerprinting approach using electrophoresis.

As evident from the description above, all these methods depend on the characteristics of the specific enzymes they util-

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ize (as they cut DNA only at specific nucleotide sequences, or restriction sites), therefore limiting the proportion of CpGs in the genome that can be recognized by these enzymes. For example, in the human genome, the proportion of CpGs located within *Hpa* II sites, used in DMH and MTA, is estimated to be 8.04% (4.14% in transposable elements and 3.90% in unique sequences), but only the unique-sequence CpGs can be tested with hybridization techniques. The usage of *Not* I (*Nocardia otitidis-caviarum*), capitalized on by RLGS, results in even more limited representation of the CpGs (although it is more CpG island specific). Yet, despite their limitations, these techniques have been successfully utilized (Day et al., 2002; Huang, Perry, & Laux, 1999; Yan et al., 2000, 2002).

The next block of technologies have been developed only recently. These use a combination of cell types as the source of DNA, so that the CpGs at which methylation occurs varies (methylation-variable positions) and can be identified from the mixed methylated/unmethylated pattern observed (Novik et al., 2002). Specifically, matrix-assisted laser desorption/ionization is a mass spectrometry technique utilizing soft ionization. Two other technologies are based on the deamination of unmethylated cytosines with sodium bisulfite, or the enrichment with targeting antibodies (Heyward & Sweatt, 2015; Laird, 2010). There are various applications of these technologies, allowing for the analyses of candidate genes and regions, as well as for whole-genome analyses, either with microarrays or via sequencing. These methods do not have the limitations characteristic of the restriction enzyme-dependent methods. For example, among high-throughput methods used to characterize DNA methylation in the central nervous system (CNS), there are whole-genome bisulfite sequencing, ten–eleven translocation (TET)-assisted bisulfite sequencing, reduced representation bisulfite sequencing, and affinity enrichment based (e.g., MeDIP-Seq), each of them has advantages and disadvantages (Heyward & Sweatt, 2015; Table 1). Although there is an evident increase in the utilization of these techniques in studies of (a)typical development, cognitive processes have unfortunately been largely neglected by such studies.

The *composition of chromatin* (i.e., in studies of histone modifications) is analyzed using a technique called chromatin immunoprecipitation (ChIP). In general, ChIP assays are designed to detect connections between the genome and proteome, that is, between specific genomic regions and specific proteins, such as transcription factors on gene promoters and other DNA-binding sites, including detection of the specific locations of various histone modifications in the genome. The appeal of ChIP assays is that they are able to provide a glimpse into transcription regulation at any given time by registering specific Protein × DNA interactions and quantifying this interaction by means of various techniques, such as quantitative PCR, hybridization on biochip, or targeted sequencing. ChIP is a multistage experiment, engaging a number of molecular biology and biochemistry techniques, such as trapping protein–DNA interacting partners (crosslinking), cell lysis, nucleic acid shearing, antibody-based immunoprecipitation of DNA-protein complexes, DNA purification, and DNA quantification (quantitative PCR). The utilization of this method in genome-wide analyses using microarrays has resulted in the development of the ChIP on chip (also known as ChIP-chip) technology, which combines chromatin immunoprecipitation (ChIP) with the DNA microarray (chip). This technology, used with whole-chromosome oligonucleotide microarrays, has resulted in the mapping of transcription-factor binding sites (Cawley et al., 2004; Martone et al., 2003). Other examples include CpG islands (Weinmann, Yan, Oberley, Huang, & Farnham, 2002; Wells, Yan, Cechvala, Huang, & Farnham, 2003) and promoters (Li et al., 2003) identified across the genome. Although highly popular, ChIP-on-chip experiments are not without challenges because they require the creation of suitable genomic microarrays and the amplification of sparse starting material for hybridization (Buck & Lieb, 2004). In this regard, ChIP-on-chip methods share their main limitation with the whole-genome cytosine methylation methods: the need for widespread availability of suitable genomic microarrays. The current state of the art method to study chromatin marks across the entire genome is ChIP followed by massively parallel next-generation sequencing (ChIP-Seq). The DNA portion obtained after ChIP is not used to study selected genes or predefined sets of loci across the genome via quantitative PCR or to study the genome via microarrays, but is subjected to massive parallel sequencing. High-throughput sequencing now enables the elucidation of the complete methylome. There are now base pair resolution data, obtained when the methylome is sampled across development from zygote to terminally differentiated adult cells (Smith et al., 2012). The limitations of the method include the fact that it yields genome-wide maps of a given chromatin mark, the data analysis of which requires intensive bioinformatics manipulations. ChIP-Seq is routinely used in other research areas (Wang et al., 2008), but has just started being applied to the study of cognitive processes. To illustrate, Park, Rehrauer, and Mansuy (2013) used a fear conditioning paradigm for associative learning and then performed a genome-wide analysis of the acetylation of the lysine residue 5 of histone 4 (H4K5ac) via ChIP-Seq. Although there was a correlation between H4K5ac and learning-induced gene expression overall, it was substantially stronger when obtained for situations in which mice received strong fear stimuli. Commenting on this result, Fischer (2014) suggested that this finding is similar to those obtained in studies of human diseases and model animals lacking specific enzymes of the epigenetic machinery (Kerimoglu et al., 2013; Peleg et al., 2010).

To study ncRNA, researchers typically utilize methods that allow the isolation and genotyping or sequencing of small ncRNAs (e.g., microRNAs) as well as long ncRNA species in order to understand the role of ncRNA in gene activation and silencing and the posttranscriptional regulation of gene expression.

Finally, it is important to acknowledge the availability of novel transgenic systems that are used to confirm functions of epigenetic processes in multiple different lineages, and

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to identify the developmental windows in which specific epigenetic processes are essential (Smith et al., 2012).

Epigenetic Mechanisms and Cognitive Processes

There is a growing literature aimed at understanding the connection between epigenetic and cognitive processes. The main quest of this literature is to identify what has been referred to as an "epigenetic code for memory" (Gräff & Mansuy, 2008). Several types of epigenetic mechanisms have been associated with cognitive processes. The relevant literature is focused primarily on DNA methylation and hydroxymethylation, histone modification by acetylation and methylation, and the action of ncRNAs (Gräff, Kim, Dobbin, & Tsai, 2011). Here the corresponding literature will be reviewed briefly for illustrative purposes. This review is structured so that the literature on epigenetic processes is summarized; followed by a brief comment on the epigenetic regulation of a single gene whose role in cognitive processes is recognized; and then completed with a brief overview of the role of epigenetic mechanisms in clinical conditions, where cognitive processes are severely challenged.

DNA methylation

The important role of DNA methylation (for a review, see Wu & Zhang, 2010, 2014) has been established in a variety of biological processes, such as chromosomal inactivation, transposable element silencing, genomic imprinting, and embryonic stem development (Jaenisch & Bird, 2003). For example, allele-specific DNA methylational profiles have been associated with X chromosome inactivation (Pfeifer, Steigerwald, Hansen, Gartler, & Riggs, 1990) and gene-specific imprinting (Bartolomei, Webber, Brunkow, & Tilghman, 1993). DNA methylation is a covalent alteration of DNA catalyzed by a family of enzymes known as DNA methyltransferases (DNMTs). They catalyze the transfer of a methyl group from *S*-adenosyl methionine to DNA. There are five DNMTs known in mammals, DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L; yet only three of them, DNMT1, DNMT3a, and DNMT3b, manifest methyltransferase activity. These three enzymes are thought to have differential functions, with DNMT3a and DNMT3b referred to as de novo and DNMT1 as maintenance DNMT (Portela & Esteller, 2010). It is important to note that DNMT1 acts to maintain methylation profiles after DNA replication (Bird, 2002), bestowing the heritability of this epigenetic mark in cell generations. However, the division of labor between de novo and maintenance methylation is not straightforward, as captured in the revision of the model proposed by Jones and Liang (2009).

Methylation of DNA (i.e., the covalent attachment of a methyl group) at the fifth position carbon (5′ or C-5) within the cytosine pyrimidine ring (5-methylcytosine [5mC]; Bird, 2002) is considered to be the best studied epigenetic modification (Fischer, 2014). It is important to understand that what results from this reaction is a very strong carbon–carbon (the 5′ carbon on the cytosine ring and the methyl carbon) bond, the breaking of which requires a high degree of energy (Day & Sweatt, 2010). In mammals, DNA methylation typically occurs at promoter regions and at CpG dinucleotides within genes (Bird, 1980). Yet recent research has indicated the presence of a relatively high amount of non-CpG methylation, including nucleotide combinations such as CHG and CHH, where H is A, C, or T (Portela & Esteller, 2010). Moreover, according to the results of a genome-wide study that sampled DNA methylation at different developmental stages in the neurons and glial cells of mice and humans (Lister et al., 2013), non-CpG, CH methylation strongly accumulates in neurons through early childhood and adolescence and becomes the more prevalent form of methylation in mature neurons. Recent research has provided further evidence that non-CpG methylation plays a critical role in neuronal differentiation by showing that non-CpG methylation is a better predictor of neuron subtype-specific gene expression patterns when human GABAergic interneurons and glutamatergic projection neurons are compared (Kozlenkov et al., 2016).

The field of studies implicating DNA methylation in cognition originated in the late 2000s (Miller & Sweatt, 2007) and is burgeoning now. Miller and Sweatt (2007) demonstrated that exposure to learning (in this particular case, through the Pavlovian fear conditioning paradigm) led to an upregulation of the mRNA of de novo DNMTs (DNMT3a and DNMT3b) in the rat hippocampus, which in turn correlated with increased DNA methylation of the protein phosphatase 1 (*PP1*) and the *reelin* (*Rln*) genes within 1 hr after memory training. It is remarkable that learning-induced locus-specific DNA methylation is dynamic and the initial status can restore spontaneously, that is, expression changes in both *PP1* and *Rln* returned to a baseline level 24 hr after fear conditioning training (Miller & Sweatt, 2007). In addition, DNA-methylation was linked to the brain-derived neurotrophic factor (*Bdnf*) gene expression within 2 hr after memory training, while no difference in DNA methylation was detectable 24 hr later (Lubin, Roth, & Sweatt, 2008), This can be stimulated forcefully, by pharmacological inhibition of DNMT via the administration of DNA-methylation inhibitors such as 5-aza-2′-deoxycytidine or zebularine (Miller, Campbell, & Sweatt, 2008; Miller & Sweatt, 2007), or via injecting enzymatic DNMT inhibitors (Lubin & Sweatt, 2007) into the hippocampus. The impact of these latter pharmacological manipulations contribute to what is now viewed as convincing evidence that DNA methylation in the adult brain is quite dynamic and related to neuronal functioning during task performance, learning and new memory formation processes, and other cognitive processes (Fischer, 2014).

Another example of the importance of DNA methylation in memory formation comes from studies of the amygdala. It has been recently demonstrated that individual differences in the methylation of the proximal promoter of the human serotonin transporter (*5-HTT*) gene (solute carrier family C6, member 4 [*SLC6A4*]) explain 6.7%–10.4% of the variability

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in threat-related amygdala activity in two unrelated cohorts of participants (Nikolova et al., 2014). Four observations are of particular interest here. First, the association was consistent, when DNA methylation was assessed in blood and saliva. Second, it was observed at different stages of development, specifically in adolescence and young adulthood. Third, the variability was comparable to or greater than that of the association between the *SLC6A4* polymorphism–*5-HTT* linked polymorphic region (*5-HTTLPR*) genotype and amygdala activity (Murphy, 2013). Fourth, the methylation of the CpG site most strongly associated with amygdala activity was negatively correlated with serotonin transporter mRNA concentrations in postmortem amygdala tissue. Similarly, numerous examples now exist illustrating that increased methylation within (particularly in the promoter region) or near candidate genes for cognitive processes is associated with reduced gene expression (Nikolova & Hariri, 2015; Table 1).

Of importance as well were findings from these studies that implicated a kind of cross talk between learning-related DNA methylation and histone acetylation, such that the latter apparently overrides the former (Miller et al., 2008). These observations contributed to the growing literature on the presence of such cross talk, which has been demonstrated in a cell culture system (Cervoni & Szyf, 2001) and in vivo, where rat maternal behavior induced epigenetic programming (changes in DNA methylation and histone acetylation) in the offspring's glucocorticoid receptor gene promoter in the hippocampus (Weaver et al., 2004). The utilization of genetic approaches also offers support to the hypothesized importance of DNA methylation in cognition (Fan et al., 2001; Feng et al., 2010; Oliveira, Hemstedt, & Bading, 2012), although this evidence is still not voluminous.

The dynamic changes in the DNA methylation of genes related to cognition (e.g., *Rln* and *Bdnf*) might offer insight into the mechanism of the association between DNA methylation and learning and memory. For example, it has been reported that contextual fear conditioning within 24 hr after training induced hypermethylation of the gene *calcineurin* in the prefrontal cortex. Moreover, the gene's methylation persisted for 30 days, even after the gene's expression had returned to a baseline level. In addition, the administration of DNMT inhibitors 30 days after conditioning impaired long-term memory. These observations were interpreted as indicative of the importance of DNA methylation for memory consolidation, shifting the newly formed memory to the cortex, and memory stability.

DNA hydroxymethylation

It was recently observed that DNA is subject not only to methylation but also to hydroxymethylation. This epigenetic mark, the attachment of a hydroxymethyl group to 5-cytosine (5hmC), was initially described in the 1970s (Penn, Suwalski, O'Riley, Bojanowski, & Yura, 1972), but gained deserved attention from the field only much later (Rudenko & Tsai, 2014), due to reports of the presence of 5 hmC in the cerebellar neurons (Kriaucionis & Heintz, 2009) and the discovery of the TET proteins and their capacity to convert 5 mC to 5 hmC in mammals (Tahiliani et al., 2009). It turned out that three TET proteins (TET 1–3) mediate DNA methylation by initially converting 5mC to 5hmC, then to 5-formylcytosine and 5-carboxylcytosine, thereby eventually mediating active DNA demethylation (Pastor, Aravind, & Rao, 2013). Given the recent nature of these findings, relatively little is known about 5mhC and its related proteins. Of interest is that one study presented findings that TET1 can regulate and be regulated by neuronal activity (Kaas et al., 2013). Of interest as well is that one of the protein readers of 5mhC is the methyl-CpG binding protein 2 (MeCP2) protein (Spruijt et al., 2013), which is well known for its role in chromatin and transcriptional regulation through 5mC binding.

Compared with other cell types, hmC is substantially enriched in CNS neurons; hydroxymethylation accounts for 25%–40% of all modified CG dinucleotides in the frontal cortex and cerebellum (Kinde et al., 2015). As the data on the role of 5hmC DNA is just accumulating, its exact functional significance is not known yet. All TET proteins are expressed in the adult brain (Kriaucionis & Heintz, 2009; Szulwach et al., 2011). An analysis of the distribution of 5hmC in both human and mouse brains identified stable and dynamically modified loci during neuronal development and ageing (Szulwach et al., 2011). The whole-genome analysis of 5hmC dynamics during mammalian brain development has generated interesting findings (Lister et al., 2013), namely, that (a) 5hmC is enriched within active genomic regions in both fetal and adult mouse brains; and (b) despite lower absolute levels of hmC in the fetal brain, adult patterns of hmCG are already forming in utero in both neurons and astrocytes, suggesting the importance of hydroxymethylation developmentally (Rudenko & Tsai, 2014). Findings from the studies of TET1 have generated hypotheses about the importance of hydroxymethylation for increased transcriptional flexibility, which might be highly relevant for the genomic regulation of cognition (Rudenko et al., 2013). It has been shown that TET1 and TET3 mRNA levels appear to change in response to neuronal activity (Kaas et al., 2013; Li et al., 2014). Only a few animal model studies of hydroxymethylation have been conducted (Kaas et al., 2013; Rudenko et al., 2013), but their results suggest that 5-hydroxymethylcytosine is simply a product of DNA demethylation. These hydroxymethylation events are thought to be critical for transcriptional regulation, as well as long-lasting cellular status changes in development and heritable traits (Guan, Xie, & Ding, 2015; Shukla, Sehgal, & Singh, 2015).

Histone acetylation

Evidence connecting histone acetylation to learning has been accumulating since the 1970s (Schmitt & Matthies, 1979), when it was shown that the acetylation of histones is altered when rats undergo memory consolidation. The process of histone acetylation constitutes the addition of a negatively charged

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acetyl group to lysine residues (K) on a histone protein, which reduces the affinity between the positively charged residue and negatively charged DNA (Brownell & Allis, 1996) and structurally opens chromatin for translational activities. Specifically, the acetylation of H2BK5, H3K14, H4K5, and H4K12 has been shown to be particularly important in substantiating cognition (Gräff & Mansuy, 2008; Puckett & Lubin, 2011).

Histone acetylation is controlled by HATs, which catalyze the addition of acetyl group, and HDACs, which catalyze the removal of the acetyl group, exerting opposing actions on histones. Both HATs and HDACs are classified into subfamilies: Gcn5-related *N*-acetyltransferases (GNATs), MYST (named after founding members MOZ, Ybf2, Sas2, and Tip60), and p300/CREB-binding protein histones (p300/CBP) for the former, and zinc-dependent Classes I, II, and IV and NAD-dependent Class III (Haberland, Montgomery, & Olson, 2009) for the latter.

Early evidence from the 1970s has been critical for the renewal of interest in epigenetic mechanisms in the 21st century. Swank and Sweatt (2001) demonstrated that the exposure of mice to a novel taste can trigger long-lasting lysine acetylation and increased histone acetyltransferase activity in the insular cortex. The connection between histone acetylation and learning and memory has been further substantiated and differentiated by studies that focus on specific histone modifications and their corresponding enzymes (Federman, Fustiñana, & Romano, 2009; Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Levenson et al., 2004). Yet, because the majority of these studies report on bulk histone modifications, the field's understanding of the specifics (pairing particular modifications with particular enzymes and particular types of learning) is still limited.

The functional meaning of histone acetylation is in the modification of a chromatin structure such that it becomes more permissive to gene transcription; it is accomplished by decreasing the electrostatic affinity between histone proteins and the DNA (Kouzarides, 2007; Li, Carey, & Workman, 2007). Moreover, there is evidence that histone acetylation intensifies following neuronal activity, thereby promoting gene expression changes and, consequently, long-term synaptic plasticity and memory (Gräff & Tsai, 2013). In other words, there are different types of cellular modulations that trigger histone acetylation, which in turn enhance ongoing or prompt other modulations. These are modulations of and by neuronal activity, synaptic plasticity, and memory formation (Gräff & Tsai, 2013).

Histone acetylation has been observed to be initiated by a number of different types of *neuronal activity*, including the stimulation of neurotransmitter pathways by receptor-specific agonists (Crosio, Heitz, Allis, Borrelli, & Sassone-Corsi, 2003), potassium chloride mediated neuronal depolarization (Maharana, Sharma, & Sharma, 2010), and, more generically, activation of the mitogen-activated protein kinase pathway (Sweatt, 2001), either directly (Levenson et al., 2004) or through cross talk with other types of histone modification (Latham & Dent, 2007). Other triggers of histone modification have also been registered (Gräff & Tsai, 2013). Of particular relevance to the discussion here is the role of brain-derived neurotrophic factor (*BDNF*) in this process (see references to this gene above and below). *BDNF* is a known agent of neuroplasticity (Cowansage, LeDoux, & Monfils, 2010). It has been demonstrated, for example, that the stimulation of cortical neurons with BDNF resulted in the nitrosylation of HDAC2 on cysteine (C) 262 and C 274, histone hyperacetylation, and a concurrent increase in neurotrophin-dependent gene expression, including *BDNF* itself (Nott, Watson, Robinson, Crepaldi, & Riccio, 2008). It is also known that the expression of the *BDNF* gene is intensified by neuronal activity-driven calcium-dependent derepression by MeCP2 (Chen, Chang, et al., 2003). Conversely, it is negatively regulated by HDAC2 (Guan et al., 2009). Based on these two observations, it can be hypothesized (Gräff & Tsai, 2013) that a surge of neuronal activity may engage a positive-feedback loop clustered around HDAC2 and BDNF, which can lead to histone acetylation mediated, self-sustaining gene expression action that in turn can result in a change in synaptic strength and thus learning.

Histone acetylation also promotes and is promoted by long-term *synaptic plasticity*. The literature contains examples of associations between long-term facilitation (LTF; transcription-dependent facilitation of electrical transmission across synapses) and enhanced histone acetylation in the genes implicated in LTF (Guan et al., 2002), and between long-term depression (transcription-dependent deterioration of electrical transmission across synapses), and reduced histone acetylation in the genes implicated in long-term depression (Hart et al., 2011). Induction of the molecular equivalent of LTF in mammals, long-term potentiation (LTP; an increase in synaptic transmission efficiency as a result of presynaptic high-frequency stimulation), has also been associated with the intensification of H3 and H4 acetylation (Levenson et al., 2004), in particular at the promoter regions of *Bdnf*, a rodent homolog of the human *BDNF* gene (Sui, Wang, Ju, & Chen, 2012).

Histone acetylation also appears to be associated with *memory formation*; moreover, it has been hypothesized that different types of memory and learning might "elicit distinct epigenetic signatures in the brain" (Gräff & Tsai, 2013, p. 99). Of particular interest here is that such memory-induced histone acetylation is specific to certain genes implicated in learning and memory, in particular, *Bdnf*, which shows an increase in expression concurrent with the increase in acetylation (McQuown et al., 2011). Once again, it is hypothesized that histone acetylation triggers a system of positive reciprocal processes involving the modulation of gene expression, and further histone modifications (Gräff & Tsai, 2013).

Because of the accumulation of evidence on the role of histone acetylation in learning in general and specific cognitive processes (e.g., memory) in particular, there have been systematic attempts to, first, differentiate the roles of HDACs and HATs as functionally specialized groups of molecules and, second, differentiate the roles of different types of HDACs and HATs within these groups.

With regard to HDACs, 11 of the mammalian HDACs require the Zn_{2+} ion as a cofactor and are often referred to as

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zinc-dependent HDACs (de Ruijter, van Gennip, Caron, Kemp, & van Kuilenburg, 2003; Gregoretti, Lee, & Goodson, 2004). Under typical circumstances, all HDAC genes are expressed in an adult brain (Broide et al., 2007). Although there are some initial data implicating them in cognition (Gao et al., 2010), there is considerably less information on the NAD-dependent Class III HDACs, or sirtuins (Bordone & Guarente, 2005; Haigis & Sinclair, 2010), with regard to learning and specific cognitive functions as compared to HDACs. The zinc-dependent HDACs include Class I (HDAC1–HDAC3 and HDAC8), Class IIa (HDAC4, HDAC5, HDAC7, and HDAC9), Class IIb (HDAC6 and HDAC10), and Class IV (HDAC11); they all are expressed in the brain. Yet, their brain expression is differentiated such that HDACs differ by subcellular localization, the frequencies at which they are expressed in different regions in the brain, and by their known (or potential) function in memory formation (Fischer, 2014; Gräff & Tsai, 2013). For example, *Class I HDACs 1–3* are known to be negatively associated with learning and memory (Guan et al., 2009; Malvaez et al., 2013; McQuown et al., 2011; Nelson, Bal, Kavalali, & Monteggia, 2011). Although HDAC1 and HDAC2 are close homologues that originated from gene duplications, their functions differ. Thus, HDAC1 has been implicated, more specifically, in memory extinction (Bahari-Javan et al., 2012) and HDAC2 and HDAC3 in memory constraints (Gupta-Agarwal et al., 2012). It appears that HDAC2 is particularly attention worthy, because the literature contains convincing evidence on its increased expression, and therefore decreased histone acetylation, in Alzheimer disease (AD; Gräff et al., 2012) and aging (Peleg et al., 2010), particularly in *Bdnf* (Gräff & Tsai, 2013; Walker, LaFerla, Oddo, & Brewer, 2013). There is also evidence, although limited, that *Class IIa HDACs 4, 5, 7,* and *9* are also relevant to cognition (Gräff & Tsai, 2013), with HDAC4 (Kim et al., 2012; Wang et al., 2011) and HDAC5 (Gupta-Agarwal et al., 2012; Koseki et al., 2012) being better researched than HDAC7 and HDAC9. Specifically, a shortage of HDAC4 in adult mice brains is associated with impaired memory formation and plasticity (Kim et al., 2012; Sando et al., 2012). Of interest also is a report that there is a subtype of intellectual disability that is associated with the haploinsufficiency of HDAC4 (Williams et al., 2010). Even less research has focused on *HDAC Classes IIb and IV*; yet, although scarce, these studies do provide some evidence of the involvement of the members of these classes, for example, HDAC11 (Gupta-Agarwal et al., 2012), in substantiating learning and memory.

Compared to the body of HDAC-related research, the literature on HATs is limited. There are at least 18 HATs encoded in the mammalian genome, which are clustered in several families: the GNAT, the MYST, and the p300/CBP families; and several other HATs that cannot be grouped (Allis et al., 2007; Lee & Workman, 2007). Of these families, the majority of studies focus on the p300/CBP HATs. Thus, one of them, CBP/KAT3A, has been shown to participate in memory consolidation (Alarcon et al., 2004; Barrett et al., 2011; Korzus, Rosenfeld, & Mayford, 2004; Wood, Attner, Oliveira, Brindle, & Abel, 2006; Wood et al., 2005), although the data are not consistent (Josselyn, 2005). Yet another member of this family, P300/KAT3B, has been demonstrated to impair memory consolidation (Maurice et al., 2008; Oliveira, Wood, McDonough, & Abel, 2007). Unfortunately, at this point, genome-wide investigations of transcriptional networks associated with CBP function are limited; yet, there is evidence connecting CBP function to synaptic plasticity (Chen, Zou, Watanabe, van Deursen, & Shen, 2010) and altered gene expression following environmental enrichment training (Lopez-Atalaya et al., 2011).

It is also important to note that numerous researchers have investigated the effects of various nonspecific histone deacetylase inhibitors, including trichostatin A, suberoylanilide hydroxamic acid, sodium butyrate, phenylbutyrate, and valproic acid on learning and memory in mice and rats. The general conclusion that has arisen from this work is that these treatments appear to be effective in ameliorating specific cognitive deficits and improving various aspects of cognitive processes in animal models in response to specific targeted mutations, triggered neurodegeneration, and traumatic brain injury (for review, see Rudenko & Tsai, 2014). Clearly, there is promise that needs to be further examined with regard to the applicability of these leads to human research.

To conclude, there is substantial evidence that histone modification enzymes HATs and HDACs are important players in various cognitive processes. As Fischer stated, pointing to these enzymes' "general role in the regulation of cellular processes, it is tempting to speculate that they coordinate gene expression programs linked to long-term memory consolidation, but a major effort in future research will be to understand the mechanisms by which HATs and HDACs regulate plasticity in specific brain cells" (2014, p. 950).

Histone methylation

Histone methylation is also regulated by the counteracting activity of HMTs and HMDs. However, unlike in acetylation, in methylation the lysine residues (K) of histones can be mono-, di-, or trimethylated (me, me2, me3). Moreover, unlike acetylation again, the impact of histone methylation on transcription can differ based on what particular K residue of the histone tail is altered (Lipsky, 2013). Each of these residues can be catalyzed by specific enzymes (Badeaux & Shi, 2013): HMTs and HDMs.

There is a growing body of studies indicating histone methylation in neuronal plasticity and, correspondingly, in cognitive functions. One popular target of these studies is H3K4 trimethylation (H3K4me3), indicating active gene promoters. H3K4me3 is catalyzed by at least 10 different enzymes (Badeaux & Shi, 2013). It has been shown that the amount of H3M4me3 increases along with unfolding fear conditioning training in rodents (Gupta et al., 2010), and that levels of H3K4 me3 and me2 correlate with the expres-

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sion of glutamate receptors in the human brain (Stadler et al., 2005). Model animals in whom the function of H3K4 HMTs is altered demonstrate impaired memory (Gupta et al., 2010; Kerimoglu et al., 2013); moreover, the mechanism for this impairment has been further traced to the deregulation of learning-relevant genes and H3K9 acetylation (Kerimoglu et al., 2013). This latter observation further substantiated evidence of some cross talk between different histone "-lations." Yet, overall, the relevant literature is relatively small and not without contradictions (Gupta-Agarwal et al., 2012; Maze et al., 2010; Neelamegam et al., 2012; Schaefer et al., 2009). The role of histone methylation is considered critical, but the direction of its impact, as documented from experiments with different methyltransferases (HMTs), is not homogeneous. Even less data are currently available for HDMs, although, as is the case with HMTs, the available data are strongly suggestive of their relevance to cognitive functions (Rujirabanjerd et al., 2012; Simensen et al., 2012). In his analyses of the available data on histone methylation, Fischer (2014) concluded that histone methylation and HMT- and HDM-related activities appear to be relevant to cognitive processes, but no clear picture of this relevance is available just yet.

Noncoding RNAs

It is well established that, in addition to coding RNAs, the transcriptome includes noncoding RNAs of various sizes and types, and ncRNAs constitute the majority of the transcriptome. Noncoding RNAs are typically differentiated by their length, so that RNAs below 200 nucleotides are referred to as small and above 200 bases as long. Numerous ncRNAs have been identified, and their diversity and prominence are particularly noticeable in the brain (Qureshi & Mehler, 2012). Best researched are small ncRNAs known as miRNAs. These RNAs are 12–22 (~21, on average) nt long and catalyze gene silencing by binding to a target messenger RNA that in turn stimulates either its degradation or the inhibition of protein translation, thereby regulating protein homeostasis (Im & Kenny, 2012). Although there is a disagreement as to whether the action of ncRNA should be viewed as one of the epigenetic mechanisms, there is clear evidence that they mediate gene expression (Fatica & Bozzoni, 2013).

In particular, in neurons, specific miRNAs regulate a number of target genes to control protein secretion. For example, miR-134 (mature miRNA, which are numbered) has been demonstrated to regulate negatively the size of dendritic spines (Schratt et al., 2006). What underlies this regulation is the inhibition by miR-134 of an mRNA encoding a protein kinase that controls spine development, Limk1. There are numerous processes that permit such long-range control of protein synthesis; one such process is mRNA trafficking (Cougot et al., 2008). The same miR-134 has been hypothesized to regulate plasticity in neurons (Bekinschtein et al., 2008). Specifically, overexpression of miR-134 in the hippocampus C1 area led to a significant impairment in long-term memory using the contextual fear conditioning model. Conversely, reducing miR-134 in vivo increased memory function.

It has been shown that miRNAs are critical regulators of synaptic plasticity (Karr et al., 2009; Lee et al., 2012; Saba et al., 2012; Schratt, 2009; Schratt et al., 2006). It has also been shown that miRNA has an important role in memory consolidation, for example, via the regulation of the cyclic-AMP-responsive element-binding protein (CREB) in serotonin-induced synaptic plasticity (Gao et al., 2010; Rajasethupathy et al., 2012). Yet, it has been acknowledged that the impact of miRNA is challenging to encapsulate, because these RNAs are functionally more volatile (Kiser et al., 2015). For miRNA to mature, the double-stranded RNA-specific endoRNA (DICER) protein is required. Interpreting available data on DICER, Fischer (2014, p. 955) suggested that miRNA "may represent a molecular brake to memory formation processes, which is critical for neuronal homeostasis."

Long noncoding RNAs (lncRNAs) represent a different class of ncRNA molecules whose functions in the regulation of neural development and functioning have rarely been explored to date. Nevertheless, emerging evidence suggests that lncRNAs are capable of inducing hypomethylation in gene promoters, thereby regulating gene expression (Kurihara, Shiraishi, Satake, & Kimura, 2014). The results from a recent study by Kaur et al. (2016) suggested that BC048612, a long ncRNA, coregulated (with miR-203) the expression of neuronal growth regulator 1 (NEGR1) cell adhesion protein in neurons. In this study, the patterns of expression of lncRNA and the coacting miRNA were specific to different neuronal maturation stages: lncRNA expression increased the amount of NEGR1 during early maturation of the neurons, with the regulatory control being "passed to" the miRNA during later stages to fine-tune the expression of NEGR1. Thus, although little is known at this point about ncRNAs comparatively speaking, it is a very promising class of agents for understanding how the brain organizes gene expression during cognitive processing.

Epigenetic regulation of the BDNF gene in rodents and humans

BDNF is a small secreted protein that is a well-studied member of the neurotrophin family of growth factors (Leibrock et al., 1989). It is essential for the establishment of LTP and is intimately involved in neuroplasticity and learning and memory (Choi et al., 2010; Park & Poo, 2012; Psotta, Lessmann, & Endres, 2013). There is a voluminous literature on BDNF, because it plays a very important role in (a)typical neural development. In the context of this review, however, what is relevant is the recent interest in the epigenetic regulation of BDNF that mediates the influence of the environment, however defined, on the expression of the *BDNF* gene (Roth & Sweatt, 2010).

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The first report focusing on the in vitro connection between neuronal depolarization and hypomethylation within the transcriptional regulatory region of the *BDNF* gene, along with the corresponding increase in *BDNF* mRNA expression, was published by Martinowich et al. in 2003. The importance of this finding cannot be overstated because it was a major step toward understanding the role of DNA methylation as a potential mediator of activity-dependent transcriptional regulation within the CNS. The results of this work have not only substantiated the hypothesized importance of epigenetic dynamic regulation (i.e., reduced methylation–increased *BDNF* gene expression; increased methylation–decreased *BDNF* gene expression) but also opened a new chapter in detailing the working model implicating transcriptional regulation as the permissive context for synaptic plasticity and memory (Kandel, 2001, 2012; Levenson et al., 2004).

The data on epigenetic mechanisms of BDNF regulation have been obtained primarily in rodent studies (for a review, see Boulle et al., 2012). For example, it has been demonstrated that MeCP2 is a repressor of *Bdnf* gene transcription (Im, Hollander, Bali, & Kenny, 2010; Klose & Bird, 2003). Moreover, promoter IV of *Bdnf* contains a specific binding site for CREB (Tao, West, Chen, Corfas, & Greenberg, 2002), which in turn can influence chromatin remodeling (Chan & La Thangue, 2001). Moreover, Miller and Sweatt (2007) demonstrated that contextual fear conditioning enhanced *DNMT* gene expression in the adult hippocampus, which in turn correlated with increases in the mRNA levels for BDNF.

The *BDNF* gene in rodents and humans has multiple promoters orchestrating transcription. It has been reported that promoters of *BDNF* undergo dynamic chromatin remodeling. One of these promoters, a major activation promoter IV, appears to be of particular importance for substantiating cognitive processes (Lipsky et al., 2001). Specifically, HDAC1, in conjunction with the corepressor molecule Sin3a, maintains the repressed state of the *Bdnf* gene (Martinowich et al., 2003). In turn, intensified *Bdnf* transcription correlates with promoter IV phosphorylation and the dissociation of MeCP2 (Chen, Chang, et al., 2003; Martinowich et al., 2003). Activity-induced DNA demethylation of *Bdnf* promoter IX has been reported to be associated with increased hippocampal neurogenesis (Ma et al., 2009). Moreover, there is evidence that methylation at lysine (K) 27 on histone H3 (H3K27) co-occurs with transcriptional repression, whereas acetylation on histones H3 and H4 is associated with transcriptional activation (Tsankova, Renthal, Kumar, & Nestler, 2007). BDNF activation leads to the dissociation of HDAC2 from the chromatin by nitrosylation on cysteines 262 and 274 of HDAC2 (Nott et al., 2008). Both acetylation and nitrosylation result in the increased histone acetylation of the promoter regions of the *BDNF* gene and other plasticity-related genes. There are numerous reports on the epigenetic regulation of the *BDNF* (Boulle et al., 2012), its reversibility (Shirayama, Chen, Nakagawa, Russell, & Duman, 2002), and the consequences of these complex relations for cognitive processes.

Epigenetic mechanisms in clinical conditions where cognitive processes are severely challenged

Another source of information relevant to understanding the role of epigenetic mechanisms in cognitive processes comes from studies of clinical conditions. Here relevant lines of research are discussed only briefly (for review, see Fischer, 2014; Rudenko & Tsai, 2014). In general, the critical information has been ascertained from studies of developmental disorders, as exemplified here by Rubinstein–Taybi syndrome (RTS; Rubinstein & Taybi, 1963), Rett syndrome (RTT; Rett, 1966), and Kabuki syndrome (KS; Niikawa et al., 1988); and by studies of neurodegenerative disorders, as exemplified here by Huntington disease (HD; Walker, 2007) and AD (Sennvik et al., 2000).

RTS is an autosomal dominant condition (Oike et al., 1999) that affects 1 in 125,000 to 1 in 720,000 births (Petrif et al., 1995) and is characterized by anatomical abnormalities and severe intellectual disability. RTS is caused by mutations in the histone acetyltransferase, HAT, protein, CREB-binding protein, CBP/KAT3A (Petrif et al., 1995) or mutations in EP300 (Roelfsema et al., 2005), a protein closely associated with CBP.

RTT is an X-linked dominant developmental disorder and is the best studied human epigenetic abnormality. It is characterized by profound developmental regression, where development progresses normally until 6–18 months of age, but then declines in severe developmental stagnation accompanied by microcephaly, hypotonia, weight loss, and severe mental retardation (Bruck, Philippart, Giraldi, & Antoniuk, 1991; Hagberg, Aicardi, Dias, & Ramos, 1983; Motil, Schultz, Brown, Glaze, & Percy, 1994). The majority of RTT cases are caused by mutations in *MeCP2* (Amir et al., 1999; Chahrour et al., 2008; Chahrour & Zoghbi, 2007).

KS is a developmental disorder, characterized by the presence of pale, chalky facial skin (reminiscent of the makeup of actors of Kabuki, a Japanese traditional theatrical form) and bodily dysmorphology, developmental delay, intellectual disability (ranging from mild to severe), and often microcephaly, hypotonia, and nystagmus or strabismus. According to the prevalence data collected in Japan, KS occurs in approximately 1 in 32,000 newborns (Niikawa et al., 1988). Observed worldwide, the syndrome is caused by various mutations in the lysine-specific methyltransferase 2D (*MLL2/KMT2D*) gene on chromosome 12q13 (Ng et al., 2010).

HD is an autosomal dominant, progressive, neurodegenerative disorder with a distinct phenotype characterized by chorea, dystonia, lack of coordination, cognitive decline, and behavioral difficulties; it is associated with progressive, selective neural cell loss and atrophy in the caudate and putamen (Walker, 2007). Given the intergenerational dynamics of HD in some families (i.e., the phenomenon of anticipation, as each subsequent generation experiences the onset of symptoms

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earlier in life than the previous generation), epigenetic influence was hypothesized (Ridley, Frith, Crow, & Conneally, 1988). The field has been gradually accumulating molecular data substantiating and detailing this hypothesis, implementing, in particular, histone-modifying enzymes (Bardai, Price, Zaayman, Wang, & D'Mello, 2012; Bates, Victor, Jones, Shi, & Hart, 2006; Cong et al., 2005; Jiang et al., 2006).

AD is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by specific alterations to the brain and its function, specifically the formation of amyloid-beta plaques, neurofibrillary tangles, and severe neuronal loss, eventually leading to dementia (Sennvik et al., 2000). Although familial early onset forms of AD (~5%) implicate specific structural variants in the human genome in the etiology of this disease (Haass & Selkoe, 2007), more typical forms of late onset AD (~95%) have been attributed to variable combinations of genetic and environmental risk factors (Goate & Hardy, 2012; Sananbenesi & Fischer, 2009). It has been shown that multiple epigenetic mechanisms (histone acetylation, DNA methylation, and ncRNAs) are relevant to both the manifestation and novel therapeutic avenues of the latter form of AD (Fischer, 2014).

Discussion

Despite the diversity of the data and findings captured in this brief overview, the critical mass of evidence pertaining to the importance of epigenetic mechanisms in the development, manifestation, and decline of cognitive functions is nevertheless clear. As indicated above, although convincing with regard to the importance of epigenetic regulation at the macroscopic level, the data are not homogeneously convergent either on the direction or the amount of epigenetic influence. Understanding the importance of these data, but finding them challenging to interpret, has led to the publication of multiple recent reviews (Boyce & Kobor, 2015; Fischer, 2014; Gräff & Tsai, 2013; Guan et al., 2015; Rudenko & Tsai, 2014) and attempts at sophisticated conceptual integrations of these data (Heyward & Sweatt, 2015; Sweatt, 2009), carving out a new field studying the (a)typical epigenetic regulation of cognitive processes also referred to as neuroepigenetics (Sweatt, 2013).

Yet, although the current attempts at integration are driven by animal data, they are still highly relevant to thinking about designs for human studies. To illustrate, two such integrations are considered here. With regard to the epigenetic machinery as a whole, Guan et al. (2015) proposed that the machinery has two functions in cognitive processes: specifically, the "gating" that triggers gene expression required for acquisition and the "stabilizing" that enables gene expression needed for long-term storage. It is argued that a staged view of cognitive information processing (i.e., from acquisition through consolidation to storage) can be paralleled by the staged engagement of the epigenetic machinery (i.e., from cellular through circuit to network molecular mechanism; Xie et al., 2014). It is important to note that it is thought that the status of chromatin conformation before and during the presentation of cognitive stimuli (i.e., before initiation and during cognitive processing) is essential for the stimuli-triggered expression (Guan et al., 2009). Specifically, according to Guan et al. (2015, pp. 33–34),

> . . . specific epigenetic regulations, which increase or dampen the activity-dependent gene expression, modulate the amount of protein expression essential for neuronal circuit modification to facilitate or block the sensory stimulus-induced changes in the epigenetically regulated neuron, resembling the "gating" of memory formation in neurons or neuron ensembles. . . . Epigenetic regulation might also take on the role of "stabilization" for long-term memory, in which specific epigenetic markers might maintain important gene expression changes for memory consolidation. The markers that achieve the role of "stabilization" are different from those that perform the role of "gating." Different epigenetic regulatory machines and marker dynamics are employed in those two conditions.

Thus, the same neuron can utilize different epigenetic mechanisms as engaged by/with different genes to perform different stages of cognitive processing. Then, if a population of neurons is involved, a developmental perspective can be considered: epigenetic machinery might be tuned in such a way that it enhances the acquisition of information early in life (i.e., boosting cognition during early sensitive periods of the development) and the maintenance of information later in life (i.e., protecting cognition from decline during later periods of life).

The second illustration comes from the presentation of a hypothetical framework designed to interpret the data accumulated on the role of DNA methylation in cognitive processes (Heyward & Sweatt, 2015). This focus is particularly driven by the desire to understand the conundrum generated by the accumulated data suggesting that the longevity of the cellular and molecular modifications substantiating the changes in synaptic plasticity is substantially more lasting than the longevity of the proteins that constitute the molecular basis of synaptic plasticity (Crick, 1984; Dash, Moore, Kobori, & Runyan, 2007; Davis & Squire, 1984; Day & Sweatt, 2010; Holliday, 1999; Lisman, 1985). This phenomenon has been referred to as a memory paradox (Heyward & Sweatt, 2015): memories have a capacity to persist through the life span whereas the very proteins that corroborate synaptic plasticity underlying the formation and maintenance of the memory trace are subject to ongoing metabolic turnover cycles. It has been argued that the solution to this paradox lies in considering DNA methylation as ". . . a self-perpetuating information storage device, . . . therefore capable of serving as the mnemogenic process underlying long-term memory" (Heyward & Sweatt, 2015, p. 2). A formalization of this consideration has resulted in the development of a model based on the comparison between basal (less energy consuming) versus neuronal activity-induced (more energy consuming) conditions. The model (Heyward & Sweatt, 2015) makes a number of assumptions. First, it is assumed that there are "memory permissive" (i.e., promoting) and "memory disrup-

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tive" (i.e., suppressing) genes. Second, in the activity-induced combined transcription of these genes, there is a balance between cellular and molecular events, which either do or do not allow the establishment of synaptic connectivity and memory formation. Third, it is assumed that the basal condition is characterized by the predominant expression of the memory disruptive genes, whereas the activity-induced condition is characterized by the predominant expression of the memory permissive genes. Fourth, levels of methylation are expected to be lower for memory suppressing genes and higher for memory promoting genes at the basal condition and higher for suppressing and lower for promoting at the activity-induced condition. Fifth, it is assumed that, as the activity-inducing stimulus (stimuli) elapses, memory permissive genes undergo DNMT1-mediated remethylation and transcriptional suppression whereas memory disruptive genes undergo TET1-mediated demethylation and transcriptional activation.

Thus, there is increasing evidence confirming that epigenetic processes constitute a critical system of mechanisms for proper neuronal functioning (Rudenko & Tsai, 2014). Of note is that one of the emerging features of epigenetic regulation is its versatility. Specifically, epigenetic control not only provides a way to convey neuronal response but also regulates gene expression. DNA (de)methylation can serve not only as a very fast response to cognitive stimuli but also as a robust and long-lasting way to create catalogues of epigenomic annotations. Perhaps of the most importance, epigenetic regulation appears to be a key ingredient of cognitive processing and control.

It is important to realize that epigenetic effects on genome composition could be subject to the signature of evolution (especially if the effect is "human specific"). Thus, when results of a descriptive (correlational) study are interpreted, such an evolutionary perspective has to be taken into account (Fazzari & Greally, 2004).

While examining genomic and epigenomic variation within and across human populations, researchers have registered epigenetic dissimilarities between samples from different human populations (Adkins, Krushkal, Tylavsky, & Thomas, 2011; Fraser, Lam, Neumann, & Kobor, 2012; Heyn et al., 2013; Moen et al., 2013). Moreover, within a population, there appear to be genomic regions that are highly variable in methylation status across individuals; these locations are referred to as variably methylated regions (Teh et al., 2014).

Of note also is that epigenetic regulation represents a well-orchestrated system of multiple co-co-occurring events, interacting and influencing each other (Lipsky, 2013). For example, an unmethylated histone H3 (H3K4) serves as a docking site for DNMTs, which in turn cause de novo DNA methylation and the silencing of gene transcription (Szyf, 2009). It appears that most epigenetic regulation is carried out through a highly interwoven network of such co-occurring epigenetic changes (referred to as "cross talks"; Gräff & Mansuy, 2008).

Epigenetic mechanisms are characterized by a high degree of "issue" or "stimuli" specificity. It actually appears that cell- and circuit-specific patterns of DNA methylation are important for differentiating the different types of cognitive processes they substantiate (Lipsky, 2013).

It is well known that stress appears to be a major trigger of epigenetic mechanisms. There are data showing that stress induces cognitive impairment by increasing the expression of HDAC(s); Levine, Worrell, Zimnisky, and Schmauss (2012); Oztan, Aydin, and Isgor (2011). Based on these data, it has been hypothesized that changes in histone acetylation might be a mechanism for translating early-life stressors into persistent effects in adulthood (Gräff & Tsai, 2013). The literature contains multiple references to such reduced acetylation mnemonics, that is, an epigenetic "blockade" (Gräff et al., 2012), "bottleneck" (Sananbenesi & Fischer, 2009), or "brake-pad" (McQuown & Wood, 2011) for cognitive functions.

A key issue that needs to be addressed is cell type specificity. Most studies still conduct their analyses in heterogeneous populations of cells, mostly from brain tissue or blood. However, the results of these investigations can be misleading if baselines are not established on how, for example, different blood cells differ from each other in their response to inflammatory processes, cell death, or specific type of cell signaling. To overcome this problem, multiple options have been developed (Fischer, 2014). First, specific cells can be isolated from complex tissue via fluorescence-activated cell sorting. However, in many cases, the utilization of fluorescence-activated cell sorting requires biochemical pretreatment of the initial cell populations/tissue; moreover, such pretreatment works only or best on freshly connected specimens, and a substantial number of cells do not survive the pretreatment. Second, it is possible to utilize laser capture microdissection, but this procedure has another drawback in that it is very time consuming. Third, there is a possibility of directly sorting nuclei using endogenous marker proteins. Yet, although having been applied extensively in animal models, these methods have not been utilized much in human research. Fourth, the rapid development of methodological bases of research in epigenomics provides an opportunity to investigate epigenetic states of a single cell, a very promising approach in neuroepigenetics due to the highly individualized function of distinct neurons and their small groups in the brain.

A related issue is the timing of epigenetic alterations in response to environmental stimuli. For example, memory training has been found to induce epigenetic modifications within 30–60 min after its administration (Levenson et al., 2004; Peleg et al., 2010). It is clear that to understand the mechanics of such modifications, researchers need to exercise procedures that allow rapid access to cell populations and tissues of interest. Otherwise, it may be difficult to establish whether the status of the cells would truly be representative of the in vivo situation.

There is a rapid accumulation of data suggesting that DNA methylation, chromatin plasticity and its underlying enzymatic machinery, and ncRNA all contribute to the biological

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foundation of cognitive processes. Yet, conclusive data are lacking; much of the available data are mostly associative (Guan et al., 2015). Thus, for example, there are limited (if any) genome-wide data to buoy the hypothesis that transient changes in gene expression that arise during training/learning or some other environmental stimulation correlate with specific epigenetic mechanistic changes.

There is an anticipation that technologies such as epigenome-wide association studies (EWAS) and epigenome-wide sequencing studies (Fischer, 2014), will become rather routine in neuroscience, as the field tries to gain insight into the biological mechanisms underlying cognitive processes. These technologies, typically available today through core facilities in academic health centers, make the molecular component of such projects almost trivial. Yet, statistical analyses of epigenomic data are complex, notwithstanding such challenging issues as autocorrelations and sample size (Fazzari & Greally, 2004). There is a lack of guidance in how to analyze the resulting epigenetic data and how to relate them to complex cognitive phenotypes. There are some informative examples of the utilization of data mining techniques, such as machine learning approaches (Bonn et al., 2012). Yet, it is clear that even when complex data-analytic techniques are applied, at best they will detect and describe epigenetic networks, but not elucidate the inner intricacies of their "workings." To do the latter, it would be necessary to devise subsequent hypothesis-driven mechanistic studies (Fischer, 2014).

A related issue is that of confounding. There is always a chance that the correlated epigenetic feature might have no direct consequences for an outcome, but might physically co-segregate in the epigenome with a particular (hidden) sequence characteristic that was not analyzed. Moreover, an outcome itself might not be a consequence, but a cause (Fazzari & Greally, 2004).

Conclusion

Given the content and length of this review, it is clear that there is a rapid and intense accumulation of literature on the role of epigenetic regulation of cognitive processes in animal models. The relevant literature in humans is scarce. A major reason for this disproportion is that most of the animal literature uses brain tissue as the substance of cognitive processes and, therefore, the matter in which the role of epigenetic regulation is investigated. This, of course, cannot be done with humans. Whereas postmortem tissue is utilized, although still rarely, for investigation of epigenetic mechanisms, these mechanisms have a time scale that is difficult to capture in the postmortem brain. Such tissues are also inherently inappropriate for the (currently lacking) studies that seek to establish developmental patterns in the functioning of the epigenome, and to map these patterns onto the alterations in the neural circuits that support relevant behavioral manifestations of cognitive development and processing.

However, the development, dispersion, and cost of EWAS methodologies has already resulted in the appearance of a sizable literature where epigenetic regulation is investigated mostly when groups of humans (i.e., case vs. controls, exposed vs. unexposed) are studied. Although currently this literature is focused primarily on noncognitive phenotypes (e.g., mental and physical health), there are some studies focused on cognitive processes. There is evidence that the number of EWAS approaches in behavior epigenetics will only increase. In this context, it is highly important to sensitize the scientific community to the methodological, statistical, and interpretational issues that are associated with EWAS approaches. Some of them have been mentioned above, but there is no doubt that in the near future the field will be near-ripe with studies that will form the foundation for many reviews and commentaries of both content and methodological character. They are coming.

References

Adkins, R. M., Krushkal, J., Tylavsky, F. A., & Thomas, F. (2011). Racial differences in gene-specific DNA methylation levels are present at birth. *Birth Defects Research: Part A, Clinical and Molecular Teratology*, *91*, 728–736.

Alarcon, J. M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E. R., et al. (2004). Chromatin acetylation, memory, and LTP are impaired in CBP+/- mice: A model for the cognitive deficit in Rubinstein–Taybi syndrome and its amelioration. *Neuron*, *42*, 947–959.

Allis, C. D., Berger, S. L., Cote, J., Dent, S., Jenuwein, T., Kouzarides, T., et al. (2007). New nomenclature for chromatin-modifying enzymes *Cell*, *131*, 633–636.

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*, *23*, 185–188.

Azevedo, F. A. C., Carvalho, L. R. B., Grinberg, L. T., Farfel, J. M., Ferretti, R. E. L., Leite, R. E. P., et al. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology*, *51*, 532–541.

Badeaux, A. I., & Shi, Y. (2013). Emerging roles for chromatin as a signal integration and storage platform. *Nature Reviews Molecular Cell Biology*, *14*, 211–224.

Bahari-Javan, S., Maddalena, A., Kerimoglu, C., Wittnam, J., Held, T., Bähr, M., et al. (2012). HDAC1 regulates fear extinction in mice. *Journal of Neuroscience*, *32*, 5062–5073.

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*, 381–395.

Bardai, F. H., Price, V., Zaayman, M., Wang, L., & D'Mello, S. R. (2012). Histone deacetylase-1 (HDAC1) is a molecular switch between neuronal survival and death. *Journal of Biological Chemistry*, *287*, 35444–35453.

Barrett, R. M., Malvaez, M., Kramar, E., Matheos, D. P., Arrizon, A., Cabrera, S. M., et al. (2011). Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. *Neuropsychopharmacology*, *36*, 1545–1556.

Bartolomei, M. S., Webber, A. L., Brunkow, M. E., & Tilghman, S. M. (1993). Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. *Genes and Development*, *7*, 1663–1673.

Bates, E. A., Victor, M., Jones, A. K., Shi, Y., & Hart, A. C. (2006). Differential contributions of Caenorhabditis elegans histone deacetylases to huntingtin polyglutamine toxicity. *Journal of Neuroscience*, *26*, 2830–2838.

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J. I., Goldin, A., et al. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proceedings of the National Academy of Sciences*, *105*, 2711–2716.

Bird, A. P. (1980). DNA methylation and the frequency of CpG in animal DNA. *Nucleic Acids Research*, *8*, 1499–1504.

Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature*, *321*, 209–213.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press

Bird, A. P. (2002). DNA methylation patterns and epigenetic memory. *Genes and Development*, *16*, 6–21.
Bird, A. P. (2007). Perceptions of epigenetics. *Nature*, *447*, 396–398.
Bird, A. P., & Wolffe, A. P. (1999). Methylation-induced repression: Belts, braces, and chromatin. *Cell*, *99*, 451–454.
Bonn, S., Zinzen, R. P., Girardot, C., Gustafson, E. H., Gonzalez, A. P., Delhomme, N., et al. (2012) Tissue specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. *Nature Genetics*, *44*, 148–156.
Bordone, L., & Guarente, L. (2005). Calorie restriction, SIRT1 and metabolism: Understanding longevity. *Nature Reviews Molecular Cell Biology*, *6*, 298–305.
Boulle, F., van den Hove, D. L. A., Jakob, S. B., Rutten, B. P., Hamon, M., van Os, J., et al. (2012). Epigenetic regulation of the BDNF gene: Implications for psychiatric disorders. *Molecular Psychiatry*, *17*, 584–596.
Boyce, W. T., & Kobor, M. S. (2015). Development and the epigenome: The "synapse" of gene–environment interplay. *Developmental Science*, *18*, 1–23.
Bradbury, J. (2003). Human epigenome project-up and running. *PLOS Biology*, *1*, E82.
Broide, R. S., Redwine, J. M., Aftahi, N., Young, W., Bloom, F. E., & Winrow, C. J. (2007). Distribution of histone deacetylases 1–11 in the rat brain. *Journal of Molecular Neuroscience*, *31*, 47–58.
Brownell, J. E., & Allis, C. D. (1996). Special HATs for special occasions: Linking histoneacetylation to chromatin assembly and gene activation. *Current Opinion in Genetics and Development*, *6*, 176–184.
Bruck, I., Philippart, M., Giraldi, D., & Antoniuk, S. (1991). Difference in early development of presumed monozygotic twins with Rett syndrome. *American Journal of Medical Genetics*, *39*, 415–417.
Buck, M. J., & Lieb, J. D. (2004). ChIP-chip: Considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics*, *83*, 349–360.
Castel, S. E., & Martienssen, R. A. (2013). RNA interference in the nucleus: Roles for small RNAs in transcription, epigenetics and beyond. *Nature Reviews Genetics*, *14*, 100–112.
Cawley, S., Bekiranov, S., Ng, H. H., Kaparonov, P., Sekinger, E. A., Kampa, D., et al. (2004). Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell*, *116*, 499–509.
Cervoni, N., & Szyf, M. (2001). Demethylase activity is directed by histone acetylation. *Journal of Biological Chemistry*, *276*, 40778–40787.
Chahrour, M., Jung, S. Y., Shaw, C., Zhou, X., Wong, S. T. C., Qin, J., et al. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science*, *320*, 1224–1229.
Chahrour, M., & Zoghbi, H. Y. (2007). The story of Rett syndrome: From clinic to neurobiology. *Neuron*, *56*, 422–437.
Chan, H. M., & La Thangue, N. B. (2001). p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *Journal of Cell Science*, *114*, 2363–2373.
Chen, C. M., Chen, H. L., Hsiau, T. H., Shi, H., Brock, G. J., Wei, S. H., et al. (2003). Methylation target array for rapid analysis of CpG island hypermethylation in multiple tissue genomes. *American Journal of Pathology*, *163*, 37–45.
Chen, G., Zou, X., Watanabe, H., van Deursen, J. M., & Shen, J. (2010). CREB binding protein is required for both short-term and long-term memory formation. *Journal of Neuroscience*, *30*, 13066–13070.
Chen, W. G., Chang, Q., Lin, Y., Meissner, A., West, A. E., Griffith, E. C., et al. (2003). Depression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science*, *302*, 885–889.
Choi, D. C., Maguschak, K. A., Ye, K., Jang, S.-W., Myers, K. M., & Ressler, K. J. (2010). Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences*, *107*, 2675–2680.
Chuang, J. C., & Jones, P. A. (2007). Epigenetics and microRNAs. *Pediatric Research*, *61*, 24R–29R.
Cong, S. Y., Pepers, B. A., Evert, B. O., Rubinsztein, D. C., Roos, R. A., van Ommen, G. J., et al. (2005). Mutant huntingtin represses CBP, but not p300, by binding and protein degradation. *Molecular and Cellular Neuroscience*, *30*, 560–571.
Cougot, N., Bhattacharyya, S. N., Tapia-Arancibia, L., Bordonné, R., Filipowicz, W., Bertrand, E., et al. (2008). Dendrites of mammalian neurons contain specialized P-body-like structures that respond to neuronal activation. *Journal of Neuroscience*, *28*, 13793–13804.
Cowansage, K. K., LeDoux, J. E., & Monfils, M. H. (2010). Brain-derived neurotrophic factor: A dynamic gatekeeper of neural plasticity. *Current Molecular Pharmacology*, *3*, 12–29.
Crick, F. (1984). Memory and molecular turnover. *Nature*, *312*, 101.
Crosio, C., Heitz, E., Allis, C. D., Borrelli, E., & Sassone-Corsi, P. (2003). Chromatin remodeling and neuronal response: Multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. *Journal of Cell Science*, *116*, 4905–4914.
Czyz, W., Morahan, J. M., Ebers, G. C., & Ramagopalan, S. V. (2012). Genetic, environmental and stochastic factors in monozygotic twin discordance with a focus on epigenetic differences. *BMC Medicine*, *10*, 93.
Dash, P. K., Moore, A. N., Kobori, N., & Runyan, J. D. (2007). Molecular activity underlying working memory. *Learning and Memory*, *14*, 554–563.
Davies, M. N., Volta, M., Pidsley, R., Lunnon, K., Dixit, A., Lovestone, S., et al. (2012). Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biology*, *13*, R43.
Davis, H. P., & Squire, L. R. (1984). Protein synthesis and memory: A review. *Psychological Bulletin*, *96*, 518–559.
Day, J. J., & Sweatt, J. D. (2010). DNA methylation and memory formation. *Nature Neuroscience*, *13*, 1319–1323.
Day, J. K., Bauer, A. M., desBordes, C., Zhuang, Y., Kim, B.-E., Newton, L. G., et al. (2002). Genistein alters methylation patterns in mice. *Journal of Nutrition*, *132*, 2419S–2423S.
Deaton, A. M., & Bird, A. (2011). CpG islands and the regulation of transcription. *Genes and Development*, *25*, 1010–1022.
de Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., & van Kuilenburg, A. B. (2003). Histone deacetylases (HDACs): Characterization of the classical HDAC family. *Biochemical Journal*, *370*, 737–749.
Duncan, B. K., & Miller, J. H. (1980). Mutagenic deamination of cytosine residues in DNA. *Nature*, *287*, 560–561.
Esteller, M. (2008). Epigenetics in evolution and disease. *Lancet*, *372*, S90–S96.
Fan, G., Beard, C., Chen, R. Z., Csankovszki, G., Sun, Y., Siniaia, M., et al. (2001). DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *Journal of Neuroscience*, *21*, 788–797.
Fatica, A., & Bozzoni, I. (2013). Long non-coding RNAs: New players in cell differentiation and development. *Nature Reviews Genetics*, *15*, 7–21.
Fazzari, M. J., & Greally, J. M. (2004). Epigenomics: Beyond CpG islands. *Nature Reviews Genetics*, *5*, 446–455.
Federman, N., Fustiñana, M. S., & Romano, A. (2009). Histone acetylation is recruited in consolidation as a molecular feature of stronger memories. *Learning and Memory*, *16*, 600–606.
Feinberg, A. P. (2001). Methylation meets genomics. *Nature Genetics*, *27*, 9–10.
Feinberg, A. P. (2011). Genome-scale approaches to the epigenetics of common human disease. *Disease*, *456*, 13–21.
Feng, J., Zhou, Y., Campbell, S. L., Le, T., Li, E., Sweatt, J. D., et al. (2010). Dnmt1 and Dnmt3a are required for the maintenance of DNA methylation and synaptic function in adult forebrain neurons. *Nature Neuroscience*, *13*, 423–430.
Fischer, A. (2014). Epigenetic memory: The Lamarckian brain. *EMBO Journal*, *33*, 945–967.
Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L. H. (2007). Recovery of learning and memory after neuronal loss is associated with chromatin remodeling. *Nature*, *447*, 178–182.
Flexner, J. B., Flexner, L. B., & Stellar, E. (1963). Memory in mice as affected by intracerebral puromycin. *Science*, *141*, 57–59.
Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., et al. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proceeding of the National Academy of Sciences*, *102*, 10604–10609.
Fraser, H. B., Lam, L. L., Neumann, S. M., & Kobor, M. S. (2012). Population-specificity of human DNA methylation. *Genome Biology*, *13*, R8.
Frigola, J., Ribas, M., Risques, R. A., & Peinado, M. A. (2002). Methylome profiling of cancer cells by amplification of intermethylated sites (AIMS). *Nucleic Acids Research*, *30*, e28.
Gao, J., Wang, W. Y., Mao, Y. W., Gräff, J., Guan, J. S., Pan, L., et al. (2010). A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature*, *466*, 1105–1109.
Goate, A., & Hardy, J. (2012). Twenty years of Alzheimer's disease-causing mutations. *Journal of Neurochemisty*, *120*, 3–8.
Gräff, J., Kim, D., Dobbin, M. M., & Tsai, L. H. (2011). Epigenetic regulation of gene expression in physiological and pathological brain processes. *Physiological Reviews*, *91*, 603–649.
Gräff, J., & Mansuy, I. M. (2008). Epigenetic codes in cognition and behaviour. *Behavioural Brain Research*, *192*, 70–87.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press

Gräff, J., Rei, D., Guan, J.-S., Wang, W.-Y., Seo, J., Hennig, K. M., et al. (2012). An epigenetic blockade of cognitive functions in the neurodegenerating brain. *Nature*, *483*, 222–226.

Gräff, J., & Tsai, L. H. (2013). Histone acetylation: Molecular mnemonics on the chromatin. *Nature Reviews Neuroscience*, *14*, 97–111.

Gregoretti, I. V., Lee, Y. M., & Goodson, H. V. (2004). Molecular evolution of the histone deacetylase family: Functional implications of phylogenetic analysis. *Journal of Molecular Biology*, *338*, 17–31.

Grewal, S. I., & Moazed, D. (2003). Heterochromatin and epigenetic control of gene expression. *Science*, *301*, 798–802.

Guan, J.-S., Haggarty, S. J., Giacometti, E., Dannenberg, J.-H., Joseph, N., Gao, J., et al. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature*, *459*, 55–60.

Guan, J.-S., Xie, H., & Ding, X. (2015). The role of epigenetic regulation in learning and memory. *Experimental Neurology*, *268*, 30–36.

Guan, Z., Giustetto, M., Lomvardas, S., Kim, J.-H., Miniaci, M. C., Schwartz, J. H., et al. (2002). Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. *Cell*, *111*, 483–493.

Gupta, S., Kim, S. Y., Artis, S., Molfese, D. L., Schumacher, A., Sweatt, J. D., et al. (2010). Histone methylation regulates memory formation. *Journal of Neuroscience*, *30*, 3589–3599.

Gupta-Agarwal, S., Franklin, A. V., Deramus, T., Wheelock, M., Davis, R. L., McMahon, L. L., et al. (2012). G9a/GLP histone lysine dimethyltransferase complex activity in the hippocampus and the entorhinal cortex is required for gene activation and silencing during memory consolidation. *Journal of Neuroscience*, *32*, 5440–5453.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nature Reviews Molecular Cell Biology*, *8*, 112–116.

Haberland, M., Montgomery, R. L., & Olson, E. N. (2009). The many roles of histone deacetylases in development and physiology: Implications for disease and therapy. *Nature Reviews Genetics*, *10*, 32–42.

Hagberg, B., Aicardi, J., Dias, K., & Ramos, O. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Annals of Neurology*, *14*, 471–479.

Haigis, M. C., & Sinclair, D. A. (2010). Mammalian sirtuins: Biological insights and disease relevance. *Annual Review of Pathology*, *5*, 253–295.

Harshman, S. W., Young, N. L., Parthun, M. R., & Freiras, M. A. (2013). H1 histones: Current perspectives and challenges. *Nucleic Acids Research*, *41*, 9593–9609.

Hart, A. K., Fioravante, D., Liu, R. Y., Phares, G. A., Cleary, L. J., & Byrne, J. H. (2011). Serotonin-mediated synapsin expression is necessary for long-term facilitation of the Aplysia sensorimotor synapse. *Journal of Neuroscience*, *31*, 18401–18411.

Hatchwell, E., & Greally, J. M. (2007). The potential role of epigenomic dysregulation in complex human disease. *Trends in Genetics*, *23*, 588–595.

He, L., & Hannon, G. J. (2004). MicroRNAs: Small RNAs with a big role in gene regulation. *Nature Reviews Genetics*, *5*, 522–531.

Heyn, H., Moran, S., Hernando-Herraez, I., Sayols, S., Gomez, A., Sandoval, J., et al. (2013). DNA methylation contributes to natural human variation. *Genome Research*, *23*, 1363–1372.

Heyward, F. D., & Sweatt, J. D. (2015). DNA methylation in memory formation: Emerging insights. *Neuroscientist*. Advance online publication.

Holliday, R. (1990). DNA methylation and epigenetic inheritance. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences*, *326*, 329–338.

Holliday, R. (1999). Is there an epigenetic component in long-term memory? *Journal of Theoretical Biology*, *200*, 339–341.

Holliday, R., & Pugh, J. E. (1975). DNA modification mechanisms and gene activity during development. *Science*, *187*, 226–232.

Hong, E. J., West, A. E., & Greenberg, M. E. (2005). Transcriptional control of cognitive development. *Current Opinion in Neurobiology*, *15*, 21–28.

Horvath, S., Zhang, Langfelder, P., Kahn, R. S., Boks, M. P., van Ekijk, K., et al. (2012). Aging effects on DNA methylation modules in human brain and blood tissue. *Genome Biology*, *13*, R97.

Huang, T. H., Perry, M. R., & Laux, D. E. (1999). Methylation profiling of CpG islands in human breast cancer cells. *Human Molecular Genetics*, *8*, 459–470.

Im, H. I., Hollander, J. A., Bali, P., & Kenny, P. J. (2010). MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nature Neuroscience*, *13*, 1120–1127.

Im, H. I., & Kenny, P. J. (2012). MicroRNAs in neuronal function and dysfunction. *Trends in Neuroscience*, *35*, 325–334.

Imhof, A. (2006). Epigenetic regulators and histone modification. *Briefings in Functional Genomics and Proteomics*, *5*, 222–227.

Jablonka, E., & Lamb, M. J. (2002). The changing concept of epigenetics. *Annals of the New York Academy of Sciences*, *981*, 82–96.

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nature Genetics*, *33*, 245–254.

Jiang, H., Poirier, M. A., Liang, Y., Pei, Z., Weiskittel, C. E., Smith, W. W., et al. (2006). Depletion of CBP is directly linked with cellular toxicity caused by mutant huntingtin. *Neurobiology of Disease*, *23*, 543–551.

Jiang, Y., Langley, B., Lubin, F. D., Renthal, W., Wood, M. A., Yasui, D. H., et al. (2008). Epigenetics in the nervous system. *Journal of Neuroscience*, *28*, 11753–11759.

Jones, P. A., & Liang, G. (2009). Rethinking how DNA methylation patterns are maintained. *Nature Reviews Genetics*, *10*, 805–811.

Josselyn, S. A. (2005). What's right with my mouse model? New insights into the molecular and cellular basis of cognition from mouse models of Rubinstein–Taybi Syndrome. *Learning and Memory*, *12*, 80–83.

Kaas, G. A., Zhong, C., Eason, D. E., Ross, D. L., Vachhani, R. V., Ming, G. L., et al. (2013). TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation. *Neuron*, *79*, 1086–1093.

Kaminsky, Z. A., Tang, T., Wang, S.-C., Ptak, C., Oh, G. H. T., Wong, A. H. C., et al. (2009). DNA methylation profiles in monozygotic and dizygotic twins. *Nature Genetics*, *41*, 240–245.

Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science*, *294*, 1030–1038.

Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Molecular Brain*, *5*, 14.

Karr, J., Vagin, V., Chen, K., Ganesan, S., Olenkina, O., Gvozdev, V., et al. (2009). Regulation of glutamate receptor subunit availability by microRNAs. *Journal of Cell Biology*, *185*, 685–697.

Kaur, P., Tan, J. R., Karolina, D. S., Sepramaniam, S., Armugam, A., Wong, P. T. H., et al. (2016). A long non-coding RNA, BC048612 and a microRNA, miR-203 coordinate the gene expression of neuronal growth regulator 1 (NEGR1) adhesion protein. *Biochimica et Biophysica Acta*, *1863*, 533–543.

Kerimoglu, C., Agis-Balboa, R. C., Kranz, A., Stilling, R., Bahari-Javan, S., Benito-Garagorri, E., et al. (2013). Histone-methyltransferase mll2 (kmt2b) is required for memory formation in mice. *Journal of Neuroscience*, *33*, 3452–3464.

Kim, J., Kollhoff, A., Bergmann, A., & Stubbs, L. (2003). Methylation-sensitive binding of transcription factor YY1 to an insulator sequence within the paternally expressed imprinted gene, Peg3. *Human Molecular Genetics*, *12*, 233–245.

Kim, M. S., Akhtar, M. W., Adachi, M., Mahgoub, M., Bassel-Duby, R., Kavalali, E. T., et al. (2012). An essential role for histone deacetylase 4 in synaptic plasticity and memory formation. *Journal of Neuroscience*, *32*, 10879–10886.

Kinde, B., Gabel, H. W., Gilbert, C. S., Griffith, E. C., & Greenberg, M. E. (2015). Reading the unique DNA methylation landscape of the brain: Non-CpG methylation, hydroxymethylation, and MeCP2. *Proceedings of the National Academy of Sciences*, *112*, 6800–6806.

Kiser, D. P., Rivero, O., & Lesch, K.-P. (2015). Annual Research Review: The (epi)genetics of neurodevelopmental disorders in the era of whole-genome sequencing—Unveiling the dark matter. *Journal of Child Psychology and Psychiatry*, *56*, 278–295.

Klose, R. J., & Bird, A. P. (2003). Molecular biology: MeCP2 repression goes nonglobal. *Science*, *302*, 793–795.

Klose, R. J., & Bird, A. P. (2006). Genomic DNA methylation: The mark and its mediators. *Trends in Biochemical Sciences*, *31*, 89–97.

Korzus, E., Rosenfeld, M. G., & Mayford, M. (2004). CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron*, *42*, 961–972.

Koseki, T., Mouri, A., Mamiya, T., Aoyama, Y., Toriumi, K., Suzuki, S., et al. (2012). Exposure to enriched environments during adolescence prevents abnormal behaviours associated with histone deacetylation in phencyclidine-treated mice. *International Journal of Neuropsychopharmacology*, *15*, 1489–1501.

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell*, *128*, 693–705.

Kozlenkov, A., Wang, M., Roussos, P., Rudchenko, S., Barbu, M., Bibikova, M., et al. (2016). Substantial DNA methylation differences between two major neuronal subtypes in human brain. *Nucleic Acids Research*, *44*, 2593–2612.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press

Kriaucionis, S., & Heintz, N. (2009). The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science*, *324*, 929–930.

Kurihara, M., Shiraishi, A., Satake, H., & Kimura, A. P. (2014). A conserved noncoding sequence can function as a spermatocyte-specific enhancer and a bidirectional promoter for a ubiquitously expressed gene and a testis-specific long noncoding RNA. *Journal of Molecular Biology*, *426*, 3069–3093.

Laird, P. W. (2010). Principles and challenges of genomewide DNA methylation analysis. *Nature Reviews Genetics*, *11*, 191–203.

Latham, J. A., & Dent, S. Y. (2007). Cross-regulation of histone modifications. *Nature Structural and Molecular Biology*, *14*, 1017–1024.

Leader, J. E., Wang, C., Popov, V. M., Fu, M., & Pestell, R. G. (2006). Epigenetics and the estrogen receptor. *Annals of New York Academy of Sciences*, *1089*, 73–87.

Lee, K., Kim, J. H., Kwon, O. B., An, K., Ryu, J., Cho, K., et al. (2012). An activity-regulated microRNA, miR-188, controls dendritic plasticity and synaptic transmission by downregulating neuropilin-2. *Journal of Neuroscience*, *32*, 5678–5687.

Lee, K. K., & Workman, J. L. (2007). Histone acetyltransferase complexes: One size doesn't fit all. *Nature Reviews Molecular Cell Biology*, *8*, 285–295.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., et al. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature*, *341*, 149–152.

Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *Journal of Biological Chemistry*, *279*, 40545–40559.

Levine, A., Worrell, T. R., Zimnisky, R., & Schmauss, C. (2012). Early life stress triggers sustained changes in histone deacetylase expression and histone H4 modifications that alter responsiveness to adolescent antidepressant treatment. *Neurobiology of Disease*, *45*, 488–498.

Li, B., Carey, M., & Workman, J. L. (2007). The role of chromatin during transcription. *Cell*, *128*, 707–719.

Li, E., Beard, C., & Jaenisch, R. (1993). Role for DNA methylation in genomic imprinting. *Nature*, *366*, 362–365.

Li, X., Wei, W., Zhao, Q.-Y., Widagdo, J., Baker-Andresen, D., Flavell, C. R., et al. (2014). Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. *Proceedings of the National Academy of Sciences*, *111*, 7120–7125.

Li, Z., Van Calcar, S., Qu, C., Cavenee, W. K., Zhang, M. Q., & Ren, B. (2003). A global transcriptional regulatory role for c-Myc in Burkitt's lymphoma cells. *Proceedings of the National Academy of Sciences*, *100*, 8164–8169.

Lipsky, R. H. (2013). Epigenetic mechanisms regulating learning and long-term memory. *International Journal of Developmental Neuroscience*, *31*, 353–358.

Lipsky, R. H., Xu, K., Zhu, D., Kelly, C., Terhakopian, A., Novelli, A., et al. (2001). Nuclear factor kappaB is a critical determinant in N-methyl-d-aspartate receptormediated neuroprotection. *Journal of Neurochemistry*, *24*, 254–264.

Lisman, J. E. (1985). A mechanism for memory storage insensitive to molecular turnover: A bistable autophosphorylating kinase. *Proceedings of the National Academy of Sciences*, *82*, 3055–3057.

Lister, R., Mukamel, E. A., Nery, J. R., Urich, M., Puddifoot, C. A., Johnson, N. D., et al. (2013). Global epigenomic reconfiguration during mammalian brain development. *Science*, *341*, 629.

Lopez-Atalaya, J. P., Ciccarelli, A., Viosca, J., Valor, L. M., Jimenez-Minchan, M., Canals, S., et al. (2011). CBP is required for environmental enrichment-induced neurogenesis and cognitive enhancement. *EMBO Journal*, *30*, 4287–4298.

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of bdnf gene transcription in the consolidation of fear memory. *Journal of Neuroscience*, *28*, 10576–10586.

Lubin, F. D., & Sweatt, J. D. (2007). The IkappaB kinase regulates chromatin structure during reconsolidation of conditioned fear memories. *Neuron*, *55*, 942–957.

Ma, D. K., Jang, M.-H., Guo, J. U., Kitabatake, Y., Chang, M.-L., Pow-anpongkul, N., et al. (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science*, *323*, 1074–1077.

Maharana, C., Sharma, K. P., & Sharma, S. K. (2010). Depolarization induces acetylation of histone H2B in the hippocampus. *Neuroscience*, *167*, 354–360.

Malvaez, M., McQuown, S. C., Rogge, G. A., Astarabadi, M., Jacques, V., Carreiro, S., et al. (2013). HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. *Proceedings of the National Academy of Sciences*, *110*, 2647–2651.

Margueron, R., Trojer, P., & Reinberg, D. (2005). The key to development: Interpreting the histone code? *Current Opinion in Genetics and Development*, *15*, 163–176.

Martinowich, K., Hattori, D., Wu, H., Fouse, S. D., He, F., Hu, Y., et al. (2003). DNA methylation-related chromatin remodeling in activity-dependent Bdnf gene regulation. *Science*, *302*, 890–893.

Martone, R., Euskirchen, G., Bertrone, P., Hartman, S., Royce, T. E., Luscombe, N. M., et al. (2003). Distribution of NF-kappaB-binding sites across human chromosome 22. *Proceedings of the National Academy of Sciences*, *100*, 12247–12252.

Maurice, T., Duclot, F., Meunier, J., Naert, G., Givalois, L., Meffre, J., et al. (2008). Altered memory capacities and response to stress in p300/CBP-associated factor (PCAF) histone acetylase knockout mice. *Neuropsychopharmacology*, *33*, 1584–1602.

Maze, I., Covington, H. E. I., Dietz, D. M., LaPlant, Q., Renthal, W., Russo, S. J., et al. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science*, *327*, 213–216.

McGowan, P. O., Suderman, M., Sasaki, A., Huang, T. C. T., Hallett, M., Meaney, M. J., et al. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. *PLOS ONE*, *6*, e14739.

McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., Alenghat, T., et al. (2011). HDAC3 is a critical negative regulator of long-term memory formation. *Journal of Neuroscience*, *31*, 764–774.

McQuown, S. C., & Wood, M. A. (2011). HDAC3 and the molecular brake pad hypothesis. *Neurobiology of Learning and Memory*, *96*, 27–34.

Mercer, T. R., & Mattick, J. S. (2013). Structure and function of long noncoding RNAs in epigenetic regulation. *Nature Structural and Molecular Biology*, *20*, 300–307.

Miller, C. A., Campbell, S. L., & Sweatt, J. D. (2008). DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. *Neurobiology of Learning and Memory*, *89*, 599–603.

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron*, *53*, 857–869.

Moen, E. L., Zhang, X., Mu, W., Delaney, S. M., & Wing, C., McQuade, J., et al. (2013). Genome-wide variation of cytosine modifications between European and African populations and the implications for complex traits. *Genetics*, *194*, 987–996.

Motil, K. J., Schultz, R., Brown, B., Glaze, D. G., & Percy, A. K. (1994). Altered energy balance may account for growth failure in Rett syndrome. *Journal of Child Neurology*, *9*, 315–319.

Murphy, S. E., Norbury, R., Godlewska, B. R., Cowen, P. J., Mannie, Z. M., Harmer, C. J., et al. (2013). The effect of the serotonin transporter polymorphism (5-HTTLPR) on amygdala function: A meta-analysis. *Molecular Psychiatry*, *18*, 512–520.

Neelamegam, R., Ricq, E. L., Malvaez, M., Patnaik, D., Norton, S., Carlin, S. M., et al. (2012). Brain-penetrant LSD1 inhibitors can block memory consolidation. *ACS Chemical Neuroscience*, *3*, 120–128.

Nelson, E. D., Bal, M., Kavalali, E. T., & Monteggia, L. M. (2011). Selective impact of MeCP2 and associated histone deacetylases on the dynamics of evoked excitatory neurotransmission. *Journal of Neurophysiology*, *106*, 193–201.

Ng, S. B., Bigham, A. W., Buckingham, K. J., Hannibal, M. C., McMillin, M. J., Gildersleeve, H. I., et al. (2010). Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature Genetics*, *42*, 790–793.

Nikolova, Y. S., & Hariri, A. R. (2015). Can we observe epigenetic effects on human brain function? *Trends in Cognitive Sciences*, *19*, 366–373.

Nikolova, Y. S., Koenen, K. C., Galea, S., Wang, C. M., Seney, M. L., Sibillie, E., et al. (2014). Beyond genotype: Serotonin transporter epigenetic modification predicts human brain function. *Nature Neuroscience*, *17*, 1153–1155.

Niikawa, N., Kuroki, Y., Kajii, T., Matsuura, N., Ishikiriyama, S., Tonoki, H., et al. (1988). Kabuki make-up (Niikawa–Kuroki) syndrome: A study of 62 patients. *American Journal of Medical Genetics*, *31*, 565–589.

Nott, A., Watson, P. M., Robinson, J. D., Crepaldi, L., & Riccio, A. (2008). S-nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. *Nature*, *455*, 411–415.

Novik, K. L., Nimmrich, I., Genc, B., Maier, S., Piepenbrock, C., Olek, A., et al. (2002). Epigenomics: Genome-wide study of methylation phenomena. *Current Issues in Molecular Biology*, *4*, 111–128.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press

Oike, Y., Hata, A., Mamiya, T., Kaname, T., Noda, Y., Suzuki, M., et al. (1999). Truncated CBP protein leads to classical Rubinstein–Taybi syndrome phenotypes in mice: Implications for a dominant-negative mechanism. *Human Molecular Genetics*, *8*, 387–396.

Oliveira, A. M., Hemstedt, T. J., & Bading, H. (2012). Rescue of aging-associated decline in Dnmt3a2 expression restores cognitive abilities. *Nature Neuroscience*, *15*, 1111–1113.

Oliveira, A. M., Wood, M. A., McDonough, C. B., & Abel, T. (2007). Transgenic mice expressing an inhibitory truncated form of p300 exhibit long-term memory deficits. *Learning and Memory*, *14*, 564–572.

Oztan, O., Aydin, C., & Isgor, C. (2011). Stressful environmental and social stimulation in adolescence causes antidepressant-like effects associated with epigenetic induction of the hippocampal BDNF and mossy fibre sprouting in the novelty-seeking phenotype. *Neuroscience Letters*, *501*, 107–111.

Park, C. S., Rehrauer, H., & Mansuy, I. M. (2013). Genome-wide analysis of H4K5 acetylation associated with fear memory in mice. *BMC Genomics*, *8*, 539–545.

Park, H., & Poo, M. M. (2012). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience*, *14*, 7–23.

Pastor, W. A., Aravind, L., & Rao, A. (2013). TETonic shift: Biological roles of TET proteins in DNA demethylation and transcription. *Nature Reviews Molecular Cell Biology*, *14*, 341–356.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Java, S., Agis-Balboa, R. C., et al. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science*, *328*, 753–756.

Penn, N. W., Suwalski, R., O'Riley, C., Bojanowski, K., & Yura, R. (1972). The presence of 5-hydroxymethylcytosine in animal deoxyribonucleic acid. *Biochemical Journal*, *126*, 781–790.

Petrif, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C. M., Masuno, M., et al. (1995). Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature*, *376*, 348–351.

Pfeifer, G. P., Steigerwald, S. D., Hansen, R. S., Gartler, S. M., & Riggs, A. D. (1990). Polymerase chain reaction-aided genomic sequencing of an X chromosome-linked CpG island: Methylation patterns suggest clonal inheritance, CpG site autonomy, and an explanation of activity state stability. *Proceedings of the National Academy of Sciences*, *87*, 8252–8256.

Plass, C., Shibata, H., Kalcheva, I., Mullins, L., Kotelevtseva, N., Mullins, J., et al. (1996). Identification of Grf1 on mouse chromosome 9 as an imprinted gene by RLGS-M. *Nature Genetics*, *14*, 106–109.

Polderman, T. J. C., Benyamin, B., de Leeuw, C. A., Sullivan, P. F., van Bochoven, A., Visscher, P. M., et al. (2015). Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nature Genetics*, *47*, 702–709.

Portela, A., & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature Biotechnology*, *28*, 1057–1068.

Psotta, L., Lessmann, V., & Endres, T. (2013). Impaired fear extinction learning in adult heterozygous BDNF knock-out mice. *Neurobiology of Learning and Memory*, *103*, 34–38.

Ptashne, M. (2007). On the use of the word "epigenetic." *Current Biology*, *17*, R233–R236.

Puckett, R. E., & Lubin, F. D. (2011). Epigenetic mechanisms in experience-driven memory formation and behavior. *Epigenomics*, *3*, 649–664.

Qureshi, I. A., & Mehler, M. F. (2012). Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. *Nature Reviews Neuroscience*, *13*, 528–541.

Rajasethupathy, P., Antonov, I., Sheridan, R., Frey, S., Sander, C., Tuschl, T., et al. (2012). A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. *Cell*, *149*, 693–707.

Rett, A. (1966). On an unusual brain atrophy syndrome in hyperammonemia in childhood. *Wiener Medizinische Wochenschrift*, *116*, 723–726.

Rideout., W. M., III, Eggan, K., & Jaenisch, R. (2001). Nuclear cloning and epigenetic reprogramming of the genome. *Science*, *293*, 1093–1098.

Ridley, R. M., Frith, C. D., Crow, T. J., & Conneally, P. M. (1988). Anticipation in Huntington's disease is inherited through the male line but may originate in the female. *Journal of Medical Genetics*, *28*, 589–595.

Riggs, A. D. (1975). X inactivation, differentiation, and DNA methylation. *Cytogenetics and Genome Research*, *14*, 9–25.

Ringrose, L., & Paro, R. (2004). Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annual Review of Genetics*, *38*, 413–443.

Roadmap Epigenomics. (2015). Integrative analysis of 111 reference human epigenomes. *Nature*, *518*, 317–330.

Roberson, E. D., & Sweatt, J. D. (2001). Memory-forming chemical reactions. *Reviews in the Neurosciences*, *12*, 41–50.

Roelfsema, J. H., White, S. J., Ariyürek, Y., Bartholdi, D., Niedrist, D., Papadia, F., et al. (2005). Genetic heterogeneity in Rubinstein–Taybi syndrome: Mutations in both the CBP and EP300 genes cause disease. *American Journal of Human Genetics*, *76*, 572–580.

Roth, T. L., & Sweatt, J. D. (2010). Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormones and Behavior*, *59*, 315–320.

Rubinstein, J. H., & Taybi, H. (1963). Broad thumbs and toes and facial abnormalities: Possible mental retardation syndrome. *American Journal of Diseases of Children*, *105*, 588–608.

Rudenko, A., Dawlaty, M. M., Seo, J., Cheng, A. W., Meng, J., Le, T., et al. (2013). Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. *Neuron*, *79*, 1109–1122.

Rudenko, A., & Tsai, L.-H. (2014). Epigenetic modifications in the nervous system and their impact upon cognitive impairments. *Neuropharmacology*, *80*, 70–82.

Rujirabanjerd, S., Nelson, J., Tarpey, P. S., Hackett, A., Edkins, S., Raymond, F. L., et al. (2012). Identification and characterization of two novel JARID1C mutations: Suggestion of an emerging genotype–phenotype correlation. *European Journal of Human Genetics*, *18*, 330–335.

Russo, V. E. A., Martienssen, R. A., & Riggs, A. D. (Eds.) (1996). *Epigenetic mechanisms of gene regulation*. Woodbury, NY: Cold Spring Harbor Laboratory Press.

Saba, R., Störchel, P. H., Aksoy-Aksel, A., Kepura, F., Lippi, G., Plant, T. D., et al. (2012). Dopamine-regulated microRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. *Journal of Molecular Cell Biology*, *32*, 619–632.

Sananbenesi, F., & Fischer, A. (2009). The epigenetic bottleneck of neurodegenerative and psychiatric diseases. *Biological Chemistry*, *390*, 1145–1153.

Sando, R. I., Gounko, N., Pieraut, S., Liao, L., Yates, J. I., & Maximov, A. (2012). HDAC4 governs a transcriptional program essential for synaptic plasticity and memory. *Cell*, *151*, 821–834.

Schaefer, A., Sampath, S. C., Intrator, A., Min, A., Gertler, T. S., Surmeier, D. J., et al. (2009). Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex. *Neuron*, *64*, 678–691.

Schmitt, M., & Matthies, H. (1979). Biochemical studies on histones of the central nervous system: III. Incorporation of [14C]-acetate into the histones of different rat brain regions during a learning experiment. *Acta Biologica et Medica Germanica*, *38*, 683–689.

Schratt, G. M. (2009). microRNAs at the synapse. *Nature Reviews Neuroscience*, *10*, 842–849.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., et al. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature*, *439*, 283–289.

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron*, *36*, 241–263.

Schwartz, Y. B., & Pirrotta, V. (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nature Reviews Genetics*, *8*, 9–22.

Sennvik, K., Fastbom, J., Blomberg, M., Wahlund, L.-O., Winblad, B., & Benedikz, E. (2000). Levels of alpha- and beta-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. *Neuroscience Letters*, *278*, 169–172.

Shahbazian, M. D., & Grunstein, M. (2007). Functions of site-specific histone acetylation and deacetylation. *Annual Review of Biochemistry*, *76*, 75–100.

Shibata, H., Hirotsune, S., Okazaki, Y., Komatsubara, H., Muramatsu, M., Takagi, N., et al. (1994). Genetic mapping and systematic screening of mouse endogenously imprinted loci detected with restriction landmark genome scanning method (RLGS). *Mammalian Genome*, *5*, 797–800.

Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S., & Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *Journal of Neuroscience*, *22*, 3251–3261.

Shukla, A., Sehgal, M., & Singh, T. R. (2015). Hydroxymethylation and its potential implication in DNA repair system: A review and future perspectives. *Gene*, *564*, 109–118.

Simensen, R. J., Rogers, R. C., Collins, J. S., Abidi, F., Schwartz, C. E., & Stevenson, R. E. (2012). Short-term memory deficits in carrier females with KDM5C mutations. *Journal of Genetic Counseling*, *23*, 31–40.

Singer, J., Roberts-Ems, J., & Riggs, A. D. (1979). Methylation of mouse liver DNA studied by means of the restriction enzymes *Msp*I and *Hpa*II. *Science*, *203*, 1019–1021.

Smith, A. K., Kilaru, V., Klengel, T., Mercer, K. B., Bradley, B., Conneely, K. N., et al. (2015). DNA extracted from saliva for methylation studies of psychiatric traits: Evidence tissue specificity and relatedness to brain. *American Journal of Medical Genetics*, *168B*, 36–44.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press

Smith, Z. D., Chan, M. M., Mikkelsen, T. S., Gu, H., Gnirke, A., Regev, A., et al. (2012). A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature*, *484*, 339–344.
Smith, Z. D., & Meissner, A. (2013). DNA methylation: Roles in mammalian development. *Nature Reviews Genetics*, *14*, 204–220.
Spruijt, C. G., Gnerlich, F., Smits, A. H., Pfaffeneder, T., Jansen, P. W., Bauer, C., et al. (2013). Dynamic readers for 5-(hydroxy) methylcytosine and its oxidized derivatives. *Cell*, *152*, 1146–1159.
Stadler, F., Kolb, G., Rubusch, L., Baker, S. P., Jones, E. G., & Akbarian, S. (2005). Histone methylation at gene promoters is associated with developmental regulation and region-specific expression of ionotropic and metabotropic glutamate receptors in human brain. *Journal of Neurochemistry*, *94*, 324–336.
Strahl, B. D., & Allis, C. D. (2000). The language of covalent histone modifications. *Nature*, *403*, 41–45.
Sui, L., Wang, Y., Ju, L. H., & Chen, M. (2012). Epigenetic regulation of reelin and brain-derived neurotrophic factor genes in long-term potentiation in rat medial prefrontal cortex. *Neurobiology of Learning and Memory*, *97*, 425–440.
Swank, M. W., & Sweatt, J. D. (2001). Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning. *Journal of Neuroscience*, *21*, 3383–3391.
Sweatt, J. D. (2001). The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry*, *76*, 1–10.
Sweatt, J. D. (2009). Experience-dependent epigenetic modifications in the central nervous system. *Biological Psychiatry*, *65*, 191–197.
Sweatt, J. D. (2013). The emerging field of neuroepigenetics. *Neuron*, *80*, 624–632.
Szulwach, K. E., Li, X., Li, Y., Song, C.-X., Wu, H., Dai, Q., et al. (2011). 5-hmC–mediated epigenetic dynamics during postnatal neurodevelopment and aging. *Nature Neuroscience*, *14*, 1607–1616.
Szyf, M. (2009). Epigenetics DNA methylation, and chromatin modifying drugs. *Annual Review of Pharmacology and Toxicology*, *49*, 243–263.
Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*, *324*, 930–935.
Takai, D., & Jones, P. A. (2002). Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proceedings of the National Academy of Sciences*, *99*, 3740–3745.
Tao, X., West, A. E., Chen, W. G., Corfas, G., & Greenberg, M. E. (2002). A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron*, *33*, 383–395.
Teh, A. L., Pan, H., Chen, L., Ong, M. L., & Dogra, S., Wong, J., et al. (2014). The effect of genotype and in utero environment on inter-individual variation in neonate DNA methylomes. *Genome Research*, *24*, 1064–1074.
Thompson, T. M., Sharfi, D., Lee, M., Yrigollen, C. M., Naumova, O. Y., & Grigorenko, E. L. (2013). Comparison of whole-genome DNA methylation patterns in whole blood, saliva, and lymphoblastoid cell lines. *Behavior Genetics*, *43*, 168–176.
Tsankova, N., Renthal, W., Kumar, A., & Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience*, *8*, 355–367.
Tylee, D. S., Kawaguchi, D. M., & Glatt, S. J. (2013). On the outside, looking in: A review and evaluation of the comparability of blood and brain "-omes." *American Journal of Medical Genetics*, *162B*, 595–603.
Van Speybroeck, L. (2002). From epigenesis to epigenetics: The case of C. H. Waddington. *Annals of New York Academy of Sciences*, *981*, 61–81.
Waddington, C. H. (1942). The epigenotype. *Endeavour*, *1*, 18–20.
Waddington, C. H. (1957). *The strategy of the genes*. London: Allen & Unwin.
Walker, F. O. (2007). Huntington's disease. *Lancet*, *369*, 218–228.
Walker, M. P., LaFerla, F. M., Oddo, S. S., & Brewer, G. J. (2013). Reversible epigenetic histone modifications and Bdnf expression in neurons with aging and from a mouse model of Alzheimer's disease. *Age*, *35*, 519–531.
Wang, D., Szyf, M., Benkelfat, C., Provençal, N., Turecki, G., Caramaschi, D., et al. (2012). Peripheral SLC6A4 DNA methylation is associated with in vivo measures of human brain serotonin synthesis and childhood physical aggression. *PLOS ONE*, *7*, e39501.
Wang, W. H., Cheng, L. C., Pan, F. Y., Xue, B., Wang, D. Y., Chen, Z., et al. (2011). Intracellular trafficking of histone deacetylase 4 regulates long-term memory formation. *Anatomical Record*, *294*, 1025–1034.
Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., et al. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature Genetics*, *40*, 897–903.
Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*, 847–854.
Weinberg, M. S., Villeneuve, L. M., Ehsani, A., Amarzguioui, M., Aagaard, L., Chen, Z. X., et al. (2006). The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells. *RNA*, *12*, 256–262.
Weinmann, A. S., Yan, P. S., Oberley, M. J., Huang, T. H., & Farnham, P. J. (2002). Isolating human transcription factor targets by coupling chromatin immunoprecipitation and CpG island microarray analysis. *Genes and Development*, *16*, 235–244.
Wells, J., Yan, P. S., Cechvala, M., Huang, T., & Farnham, P. J. (2003). Identification of novel pRb binding sites using CpG microarrays suggests that E2F recruits pRb to specific genomic sites during S phase. *Oncogene*, *22*, 1445–1460.
Williams, S. R., Aldred, M. A., Der Kaloustian, V. M., Halal, F., Gowans, G., McLeod, D. R., et al. (2010). Haploinsufficiency of HDAC4 causes brachydactyly mental retardation syndrome, with brachydactyly type E, developmental delays, and behavioral problems. *American Journal of Human Genetics*, *87*, 219–228.
Wood, M. A., Attner, M. A., Oliveira, A. M., Brindle, P. K., & Abel, T. (2006). A transcription factor-binding domain of the coactivator CBP is essential for long-term memory and the expression of specific target genes. *Learning and Memory*, *13*, 609–617.
Wood, M. A., Kaplan, M. P., Park, A., Blanchard, E. J., Oliveira, A. M., Lombardi, T. L., et al. (2005). Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learning and Memory*, *12*, 111–119.
Wu, C.-T., & Morris, J. R. (2001). Genes, genetics, and epigenetics: A correspondence. *Science*, *293*, 1103–1105.
Wu, H., & Zhang, Y. (2014). Reversing DNA methylation: Mechanisms, genomics, and biological functions. *Cell*, *156*, 45–68.
Wu, S. C., & Zhang, Y. (2010). Active DNA demethylation: Many roads lead to Rome. *Nature Reviews Molecular Cell Biology*, *11*, 607–620.
Xie, H., Liu, Y., Zhu, Y., Ding, X., Yang, Y., & Guan, J.-S. (2014). In vivo imaging of immediate early gene expression reveals layer-specific memory traces in the mammalian brain. *Proceedings of the National Academy of Sciences*, *111*, 2788–2793.
Yan, P. S., Chen, C. M., Sji, H., Rahmatpanah, F., Wei, S. H., & Huang, T. H. (2002). Applications of CpG island microarrays for high-throughput analysis of DNA methylation. *Journal of Nutrition*, *132*, S2430–S2434.
Yan, P. S., Perry, M. R., Laux, D. E., Asare, A. L., Caldwell, C. W., & Huang, T. H. (2000). CpG island arrays: An application toward deciphering epigenetic signatures of breast cancer. *Clinical Cancer Research*, *6*, 1432–1438.
Yang, J., Lee, T., Kim, J., Cho, M.-C., Han, B.-G., Lee, J.-Y., et al. (2013). Ubiquitous polygenicity of human complex traits: Genome-wide analysis of 49 traits in Koreans. *PLOS Genetics*, *9*, e1003355.

https://doi.org/10.1017/S0954579416000857 Published online by Cambridge University Press