



**PAIN & ADDICTION
GENOMICS & EPIGENETICS**

OBJECTIVES

- Discuss the difference between epigenetics and genomics
- History and significance of epigenetics
- Identify genetic factors that affect or increase the potential for addiction
 - Single nucleotide polymorphisms
 - Epigenetic impact on addiction



GENETICS VERSUS GENOMICS

- Genetics: scrutinizes the functioning and composition of the single gene where as genomics addresses all genes and their inter relationships in order to identify their combined influence on the growth and development of the organism.¹
- Genomics: study of genes and their functions, and related techniques.¹



PAIN GENOMICS²

- Strategies should focus on substances modulating:
 - Intracellular signal transduction
 - Ion transport
 - Anatomical structure development
- Processes involved in the genetic-based absence of pain provide promising fields for curative or preventive treatments.

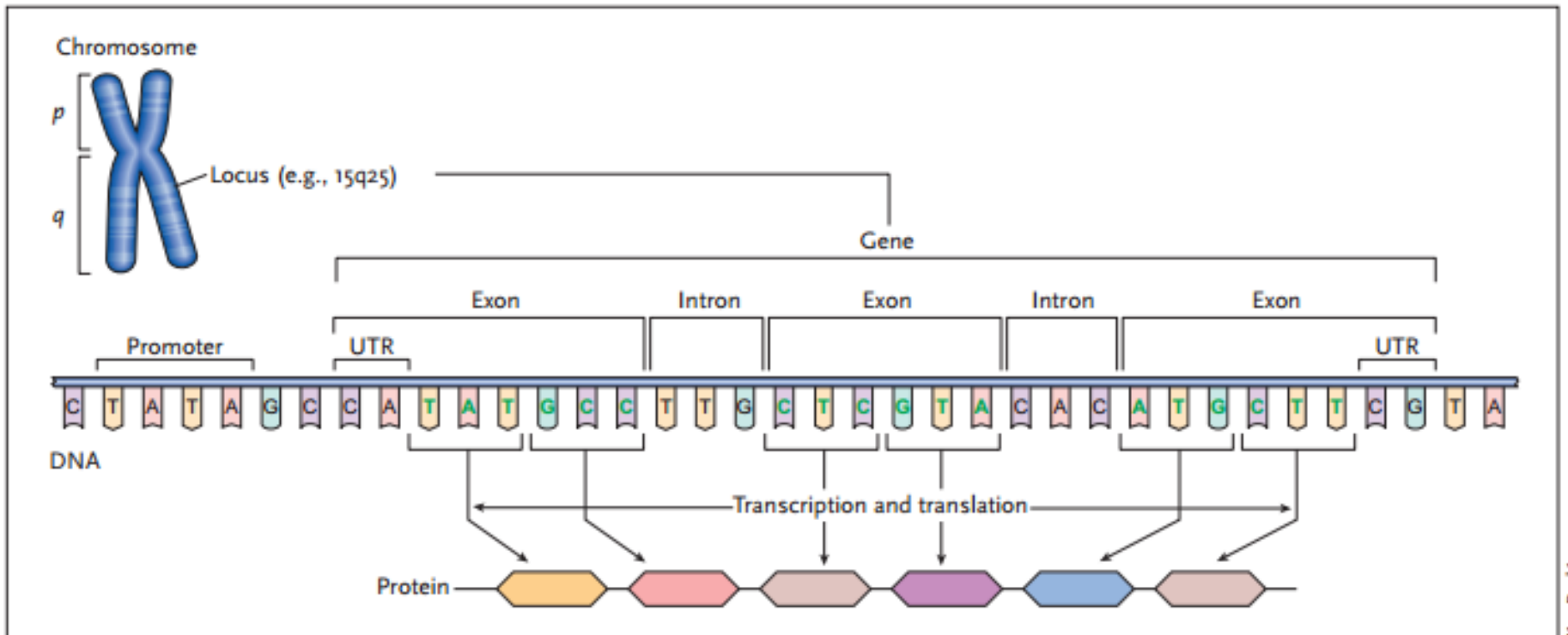


PAIN GENOMICS²

- Substances that interact with G-protein coupled receptor pathways provide symptomatic, not preventative relief of pain.
- Biological functions accessed either by analgesic drugs or microRNAs may be a future direction for drug development.



STRUCTURE OF A GENE³



GENOMICS VERSUS EPIGENETICS

- Genomics: Applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes.¹
- Epigenetics: refers to external modifications to DNA that turn genes “on” or “off” that do not change the DNA sequence, but affects the reading of genes.⁴



HISTORY OF EPIGENETICS⁵

- 1868: Charles Darwin conceptually described the biological process for epigenetic inheritance
- 1809: Jean-Baptiste Lamarck proposed a possible mechanism for the inheritance of acquired characteristics
- 1942: Conrad Waddington coined the term “epigenetics”
- Several decades later: 5-methyl cytosine was suggested to have a role in controlling gene activity



EPIGENETICS

- Alteration of DNA accessibility and chromatin structure resulting in changes of an organism's phenotype that could not be attributed to modifications in its genotype⁶
- Regulation of gene expression
- Modification include:
 - Histone deacetylation
 - Histone acetylation
 - DNA methylation
 - microRNAs (miRNA)
 - noncodingRNA (ncRNA)



REGULATORY RNA⁷

- MicroRNAs (miRNAs)
 - Comprised of short noncoding RNA that regulate gene expression post-transcriptionally
 - Control the expression of important epigenetic regulators, including DNA methyltransferases, histone deacetylases and polycomb group genes.
- Complicated network of feedback between miRNAs and epigenetic pathways appears to form an epigenetics-miRNA regulatory circuit, and to organize the whole gene expression profile



DNA METHYLATION⁵

- DNA methylation is involved with silencing of gene activity in multiple biological systems
- Hypermethylation at promoters blocks access of transcriptional machinery and thus inhibits gene expression
- Hypomethylation facilitates gene activation as a result of increased accessibility of DNA by polymerase.



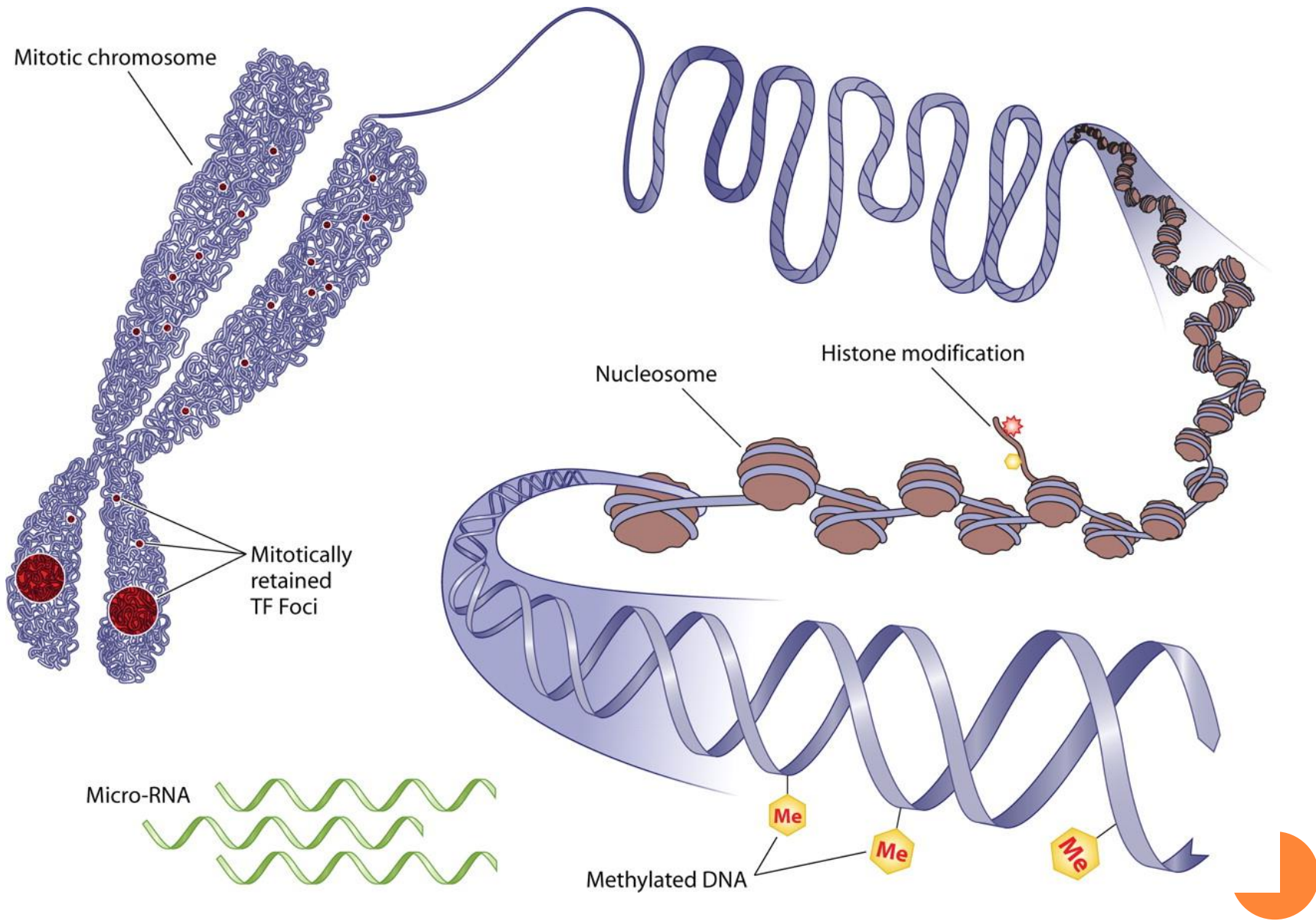
DNA METHYLATION⁸

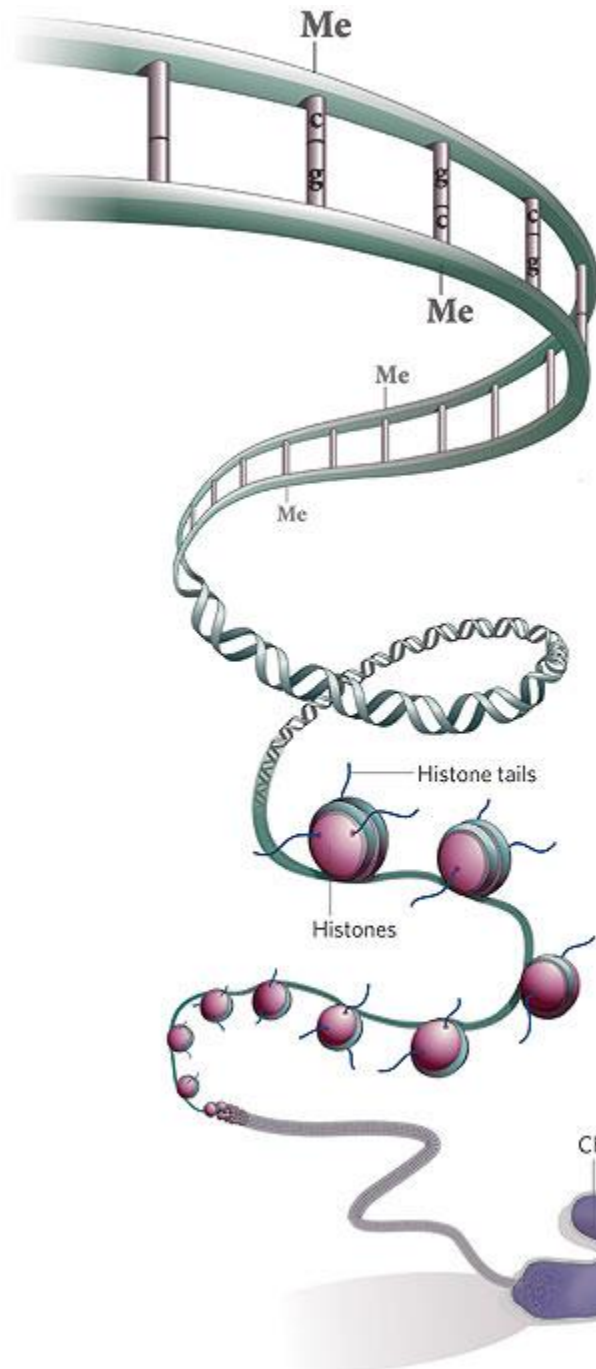


HISTONE MODIFICATION⁵

- Post-translational mechanisms including lysine and arginine methylation, serine and threonine phosphorylation, lysine acetylation, and lysine ubiquitination and sumoylation.
- Occur primarily within the histone amino-terminal tails protruding from the surface of the nucleosome as well as on the globular core region
- Impact chromosome function through
 - electrostatic charge of the histone causing a structural change within the histone or their binding to the DNA
 - modifications to binding sites for protein recognition modules that recognize acetylated or methylated lysines







The two main components of the epigenetic code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

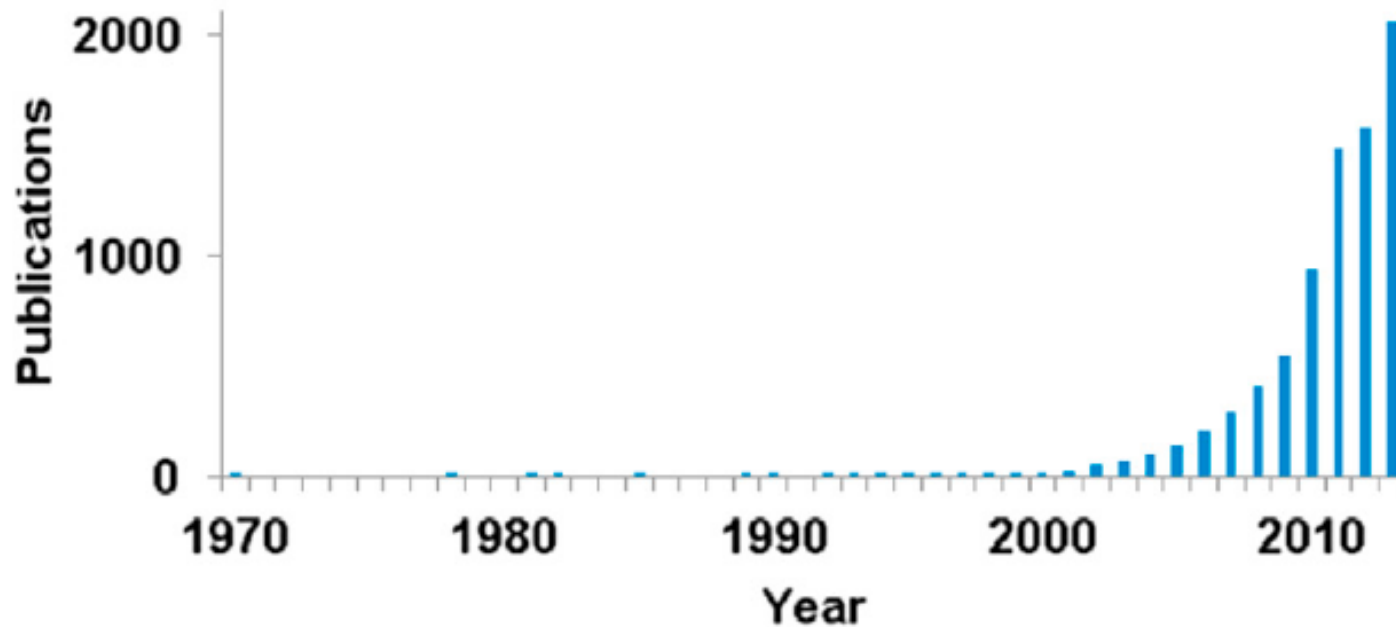
Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.



WHERE IS EPIGENETICS HEADED?¹

Epigenetics



EPIGENETICS IS A BALANCING ACT

- Regulation and stimulation of gene expression must be in balance in order for the cell to act “normally”
- This balancing act allows for treatment therapies that tip the scale one way or another by creating treatments that increase or decrease gene transcription

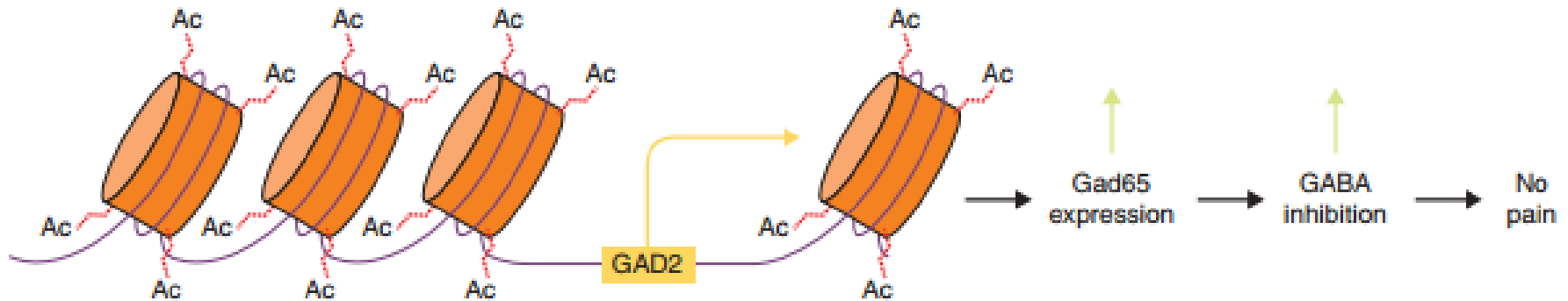


EPIGENETICS HAS BEEN IMPLICATED IN PAIN PROCESSING¹¹

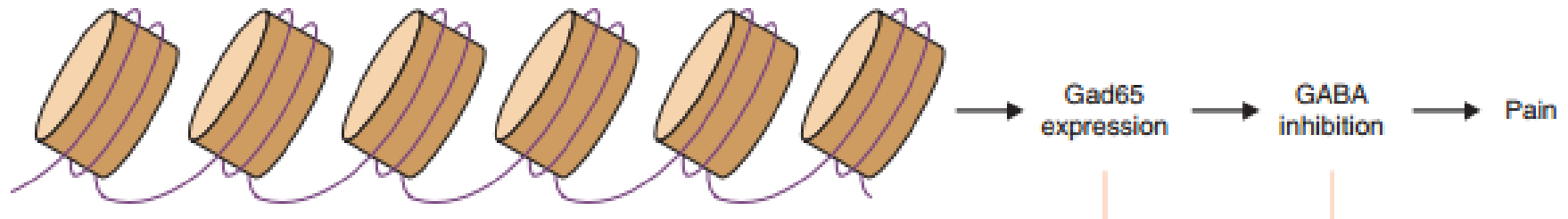
- “Point mutations have been identified in various genes as the underlying cause of different hereditary and sensory autonomic neuropathy types, most notably the gene encoding the TrkA receptor.”^{11,12}
- GCH1, which encodes GTP cyclohydrolase; COMT, an enzyme that eliminates catecholamines; and OPRM1, the μ -opioid receptor gene have been implicated in chronic pain conditions although study results have been difficult to replicate.¹¹



(a) Active (open)



(b) Inactive (closed)



How epigenetic mechanisms can influence pain processing¹. (a) Under normal conditions, histone tails are acetylated at the GAD2 promoter in the nucleus raphe magnus (NRM). (b) After application of complete Freund's adjuvant (CFA), Gad65 expression is suppressed through hypoacetylation of the GAD2 promoter, leading to loss of descending inhibition from the NRM [84]. GABA, γ -aminobutyric acid.



TRANSCRIPTIONAL REGULATION- EPIGENETICS

- DNA methylation prevents the association of DNA-binding factors with their target sequence or bind to methyl-CpG-binding proteins to recruit transcriptional co-repressors to modify the surrounding chromatin into a silenced state.¹³



TARGETING TREATMENT¹¹

- Anti-tumor necrosis factor alpha (anti-TNF- α) therapy
- Tropomyosin receptor kinase (Trk) inhibitors
- Histone deacetylase inhibitors (HDAC inhibitors)
- Na_v1.7 gene implicated in inflammatory pain
 - Treatments have been difficult to identify because of homology of isoforms.



ANTI-TUMOR NECROSIS FACTOR ALPHA (ANTI-TNF-ALPHA) THERAPY¹⁴

- Anti-TNF therapy suppresses tumor necrosis factor resulting in a decrease in inflammatory mediators
 - Tanezumab (not FDA approved)
 - Undergoing Phase II and Phase III clinical trials to evaluate the treatment of various pain diseases, such as osteoarthritis, chronic low back pain, and bone cancer



TROPOMYOSIN RECEPTOR KINASE (TRK) INHIBITOR

- Trk dimerization and tyrosine phosphorylation provide docking sites for adapter molecules, internalization, and intracellular signaling of pain stimuli.¹⁵
- Essential for neurotrophin formation and function
- Neurotrophins are pain mediators and modulators.



TROPOMYOSIN RECEPTOR KINASE (TRK) INHIBITOR

- TrkA¹⁶
 - Cognate receptor to nerve growth factor
 - Attenuates sarcoma induced nerve sprouting, neuroma formation and bone cancer pain
- TrkB
 - Mediates the effects of brain derived neurotrophic factor (BDNF) (related to nerve growth factor)
 - BDNF is known to be altered persistently for several weeks after the last drug administration, and manipulation of these genes in rodent models has been shown to be associated with the regulation of drug relapse behavior⁸



HISTONE DEACETYLASE INHIBITORS (HDAC INHIBITORS)

- HDAC inhibitors have been shown to have anti-inflammatory properties in other models¹⁷
- In animal studies, data has suggested that HDAC inhibitors, *N*-(2-aminophenyl)-4-[*N*-(pyridine-3-ylmethoxy-carbonyl)aminomethyl]benzamide (MS-275) and suberoylanilide hydroamic acid (SAHA), have provided analgesia and suggest that HDACs are potential targets for the epigenetic treatment.¹⁸



DRUG INDUCED NEURONAL PLASTICITY

- Drug-induced neuronal plasticity mediated via alterations in gene expression has long been viewed as a major molecular mechanism for the development of drug addiction and relapse⁸



GENETIC FACTORS INVOLVED IN DRUG DEPENDENCE AND RESPONSE TO TREATMENT³

- Contributors to drug vulnerability and dependence:
 - CYP450, dopamine beta-hydroxylase, and monoamine oxidase enzymes
 - Dopamine D2, mu opioid receptors
 - Serotonin and dopamine transporters
 - Δ FosB genes
 - DARPP-32



TRANSCRIPTION FACTOR Δ FOSB⁸

- Expression is increased several fold in the striatum during repeated drug exposure, and often persists long after drug exposure ceases.
- The extraordinary stability of Δ FosB in neurons has led to the theory that it plays a significant role in the onset of addiction.
- Upon acute exposure to drugs of abuse, transient increases in the nucleus accumbens occur in:
 - c-fos
 - FosB Fra-1
 - FosB Fra-2



ALTERATIONS IN CYP450¹⁹

- Rapid Metabolizers
 - 2A6: Respond poorly to nicotine replacement
- Intermediate Metabolizers
- Slow Metabolizers
 - 2A6: Have higher relapse after nicotine replacement treatment and have higher levels of nicotine than normal metabolizers
 - 2D6: Have lower opiate dependence



ALTERATIONS IN CYP450³

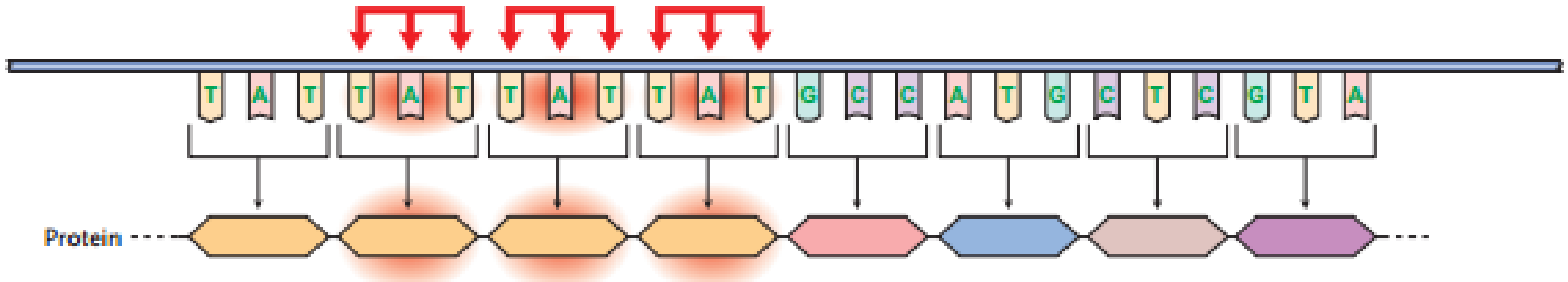
- CYP2A6 metabolizes nicotine to cotinine. Variation influences aspects of smoking dependence.
- Defective CYP2D6 results in poor metabolism of opiates suggesting a pharmacogenetic protection factor against oral opioid dependence.



TANDEM REPEATS³

Tandem Repeats

Addition of multiple copies of a DNA triplet (here, TAT), end-to-end; each new triplet can add one more copy of the amino acid it encodes to the protein product; the resulting elongated protein product may function differently than that produced by the gene's wild-type variant.



ALTERATIONS IN DOPAMINE PATHWAYS³

○ Gene SLC6A3

- Encodes dopamine transporter, DAT1
- Genetic variants containing a variable number of tandem repeats can occur in exon 15
 - Associated with cocaine induced paranoia but not dependence
 - Associated with methamphetamine psychosis but not abuse



ALTERATIONS IN DOPAMINE PATHWAYS³

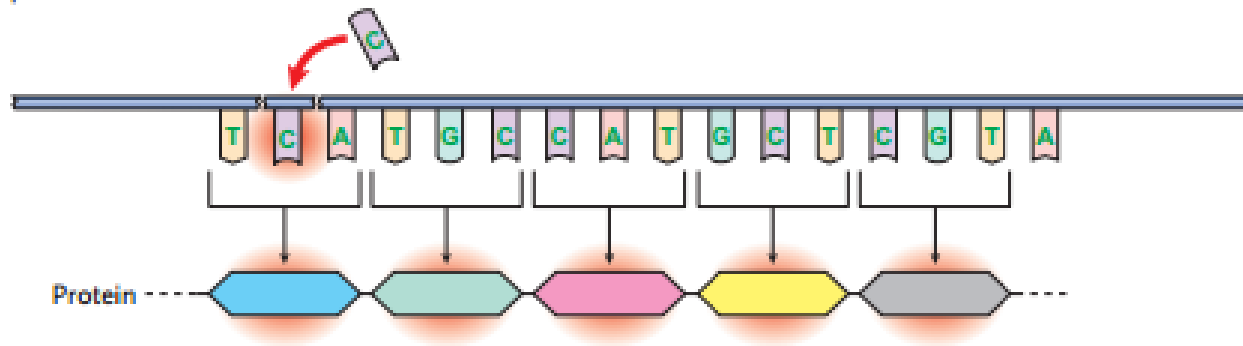
- Monoamine oxidase-A
 - Metabolizes serotonin, dopamine and norepinephrine
 - Genetic variants containing a variable number of tandem repeats has been associated with risk for substance use disorders.
- Dopamine receptor D2 variant, TaqIA
 - Associated with lower density of D2 receptors
 - TaqIA polymorphism may contribute to:
 - Substance abuse
 - Polysubstance abuse



INSERTION AND DELETION³

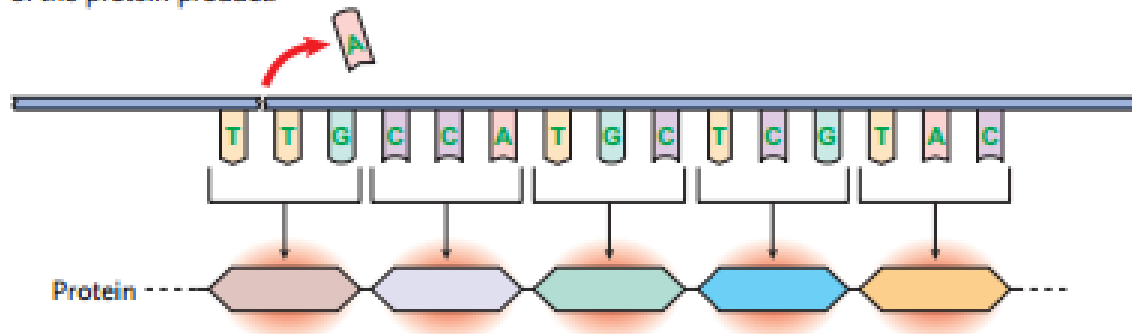
Insertion

Intrusion of an additional DNA unit or units into the gene sequence (here, a C between the T and A in wild-type triplet 1); this may change not only the sequence of the triplet in which it occurs, but of all subsequent triplets, producing alterations in many amino acids of the protein product.



Deletion

Excision of a DNA unit or units (here, an A from wild-type triplet 1); this may change all subsequent triplets and the corresponding amino acids of the protein product.



ALTERATIONS IN DOPAMINE PATHWAYS³

- Dopamine beta-hydroxylase metabolizes norepinephrine.
 - Two polymorphisms, an insertion and deletion, often inherited together have been associated with cocaine induced paranoia.

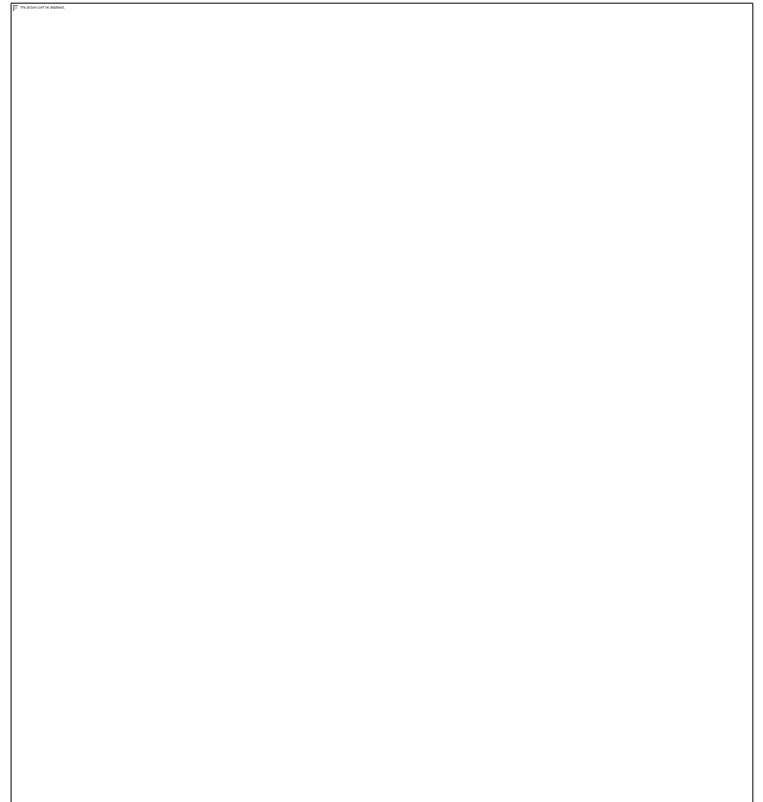


SINGLE NUCLEOTIDE POLYMORPHISMS³



COPY NUMBER VARIATION

- Alterations of DNA genomes that result in the cell having an abnormal variation in the number of copies of one or more section of the DNA.
- They results from deletions or duplications of large portions of genome.
- CNVs differ from SNPs because they affect large regions of the genome where SNPs affect only one nucleotide base pair.



ALTERATIONS IN OPIOID SYSTEM³

- Mu opioid receptor gene, OPRM1, encodes the receptor for beta endorphin.
 - Variation has been implication in heroin addiction
 - SNP (A118G) has been associated with increased risk of developing alcoholism
 - SNPs in exon 1 analysis has resulted in contradictory results including:
 - An increased affinity for beta-endorphin and greater receptor activation upon binding
 - No change in receptor function, signaling or binding affinity for various opioids



COMBINE STUDY

- Multisite study
- 911 subjects
- Results: those who were homozygous for the OPRM1 gene variant showed greater response to naltrexone treatment compared to those who did not have the gene variant²⁰
- OPRM1 genotyping in patients with alcohol dependence could be useful in determining the best treatment¹⁹



EARLY PHASES OF WITHDRAWAL

- “CREB mediates a form of tolerance and dependence, which dampens an individual’s sensitivity to subsequent drug exposure and contributes to a negative emotional state during early phases of withdrawal.”^{21,22}



INCREASED DRIVE AND MOTIVATION FOR DRUG

○ Δ FosB^{21,22}

- Mediates a state of relatively prolonged sensitization to drug exposure
- May contribute to the increased drive and motivation for drug, a core symptom of addictive disorders



DARPP-32, A PROTEIN ASSOCIATED WITH ADDICTIVE BEHAVIORS¹⁹

- DARPP-32 is a phosphoprotein and important media to of the extracellular signal-regulated kinase activity.²³
- Opiates modulate the expression of DARPP-32 which leads to drug-induced changes in neural synaptic plasticity.²³
- Silencing DARPP-32 using an siRNA may provide a novel gene therapy strategy to overcome drug addiction.^{19,23}



ALTERATIONS IN SEROTONIN PATHWAY³

- Gene SLC6A3
 - Encodes serotonin transporter, 5-HTT
 - Directs serotonin reuptake into the presynaptic neuron
- Genetic variants containing short variants reduce the transcriptional efficacy leading to decreased production of 5-HTT
 - Associated with heroin dependence in particular populations
 - Implicated in nicotine dependence



ALTERATIONS IN ALDH¹⁹

- ALDH * 2/ * 2 encodes for a dysfunctional enzyme
- More common in Asian populations
- Results in decreased metabolism of alcohol due to a lack of acetylaldehyde dehydrogenase that converts acetylaldehyde to acetate.
- Symptoms include:
 - Tachycardia
 - Flushing
 - Headache
- This is protective against alcohol dependence



ALTERATIONS IN CHROMATIN^{19,24}

- Changes in histones leading to alterations in chromatin have been correlated cocaine's direct action on genes leading researchers to believe this remodeling causes increased production of proteins associated with drug-seeking behaviors.
- Blocking histone alteration may prevent drug seeking behavior.



GENOME WIDE ASSOCIATION STUDIES³

- Studies have identified generic variants that may contribute to addiction vulnerability through unsuspected mechanisms including:
 - Cell adhesion
 - Protein translation
 - Trafficking
 - Degradation
 - Transcriptional regulation
 - Transport processes
 - Cell structures
- The above are now genes that are candidates for studies and biochemical pathway analysis



GENOME WIDE ASSOCIATIONS IN ALCOHOL DEPENDENCE²⁵

- Associations with canonical pathways previously shown to be involved in alcohol dependence were observed in a genome-wide DNA methylation analysis in alcohol dependence
 - Dehydrogenases
 - ADH1A
 - ADH7
 - ALDH3B2
 - Cytochrome P450 2A13
- Regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases along its length in the proopiomelanocortin (POMC) gene showed a significant association with alcohol craving



DRUG INDUCED EPIGENETIC CHANGES: ALCOHOL⁸

○ Human

- A significant increase in global DNA methylation has been reported in alcoholic patients compared to normal controls

○ Rat

- Exposure to excessive ethanol, at levels equivalent to human binge drinking, demonstrated tissue-specific alteration in histone H3 acetylation at lysine⁹
- Enhanced levels of histone H3 and H4 acetylation, CBP and neuropeptide Y (NPY), as well as decreased levels of HDAC activity, in rat amygdala were found to be involved in the anxiolytic effects of acute ethanol
- Moreover, the development of anxiety in alcohol withdrawal was associated with decreased histone acetylation and increased HDAC activity in the amygdala
- The administration of a known HDAC inhibitor, trichostatin A, prevented the development of alcohol withdrawal-related anxiety in rats by recovering the diminished levels of histone acetylation and inhibiting the augmented HDAC activity following chronic alcohol exposure

○ Mouse

- Exposure to chronic ethanol treatment altered DNA methylation at the NMDA receptor Nr2b gene in mice



DRUG INDUCED EPIGENETIC CHANGES: ALCOHOL AND NICOTINE⁸

○ Human

- DNA methylation at the monoamine oxidase-A (MAOA) gene was shown to be associated significantly with alcohol and nicotine dependence in women



DRUG INDUCED EPIGENETIC CHANGES: NICOTINE⁸

○ Human

- A DNA methylation status signature at the monoamine oxidase-A (MAOA) gene is associated with smoking
- Significant and persistent demethylation of MAOB promoter was found in former smokers (abstinent for over 10 years) and current smokers when compared to non-smokers
- The role of tobacco smoke in mediating DNA methylation change is supported by significantly higher level of nucleic acid demethylase activity in mice exposed to cigarette smoke compared to controls

○ Mouse

- Injection of nicotine down-regulated Dnmt1 mRNA and protein expression in mice, as well as decreasing DNA methylation level of the promoter region of glutamic acid decarboxylase 67 (GAD67), one of the two enzymes that synthesize GABA in the brain



DRUG INDUCED EPIGENETIC CHANGES: AMPHETAMINE⁸

○ Rat

- Rats exposed to subchronic amphetamine treatment showed differential DNA methylation and mRNA expression of Dnmt1

○ Mouse

- Alteration in expression of Mecp2 in the nucleus accumbens (NAc) altered both amphetamine-induced locomotion and the rewarding properties of amphetamine in conditional place preference
- Following chronic amphetamine administration, DFosB, a key transcription factor involved in the behavioural responses to cocaine, has been shown to accumulate in the striatum in mice
- The up-regulation of DFosB desensitizes c-fos mRNA induction to a subsequent drug exposure by binding to the c-fos promoter and recruits histone deacetylase 1 (HDAC1), which deacetylates nearby histones and attenuates gene activity



DRUG INDUCED EPIGENETIC CHANGES: AMPHETAMINE⁸

○ Mouse

- The administration of HDAC inhibitors butyric acid (BA) and valproic acid (VPA) was shown to potentiate amphetamine-induced behavioral sensitisation in mice
- An increased level of H4 hyperacetylation in the striatum was observed upon administration of VPA and amphetamine, while their co-treatment induced an additive effect on histone H4 acetylation
- The occurrence of the chronic amphetamine induced-sensitized response following amphetamine challenge was blocked by repeated administration of butyric acid (BA) and VPA
- The enhanced amphetamine-induced behavioral sensitization caused by HDAC inhibitors has also been shown to be associated with increased associative learning and memory, suggesting that histone deacetylation accounts at least partially for the induction and maintenance of the behavioral responses to amphetamine



DRUG INDUCED EPIGENETIC CHANGES: COCAINE⁸

○ Mouse

- Repeated cocaine administration reduced global levels of histone 3 lysine 9 (H3K9) demethylation in the nucleus accumbens of mice
- This reduction in H3K9 level was mediated by down-regulation of histone methyltransferase G9a, one of the key histone modification enzymes
- Repression of G9a after repeated cocaine administration increased the plasticity of nucleus accumbens neurons and enhanced the preference for cocaine
- Cocaine sensitivity was mediated via histone acetylation at the c-fos gene promoter in the striatum in mice
- Decreased cerebral volume and significantly lower sustained visual–spatial attention and spatial working memory were observed in the offspring of male mice exposed to cocaine
- Such phenotypical changes might be caused by the observed altered expression levels of DNMT1 and DNMT3 in the germ-cell rich seminiferous tubular tissue of the male mice exposed to cocaine
- The levels of global DNA methylation and expression of selected genes in hippocampal neurones were altered significantly in the male offspring of cocaine-exposed mothers at 3 and 30 days postnatum, compared to the offspring of non-exposed mothers

DRUG INDUCED EPIGENETIC CHANGES: COCAINE⁸

○ Rat

- MeCP2, a key transcriptional repressor, was shown to control the effects of cocaine on striatal brain-derived neurotrophic factor (BDNF) levels via homeostasis interactions with microRNA-212
- Acute cocaine administration induced transient H4 hyperacetylation of various genes, e.g. cFos and FosB
- Chronic cocaine injection did not have an effect on the c-fos promoter, but resulted in H3 hyperacetylation of the FosB promoter, as well as the Cdk5 and BDNF genes
- These histone modifications were shown to be long-lasting, persisting for at least 1 week after the last cocaine injection
- Administration of HDAC inhibitors extinguished cocaine-induced conditioned place preference in mice
- Administration of HDAC inhibitors decreased cocaine self-administration in rats



DRUG INDUCED EPIGENETIC CHANGES: OPIATE⁸

○ Human

- Increased DNA methylation in the promoter of opioid receptor mu 1(OPRM1) gene was seen in methadone-maintained former heroin addicts compared to controls



EPIGENETICS HAVE BEEN IMPLICATED IN ADDICTION AND REWARD BEHAVIOR^{10,26}

- By regulating expression of gene networks, epigenetic mechanisms contribute to:
 - Drug-induced structural changes in synapses
 - Synaptic plasticity
 - Addictive behavior
 - Complex drug-induced neuroadaptations in the brain
- Chronic drug exposure
 - Alters gene expression in the brain
 - Produces long-term changes in neural networks that underlie compulsive drug taking and seeking behaviors



ROLE OF ENVIRONMENT IN ADDICTION AND PAIN MANAGEMENT

- Environmental exposures lead to active regulation or changes of the chemical and DNA structure in the nervous system.²⁷
- Changes in the structure of DNA leads to alterations in gene readout and ultimately changes in neural function.²⁴



ROLE OF ENVIRONMENT IN ADDICTION AND PAIN MANAGEMENT²⁷

- Common environmental influences include:
 - Pregnancy
 - Maternal behavior
 - Nutrition of mother
 - Nicotine use
 - Alcohol use
 - Drug use
 - Infection
 - Abuse
 - Trauma
 - Illness
 - Psychological or physical stress
 - Toxins
 - Drug exposure
 - Psychotrauma



FUTURE DIRECTIONS: TARGETED DRUG THERAPY

- Silencing DARPP-32
 - Gold Nanorod short interfering Ribonucleic Acid (GNR siRNA) silenced DARPP-32 proteins without causing cell death^{3,28}
- Vaccines³
 - Multiple vaccines are being studied to treat addiction
 - Take advantage of the immune response and production of antibodies against the substance being abused
 - Example: NicVax
 - Phase 3 clinical trial
 - Composed of a nicotine-like molecule and an inactivated bacterial surface protein
 - Anti-nicotine vaccine expected to be the first in its class



FUTURE DIRECTIONS: TARGETED DRUG THERAPY^{3,29}

○ Other vaccine research

- Vaccines targeting the enzyme aldehyde dehydrogenase to prevent alcohol abuse
- Phase 2 trial by researchers at the Baylor College of Medicine
 - Vaccine that combines inactivated cholera toxin protein with a cocaine-like molecule for treatment of cocaine addiction
 - Chi and colleagues showed that mice had less hyperactivity after treatment with a disabled cold virus combined with a cocaine analog



FUTURE DIRECTIONS

- Understanding implication of epigenetic impact on addiction and response to pain management
- Discovering other transcription factors implicated in addiction¹³:
 - Glucocorticoid receptor
 - Nucleus accumbens 1 transcription factor (NAC1)
 - Early growth response factors (EGRs)
 - Signal transducers and activators of transcription (STATs)



TARGETED DRUG THERAPY CONSIDERATIONS³

- Protect against genetic discrimination
- Ensure fair access
- Risk versus benefit
- Assess healthcare costs



DISCUSSION QUESTIONS

1. What other environmental factors could influence epigenetics in the realm of addiction?
2. Discuss how the findings in mice and rats may demonstrate drug induced epigenetic changes in humans.
3. What do you think the role of Genome Wide Associations will play in discovering variants implicated in pain and addiction?
4. In what situations will copy number variations and single nucleotide polymorphisms will impact epigenetics?
5. How is epigenetics a balancing act? Discuss the importance of methylation and histone modifications and their affect on transcription.
6. Do you think vaccines are the answer?



MULTIPLE CHOICE QUESTIONS

1. External modifications to DNA that turn genes “on” or “off” and do not change the DNA sequence, but affect the reading of genes is:
 - a. Epigenetics
 - b. Genomics
 - c. Genetics

2. Which of the following are not implicated in epigenetics?
 - a. DNA methylation
 - b. Histone modification
 - c. Alterations in neurotransmitter pathways
 - d. Gene sequencing



MULTIPLE CHOICE QUESTIONS

3. True/False. Blocking histone alteration may prevent drug seeking behavior.
4. The following are environmental factors that can alter gene expression:
 - a. Drug use
 - b. Nutrition
 - c. Infection
 - d. All the above



MULTIPLE CHOICE QUESTIONS

5. Which of the following are not implicated in addiction?
- a. CYP2D6
 - b. CYP2B6
 - c. DARPP-32
 - d. OPRM1
 - e. All the above
6. Which of the following are potential treatment targets for pain or addiction?
- a. DARPP-32
 - b. Tropomyosin receptor kinase
 - c. Histone deacetylase
 - d. Tumor necrosis factor
 - e. All the above



QUESTIONS?

Contact information:



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