Review

Biological Psychiatry

Epigenetic Effects of Cannabis Exposure

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ABSTRACT

The past decade has witnessed a number of societal and political changes that have raised critical questions about the long-term impact of marijuana (*Cannabis sativa*) that are especially important given the prevalence of its abuse and that potential long-term effects still largely lack scientific data. Disturbances of the epigenome have generally been hypothesized as the molecular machinery underlying the persistent, often tissue-specific transcriptional and behavioral effects of cannabinoids that have been observed within one's lifetime and even into the subsequent generation. Here, we provide an overview of the current published scientific literature that has examined epigenetic effects of cannabinoids. Though mechanistic insights about the epigenome remain sparse, accumulating data in humans and animal models have begun to reveal aberrant epigenetic modifications in brain and the periphery linked to cannabis exposure. Expansion of such knowledge and causal molecular relationships could help provide novel targets for future therapeutic interventions.

Keywords: Addiction, Cannabinoids, CB_1 receptor, DNA methylation, Epigenetics, Neurodevelopment

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Extensive political and societal debates are currently being waged at state and federal levels regarding the legalization of marijuana (*Cannabis sativa*), which remains today the most commonly used illicit substance in the United States and in many countries worldwide. As evident in Figure 1, there has been a dramatic exponential increase of cannabis studies over the past two decades in response to the transformative implications resulting from the growing discussions and laws passed regarding legalization of recreational and medical marijuana use. Of the published studies to date in the PubMed database, about 13% relate to the neurobiological effects of cannabis and approximately 27% are directed toward obtaining behavioral insights. Despite the perceived low health risk of cannabis use by the general public, there is growing clinical awareness about the spectrum of behavioral and neurobiological disturbances associated with cannabis exposure, such as anxiety, depression, psychosis, cognitive deficits, social impairments, and addiction (1–7). The acute intoxication induced by cannabis consumption is strongly linked with concerns about its direct effects on cognition and motor function, but a central issue relates to its long-term impact, especially when exposure occurs during critical periods of brain development. Key gaps of scientific knowledge pertain to the biological mechanisms that maintain persistent phenotypic and molecular alterations long after its acute use.

The major psychoactive cannabinoid within cannabis, Δ^9-tetrahydrocannabinol (THC), targets the endocannabinoid (eCB) system, which plays a key role in the development of the brain and several other organs. In recent years, various human and experimental animal studies have evaluated the long-term impact of cannabis and cannabinoids on neurodevelopment, behavior, and several biological systems such as immunological mechanisms and reproductive processes [reviewed in (7–10)]. Moreover, behavioral abnormalities and molecular impairments in the brain have also been demonstrated to extend even into subsequent generations of offspring whose parents were exposed to cannabinoids before mating (11–15).

The epigenome provides a cellular fingerprint of environmental experiences, including drug exposure history, and thus is a highly relevant biological candidate expected to maintain persistent abnormalities and aberrant neuronal processing over time. The role of epigenetics in psychiatric disorders has been a major scientific focus during the past few years. According to the classic definition, "an epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" [as proposed by Conrad Waddington in the 1950s (16,17)]—this view implies heritability resulting in a phenotype. In the molecular biological era of recent years, "epigenetic" typically has been used to refer to mechanisms that modulate gene expression without altering the genetic code. Our article provides an overview of research endeavors relevant to cannabis-related epigenetic mechanisms that could shed light about the biological processes that establish the molecular platform that maintains marijuana's protracted effects on gene expression and ultimately behavior.

EPIGENETIC MECHANISMS

In a biological mechanistic context, knowledge of how gene expression is regulated by the cellular network of *cis*-acting elements and *trans*-acting factors has evolved substantially during the past decade. Generally, the interaction between

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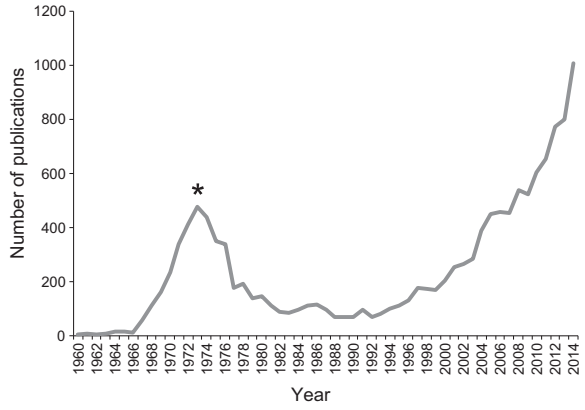

Figure 1. Number of publications in PubMed between 1960 and 2014 related to cannabis research. The data show the exponential increase in research studies over recent decades that coincides with changes in the legalization status (starting ~1996) and debates of recreational and medical marijuana use. The drop in publications in the 1970s marks changes in state laws and local regulations banning possession or sale of cannabis and cannabis becoming a schedule I drug (*).

genomic DNA elements (specific sequences with regulatory function), epigenetic modifiers, and transcription factors determines the expression state of genes. This network of processes is tightly coordinated in space and time; in the specification of different cell, tissue, and organ types; and throughout the life span of the individual (18–21).

Some of the most important ontogenetic regulatory decisions take place in early development and thus have critical implications for drug exposure during this period. Epigenetic modifications that can regulate gene expression levels include DNA methylation, nucleosomal structure and positioning, posttranslational modifications of nucleosomal histones, histone replacement, and small RNA molecules that influence protein production (Figure 2A). Mechanistic implications of the specific epigenetic processes that have thus far been linked to the effects of cannabis are briefly summarized below.

DNA Methylation

The role of DNA methylation (Figure 2B) in the regulation of gene expression is still controversial and highly dependent on genomic location, developmental stage, cell type, or disease state. Historically, CpG methylation in promoter regions and transcriptional regulatory sequences has frequently been associated with gene silencing, whereas methylation within the gene body is less understood and may act as either a positive or a negative effector (21,22). Accumulating evidence now also indicates that DNA methylation in the brain is reversible and its distribution changes throughout neuronal maturation and aging in neurodevelopmental disorders, including addiction to drugs such as cocaine (23,24). Mechanistically, DNA methylation (5-methylcytosine [5mC]) is generated by DNA methyltransferases. At promoter regions, 5mC is often associated with the binding of methyl-CpG binding domain-containing proteins (e.g., methyl-CpG-binding protein 2 [MeCP2]). The oxidation of 5mC to 5-hydroxymethylcytosine by ten-eleven translocation proteins can prevent access to DNA methyltransferases and thereby can maintain an

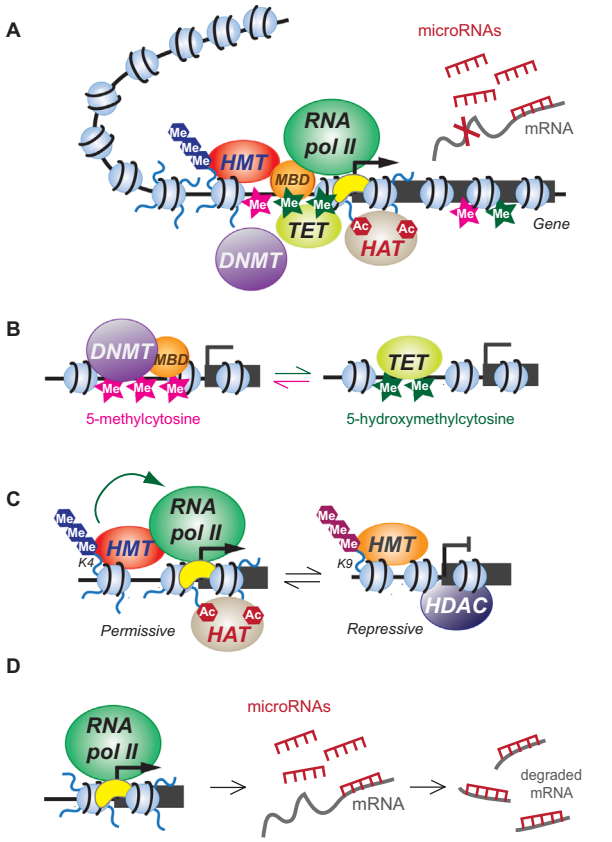

Figure 2. Several epigenetic mechanisms relevant to the effects of exogenous cannabinoids. **(A)** Gene expression is regulated by a network of DNA elements (e.g., promoters) and *trans*-acting factors (proteins that bind to the DNA) that interact physically and functionally to generate appropriate messenger RNA (mRNA) transcript levels from a gene. The resulting balance can be disrupted by drug exposure. Regulatory mechanisms include DNA methylation (Me), positioning and posttranslational modifications of nucleosomes (small blue balls), recruitment of sequence-specific and basal transcription factors and RNA polymerase II, and noncoding RNAs. The DNA-protein structure forms three-dimensional structures (represented by the chromatin loop) that influence the expression of associated genes. **(B)** DNA methyltransferases (DNMT) generate 5-methylcytosine (pink stars) at CpG sites, facilitated by methyl-CpG binding domain (MBD)-containing proteins. Ten-eleven translocation (TET) proteins mediate the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (green stars), leading to demethylation of the DNA. **(C)** Modifications of nucleosomal histone tails such as methylation (Me) and acetylation (Ac) are mediated by histone methyltransferases (HMT) and histone acetyltransferases (HAT), respectively. Depending on modified amino acid residue, methylation can have either permissive (e.g., on lysine 4 [K4]) or repressive (e.g., on lysine 9 [K9]) effects on transcription. Permissive modifications facilitate gene activation via the recruitment of the RNA polymerase II machinery. Acetylation is removed by histone deacetylases (HDAC) and can lead to transcriptional repression. **(D)** MicroRNAs are produced from specific genes and target protein-coding mRNAs for degradation, thereby prevention protein production.

unmethylated state of the promoter, leading to transcriptional activation (25). Interestingly, DNA methylation marks at specific gene loci have been shown to persist even during the maturation of germ cells (26,27) and thus are interesting candidates for the propagation of the long-term effects of cannabis throughout multiple generations.