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Teaching Links between Epigenetics and Anesthesia to Anesthesia Providers

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Abstract

Background

Epigenetics has been proposed as the epicenter of anesthesia research. The epigenetic landscape developed by Waddington reflects that human diversity is likely the result of a myriad of complex interactions between both intracellular and environmental environments, rather than a simple one-to-one protein coding of the genome. A gap in the literature identifies a need to better explore links between epigenetics and anesthesia. No formal educational program on this topic geared toward anesthesia providers was identified based on the comprehensive literature review completed.

Objectives

This study aimed to describe the knowledge of the anesthesia providers including Certified Registered Nurse Anesthetists (CRNAs), Anesthesiologists (Medical Doctors or Doctors of Osteopathy), and Student Registered Nurse Anesthetists (SRNAs) on the identified links between epigenetics and anesthesia after a 1 hour educational offering on epigenetics and anesthesia.

The secondary objective was to gauge the acceptability of such an educational offering amongst anesthesia providers.

Methods

A post-test only study design was utilized for this study. A validated, one hour educational offering on epigenetics and anesthesia linkages was delivered to anesthesia providers followed with the subsequent administration of a posttest exam. The posttest was an assessment of learning based upon the ten objectives of the educational presentation with two questions per objective and was aligned with the contents of instruction. Also, the posttest

included six modified Likert-type questions to gauge the 1 hour educational program's acceptability.

Results

All 34 questions were answered by the 41 study participants with a Kuder-Richardson 20 value of .41. The mean correct scores on the 20 knowledge questions was $M = 13.05$ with a $SD = 2.76$. The six Acceptability Scale questions had a Cronbach's Alpha value of .778. Most participants had a neutral to acceptable rating for the program on the acceptability portion of the posttest. No statistically significant association was found between gender, age group, employment status, position/role, and education level with the post-test epigenetic exam mean scores.

Conclusion

Forty one anesthesia providers learned key concepts of epigenetics in anesthesia. The educational program expanded the knowledge of anesthesia providers to an emerging topic, epigenetics in anesthesiology that has the potential to change anesthesia theory, research, and practice.

Background and Significance

Epigenetics has been proposed as the epicenter of anesthesia research. The epigenetic landscape developed by Waddington reflects that human diversity is likely a result of a myriad of complex interactions, both intracellular and environmental, rather than a simple one-to-one protein coding of the genome (Baedke, 2013). Knowledge is lacking about anesthetic-induced epigenomic effects. Also, not understood is how the epigenome affects the ways in which an anesthetic agent acts. Epigenetics and epigenomics in anesthesiology is an important avenue of learning to pursue because humans and other animals such as rats, have an epigenome. It is likely that at some point, anesthetic intervention outcomes will be influenced by epigenomic changes caused by environmental factors (Cortessis et al., 2012; Skipper, 2011). Although beyond the scope of this proposed study, Csoka and Szyf (2009) propose that epigenetic side effects of pharmaceuticals are involved in the etiology of heart disease, cancer, neurocognitive disorders, obesity, diabetes, infertility, and sexual dysfunction.

Out of the first year's lectures at NorthShore University School of Nurse Anesthesia, the primary researcher grew an appreciation for exploring advances in epigenetics and anesthesiology and slowly came to realize that there existed a knowledge gap in how these two branches of science might be closely linked with one another. Although much has been written about anesthesiology, there still are many unknown mechanisms of action and effects of anesthetic medications, which epigenetics could provide scientific explanations for in the future (Bain & Shaw, 2012; Biel, Wascholowski, & Giannis, 2005). Furthermore, the scientific community's burgeoning inquiry into epigenetics (Egger, Liang, Aparicio, & Jones, 2004; Feinberg, 2008; Lirk, Fiegl, Weber, & Hollmann, 2015; Naguib, Bie, & Ting, 2012) fueled my own investigation and continued interest on this research topic.

In 2014, the primary researcher's Master of Science in Nursing thesis was an integrative literature review (ILR) on epigenetics in anesthesiology using Whittemore and Knafl's (2005) methodology for ILR. The ILR was done primarily to critically analyze the seminal findings in epigenetics and epigenomics, which have practice implications for anesthesia providers. This body of work was updated in order to have it published in a peer reviewed, professional journal and served as the backbone for the development of a teaching module on epigenetics for consideration in anesthesiology. The educational activity on epigenetics in anesthesiology was evaluated for its acceptability among anesthesia providers (Anesthesiologists, CRNA's, and SRNA's).

Deficiencies in Past Studies

Although anesthetic-epigenetics linkages are discussed both theoretically and more concretely in the literature, no previous study had examined the anesthesia providers' knowledge on this topic. There was an unmet need for a well-developed educational module for the anesthesia provider on the topic of epigenetics for consideration in anesthesiology. Integration of epigenetics into current nursing anesthesia curriculum is critical for the translation of key epigenetic research findings to anesthesia practice.

Research Problem

Epigenetics in anesthesiology is a novel area of science. Anesthesia providers, including CRNAs, SRNAs, Anesthesiologists, Anesthesiology Residents, and Anesthesiology Assistants (AAs), know little about the interplay between epigenetics and anesthetic medications. There was no identifiable teaching program/module available in the literature on the topic to date. Developing, instituting, and evaluating such a program expanded the knowledge of anesthesia providers and fostered further investigation and discourse on the fundamental concepts and

seminal findings in epigenetics for consideration in anesthesiology. Ultimately, improvement in anesthesia providers' knowledge on these topics could lead to improved patient outcomes related to anesthesia care.

Purpose of the Study

This study's purpose was to expand the knowledge base of the anesthesia providers on both theoretical and practical applications of epigenetics to anesthesia practice. It is important that anesthesia providers continue to expand their knowledge on epigenetics and epigenomics in order to optimize patient safety by preventing iatrogenic anesthetic outcomes caused by epigenetic changes (Smith, 2011). Moreover, epigenetics is critical to the advancement of the nursing anesthesia profession as a whole. Equipped with a greater understanding of how the epigenome and anesthetic agents interact may one day lead to more customized anesthetic delivery to each individual patient (Smith, 2011). Furthermore, it was also this study's purpose to explore how the anesthesia providers' knowledge on this topic has changed after undergoing the teaching/learning module.

Research Questions

Using the PICOT (population, intervention or issue, comparison intervention or group, outcome, and time) format to devise the research questions for this DNP project, the following research questions were developed:

- Are anesthesia providers knowledgeable about fundamental concepts and seminal research findings relevant to epigenetics in anesthesiology?
- Is a 1 hour educational offering consisting of didactic lecture on epigenetics in anesthesiology usable and acceptable in meeting the knowledge and information needs of anesthesia providers?

- P – Anesthesia Providers
- I – Epigenetics in anesthesiology teaching module
- C - None
- O - Acceptability of the module and knowledge level using mean scores
- T - One hour session included the delivery of the teaching/learning module and the post survey

Literature Review

Literature Review Methodology

The Whittemore and Knafl (2005) methodology for performing an integrative literature review (ILR), which is the broadest type of research review method, was used to diversify and capture both experimental and non-experimental designs. This approach includes both theoretical and empirical sources, which increases the potential for findings to have a greater role in evidence-based practice (Whittemore & Knafl, 2005).

Literature Search Procedure

In seeking out the background and research articles for this literature review, a number of computerized databases were searched including DePaul WorldCat and MEDLINE/PubMed. Articles where the keywords “epigenetics” and “anesthesia” both appeared within the text were chosen initially. Both the DePaul University Library (depaul.worldcat.org) and MEDLINE/PubMed were key in finding articles as they offered access to multiple databases (see Table 1). A 13-year (2004-2017) search range was used initially, but because of the relatively small number of studies on this topic, this search was expanded to articles written as far back as 1980. Articles were excluded if the use of anesthesia was for purposes of anesthesia only and

therefore, not linked to the study of epigenetics. Articles were also excluded if they did not specifically discuss epigenetics, as was the case, where proteomics or pharmacogenetics was only discussed. Also excluded were studies that simply identify epigenetic changes in certain pathophysiological conditions and those that manipulate epigenetic markers without any pharmacological influence. A total of 33 articles were read in the pursuit of how anesthesia links to epigenetics, which yielded 16 highly relevant articles for the proposed study.

Conceptual Categories of Linkage Between Epigenetics and Anesthesiology

According to Biel, Wascholowski, and Giannis (2005), the key regulatory concepts and mechanisms of epigenetics are acetylation, methylation, phosphorylation, ribosylation, sumoylation, and ubiquitinylation. These processes act upon the histone code to bring about downstream biological processes. By exploring the literature in order to find linkages between epigenetics and anesthesiology, a number of conceptual categories emerged, which include:

- Ketamine-induced unknown epigenetic mechanism in learning, memory, and depression involving the synaptic (glutamate) and activity-regulated cytoskeleton (Arc) proteins
- Histone methylation and DNA methylation effects on learning and memory
- Reprogramming of infant brain after general anesthesia
- Epigenetic allele silencing in malignant hyperthermia (MH)
- Carcinogenicity of anesthetics
- Single nucleotide polymorphism (SNP) of methylanetetrahydrofolate reductase (MTHFR) 677T, homocysteine elevation and nitrous oxide-related myelopathy
- Hypermethylation of hippocampal synaptic plasticity-related genes with sevoflurane exposure

- MicroRNA-21-mediated cardioprotective effect of isoflurane gas
- H4 histone acetylation and pain
- Epigenetic gene silencing-related c-fiber dysfunctions in neuropathic pain

Ketamine-Induced Unknown Epigenetic Mechanism in Depression

According to Duman and Aghajanian (2012), ketamine (a synthetic N-Methyl D-Aspartate aka NMDA) is a medication commonly used to induce general anesthesia and to treat pain. It works on the NMDA receptor (NMDAR) as an antagonist. Usually, glutamate and aspartate, both excitatory neurotransmitters, engage NMDAR, as well as, D-serine (possibly through phosphorylation), which acts as a co-agonist thereby increasing neurotransmitter excitation (Jenuwein & Allis, 2001; Strahl & Allis, 2000). NMDAR is involved in cellular membrane depolarization, rapidly increasing permeability to magnesium and calcium, linked to synaptic plasticity of neurons and hence, affecting how organisms learn and memory development. Depression is characterized by pleasure loss, decreased cognition and memory, and alterations in sleeping, eating, ambulation, and sexual behaviors. In depression, there appears to be atrophy of the neuron, decreased neuron number in the brain's cortex and limbic regions, and slowed neurotransmission across neurons. Commonly used antidepressants such as selective serotonin reuptake inhibitors (SSRI's) are used to flood the synapse with neurotransmitter to aid in neuronal communication and to treat symptoms of depression; however, the effects SSRIs take weeks to months to materialize and often only lead to moderate symptom relief. Here, ketamine shows promise as an effective antidepressant, due to its seemingly rapid synaptogenesis and reversal of the atrophy caused by chronic stress, a known depressive factor, in rodents. Reversal of depressive symptoms via a single dose of ketamine has been seen within as little as a few hours in treatment-resistant patients lasting seven to ten days (Duman & Aghajanian, 2012).

Duman and Aghajanian (2012) describe that chronic stress leads to decreased brain-derived neurotrophic factor (BDNF), necessary for neuronal development early in life and for the survival and function of neurons in adulthood. Fluoxetine, an SSRI, acts on the BDNF molecule to increase excitatory glutamate activity, but only modestly in comparison to ketamine. In activating BDNF release, ketamine leads to increased signaling and subsequent translation of synaptic proteins such as glutamate A1 (GluA1) and activity-regulated cytoskeleton-association protein (Arc) (Duman & Aghajanian, 2012). In this way, we see some evidence of an unknown epigenetic mechanism activated by ketamine turning on a specific physiologic pathway.

Histone Methylation, DNA Methylation and Long-Term Memory

Gupta et al. (2010) discuss the ways in which histone methylation may be dysregulated in long-term memory formation and in cognitive impairments like schizophrenia. It is likely that both silencing of genes and transcriptional activation, specifically, oppositely focused processes, acting on different histone areas may be necessary in consolidating memory. Methyltransferase is an important enzyme in the methylation process (Gupta et al., 2010). It stands to reason that if a given anesthetic could affect methyltransferase activity or some other yet unknown mechanism, then memory could be negatively impacted.

Ji et al. (2014) expand on how epigenetic mechanisms impact long-term memory formation. They explain that histone acetylation and histone deacetylation as controlled by histone acetyltransferase and histone deacetylase (HDAC) activity, respectively. The presence of HDACs, specifically HDAC2, have a negative impact on long term memory formation by muffling key genes in learning and memory such as brain-derived neurotrophic factor (BDNF), calmodulin-dependent protein kinases (CaMKII), and cAMP response element binding protein (CREB). Their research also shows cellular apoptosis, also known as programmed cell death, as a

result of exposure to inhaled anesthetic. On the other hand, neuroprotection can occur by using HDAC inhibitors like valproic acid (Ji et al., 2014).

Chestnut et al. (2011) further studied how epigenetic regulation via DNA methyltransferase (Dnmt) is part of motor neuron cell death. Used to inhibit Dnmt catalytic activity, RG108 and procainamide (a sodium channel blocking antiarrhythmic) protected cultured neurons from excessive DNA methylation and apoptosis (programmed cell death). Dnmt-influenced apoptosis is linked to human amyotrophic lateral sclerosis (ALS) (Chestnut et al., 2011). So, if procainamide is taken, the motor neuron cell may be protected, but what of the drugs that may conversely affect these same cells? And, what inherent individual epigenetic traits does the one undergoing anesthesia bring to the equation?

Reprogramming of Infant Brain after General Anesthesia

General anesthesia (GA) "is intended to bring about five distinct states during surgery: analgesia, amnesia, loss of consciousness, motionlessness, and weakening of autonomic responses" (Surgeryencyclopedia.com, 2014). Culley, Maze, and Crosby (2014) note that around the year 2000, scientists began to question the return of the brain's functionality after having undergone general anesthesia. This concern is particularly poignant in the infant brain, which when exposed to GA experienced apoptotic neurodegeneration, loss of neural synapses, and cognitive/behavioral deficits moving into maturity as seen in animal models. Synaptogenesis in humans is believed to occur between late gestation and 3-4 years of age, so extrapolating from animal studies and applying to humans, developmental deficits in cognition and behavior may be an inadvertent result of undergoing GA (Culley et al., 2012).

Culley, Maze, and Crosby (2010) note that there is a greater than twofold increase in the incidence of Attention Deficit Hyperactivity Disorder (ADHD) in young adults (< 19 years old),

who underwent two or more GA's before the age of 2 years old. There is some discrepancy in findings as they are not consistently reported between studies and male infants over-represent the infants undergoing GA. Furthermore, it is possible that the surgical stress itself with release of cytokines and other pro-inflammatory mediators may be responsible for the decrease in neurotrophic factors, neurogenesis, and formation of synapses; an example of epigenetic dysregulation. This then, lends credence to the use of anti-inflammatory medications such as non-steroidals to prevent negative effects on neurological dysfunction. So, the consideration of seemingly less offensive anesthetics such as alpha-2 adrenergic agonists (dexmedetomidine) and xenon also warrants investigation (Culley, et al., 2012). Csoka and Szyf (2008) corroborate that GA agents must further be studied for their effects on return to normal cognition.

MTHFR 677T Polymorphism, Homocysteine Elevation and Nitrous Oxide-Related Myelopathy

Lacassie et al. (2006) that the enzyme 5,10 methylenetetrahydrofolate reductase (MTHRF) is the catalyst that reduces 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant type of circulating folate and donor of carbon for remethylation of homocysteine to methionine. Methionine is methylated throughout biochemistry and important in many ways including myelin sheath formation and in DNA synthesis. Nitrous oxide (N2O), a volatile gas used in general anesthesia, oxidizes the cobalt atom of vitamin B12 irreversibly, which inhibits the cobalamin-dependent enzyme methionine synthase. Hence, N2O leads to decreased MTHRF activity (Lacassie, 2006).

Lacassie et al. (2006) go on to explain that the autosomal recessive inheritance of the thermolabile form of MTHRF, a genetic mutation resulting from a 677 cytosine to thymine

altered pairing such that valine instead of alanine is expressed. The thermolabile MTHRF is associated with elevated plasma homocysteine levels (hyperhomocysteinaemia) and decreased MTHRF activity. Coronary artery disease, neurological deficit, neural tube defects have been investigated as linked to low MTHRF activity (Lacassie, 2006).

Lacassie et al. (2006) describe in their case report, a patient had been exposed to N20 serially for surgical repair of cervical spinal stenosis. Their patient presented with increasingly severe neurological deficit up to and including paralysis, which they hypothesized was from decreased MTHFR activity due to the irreversible oxidation of the cobalt atom of vitamin B12 (folate) from N20 administration, an inherited MTHFR gene mutation, and hyperhomocysteinaemia. They were able to slowly restore the patient's neurological dysfunction with folic acid and vitamin B12 supplementation (Lacassie, 2006).

Although the genetic screening for MTHRF mutation is not routinely practiced, it may be perhaps influential in the practice of anesthesia in upcoming years, particularly in context of N2O administration. Lacassie et al. (2006) do not identify a specific epigenetic mechanism in their article, but there is one (or more) in play because the mutated MTHFR is expressed phenotypically.

Sevoflurane-Induced Down-Regulation of Hippocampal Oxytocin and Arginine Vasopressin

Zhou et al. (2015) exposed neonatal, 20 day old mice to either air or sevoflurane gas for six hours. After mice were exposed to their respective gases, they were introduced to the same stimulus mouse for five minutes with a thirty-minute break for a total of four sessions. The length of time spent smelling within two centimeters of the introduced mouse was recorded. At the fifth session, the same stimulus mouse and a novel mouse were introduced. The sevoflurane-exposed

mice took longer at each introduction to become familiar with the stimulus mouse and then, at the fifth session, to discriminate between the mouse they already were familiar with and the novel one, showing a slowed learning response. Mice were then euthanized and the hippocampus of each was resected. The amount of oxytocin and arginine vasopressin was markedly different between groups, where the sevoflurane group had less of both hormones present and down-regulation of their respective messenger RNA (mRNA). Zhou et al. (2015) based upon previous related research and their own findings, question whether methylation of the oxytocin and arginine vasopressin gene promoters leads to down-regulation of their transcription (Zhou et al., 2015).

Hypermethylation of Hippocampal Synaptic Plasticity-Related Genes with Sevoflurane Exposure

Sevoflurane is an inhaled anesthetic used to induce GA. Ju and colleagues studied how neonatal rats exposed to sevoflurane functioned cognitively via the open field test, fear conditioning, and the Morris water maze. Neonatal rats (male only) were separated into five groups: control, control + 5-aza-deoxycitidine (5-AZA), sevoflurane, sevoflurane+dimethyl sulphoxide (DMSO, vehicle), and sevoflurane+5-AZA. Experimental rats were exposed to 3% sevoflurane for two hours on three consecutive days and then, euthanized serially at 1 hour, 6 hours, 24 hours, and 30 days after the last sevoflurane exposure. The hippocampus was then dissected, DNA isolated, and the DNA methylation status of the synaptic plasticity genes, Brain-derived neurotrophic factor (BDNF) and Reelin genes, assessed. There was a decreased expression of both BDNF and Reelin due to DNA hypermethylation in the Sevoflurane and Sevoflurane + vehicle groups, which manifested as a decreased number of dendritic spines in the pyramidal neurons of the hippocampus – an indication of decreased synaptic plasticity. Also,

found was increased DNA methyltransferases (DNMTs), specifically DNMT 3a and DNMT 3b, and decreased methyl CpG binding protein 2 (MeCP2). This further coincided with decreased cognition on behavioral exams. In those rats, which were injected with 5–aza-2-deoxycytidine (5-AZA), a DNA methyltransferase inhibitor, and in the control group the above changes/manifestations were not seen after sevoflurane exposure and subsequent DNA analysis (Ju et al., 2016).

Isoflurane is another inhaled anesthetic used for GA induction. Ji et al. (2014) studied the effect of isoflurane on rats' cognition and histone acetylation and found both decreased with repeated isoflurane exposure. Also, the hippocampal BDNF-tyrosine kinase receptor B (TrkB) pathway needed for memory consolidation, CREB, CaMKII as well as, downstream signaling, phospho-calmodulin-dependent protein kinase and phospho-cAMP were diminished. Sodium butyrate (NaB), when intraperitoneally injected into study rats, inhibited histone deacetylase (HDAC) and increased neuronal histone acetylation, actively preventing cognitive decline. Repeated exposures to Isoflurane further led to increased hippocampal inflammation, as evidenced by increased levels of interleukin 1*B* (IL-1*B*) and interleukin 6 (IL-6), to cellular apoptosis (programmed cell death), and to diminished cognition on behavioral tests. Again, NaB ameliorated these effects (Ji et al., 2014) and potentially may serve an important role in future anesthesia practice.

MicroRNA-21-Mediated Cardioprotective Effect of Isoflurane Gas

Qiao et al. (2015) write about the unknown mechanism by which isoflurane induces cardioprotection in mice. Isoflurane is another inhaled anesthetic used for GA induction. Microribonucleases (RNAs) are single stranded, non-coding, endogenous molecules

approximately 22 nucleotides long and key regulators of gene expression. Micro RNA-21 is inherent to cardiomyocytes, the vascular endothelium, vascular smooth muscle cells, and cardiac fibroblasts (Qiao et a., 2015).

Qiao and colleagues (2015) explain that evidence points towards the role of micro RNA-21 in cardiac cell and vascular smooth muscle cell proliferation and apoptosis, as well as, in the functionality of cardiac fibroblasts. Pathophysiological events like myocardial infarction (MI) and congestive heart failure are being investigated with linkages to micro RNA-21 (Qiao et al., 2015).

Qiao et al. (2015) found that treatment with isoflurane protected mice from ischemia/reperfusion injury by a microRNA-dependent mechanism and that the protein kinase B/nitric oxide/mitochondrial permeability transition pore (Akt/NOS/mPTP) pathway is in involved in this phenomenon. Isoflurane is cardioprotective because it upregulates micro RNA-21 expression, downregulates the expression of RHOA (micro RNA-21 target), and lessens MI size and improves recovery of cardiac cells after ischemia/reperfusion by delaying mPTP opening, slowing cell death, and increasing phosphorylation of Akt and NOS in the ischemic/reperfused myocardium (Qioa at al., 2015).

Isoflurane-Induced Decreases in Histone Acetylation and Cognition

Ji et al. (2014) studied the effect of isoflurane on aged rat's cognition and histone acetylation and found both decreased with repeated isoflurane exposure. Also, the hippocampal BDNF-tyrosine kinase receptor B (TrkB) pathway needed for memory consolidation, CREB, CaMKII as well as, downstream signaling, phospho-calmodulin-dependent protein kinase and phospho-cAMP were diminished. Interestingly, sodium butyrate (NaB), when intraperitoneally

injected into study rats, inhibited histone deacetylase (HDAC) and increased neuronal histone acetylation, actively preventing cognitive decline. Repeated exposures to Isoflurane further led to increased hippocampal inflammation, as evidenced by increased levels of interleukin 1*B* (IL-1*B*) and interleukin 6 (IL-6), to cellular apoptosis (programmed cell death), and to diminished cognition on behavioral tests. Again, NaB ameliorated these effects (Ji et al., 2014) and potentially may serve an important role in future anesthesia practice.

Carcinogenicity of Anesthetics

It has been proposed that pharmaceutical agents have carcinogenic effects both by genotoxic and epigenetic mechanisms. Williams, Mazue, McQueen, and Shimada (1980) found that hydralazine, a vasodilating antihypertensive agent, had direct genotoxic (damage to DNA structure itself) effects in rabbit hepatocytes in those rabbits, which were slow acetylators. Hydralazine is oftentimes used intraoperatively to control increased blood pressure. Humans, like rabbits, are polymorphic in their activity of N-acetyltransferase (NAT), a key enzyme in the metabolism of hydralazine (Williams, Mazue, McQueen, & Shimada, 1980). Unknown at the time was that an epigenetic mechanism also was at work. Hydralazine inhibits DNA methylation with the potential of triggering a lupus-like autoimmune response. This reaction may be related to the body's global genomic hypomethylation yielding an under-expression of proteins, and hence, recognition of a non-self/foreign body state (Csoka & Szyf, 2008).

Csoka and Szyf (2008) write that no longer in use as an anesthetic, due to its now known teratogenic effects, is thalidomide, a sedative-hypnotic. Yet, it remains in use as a chemotherapeutic agent. Historically, fetuses exposed to thalidomide from the 21st through the 40th days of gestation often died of bowel atresia, kidney or heart malformations. These thalidomide-exposed fetuses, once born, often had truncated upper limbs and some of those who

bore offspring also had offspring with shortened limbs. The fact that it is a heritable trait may point toward epigenetic mechanisms at work such as altered DNA methylation or an alteration of sequence-specific DNA binding proteins (Csoka & Szyf, 2008).

Biel, Wascholowski, and Giannis (2005) describe the potential of HDAC inhibitors in the treatment of cancer. Although histone acetylation globally regulates gene expression, HDACs affect a smaller subset of genes associated with cell growth control. HDAC inhibitors are proving effective in stopping leukemia and solid tumor growth (Biel, Wascholowski, & Giannis, 2005).

Malignant Hyperthermia (MH)

Robinson et al. (2009) analyzed human parent-offspring groups, where the father or mother was deemed malignant hyperthermia susceptible (MHS) or malignant hyperthermia equivocal (MHE) based upon muscle biopsy exposure to halothane and caffeine. MHS individuals responded to both agents. MHE individuals only responded to one agent. Either group was considered high risk for developing MH. Found consistently across groups was the presence of ryanodine receptor protein (RYR1) mutations. RYR1 encodes for the calcium release channel in skeletal muscle's sarcoplasmic reticulum and known mutations are associated with clinical MH presentation. To explain this, one epigenetic mechanism, allele silencing, was researched. This mechanism recognizes that an allele from one parent can be turned off, or silenced, and as a result the genome of the offspring is functionally imprinted with only one parent's DNA. They found that children of MHS/MHE fathers were less affected than of MHS/MHE mothers with a total of 2113 transmissions of the RYR1 mutated gene and seen in 49% of the total study sample. Daughters were also less likely to inherit the gene than were sons. It was deemed that monoallelic silencing was a relatively rare epigenetic factor in transmission of

MH susceptibility, but could be prominent with core myopathy phenotypes (Robinson et al., 2009).

Klingler et al. (2014) also investigated the mutated RYR1 receptor type in MH. They identified and studied the classical MH triggering agents such as succinylcholine and volatile anesthetics. In looking at 200 confirmed MH cases, 81% occurred where both a volatile agent and succinylcholine were used, 1% where only succinylcholine was used, and the remaining 18% where only a volatile anesthetic was used. The most often seen cases were in young males with a RYR1 mutation. Only 103 subjects carried RYR1 variants/mutations, leaving room for future pathophysiological study including looking at potential epigenetic factors (Klingler et al., 2014).

H4 Histone Acetylation and Pain

Lessans and Dorsey (2013) describe chromatin remodeling and modification as the key ways that genes express themselves. If this process takes on a pathological shape, then the person will present with an unfavorable health state, such as pain. Identifying pathological shapes and mechanisms may lead to exciting breakthroughs in how pain is treated. Another epigenetic mechanism is methylation of histones, the chief positively-charged, compacting proteins within the cell's nucleus, to inhibit gene expression or histone acetylation, yielding gene transcription. Histones linked to negatively charged proteins along the DNA backbone form chromatin. The authors note that pain is the most frequent cause for seeking out health care provision and over 350 genes are currently relevant in clinical and experimental pain with countless more involved in analgesia regulation. Yet, the genes themselves are not the only answer in how people experience pain. Transcriptional and translational epigenomic mechanisms guide how one progresses through the pain continuum (Lessans & Dorsey, 2013).

As mentioned, one of anesthesia's goals is to provide analgesia. Known pathology involved during the transition from acute to chronic pain can be supplemented by the study of epigenetic mechanisms involved in the nervous system's plasticity. Moving from the periphery to the brain's cortex, nerve or tissue damage leads to pathologic connections developing, leaving some people with chronic pain and dysfunction. Furthermore, life's environmental factors may serve as epigenetic primers for how individuals experience pain and analgesia (Lessans & Dorsey, 2013). In the meantime, some consensus exists on neurogenic pain's development being related to NMDAR - mediated plasticity. So, agents like Ketamine, already proven beneficial in treating acute pain, may be helpful in the prevention of chronic pain (Javitt et al., 2011).

Lirk et al. (2015) further describe how pain is tied to epigenetic mechanisms. Both opiates and local anesthetics are fundamental parts of perioperative pain management. Opiates seemingly lead to global DNA methylation whereas local anesthetics (LAs), as a class, lead to global DNA demethylation. Lirk et al. (2015) explain the work of Hwang et al., who showed that the expression of the mu opioid receptor is mediated by DNA methylation. In P19 embryonic cells, proximal promoter regions for the mu receptor were hypermethylated and gene silencing ensued. But, when exposed to retinoic acid, the mu receptor gene is expressed stably in mature astrocyte/neuronal cells and coincidentally with decreased MeCP2 binding in the promoter region. When methylated DNA is bound by MeCP2, there is histone deacetylation, histone compacting, and decreased transcription of the mu receptor (Lirk et al., 2007) and hence, without appropriate MeCP2 binding there is an overexpression of the mu receptor.

Lirk et al. (2007) further describe that among the negative effects of opiates are nausea, vomiting, respiratory depression, slowed bowel peristalsis, and opioid-induced hyperalgesia (OIH), an excessive sensitivity to pain. Development of OIH varies with the type of opioid used.

OIH seems the worst with Remifentanil and least with buprenorphine, a partial mu receptor agonist and K receptor agonist, and is prevented by Ketamine, as described earlier, a NMDA receptor antagonist. In an article by Liang, Li, and Clark (2013) inhibiting histone acetyltransferase when morphine, the model opiate, was present in mice led to less signs of OIH, whereas inhibiting histone deacetylase worsened OIH (Liang, Li, & Clark, 2013).

Lirk and colleagues (2015) explain that systemic absorption of LAs after local injection, such as lidocaine and ropivicaine, and the direct intravenous injection of them have been under investigation for both analgesic and anti-inflammatory effects. Local anesthetics demethylate by at least one identified mechanism, inhibition of DNA methyltransferase. It stands to reason that preventing methylation-induced hyperalgesia, as described above, may be a potential target for the demethylating effects of LAs. Beyond this, the perioperative period is associated with increased inflammatory mediator release, which is regulated by histone methylation. Here, the epigenetic demethylating property of LAs may potentially serve to prevent/treat surgical inflammation (Lirk, et al, 2015).

Alvarado et al., (2015) hypothesize that changes to promoters and enhancers of genes via DNA methylation lead to alterations in gene expression resulting in brain structure and function because of pain. Synaptotagmin 2 is a membrane-trafficking protein and a regulator of synaptic function. Increased synaptotagmin is present in synaptogenesis reflecting synaptic density and plasticity (Alvarado et al., 2015). Virok et al. (2011) showed that increased chronic restraint stress positively correlated with Synaptotagmin 2 expression in the PFC of rats (Virok et al., 2011). This supports the hypothesis of Alvarado and colleagues (2015), that chronic anxiety/stress, whatever its source, may result in profound changes in synaptic structure +/- function. The rats, which were subjected to peripheral nerve injury had hypomethylated CpG (5'-

Cytosine-phosphate-Guanine-3') promoter sites for Synaptotagmin 2 expression in their PFC, which coincided with signs of chronic pain 6 months post injury as compared to control rats, which had neither (Alvarado et al., 2015).

Sahbaie, Liang, Shi, Sun, and Clark (2016) used mice to test how chronic and escalating morphine doses would lead to OIH and long-standing epigenetic changes after a surgical insult to one hindpaw of each mouse. The mice that received continuous opioids were more tolerant to the analgesic effects of morphine and also experienced greater pain sensitization than the control group. Two proteins, BDNF and Prodynorphin (Pdyn), both present in the spinal cords of test mice were upregulated via acetylation of the promoter region of the opioid exposed mice. When anacardic acid, an acetyltransferase inhibitor, was co-administered with morphine, these mice had less OIH and attenuated enhanced hyperalgesia. To explore other ways to also diminish hyperalgesia, the researchers also trialed the effects of a selective tropomyosin-related kinase B (ANA-12) and K-opioid receptor antagonist (nor-binaltorphimine). Both were administered intrathecally and found to limit hyperalgesia one or three days after surgery (Sahbaie, Liang, Shi, Sun, & Clark, 2016).

In summary, there are linkages between epigenetics and anesthesia that have significant implications for practice as seen in Table 2. Anesthesia providers must be educated on epigenetics in order to optimize patient outcomes related to anesthesia care.

Instructional Development Framework

According to Clark (2011), ADDIE is an acronym for **a**nalysis, **d**esign, **d**evelopment, **i**mplementation and **e**valuation and a model of instructional design (ID) used in many realms (Clark, 2011). Molenda (2015) corroborates with Clark and describes ADDIE as an umbrella term used in ID and instructional systems development (ISD). The term "ADDIE" is used

colloquially to mean the iterative and sequential process that drives ID/ISD. The ADDIE model is customizable to specific needs of the ID team (Molenda, 2015). The ADDIE model served as the instructional development framework for the "Teaching Links between Epigenetics and Anesthesia to the Anesthesia Provider" 1-hour teaching/learning seminar.

Clark (2011) in his video series lists the following segments and subsegments of the ADDIE model succinctly described below:

- **A**nalysis consists of four phases:

1. Development of instructional goals – to engage the audience in a poorly understood, but increasingly important topic in anesthesia practice
2. Instructional analysis – consideration of best way to divulge information deemed to be live-learning format
3. Learner analysis – students are all engaged in the practice of anesthesia delivery and experienced healthcare providers
4. Developing learning objectives – based upon key concepts gathered from integrative literature review

- **D**esign consisted of three phases:

1. Design assessments – Were accurate to what was being taught and what was being gauged
2. Chosen a course format – Power Point, live-teaching/learning
3. Created an instructional strategy to help students learn course content – Considered content presentation, learner participation/discussion, assessment and follow through activities including post-test and statistical analysis. Pre-

instructional materials included an introductory letter explaining the core concepts of the seminar offering and volunteerism in the research study. Plan was to present content concisely and adhere closely to learning objectives.

- **D**evelopment consisted of three phases:

  1. Created a sample instruction – Presented five rudimentary versions of this program's content to different academic audiences at DePaul University
  2. Developed the course materials – Power Point slides
  3. Conducted a run-through – Practiced teaching session to confirm succinctness, timeliness, and appropriateness

- **I**mplementation had three phases:

  1. Trained the instructor – this was myself and intimate knowledge of topic allowed me to present in an expert manner
  2. Prepared the learners – sent letter to all students about intent of research and voluntary participation, explain meeting time, and expectations for attendance
  3. Arranged the learning space - tested AV equipment, printed out slides

- **E**valuation has two phases:

  1. Formative evaluation (focused on clarity, impact and feasibility) – Used three-member DNP project committee to ensure the project's growth and development. Conducted field trial in the classroom of St. Alexius Medical Center alone. Was already familiar with the environment of NorthShore Evanston Hospital's Frank Auditorium from past teaching experiences there
  2. Summative evaluation (established the worth of instruction) – Used the posttest

with both multiple choice questions and a Likert score acceptability scale

Methods

Study Design

This study utilized a posttest only study design.

Sample and Settings

There were two unique environments in which this teaching and learning seminar took place. Firstly, this seminar was taught on the 4th floor of St. Alexius Medical Center in Hoffman Estates, IL in an educational classroom with onsite audiovisual equipment and a podium, where the presenter/primary researcher stood. The room was well-lit, temperature controlled, and had tables and chairs.

Secondly, the second seminar offering took place in the Frank Auditorium located on the second floor of NorthShore University Health System's Evanston Hospital in Evanston, IL. The environment also had AV equipment, appropriate lighting, and was temperature controlled. Each attendant had an individual seat and desktop at which to sit. The presenter was at the front of the classroom, immediately in front of and at the base of a stage with learners seated in a rising stepwise fashion above the presenter allowing unobstructed views for all parties.

Target Population

As a group, the target population was "anesthesia providers", which further subdivides into five primary sets of people, CRNAs, SRNAs, Anesthesiologists, Anesthesiology Residents, and Anesthesiology Assistants. At the St. Alexius presentation, seminar attendees were a combination of Certified Registered Nurse Anesthetists (CRNAs) and Anesthesiologists (Medical Doctors or Doctors of Osteopathy). At the Evanston Hospital offering, there was a mixture of

CRNAs and SRNAs. These individuals were the ones to whom the seminar's content was tailored.

Protocol Description

The teaching/learning seminar delivered was intended to augment the knowledge base of the anesthesia provider. Through informal conversations with anesthesia provider colleagues, it was noted by myself, that the topic of epigenetics is poorly understood. This lent credence to what was found in the literature review conducted, that the topic of epigenetics in anesthesiology (Anesthetic Epigenetics) is not being taught (or at least not being explored deeply in formal anesthesia training).

This educational teaching project was a one-hour seminar session and the study design was quasi-experimental in nature as there was no randomization of study subjects (Trochim, 2006). Teaching took approximately 40 minutes, which allowed the remaining time to complete the posttest. Content was provided in a live, PowerPoint and consisted of 45-50 slides (see Appendix D). Included in the presentation was the program's objectives, the background data required to understand genomics and epigenomics, the theoretical framework of Anesthetic Epigenetics, the explanations of each of the links between epigenetics and anesthesia identified in the literature, a description of the summative analysis, and future recommendations for research and practice.

Those who attended volunteered to take part in the educational seminar and to be part of the study. Sampling was hence convenience in nature. There were potentially 13 Anesthesiologists and 26 CRNAs at St. Alexius offering of which there were actually 5 from each group that took part in the study. The teaching seminar took place in the morning, prior to the

day's surgical cases, and served as that month's educational meeting. At NorthShore, the presentation took place immediately following a Saturday Seminar Club, where SRNAs and CRNAs came to earn continuing education credits from the American Association of Nurse Anesthetists (IANA). There were approximately 60 students and seven primary faculty members at NorthShore. Second and third year SRNAs were required to come (approximately 40 students). CRNA attendance at Seminar Club is variable ranging between 1 and 15 CRNAs. There were actually 26 SRNAs and 5 CRNAs that participated at NorthShore. The Links between Epigenetics and Anesthesia presentation was not approved for CE credit, but was DePaul University IRB approved as a research protocol.

Validity and Reliability Procedures for Posttest Knowledge and Acceptability Questionnaires

All items on posttest were analyzed by the three-member, expert, DNP research committee, Drs. Tariman, Simonovich, and Drantz for validity and reliability. The items included in the acceptability questionnaire were modified from the Acceptability e-scale and were reviewed by Dr. Tariman, one of the developers of the original version, for applicability and appropriateness to this research study. There was a high level of inter-rater reliability between the committee members and each agreed that the posttest was a strong tool.

Phelan and Wren (2006) describe face-validity, where questions appear to measure what they are supposed to measure; construct validity, where questions actually measure what they are supposed to be measuring; and formative validity, where questions are used to gather information to guide program improvement (Phelan & Wren, 2006). The program's objectives, content, and

evaluation were aligned throughout its' development, which aided to maintain overall high quality and validity.

Data Collection Procedures

Paper questionnaires were administered and collected in real-time. Immediately following the completion of the PowerPoint presentation, participants were handed one questionnaire each and asked to complete it fully before leaving the classroom. In addition, consent forms, were also handed out with the posttest. Upon completion, questionnaires were returned directly and students left the classroom.

Evaluation Plan

Evaluation of learning and program acceptability was via a posttest only (see Appendix A). The one-hour time limit to complete instruction and testing was the primary reason that there was not also a pretest, which would strengthen the overall findings and internal validity. The posttest survey design aligned the 10 program objectives with 20 corresponding multiple-choice questions. Also included in the posttest, was a brief demographic questionnaire (see Appendix C) and six additional Likert score questions assessing the program's acceptability based upon the *Acceptability E-scale* developed by Tariman, Berry, Halpenny, Wolpin, and Schepp (2011) as seen in Appendix B, but modified to meet the context of my study.

Data Analysis

The data gathered from the posttest was analyzed using the International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) version 22 (IBM, 2017). The

twenty multiple-choice questions provided interval data. Each question had only one correct answer and hence, the test taker earned either a correct or an incorrect on these questions.

The data gathered from the 20-item posttest was interval in nature, a type of quantitative variable. Trochim (2006) explains that interval-type data allows the application of descriptive statistics, where findings are represented in terms of central tendency, mean (average score), median (center most score), mode (most frequently occurring score). Mean, median, and mode are further characterized by dispersion, the spread of values around central tendency, through range, the highest value minus the lowest value and through standard deviation, which shows the relation that individual scores have to the sample mean (Trochim, 2006).

Trochim (2006) further explains that when two groups are available to compare to one another, interval-type data also is amenable to inferential statistical analysis. By using the *t* test for differences between groups, an assessment of whether the means of two groups are statistically different (alpha level of 0.05 or less) from one another was planned. Furthermore, yielding the mathematical equivalent of the *t* test, a one-way Analysis of Variance (ANOVA) was considered as another option for analysis to assess statistically significant differences in three groupings of sociodemographic variables. Both *t* test and ANOVA are applicable in the posttest only research design (Trochim, 2006).

Next, the demographic data gathered was nominal and ordinal in nature and the six Likert questions regarding acceptability yielded ordinal data. Trochim teaches that in nominal measurement the numerical values simply name the attribute distinctively (i.e. CRNA = 1, MD/DO = 2, SRNA = 3) and there is no ordering or greater value implied. Ordinal measurement allows rank ordering of attributes, but there is no meaning inherent in the distances between

attributes (i.e. Years of clinical nursing experience: 1-5 = 1, 6-10 = 2, 11-15 = 3). Pearson's Chi square was considered to analyze any association between sociodemographic categorical variables and categorical variables from post-test knowledge scores using quartiles.

Completed posttests were compiled and stored in a locked space. Paper forms were kept in a safe when not used and stayed in one primary location. All tests were in the personal possession of the primary researcher for continued analysis. No names were on the tests so as to maintain confidentiality. The posttest results were not discussed with study participants. Computer data was password protected, backed up to a removable, compact, hard drive, and also locked in a safe.

Ethics and Human Subjects Protection

Educational and human subjects protection training was completed online via the Collaborative Institutional Training Initiative (CITI Program) on October 26, 2016. Strict adherence to the CITI program principles was followed for the duration of the Links between Epigenetics and Anesthesia research protocol. DePaul University's IRB and my research committee oversaw the maintenance of these standards of research.

Results

Study Participants

Forty-one participants participated in the epigenetic seminar and completed the post-test examination and acceptability questionnaire. Of these, 100% completed all the questions. As seen in Table 3, most of the participants were SRNAs and CRNAs (n=36, 87.8%) and only a few anesthesiologists (n = 5, 12.2%) participated in this study. A majority of participants were White (n = 32, 78%) and work in Urban, Academic, Teaching institutions (n = 31, 75.6%). There was a

greater number of female participants (n = 28, 68.3%) than male (n = 13, 31.7%) and most have 1-5 years of clinical experience (n= 27, 65.9%).

Epigenetics Post-test Exam Scores

The Epigenetics Exam has a good reliability score with a Kuder-Richardson value of .41, indicating adequate discriminatory property to identify those participants who learned from the epigenetics seminar. The mean scores for the epigenetics post-test examination was *M* = 13.05 with *SD* of 2.37 as seen on Table 4. Only seven (17%) out of 41 participants scored at least 80% or higher in the test, but at least twenty five (60.9%) achieved the overall mean score of 13 or higher. Sixteen participants (39%) had a score lower than the overall mean score of 13.05 and four (9.8%) participants scored less than half of the highest possible score of 20 as shown in Table 5. Among the 20 questions in the Epigenetics Exam (Appendix B), Question #7 (key concepts in the study of genomics versus the study of epigenomics), Question #9 (epigenetic mechanism of opioid induced analgesia), Question #10 (epigenetic process of acute and chronic pain sensation), Question #14 (epigenetic mechanism in which ketamine improves depression), and Question #15 (epigenetic mechanism clinical of malignant hyperthermia presentation and Ryanodine Receptor Protein (RYR1) had the most incorrect answers selected by more than half of study participants (78%, 82.9%, 78%, 56.1%, 58.5%, respectively) and therefore had the lowest sum scores among all the 20-items Epigenetics Exam as highlighted in Table 6.

Acceptability of the Epigenetics Seminar

Overall, the Epigenetics Seminar has been rated by the study participants in terms of six items in the Acceptability Scale as neutral to acceptable with a total sum score 837. The threshold for the overall neutral score is 738. Individually, Question #1 asked the participants on how easy is the epigenetics seminar to understand. This question had the lowest sum score of 103, which is

below the sum score threshold of 123 for a neutral response. The neutral point of the scale has a 3 point weight, and with 41 participants, a sum score of 123 (3x41) must be achieved to conclude that the participants rated the individual question of the Acceptability Scale at the neutral response point. Questions #2, #3, #4, #5, and #6 had sum scores of 122, 148, 157, 147 and 150, respectively as shown in Table 7.

Association of Sociodemographic Variables with Epigenetics Exam Mean Scores

Five sociodemographic variables such as gender (male or female), age groups (20-29 y.o., 30-39 y.o., or 40-49 y.o.), employment status (full time or unemployed), role (CRNA, SRNA, or anesthesiologist) and educational level (Bachelor, Master or Doctorate) were examined for statistically significant differences in the Epigenetic Exam mean scores. Inferential *t* test or one-way ANOVA test statistics showed *p* value greater than the 0.05 significance level as shown in Table 8; therefore, the null hypothesis has been retained and we can conclude that there is no difference in the mean scores between and among groups based on gender, age, employment status, role or educational level.

Discussion

Overall, the primary researcher expected and received similar results from this study. Participants did learn from the seminar and for the most part had a neutral to acceptable rating of the overall epigenetics educational program. At the outset, in designing this study, it was thought that perhaps different demographic factors would impact overall posttest performance. This proved to be false. The results of ANOVA showed *p* values >.05. Three categories based on age, role/position, and education were demographic factors that were found to be not statistically significant, which meant the null hypothesis that there is no difference in the mean scores among these three groups had to be retained. Similarly, the independent samples *t* test *p* values of >.05

deemed Employment Status and Gender to have no statistically significant association with the mean scores in the epigenetics exam. Race, Years of Experience, and Practice Setting could not be statistically evaluated due to extreme unequal sample sizes.

The study's theoretical framework interconnected multiple factors to epigenetics. These related items included genetics, environment, anesthesia, Ketamine, learning and memory, depression, glutamate, N-methyl D aspartate, histone methylation, DNA methylation, reprogramming of infant brain after general anesthesia, malignant hyperthermia (MH), carcinogenicity of anesthetics, pain, and single nucleotide polymorphism (SNP) of methylenetetrahydrofolate reductase (MTHFR) 677T with homocysteine elevation and nitrous oxide-related myelopathy. These concepts appear to be "not easy to understand" according to the study participants.

It was the intent of this study to educate anesthesia providers on the above interconnections. Each of the 20 knowledge-based questions was aligned with learning objectives and the material was covered during the PowerPoint lecture. Three questions were particularly difficult on the knowledge portion of the posttest. Questions 7, 9, and 10 had the lowest mean scores; .22, .19, and .22, respectively. For question 7, only 9 out of 41 (21.9% of participants) participants answered it correctly; for question 9, only 7 out 41 (17% of participants) answered it correctly; and for question 10, only 9 out of 41 (21.9% of participants) answered it correctly. The low scores could be interpreted in two ways: first, these questions truly have the discriminatory power to sort out participants who actually attended, understood, and learned from the lecture and therefore, would likely have answered the questions correctly. On the other hand, these questions could also be interpreted as being extremely difficult, complex or confusing to the participants and further refinement and re-testing is required to test its reliability. On the knowledge portion of

the posttest, participants earned a mean score of $M = 13.05$ with a $SD = 2.736$. Considering the novelty of the presented material, this is considered a successful reflection of learning.

Marriott (2017) described a survey of middle school teachers and students and an intervention including a computer game to increase knowledge about epigenetics. Educational goals for students was to be able to recognize the term "epigenetics," to understand that epigenetics mechanisms are able to modify health outcomes, and to understand the epigenetic scientific process. After completing the computer game, (n=1000) self-reported that they were approximately three times more able to explain epigenetics to someone else, 55.8% enjoyed the game, and 73.6% said the game made them think more about how their choices about diet, stress, health, and air quality affected their health. However, only 9.1% of teachers could explain epigenetics to someone else "well" or "completely". Moore interestingly, only 14.6% of middle school teachers and students have heard of epigenetics before the epigenetic educational program was delivered (Marriott, 2017).

The Acceptability e-Scale by Tariman et al. (2011) has been consistently shown to be a valid and reliable measure of the concept of "acceptability" (Tariman et al., 2011) and in this current study, its reliability has proven to be adequate. The results showed an overarching neutral to acceptable ratings for the epigenetic educational program offered. Although, the target population for this study was different than the one described by Marriott, both studies showed increases in knowledge after the epigenetics educational offering, as well as, moderate satisfaction with it.

Kirk et al. (2011) in a 10 country, small-scale international study aimed to develop a framework that could inform strategic planning relating to international nursing genetics-genomics practice and education. They found no country had thoroughly entwined genetics-

genomics competence into education, registration standards, or licensure. Significant differences existed where some countries had defined genetics counseling roles for nurses, public and educational initiatives for genetics curricula in nursing, standards for certification, and governmental influence, but others had parts of or none of the above. It was deemed that definitive leadership from genetic specialists and senior nursing professionals in government and regulatory bodies would need to promote the role of nurses in genomic healthcare and the expansion of genomics into the teaching programs (Kirk, et al., 2011).

Five significant barriers were identified by Kirk et al. (2011) limiting the full integration of genetics-genomics into nursing education. These were: "(a) deficits in awareness and knowledge among educators and practitioners (including those at senior levels) result in a lack of professional engagement in genetics-genomics; (b) lack of awareness at government and regulatory body levels; (c) limitations in resources included time, funding, availability of appropriate education resources, and capacity to deliver genetics-genomics education; (d) lack of attention paid to the 'patient voice,' and (e) lack of outcome evidence, compounded by the limited integration of genetics-genomics into practice" (Kirk, et al. 2011).

Similarly, the barriers identified by Kirk et al. (2011) also applied to epigenetics-epigenomic research and education as it related to the anesthesia community. Every anesthesia provider with whom the topic of epigenetics was breached did not know what the term meant or how it applied to anesthesia practice. No evidence was found in the ILR of either governmental or regulatory body involvement in epigenetic discussion, of any definitive steps taken to incorporate epigenetics into anesthesia curricula, of how epigenetics could be understood from a patient's perspective, and of few advances into how epigenetics applies to anesthesia practice. In

this way, we see that however far genomics in nursing has yet to travel, epigenomics has ever further to go in the anesthesia.

This study has found value and merit in educating anesthesia providers on a novel topic, epigenetics in anesthesiology, or as termed by the primary researcher, "Anesthetic Epigenetics." Those who took part in this study gained knowledge from the PowerPoint presentation. Most also felt the program was acceptable. No demographic variables seemingly affected the mean scores of study participants, but with expansion of this study to wider audiences of anesthesia providers perhaps this could change. These findings yield a definitive foundation for future research.

Strengths and Weaknesses

In reviewing this study, both strengths and weaknesses need to be discussed. One of the weaknesses is that the findings are limited in generalizability due to the small sample size, n=41 in this study. Although the sample is comprised of three types of anesthesia providers, future studies should also include Anesthesiology Resident Physicians and Anesthesia Assistants. There are over 50,000 members in the American Association of Nurse Anesthetists (AANA) (2016). This membership includes about 90% of the U.S.'s CRNAs and also has SRNA members. Male CRNAs account for over 40% of this total (AANA, 2016). According to Statista.com (2018), which compiles data from multiple sources, there are approximately 47,000 anesthesiologists in the U.S. (Statista.com, 2018). Hence, more demographically diverse and larger future study groups, ideally of hundreds or thousands, would allow better generalizability of the study findings.

Striving to expand scientific inquiry, this study is novel and pioneering in nature, which are both strength and weakness. The articles by Kirk et al (2011) and Marriott (2017) were somewhat related to this study and did yield interesting insights. But, ultimately, "Teaching Links between Epigenetics and Anesthesia to Anesthesia Providers" had no close equivalent to compare against and therefore, must be judged on its own merit. More research is required to build a larger body of knowledge, where this study could potentially be used as a model or for contrast by others in the future.

There was a 1 hour limit to complete instruction, to pass out consents and posttests, and for students to take posttests and return them to the primary researcher. Convenience sampling was used. The posttest-only, non-experimental design was best suited to this study, but it cannot gauge if a study participant had prior knowledge of a learning objective and its related test question. Which could mean that a participant's posttest does not actually reflect gained knowledge from the links between epigenetics and anesthesia PowerPoint presentation. Conversely, a limitation of using a pretest/posttest design is the potential introduction of "priming," a type of bias, where an external stimulus (such as a pretest) passively and subtly influences how one mentally interprets future exposures (Shanks et al., 2014). Future studies should consider alternate timelines, sampling types, randomization, blinding, and designs.

Implications for Practice

It is difficult to explain how this study will impact practice concretely. What it has already done and will continue to do is open the minds and discussions of anesthesia providers to considering Anesthetic Epigenetics in their practice. Through this emerging science, CRNAs, Anesthesiologists, and others will be able to consider the implications of how general anesthesia affects learning and memory, how surgical stress impacts the developing brain, how ketamine affects both pain and depression, how the use of local anesthetics can play a larger role in acute management and the prevention of chronic pain, how carcinogenicity and anesthetic drugs intertwine, and how malignant hyperthermia prevention could materialize through epigenetic research.

Acting as the interface between what is inside and that which is outside, epigenetic mechanisms mediate what will come. The learning we do, the memories we hold, the behavior we show, the tumors we grow, and the depression we feel are all linked to epigenetic controls. Perhaps by using methyltransferase inhibitors or by administering local anesthetics instead of general anesthetics, the anesthesia provider can bring about better patient outcomes. Studying how to offset negative epigenetic anesthetic effects and to highlight positive ones can someday bring more customized anesthetics to patients.

Conclusion

It became clear in researching how epigenetics is tied to anesthesia, that this is a new and exciting topic of study. Although a paucity of applicable research somewhat limited the literature review's scope, the ILR did identify multiple linkages between epigenetics and anesthesia, which proved to be its' greatest finding. Examples of these links are that environmental factors, like

stress and pain, lead to methylation of DNA and the subsequent transcription of proteins for inflammation and that inhaled anesthetics seem to negatively affect postoperative cognition through epigenetic influence. This ILR served as the basis for educating anesthesia providers including Anesthesiologists, Certified Registered Nurse Anesthetists, and Student Registered Nurse Anesthetists on the connections between epigenetics and anesthesia.

The 41 anesthesia provider participants in this study learned to name epigenetic mechanisms and to describe their characteristics, to identify links between epigenetics and anesthesia, to explain fundamental differences between genetics and epigenetics, to identify epigenetic mechanisms investigated in pain research, to understand how anesthetic agents affect neurocognitive dysfunction, to explain how epigenetics, anesthetics, and depression are linked, to identify epigenomic mechanisms tied to malignant hyperthermia, to discuss positive and negative epigenomic effects of general anesthesia, and to name drugs used in anesthesia practice with carcinogenic effects. They additionally explored how epigenetics and anesthesia related to stress and to the environment. Study participants were further able to gauge the learning program's acceptability, the understandability of the lecture and questions, the enjoyability of the attending program, the helpfulness in increasing topical knowledge, the time frame appropriateness, and their overall satisfaction.

In sum, this research has expanded the knowledge of anesthesia providers to an emerging topic, epigenetics in anesthesiology that has the potential to change anesthesia theory, research, and practice.

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Table 1. Computerized Databases Search Results		
Number	**MEDLINE/PubMed**	**WorldCat**
Total	27	121
Applicable	17	16

Table 2. Evidence-based Table on Studies Related to Epigenetics in Anesthesiology

Source	Study Objectives	Methods (Design, Sample Size, Setting	Study Variables	Instruments	Statistics Used	Results/ Findings	Conclusion
Alvarado et al. (2015)	To test the hypothesis: Alterations in DNA methylation in the prefrontal cortex (PFC) following nerve injury mediate the long-term genomic impact of pain as a fundamental mechanism contributing to the chronicity of pain. This mechanism raises the potential of dna methylation-modulating therapy for reversing chronic pain and also, that pain-related changes in methylation within the PFC are dynamic	Experimental, rat (N=6) study, peripheral nerve injury with an epigenetic analysis 6 months post injury	Prefrontal cortex (PFC), synaptotagmin, DNA methylation, nerve injury	Pyrosequencing analysis of methylation state of CpG binding sites distal to synaptotagmin transcriptional start site.	Standard error of the mean (SEM) p=< 0.05, 0.005, and 0.001	Increased synaptoptagmin levels seen in PFC of rats exposed to nerve injury	Study supports theory that Chronic strain/stress can lead to profound changes in synaptic structure and function
Chestnut et al. (2011)	To test hypothesis that DNA methylation via DNA methyltransferase (Dnmt) can lead to gene silencing and neuronal cell death	Empirical, use of mouse model to create Dnmt driven neuronal cell death, control groups	DNA methylation, Dnmt influenced apoptosis and linkage to human amyotrophic lateral sclerosis (ALS)	Biochemical assay for total DNMT activity in mice and human cadaver ALS brain/spinal cord	Means/variances, one way ANOVA, Newman-Keuls posthoc tests, p values of 0.05 or less	Neuronal apoptosis is present under Dnmt influence, Dnmt inhibition protects against apoptosis of motor neurons, Dnmts and methylated 5-Cytosine (5-mC) are upregulated in human sporadic ALS motor cortex	Neurodegeneration seen when Dnmt3a overexpressed, DNA methylation increases in mouse motor neurons during apoptosis
Chidambaran, Ngamprasertwong, Vinks, and Sadhasivam (2012)	Describe serious adverse drug effects as tied into genetics, epigenetics, and functional genomics	Descriptive, literature review	Pharmacogenetics, epigenetics, and genomics	Thematic analysis of literature	Not - applicable (N/A)	Robust clinical study designs needed to be able to individualize perioperative patient care	Promising future for the study of pharmacogenomics
Csoka and Szyf (2009)	Presentation of hypothesis commonly-used pharmaceutical drugs may cause continued epigenetic changes	Descriptive, evaluation of known or postulated direct and indirect effects of common	Chromatin remodeling, DNA methylation, transcription, replication, histone acetylation, DNA	Thematic analysis of literature	N/A	Identification of DNA methylation alterations, teratogenic changes due to meds	Pharmacogenomics and anesthetic epigenetic links promise to be interesting avenues of inquiry in the

		pharmaceutical drugs	methyltransferase				changing way we understand pharmacology
Fraga et al. (2005)	To compare X chromosome inactivation patterns in monozygotic twins	Empirical, questionnaire of 80 Caucasian twins from Spain, informed consent from parents, epithelial skin cell and muscle tissue biopsy DNA and RNA analysis	DNA methylation and acetylation, gene expression, epigenetic differences across different tissue types, variance in 5mC DNA conte	X-inactivation analysis, capillary electrophoresis, quantification of global histone acetylation, quatification of DNA methylation	SPSS, ANOVA, Pearson	Monozygotic twins, although genotypically identical, phenotypically differ due to external and internal factors leading to epigenetic drift	As twins grow older, their epigenetic expression changes such that there are greater numbers of differences seen in 50-year-old twins as compared to 3-year-old twins
Gupta (2010)	To explore histone H3 methylation, a possible experience-driven mechanism of transcriptional regulation in the hippocampus during consolidation of fear-conditioned memories and providing insight into epigenetic mechanisms	Experimental, use of the null allele in genetically altered mice to perform fear conditioning, then histone extraction and analysis	Altered regulation of epigenetic mechanisms may lead to cognitive impairments.	Fear conditioning with electric shock, behavioral testing for fearful expression, histone extraction from hippocampus and western blotting, PCR analysis	SPSS statistical analysis, 2 way ANOVA with statistical significance set at p= </= 0.05	Deficits in memory formation were found when M11, a known regulator of histone methylation was heterozy-gously deleted.	Histone methylation and learning are positively correlated, H3K4-specific methyltransferase is essential for hippocampally-mediated long-term memory formation.
Javitt et al. (2011)	To investigate the stimulatory effects of glutamate in both pathophysiology and in potential treatment	Investigative report, literature search of how glutamate is linked to multiple physiologic and pathophysiologic factors	N-Methyl D-aspartate receptor (NMDAR), glutamate receptor dysregulation leading to disease, and biomarkers	Thematic analysis of literature review and review of Institute of Medicine meeting discussing glutaminergic therapy possibilities and barriers to development of said therapies	N/A	Glutamate is important in neurotransmission with dysregulation of glutamate linked to schizophrenia, addiction, and depression/anxiety	Glutamate activity as a target for pharmacological research and intervention can lead to promising advances in the treatment of various disease states like using Ketamine to treat depression
Ji et al. (2014)	To investigate how long-term memory defcit induced by Isoflurane may be a function of dyregulation of histone methylation and the BDNF-TrkB signaling pathway	Experimental using aged (22 month old) rats, serial exposure of rats to Isoflurane with injection of either sodium butyrate or phosphate-	Post operative cognitive dysfunction, histone acetylation/deacetylation, BDNF, BDNF-TrkB, CaMKII, CREB, HDAC inhibitors	Open-field and fear conditioning tests, Western blotting, enzyme-linked immunosorbent assay, immunohistochemistry analysis	SPSS, mean +/- SEM, independent t sample t test, one way analysis of variance, Bonferroni, p value <0.05	Isoflurane exposure did not affect arterial blood gas, but did induce cognitive dysfunction, induce hippocampal HDAC2 expression, decrease local histone acetylation, and	Serial exposure to isoflurane increased proinflammatory cytokine exposure via chromatin remodeling, this effect can be blocked by an HDAC inhibitor and hence,

		buffered saline				reduce BDNF and p-TrkB expression. NaB pretreatment attenuated hippocampal inflammation, apoptosis, and cognitive impairment	enhance memory/limit loss of memory
Ju et al. (2016)	To test if sevoflurane-induced cognitive impairment is linked to the dysregulation of hippocampal DNA methylation of synaptic plasticity genes	Experimental using neonatal rats, serial exposure of rats to sevoflurane, behavioral testing, hippocampal analysis at different time intervals, SPSS statistical analysis, open field, fear conditioning, and Morris water maze tests, PCR analysis, Western blotting, messenger RNA measurement, Golgi staining	Cognition, behavior, DNA methylation in hippocampus, gene expression, sevoflurane exposure and subsequent neurotoxic changes (altered dendritic spine morphology/synaptic plasticity) in hippocampus, BDNF, Reelin, PP1, 5-AZA	Fear conditioning test, open field test, mRNA measurement, Morris Water Maze test, methylation specific qPCR, Western botting	SPSS, Mean =/- SEM, Shapiro-Wilk, one way Anova followed by Bonferroni, 2 way ANOVA, p value <0.05	Repeated neonatal exposure to sevoflurane increased DNMTs, decreased MeCP2, and fostered hypermethylation of BDNF and Reelin genes leading to lesser synaptic plasticity-related gene expression, and cognitive impairment. Pretreatment with 5–AZA, a DNA methylation inhibitor, reversed/prohibited sevoflurane-induced abnormalities	Established that neurocognitive impairment due to sevoflurane exposures an epigenetically mediated process
Klingler et al. (2014)	To identify both the functional and genetic characterization of MH	Retrospective and experimental, in-vitro contracture tests and clinical grading scale of MH crisis	Anesthetic agent used, age, sex, and RYR1 mutations	Multi-centre analysis, muscle biopsy, genetic screening,	Statistical analysis with expression of mean with standard deviation, median, differences between groups Mann-Whitney, statistical significance of $p<0.05$	Most MH crises occurred where succinylcholine and volatile agent used together	RyR1 mutations are linked to MH crisis, these are heritable and severity of crisis is linked to which site of RyR1 gene location
Lacassie et al. (2006)	To study reversibility of nitrous oxide (NO2) myelopathy and 5,10 methylenetetrahydrofolate reductase (MTHRF) polymorphism's role	Case study analysis, DNA analysis for homozygous 677T to C mutation	MTHRF remethylation of homocysteine to methionine, Nitrous oxide (N2O), autosomal recessive inheritance of the thermolabile form associated with elevated	Patient's clinical presentation, homocysteine and B12 levels, CT scanning, genomic DNA analysis	N/A	Thermolabile form of MTHFR leads to hyperhomocysteinaemia, vitamin B12 deficit under the influence of N2O	No specific epigenetic mechanism in article, but there is one (or more) involved because the mutated MTHFR is expressed phenotypicall

			plasma homocysteine levels and decreased MTHRF activity				y, N2O leads to decreased MTHRF activity
Lessans and Dorsey (2013)	To consider epigenetics in the study of pain	Report, literature review, descriptive investigation of known and potential epigenetic factors linked to pain	Epigenomic remodeling, environment, pain pathology, potential therapeutic potential of epigenetics in pain management	Thematic analysis and description	N/A	Potential epigenetic mechanisms linked to pain	Pain management must be understood more mechanistically with epigenetic consideration involved
Lirk, Fiegl, Weber, and Hollmann (2015)	To explore if epigenetic mechanisms influence one's response to surgery and anesthesia	Descriptive, literature review	Inflammation related to surgery, pain, wound healing, tumour growth, opiates, local anesthetics	Thematic analysis of literature	N/A	Epigenetics linked to opioid receptor expression, to opioid-induced hyperalgesia, to wound healing, perioperative inflammation, and tumour recurrence	Future perioperative patient management will likely incorporate epigenetic consideration
Qiao et al. (2015)	To explore the role of microRNA-21 in the cardioprotection induced by isoflurane gas	Standard and microRNA-21 knockout mice received echocardiograms, 30 min ischemia, 2 hour reperfusion, with or without isoflurane gas, cardiac Akt, eNOS, and nNOS analysis, mitochondrial permeability transition pore (mPTP) opening in cardiomyocytes	microRNA-21, isoflurane, cardiac proteins, mPTP,	Echocardiogram, central hemodynamic measurement, real –time reverse transcriptional-polymerase chain reaction, miR-21 extraction and qRT-PCR analysis, PCR of muRNA-21 target mRNAs, occlusion of left coronary artery, mitochondrial NADH measurements, immunoblotting of harvested hearts, mPTP opening analysis with laser-scanning confocal microscope,	Data expressed as mean =/- S.D., two-way ANOVA followed by Bonferroni's multiple comparison, also one-way ANOVA followed by Bonferroni, post-hoc test, analyses with GraphPad Prism	microRNA 21 disruption shows change ventricular phenotype, cardiac function, risk area, or ratios of p-AKT/Akt, p-NOS/eNOS, pnNOS/ nNOS, isoflurane decreased size of infarct, cardiac function and protein rations, MicroRNA-21 knockout mice were not protected from myocardial ischemia effects	Isoflurane is cardioprotective lessening the negative effects of ischemia/reperfusion
Robinson, R., Carpenter et al. (2009)	To investigate if and how allele silencing affects malignant hyperthermia	Experimental, evaluation of different familial genetic MH-related mutations, analysis of parent-offspring data	Mendelian inheritance, epigenetic allele silencing, MH inheritability	Muscle biopsy and blood samples for RyR mutation analysis, transmission data for RyR mutation	Chi squared confidence intervals for proportion assuming binomic distribution	Fewer fathers with affected daughters than sons	Allele silencing may play role in inheritance of malignant hyperthermia susceptibility, but not likely to silence RyR1

Sabhaie, Liang, Shi, Sun, and Clark (2016)	To test if prior opioid administration with subsequent painful stimulus, hindpaw incision, came together to acetylate H3K9, near Bdnf and Pdyn promoters, leading to their greater expression, to OIH, and to allodynia	Experimental, mice received escalating morphine doses vs. saline twice daily for 4 days, brief anesthesia with isoflurane and then, hindpaw incision, behavioral testing for withdrawal to pain, ANA-12, a BDNF receptor antagonist or norbilatorphimime (nor-BNI), a selective K-opioid receptor antagonist, administered intrathecally, anacardic acid given intraperitoneally, analysis of spinal cord segments for BDNF and dynorphin levels, statistical analysis	Bdnf, Pdyn, nociceptive sensitization, analgesic tolerance, TrkB, K-opioid receptor antagonist	Behavioral test for withdrawal to pain, chromatin immunoprecipitation assay, quantification of mRNA, immunoassay for BDNF and Dynorphin levels	Means+/- SEM, multiple t tests with Hol-Sidak method to correct for multiple comparisons, one way ANOVA followed by Sidak post hoc, and one way ANOVA with Fisher Least Significance Difference	Opioids administered continuously lead to more postop pain sensitization and tolerance to analgesics with epigenetic changes, (acetylation of promoter region) and upregulation Bdnf and Pdyn. Use of ANA-12 and nor-BNI led to less hyperalgesia. Anacardic acid led to less OIH and attenuated enhanced hyperalgesia	OIH is demonstrated to be a epigenetically mediated phenomenon
Uchida, Ma, and Ueda (2010)	To investigate how nerve injury induces long-lasting neuron-restrictive silencer factor (NRSF) in the dorsal root ganglion (DRG) epigenetically silencing the mu opoiod receptor gene	Male mice weighing 20-25 grams,	Sensation of pain, C-fiber dysfunction, nerve injury, $Na_V1.8$ sodium channels, mu-opoiod receptor, DRG, peripheral morphine analgesia, peripheral hypoesthesia	oligonucleotide treatment to knockout NRSF, thermal nociception test, morphine administered, quantitivative PCR for total RNA counts, lumbar 4-6 DRG western blot, immunohistochemistry, and chromatin immunoprecipitation	One-way ANOVA with Tukey-Kramer multiple comparison post hoc analysis, data analyzed using Student's t-test, $p<0.05$, results shown as means =/- SEM	peripheral nerve injury manifested with neuron restrictive silencer factor (NRSF) upregulation in the DRG via epigenetic mechanisms and also that NRSF binding to neuron-restrictive silencer element is promoted in both $Na_v1.8$ and MOP genes leading to epigenetic gene silencing. NRSF	Pain sensation and loss of it are epigenetically mediated, morphine analgesia less effective in mice after an injury has occurred if NRSF upregulated

						upregulation correlated with lesser efficacy in treating pain with morphine, NRSF knockdown showed opposite effect	
Williams, Mazue, McQueen, and Shimada (1980)	To research the genotoxicity of hydralazine and dihydralazine	Experimental using rat hepatocytes and bacteria, cultured cells exposed to two test drugs	Genotoxicity, rapid versus slow acetylation, DNA damage	Ames salmonella microsome test, hepatocyte primary culture/DNA test, acetylation rate analysis	Means +/- SEM, no further description of how values were derived	Both drugs are potential carcinogens to humans	Acetylation may influence how genotoxix both drugs are, although an old study, this may be mediated through epigenetics
Zhou et al. (2015)	To investigate the hypothesis that sevoflurane exposure leads to down-regulation of arginine vasopressin (AVP) and oxytocin (OT) manifesting with deficits in social memory formation and social discrimination	Experimental, single 6 hour exposure of neonatal mice (20 days old) to sevoflurane or air, measurement of social memory formation and social discrimination, measurement of hippocampal levels of arginine vasopressin and oxytocin, SPSS statistical analysis	Sevoflurace, hippocampus, the hormones oxytocin and arginine vasopressin, behavior,	Social behavioral tests, recognition trial, discrimination trial, immunohistochemichal analysis of OT and AVP distribution, real time quantitative PCR for OT and AVP mRNA levels, Western Blot for OT and AVP protein levels	SPSS, independent t tests to compare groups, statistical significance p</= 0.05, expressed as mean +/- SD	Mice exposed to sevoflurane lower vasopressin and oxytocin values. These 2 hormones are important in social development. Sevoflurane mice took longer to identify the same stimulus mouse on serial introductions and also to discriminate between the first stimulus mouse and a novel one	Sevoflurane exposure leads to socio-behavioral changes and in the expression of OT and AVP levels

Table 3. Sociodemographic Features of Study Participants (N=41)

Sociodemographic Variables	Frequency	Cumulative Percent
Sex		
Male	13	31.7
Female	28	100
Age Groupings		
20-29	16	39
30-39	16	78
40 and above	9	100
Race		
White	32	78
African American	2	82.9
Asian	3	90.2
Mixed Race	4	100
Years of Clinical Anesthesia Experience		
1-5 years	27	65.9
6-10 years	3	73.2
11-15 years	2	78.0
16-20 years	4	87.8
21 years and above	5	100
Employment Status		
Full time	15	36.6
Part time	1	39.0
Per diem	5	51.2
Unemployed	20	100
Role		
CRNA	10	24.4
SRNA	26	87.8
Anesthesiologist	5	100
Level of Education		
Bachelor	24	58.5
Master	11	85.4
Doctorate	6	100
Practice Sites		
Urban, Academic and Teaching	31	75.6
Urban, Non-academic, Non-teaching	6	90.2
Urban and Private	4	100

Table 4. Descriptive and Reliability Statistics for Epigenetics and Acceptability Questionnaires

All questions had valid answers	Cronbach's alpha	Kuder-Richardson 20	Mean Score (SD)	Minimum to Maximum	Sum Scores	Highest Possible Sum Score
1-20 Epigenetics Post-test Exam Questions	N/A	.41	13.05 (2.37)	8 to 18	535	820
21-26 Acceptability Questionnaire Questions	.778	N/A	3.08 (.74)	2 to 4.5	126.5	205

Table 5. Distribution of the Mean Scores on Epigenetics Examination

Actual Mean Scores of Study Participants	Frequency	Percent	Valid Percent	Cumulative Percent
8.00	1	2.4	2.4	2.4
9.00	3	7.3	7.3	9.8
10.00	3	7.3	7.3	17.1
11.00	4	9.8	9.8	26.8
12.00	5	12.2	12.2	39.0
13.00	4	9.8	9.8	48.8
14.00	10	24.4	24.4	73.2
15.00	4	9.8	9.8	82.9
16.00	6	14.6	14.6	97.6
18.00	1	2.4	2.4	100.0
Total	41	100.0	100.0	

Table 6. Distribution of Sum Scores for Individual Questions in the Epigenetics Exam

	Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q* 7	Q 8	Q* 9	Q* 10	Q 11	Q 12	Q 13	Q* 14	Q* 15	Q 16	Q 17	Q 18	Q 19	Q 20
Valid	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
Missing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sum	39	30	36	31	26	40	9	27	7	9	24	28	39	18	17	33	28	25	38	31

*Questions with the lowest sum scores.

Table 7. Descriptive Statistics of the Acceptability of the Epigenetics Seminar						**Sum Score**
How easy was the Epigenetics in Anesthesiology lecture for you to understand?	Easy n=6 (14.2%)	Neutral n=14 (34%)	Difficult n=16 (39%)	Very difficult n=5 (12.2%)		103/205 Below the neutral score of 123
How understandable were the questions?	Easy n=5 (12.2%)	Less easy n=6 (14.6%)	Neutral n=16 (39%)	Less difficult n=11 (26.8%)	Difficult n=3 (7.3%)	122/205 Very near the neutral score of 123
How much did you enjoy attending the Links between Epigenetics and Anesthesia seminar?	Very much n=8 (19%)	Enjoyed it n=11 (26.8%)	Neutral n=20 (48.7%)	Did not enjoy it n=2 (4.8%)		148/205 Above the neutral score of 123
How helpful was the Links between Epigenetics in Anesthesia seminar in increasing your knowledge on the topic?	Very helpful n=10 (24.4%)	Helpful n=17 (40.4%)	Neutral n=11 (26.8%)	Unhelpful n=3 (7.3%)		157/205 Above the neutral score
Was the amount of time it took to complete this seminar acceptable?	Very acceptable n=11 (26.8%)	Acceptable n=12 (29.2%)	Neutral n=10 (24.4%)	Unacceptable n=6 (14.6%)	Very unacceptable n=2 (4.8%)	147/205 Above the neutral score
How would you rate your overall satisfaction with the Links between Epigenetics and Anesthesia seminar?	Very satisfied n=7 (17%)	Satisfied n=15 (36.5%)	Neutral n=17 (41.4%)	Dissatisfied n=2 (4.8%)		150/205 Above the neutral score
Overall Score						827/1230 Above the overall neutral score of 738

Table 8. Results of the Subset Analyses Examining Statistically Significant Differences in Epigenetics Post-Test Mean Scores Based on Sociodemographic Variables

Sociodemographic Variable	Amenable to Statistical Analysis	Mean Scores (SD)	Statistically Significant	Statistical Test
Gender Male (n=13) Female (n=28)	Yes	13.92 (2.39) 12.64 (2.29)	No *F* = .006 *t* =1.639 *df* = 39 ***p* = .109 (2-tailed)**	T Test
Age Group 20-29 y.o. (n=16) 30-39 y.o. (n=16) 40-49 y.o. (n=9)	Yes	13.75 (2.29) 12.37 (2.47) 13.00 (2.23)	No *F* = 1.366 *df* = 2 ***p* = .267**	One-way ANOVA
Employment Status Full time (n=15) Unemployed (n=20)	Yes	12.8 (2.48) 13.65 (2.18)	No *F* = .384 *t* = -1.075 *df* =33 ***p* = .29 (2-tailed**	T Test
Position/Role CRNAs (n=10) SRNAs (n=26) Anesthesiologists (5)	Yes	12.5 (2.46) 13.19 (2.34) 13.4 (2.70)	No *F* = *.357* *df* = *2* ***p* = .702**	One-way ANOVA
Education Bachelor's degree (24) Master's degree (n=11) Doctoral degree (n=6)	Yes	24.0 (13.37) 12.09 (2.38) 13.5 (2.42)	No *F* = 1.243 *df* = 2 ***p* = .30**	One-way ANOVA
Race	No, highly homogenous sample; 78% Whites	N/A	N/A	N/A
Years of Experience	No, highly skewed with 67% of sample having 1-5 years of experience	N/A	N/A	N/A
Type of Practice Setting	No, highly skewed sample with75% work in urban, academic, teaching	N/A	N/A	N/A

Appendix A

Conceptual Framework

Appendix B

Learning Objectives

At the completion of this teaching/learning seminar, the student will be able to

1. Name epigenetic mechanisms
2. Describe characteristics of epigenetic mechanisms
3. Identify links between epigenetics and anesthesia
4. Explain fundamental differences between genetics and epigenetics
5. Identify epigenetic mechanisms investigated in pain research
6. Understand how anesthetic agents affect neurocognitive dysfunction
7. Explain how epigenetics, anesthetics, and depression are linked
8. Identify epigenomic mechanisms tied to malignant hyperthermia
9. Discuss positive and negative epigenomic effects of general anesthesia
10. Name drugs used in anesthesia practice with carcinogenic effects

Links between Epigenetics and Anesthesia for the Anesthesia Provider posttest

I. Name epigenetic mechanisms

1. What is an example of an epigenetic mechanism?

A. DNA methylation

B. Purine base pairing

C. Homogenization

D. Glycosylation

2. Which of the following reflects an epigenetic control as described by Biel, Wascholowski, and Gianni (2005) in their histone modification model?

A. Histone verification

B. Histone core protein formation

C. Histone phosporylation

D. Histone variegation

II. Describe characteristics of epigenetic modifications

3. The epigenome is:

A. non-heritable

B. genotypic in nature

C. reflective of how DNA bases pair

D. affective on how a gene is expressed

4. Epigenetic mechanisms are:

A. permanent

B. reversible

C. non-perpetuating

D. well-established in pharmacology

III. Identify links between epigenetics and anesthesiology

5. In the literature, epigenetics and anesthesiology are linked in which of the following topics?

A. neuronal regeneration

B. pain

C. pharmacotherapeutics

D. genetic testing

6. What area of anesthesia practice is linked to cognitive delay and relates to decreased histone acetylation in the hippocampus of mice?

A. regional anesthesia

B. local anesthesia

C. monitored anesthesia care

D. general anesthesia

IV. Explain fundamental differences between genetics and epigenetics

7. The study of genomics versus the study of epigenomics refers to what concepts, respectively?

a. phenotype versus genotype

b. expression of trait versus underlying DNA structure

c. control mechanisms versus foundational mechanisms

d. what something is versus what something becomes

8. The epigenome is affected by what factor?

a. the pairing of cytosine to guanine

b. the pairing of adenine to thymine

c. the environment

d. the homozygous state of a cell

V. Identify epigenetic mechanisms investigated in pain research

9. Opioid induced hyperalgesia is associated with remifentanil and other narcotics via what epigenetic mechanism?

a. demethylation

b. deacetylation

c. gene silencing

d. methylation

10. Acute and chronic pain sensation and development may be a reflection of what epigenetic process?

a. chromatin remodeling

b. chromatin formation

c. histone tail presence

d. uracil phosphorylation

VI. Understand how anesthetic agents affect neurocognitive dysfunction

11. Neurocognitive dysfunction is tied to Sevoflurane exposure and a decrease in which hormones?

a. arginine vasopressin and oxytocin

b. prolactin and luteinizing hormone

c. gastrin and ghrelin

d. aldosterone and corticotropin-releasing hormone

12. _____ have a negative impact on long term memory formation by muffling key genes in learning and memory such as brain-derived neurotrophic factor (BDNF), calmodulin-dependent protein kinases (CaMKII), and cAMP response element binding protein (CREB).

a. chromatin remodelers

b. kinases

c. histone deacetylases (HDACs)

d. phosphorylating enzymes

VII. Explain how epigenetics, anesthetics, and depression are linked

13. Brain-derived neurotrophic factor (BDNF) is necessary for neuronal development early in life and for the survival and function of neurons in adulthood. Which anesthetic agent increases excitatory glutamate activity by activating BDNF.

a. propofol

b. brevital

c. ketamine

d. midazolam

14. Ketamine acts on the N-methyl D aspartate receptor to rapidly increase permeability to magnesium and calcium fostering synaptic plasticity. By what epigenetic mechanism does ketamine improve depression?

a. unknown

b. ubiquitination

c. histone tail acetylation

d. histone tail methylation

VIII. Identify epigenomic mechanisms tied to malignant hyperthermia

15. Ryanodine receptor protein (RYR1) encodes for the calcium release channel in skeletal muscle's sarcoplasmic reticulum and known mutations are associated with clinical malignant hyperthermia presentation. What epigenetic mechanism has been researched to explain this?

a. histone ribosylation

b. allele silencing

c. histone acetylation

d. histone phosporylation

16. Succinylcholine, inhaled anesthetics, and RYR1 mutation are linked to malignant hyperthermia, but not consistently. Promising ways to explore this problem include:

a. histocompatibility examination

b. immunoassay

c. live trials

d. epigenomic research

IX. Discuss positive and negative epigenomic effects of general anesthesia

17. A positive effect of isoflurane gas is cardioprotection due to what pathway?

a. gene silencing

b. down-regulation of mu receptors and sodium channels

c. histone hypoacetylation

d. microRNA-21 influence on the Akt/nitric oxide synthase/mitochondrial permeability transition pore pathway

18. A negative epigenetic effect of general anesthesia?

a. pain remediation via ubiquination

b. greater post-operative nausea/vomiting through chemoreceptor trigger zone histone methylation

c. hypermethylation of hippocampal synaptic plasticity-related genes

d. chromatin consolidation

X. Name drugs used in anesthesia practice with carcinogenic effects

19. Which antihypertensive agent is affiliated with DNA structural changes and possible subsequent carcinogenicity?

a. Diltiazem

b. Labetalol

c. Losartan

d. Hydralazine

20. What sedative-hypnotic is used as a chemotherapeutic agent linked to truncated limbs in patients and their offspring?

a. Propofol

b. Thalidomide

c. Midazolam

d. Ketamine

Appendix C

Acceptability Scale Measures

21. How easy was the Epigenetics in Anesthesiology lecture for you to understand?

    a. 1 – very difficult

    b. 2

    c. 3

    d. 4

    e. 5 – very easy

22. How understandable were the questions?

    a. 1 – difficult to understand

    b. 2

    c. 3

    d. 4

    e. 5 – easy to understand

23. How much did you enjoy attending the Links between Epigenetics and Anesthesia seminar?

    a. 1 – not at all

    b. 2

    c. 3

d. 4

e. 5 – very much

24. How helpful was the Links between Epigenetics in Anesthesia seminar in increasing your knowledge on the topic?

a. 1 – very unhelpful

b. 2

c. 3

d. 4

e. 5 – very helpful

25. Was the amount of time it took to complete this seminar acceptable?

a. 1 – very unacceptable

b. 2

c. 3

d. 4

e. 5 – very acceptable

26. How would you rate your overall satisfaction with the Links between Epigenetics and Anesthesia seminar?

a. 1 – very dissatisfied

b. 2

c. 3

d. 4

e. 5 – very satisfied

Appendix D

Socio-demographic Questionnaire

27. What is your gender?

    a. Male

    b. Female

28. What is your age group?

    a. 20-29

    b. 30-39

    c. 40-49

    d. 50-59

    e. 60 and above

29. What is your ethnicity or race?

    a. White

    b. Hispanic/Latino

    c. Black/African American

    d. Native American/American Indian

    e. Asian/Pacific Islander

    f. Mixed Race

30. How many years of clinical anesthesia practice do you have (residency included)?

a. 1-5 years

b. 6-10 years

c. 11-15 years

d. 16-20 years

e. 21-25 years

f. 26 years and above

31. What is your employment status

a. Full-time

b. Part-time

c. Per-Diem

d. Retired

32. What is your role?

a. CRNA

b. SRNA

c. Anesthesiologist

d. Other _____

33. What is your highest level of education attained?

a. Associate degree

b. Bachelor's degree

c. Master's degree

d. Doctoral degree (MD, DO, PhD, DNP, EdD)

34. What is your primary practice setting?

a. Urban and Academic/Teaching Institution

b. Urban and Non-academic/Non-teaching Institution

c. Urban and Private Practice

d. Rural and Academic/Teaching Institution

e. Rural Non-academic/Non-teaching Institution

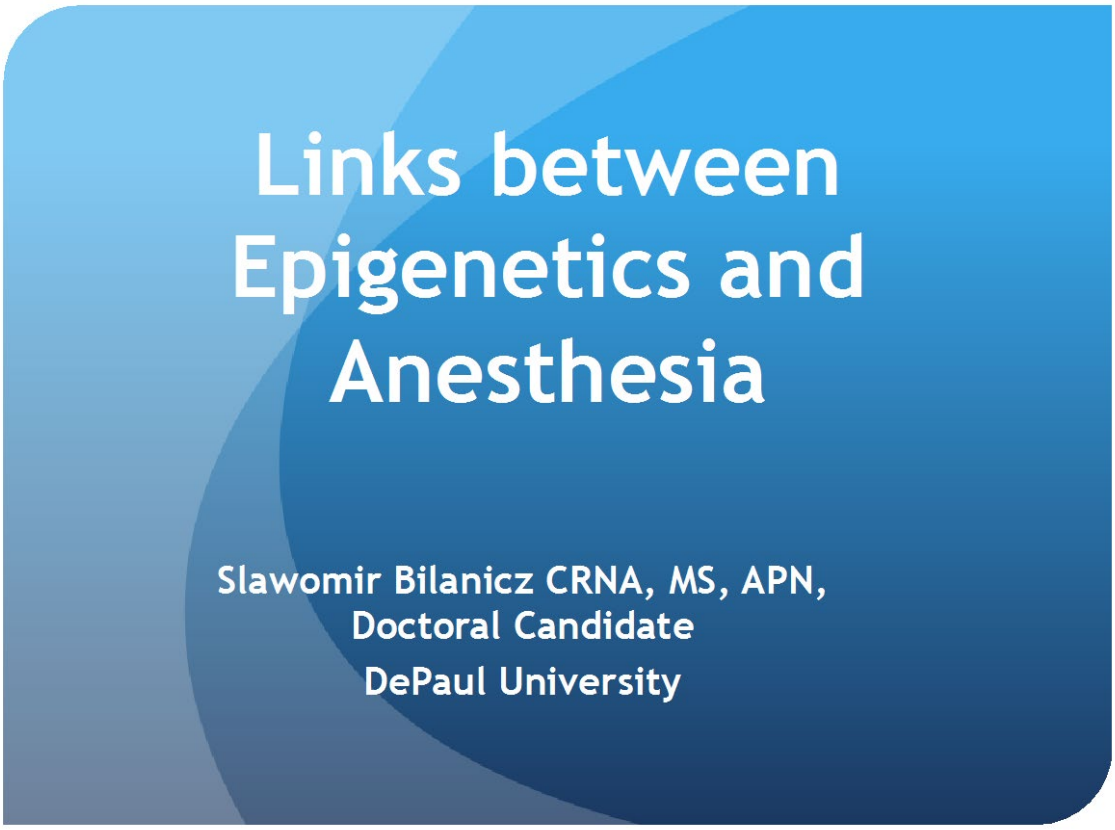
f. Rural and Private Practice

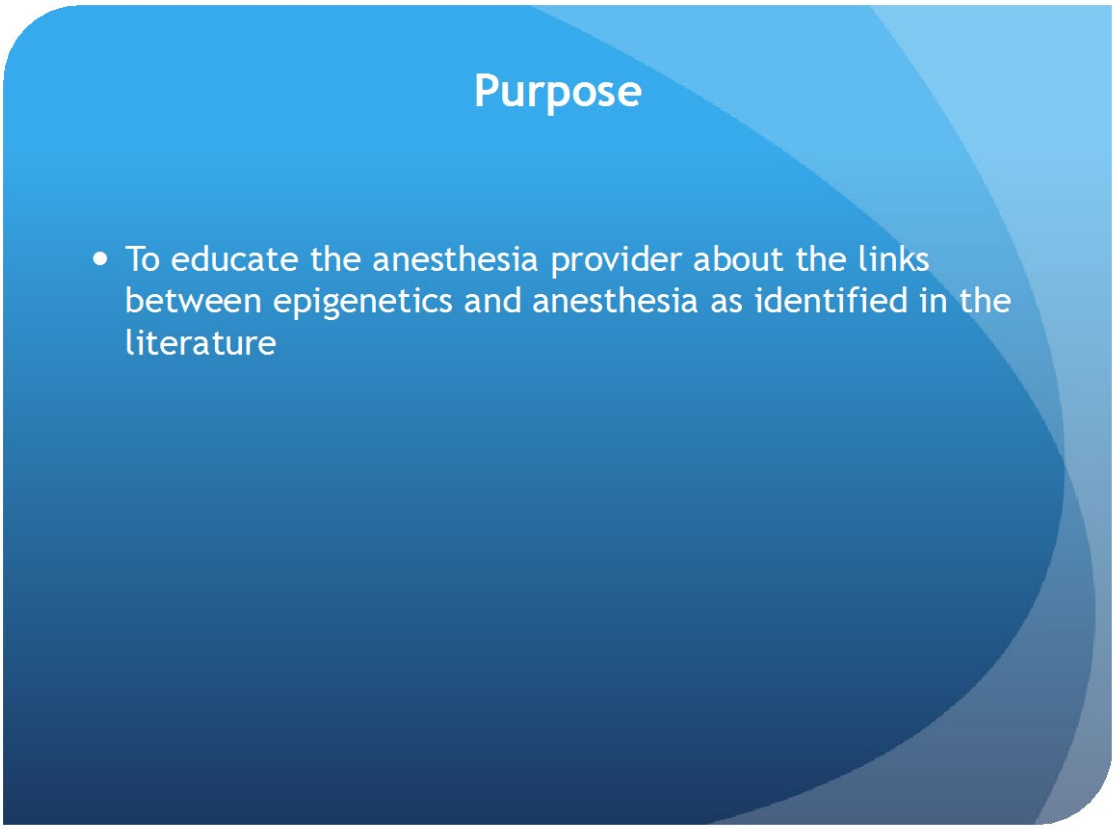
g. Urban and Veteran's Administration Institution

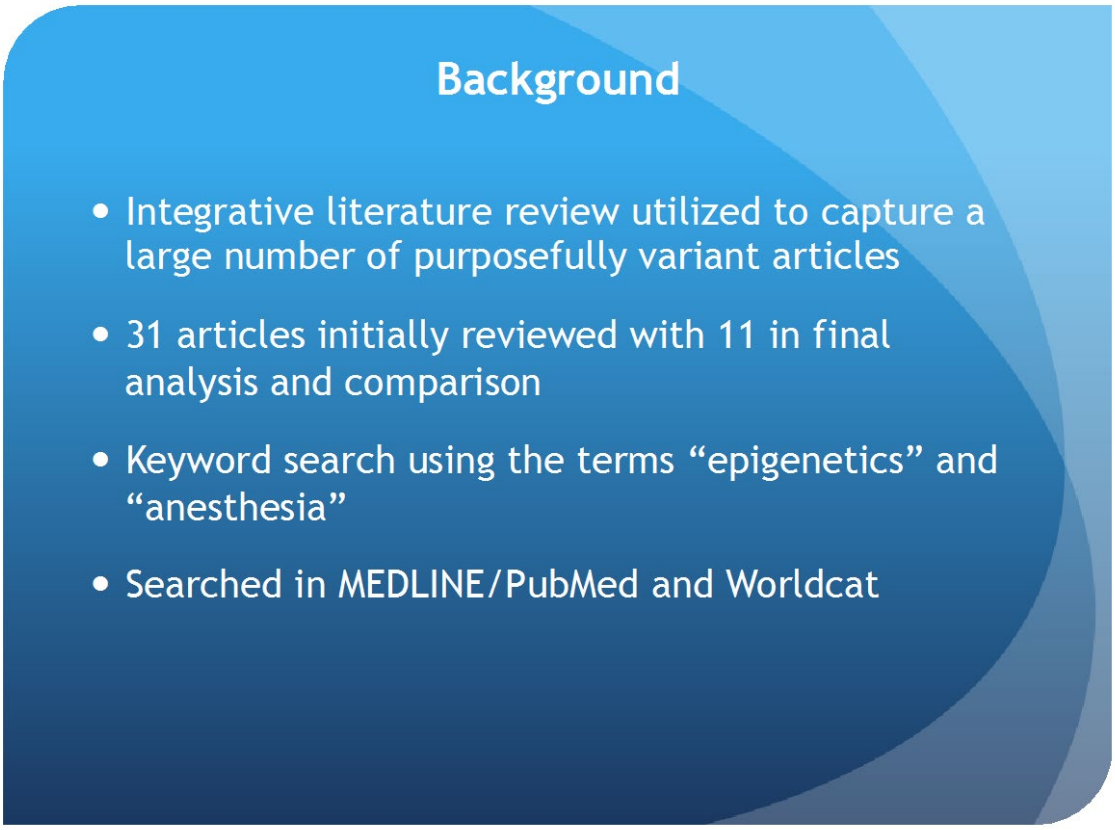
e. Rural and Veteran's Administration Institution

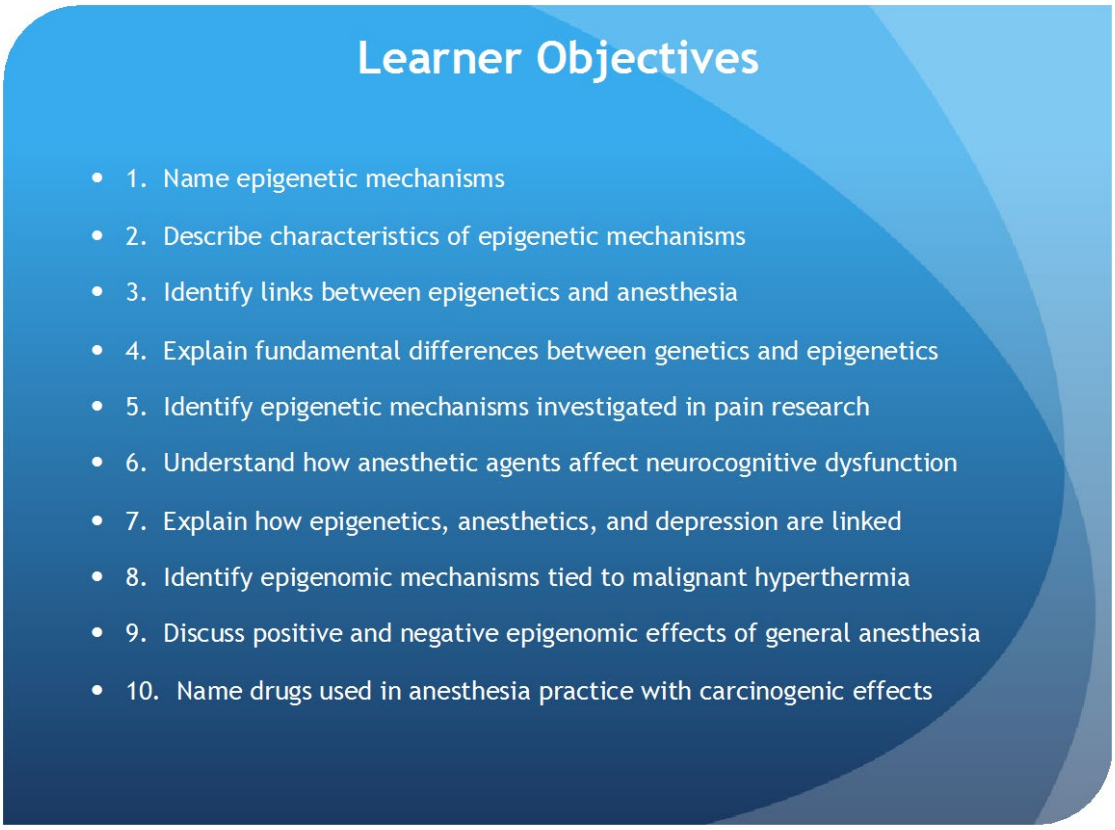
Appendix E

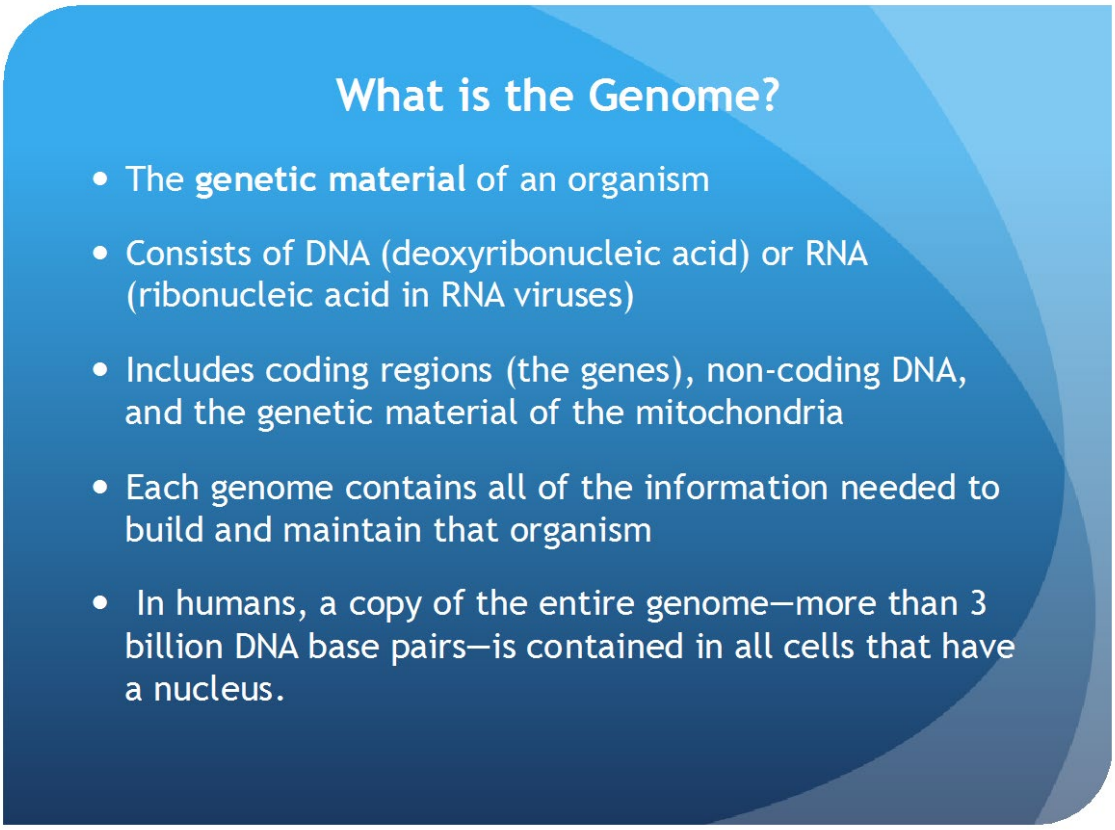
Slide Set for Presentation

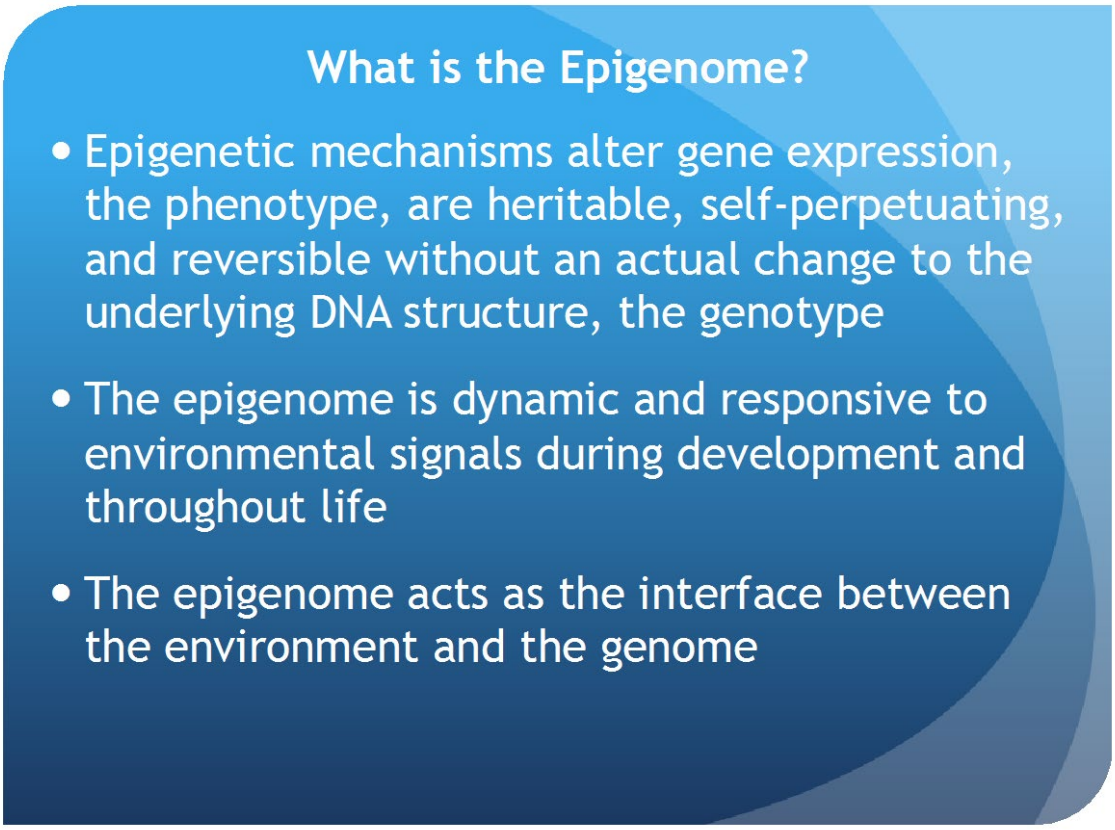

Links between Epigenetics and Anesthesia
Slawomir Bilanicz CRNA, MS, APN,
Doctoral Candidate
DePaul University

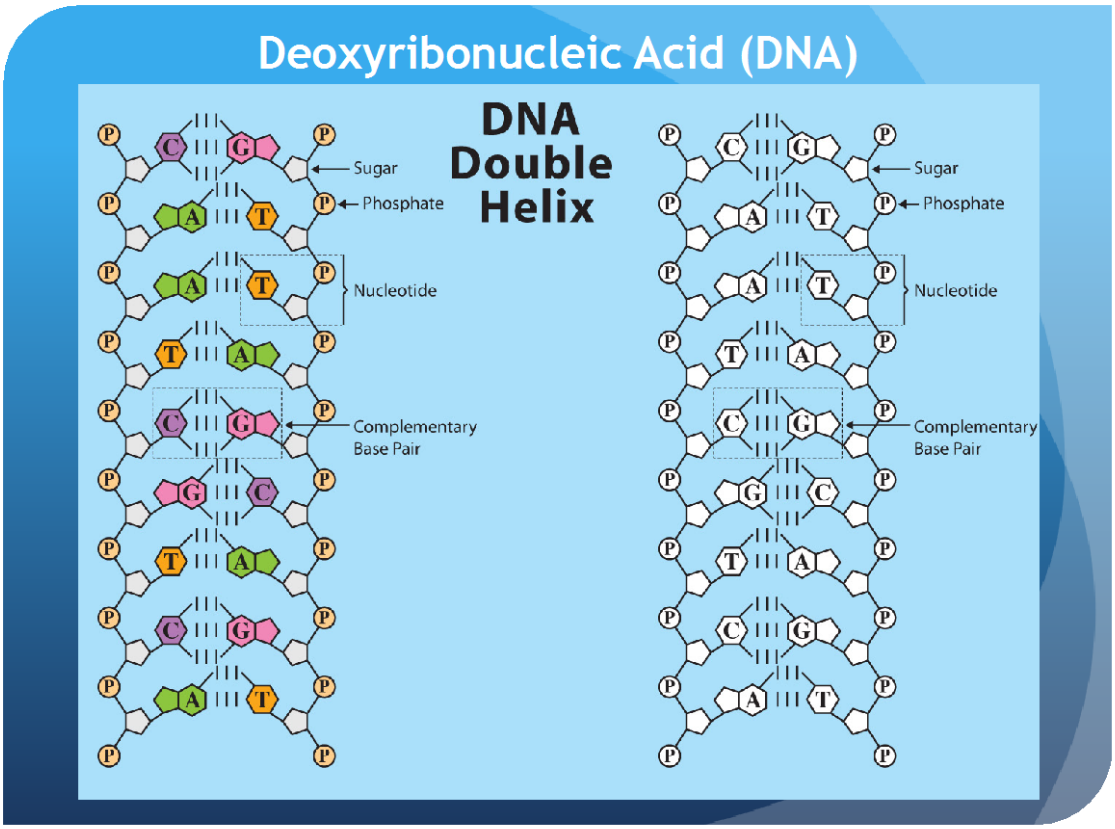

Purpose
To educate the anesthesia provider about the links between epigenetics and anesthesia as identified in the literature

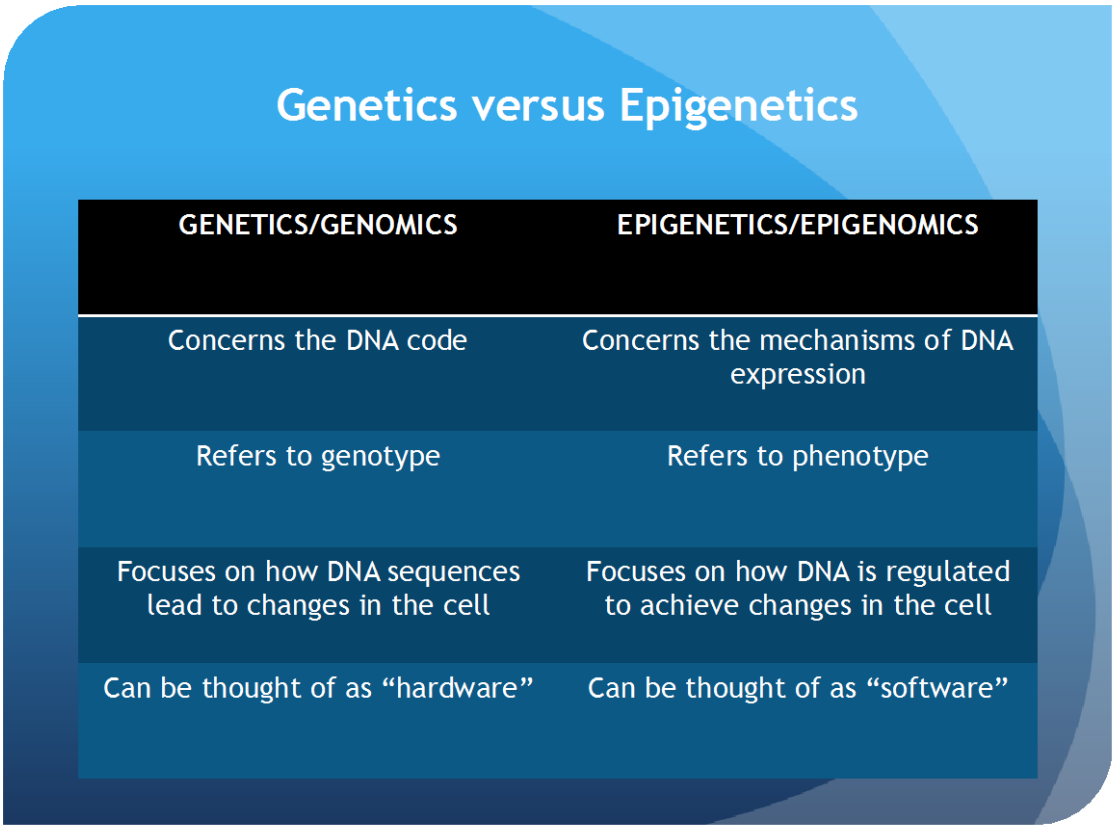

Background
• Integrative literature review utilized to capture a large number of purposefully variant articles
• 31 articles initially reviewed with 11 in final analysis and comparison
• Keyword search using the terms “epigenetics” and “anesthesia”
• Searched in MEDLINE/PubMed and Worldcat

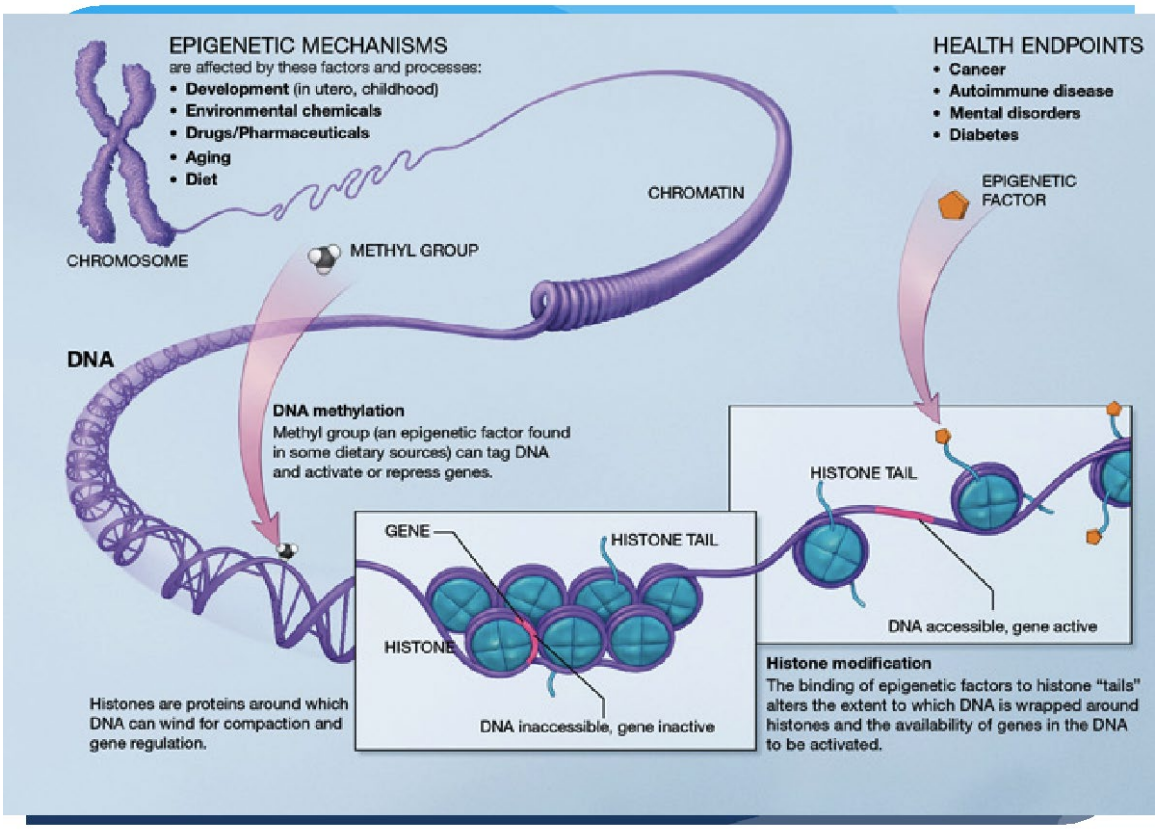

Learner Objectives
1. Name epigenetic mechanisms
2. Describe characteristics of epigenetic mechanisms
3. Identify links between epigenetics and anesthesia
4. Explain fundamental differences between genetics and epigenetics
5. Identify epigenetic mechanisms investigated in pain research
6. Understand how anesthetic agents affect neurocognitive dysfunction
7. Explain how epigenetics, anesthetics, and depression are linked
8. Identify epigenomic mechanisms tied to malignant hyperthermia
9. Discuss positive and negative epigenomic effects of general anesthesia
10. Name drugs used in anesthesia practice with carcinogenic effects

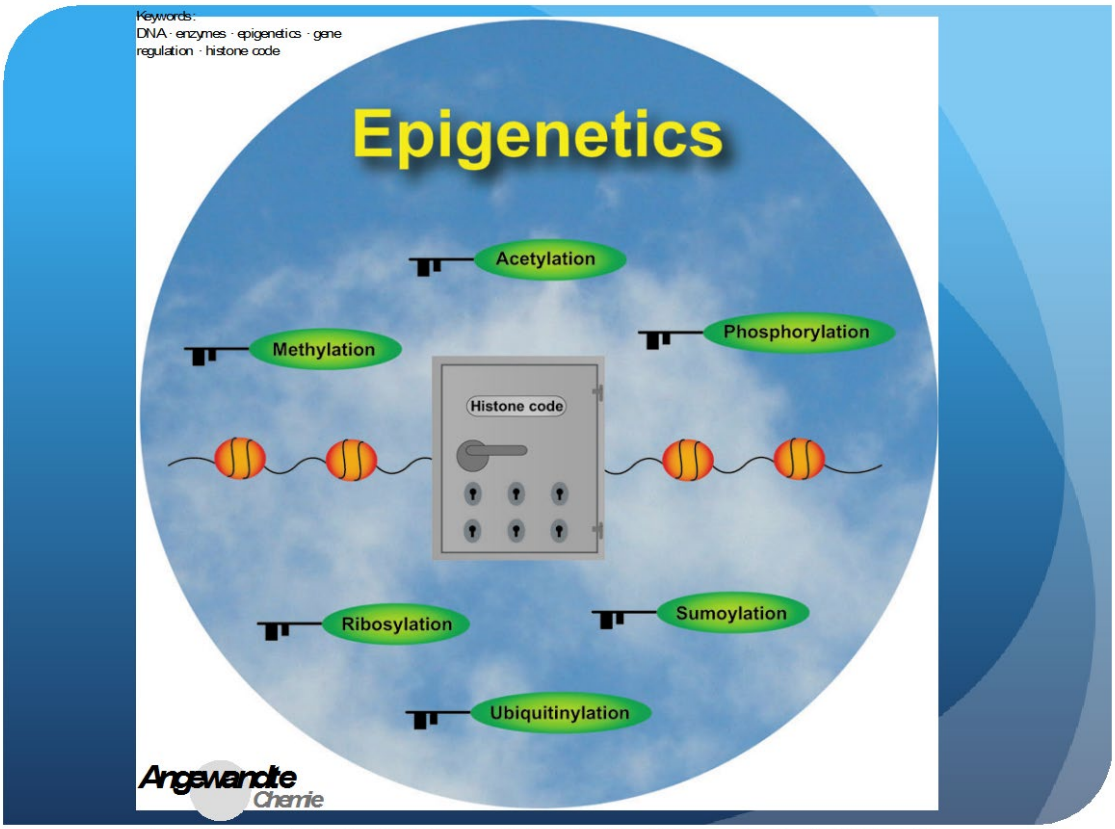

What is the Genome?
The genetic material of an organism
Consists of DNA (deoxyribonucleic acid) or RNA (ribonucleic acid in RNA viruses)
Includes coding regions (the genes), non-coding DNA, and the genetic material of the mitochondria
Each genome contains all of the information needed to build and maintain that organism
In humans, a copy of the entire genome—more than 3 billion DNA base pairs—is contained in all cells that have a nucleus.


What is the Epigenome?
• Epigenetic mechanisms alter gene expression, the phenotype, are heritable, self-perpetuating, and reversible without an actual change to the underlying DNA structure, the genotype
• The epigenome is dynamic and responsive to environmental signals during development and throughout life
• The epigenome acts as the interface between the environment and the genome

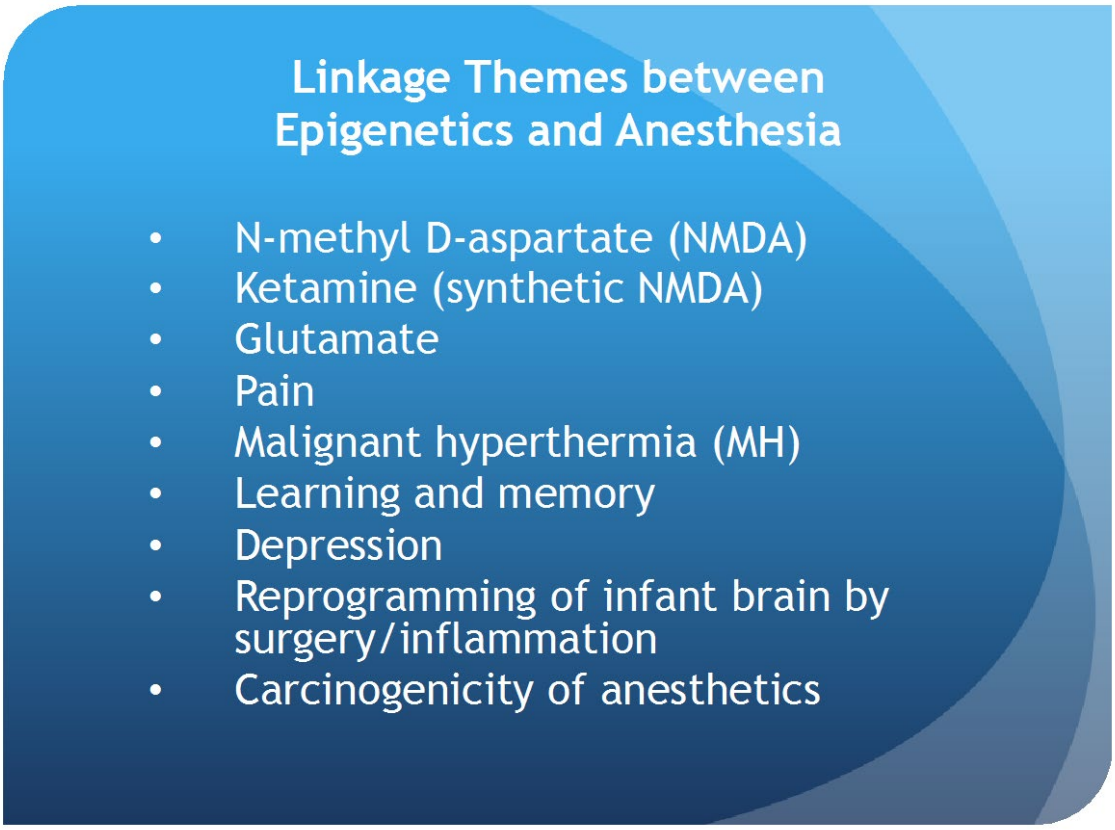

Deoxyribonucleic Acid (DNA)
DNA Double Helix
Sugar
Phosphate
Nucleotide
Complementary Base Pair
Sugar
Phosphate
Nucleotide
Complementary Base Pair

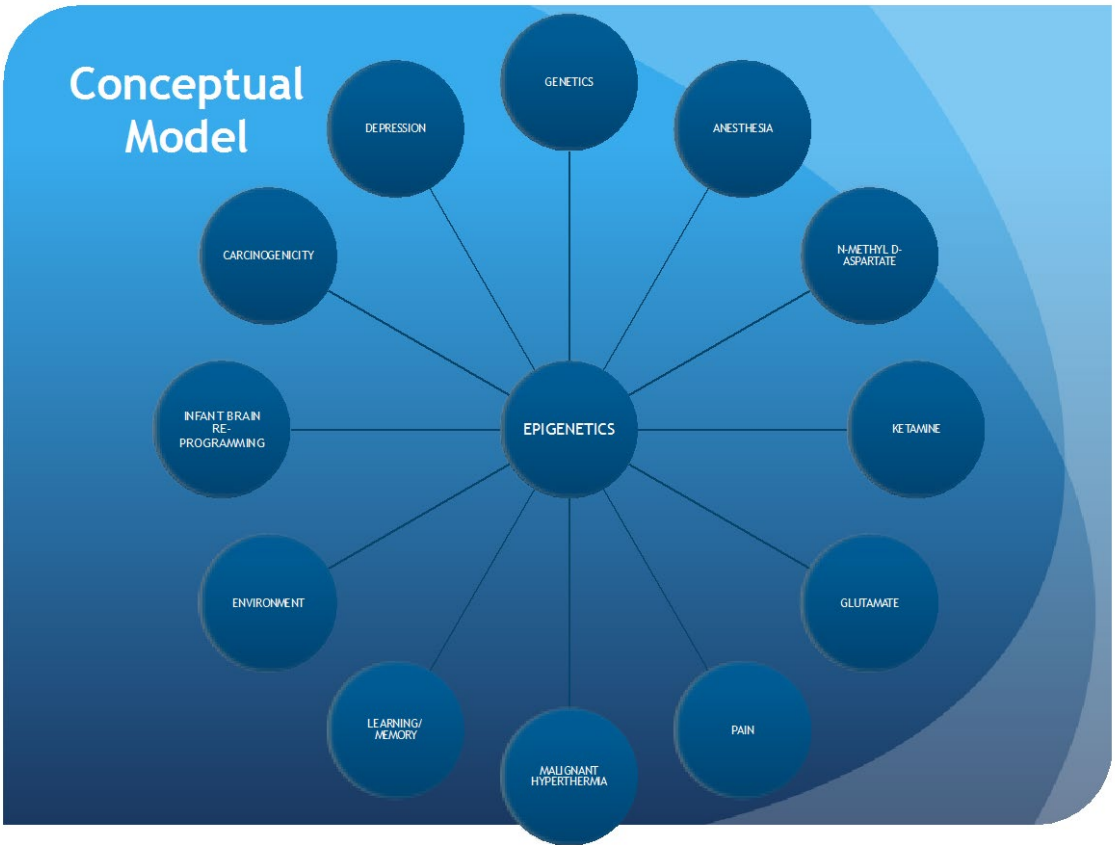

Genetics versus Epigenetics
GENETICS/GENOMICS
EPIGENETICS/EPIGENOMICS
Concerns the DNA code
Concerns the mechanisms of DNA expression
Refers to genotype
Refers to phenotype
Focuses on how DNA sequences lead to changes in the cell
Focuses on how DNA is regulated to achieve changes in the cell
Can be thought of as "hardware"
Can be thought of as "software"

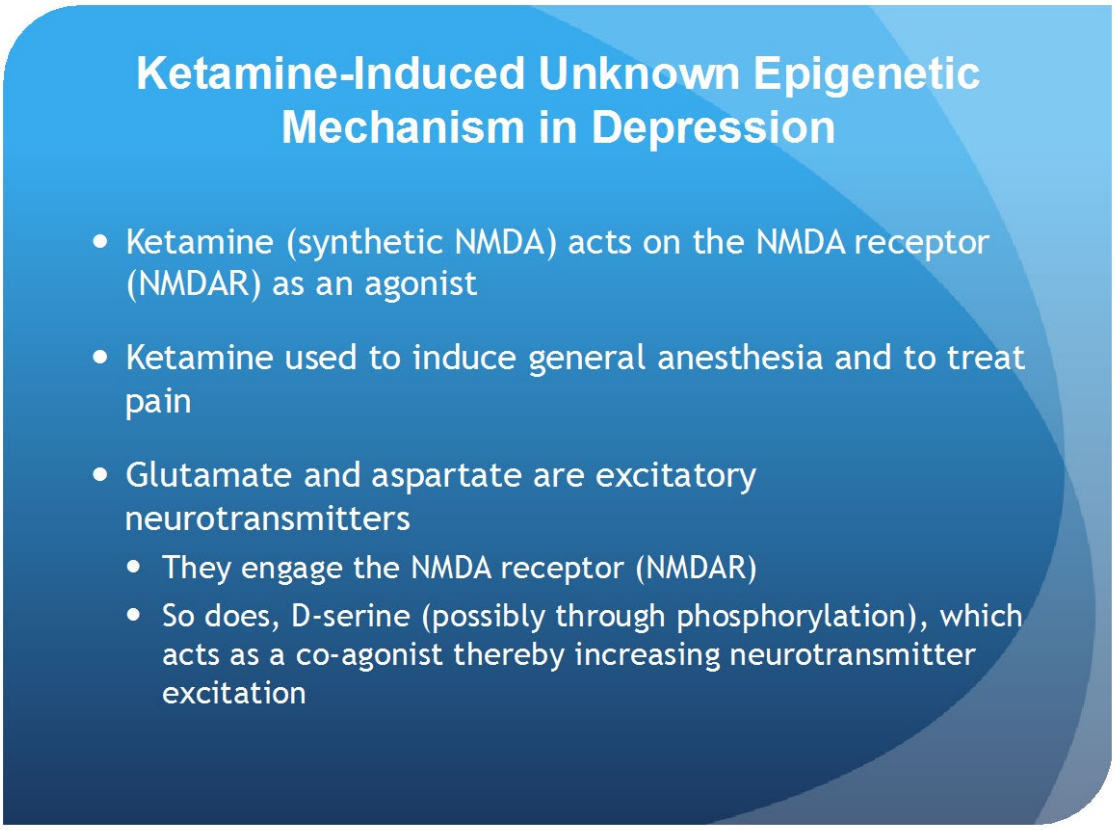

EPIGENETIC MECHANISMS
are affected by these factors and processes:
• Development (in utero, childhood)
• Environmental chemicals
• Drugs/Pharmaceuticals
• Aging
• Diet
HEALTH ENDPOINTS
• Cancer
• Autoimmune disease
• Mental disorders
• Diabetes
CHROMOSOME
CHROMATIN
METHYL GROUP
EPIGENETIC FACTOR
DNA
DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.
HISTONE TAIL
GENE
HISTONE TAIL
HISTONE
DNA accessible, gene active
DNA inaccessible, gene inactive
Histones are proteins around which DNA can wind for compaction and gene regulation.
Histone modification
The binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.

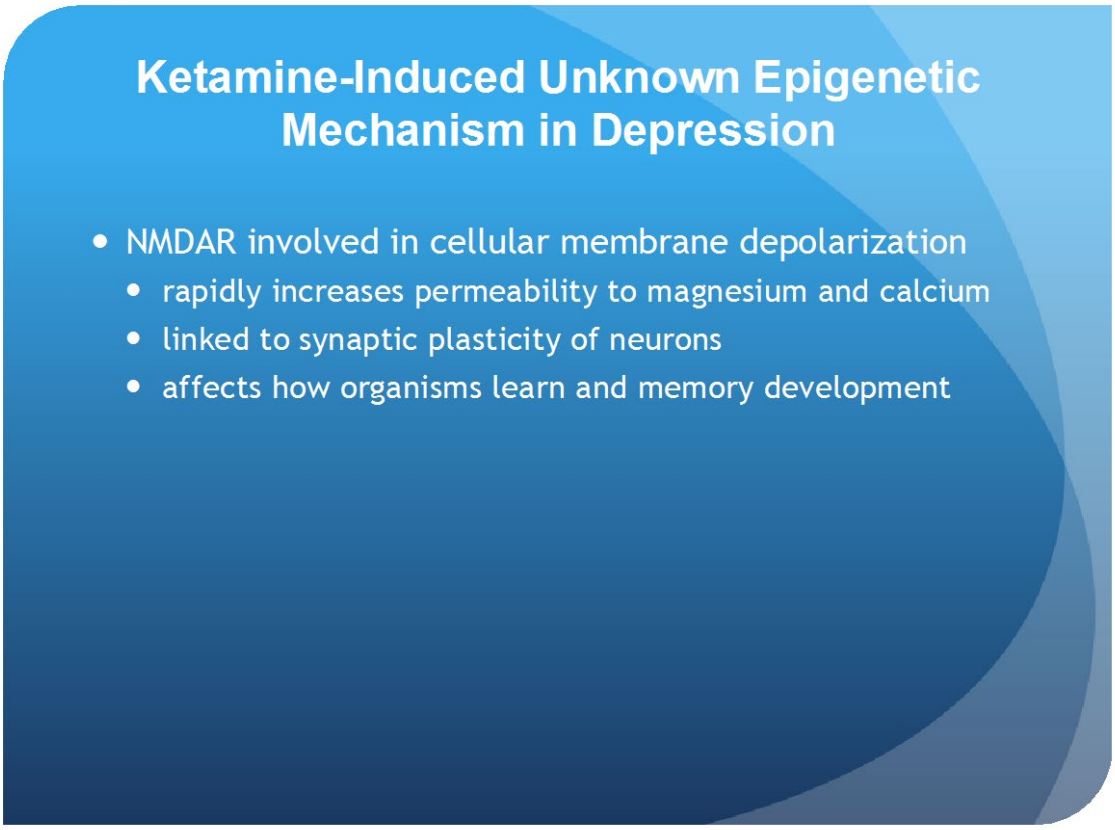


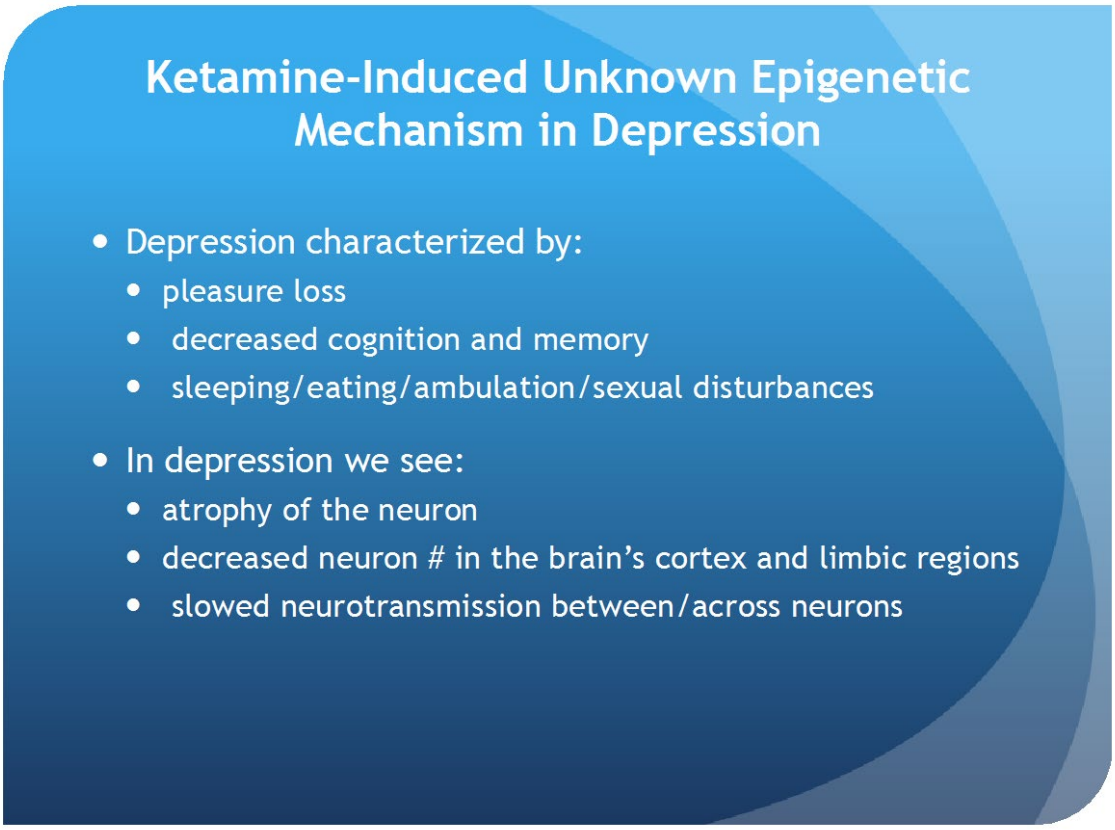
3186 © 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim DOI: 10.1002/anie.200461346 Angew. Chem. Int. Ed. 2005, 44, 3186 – 3216

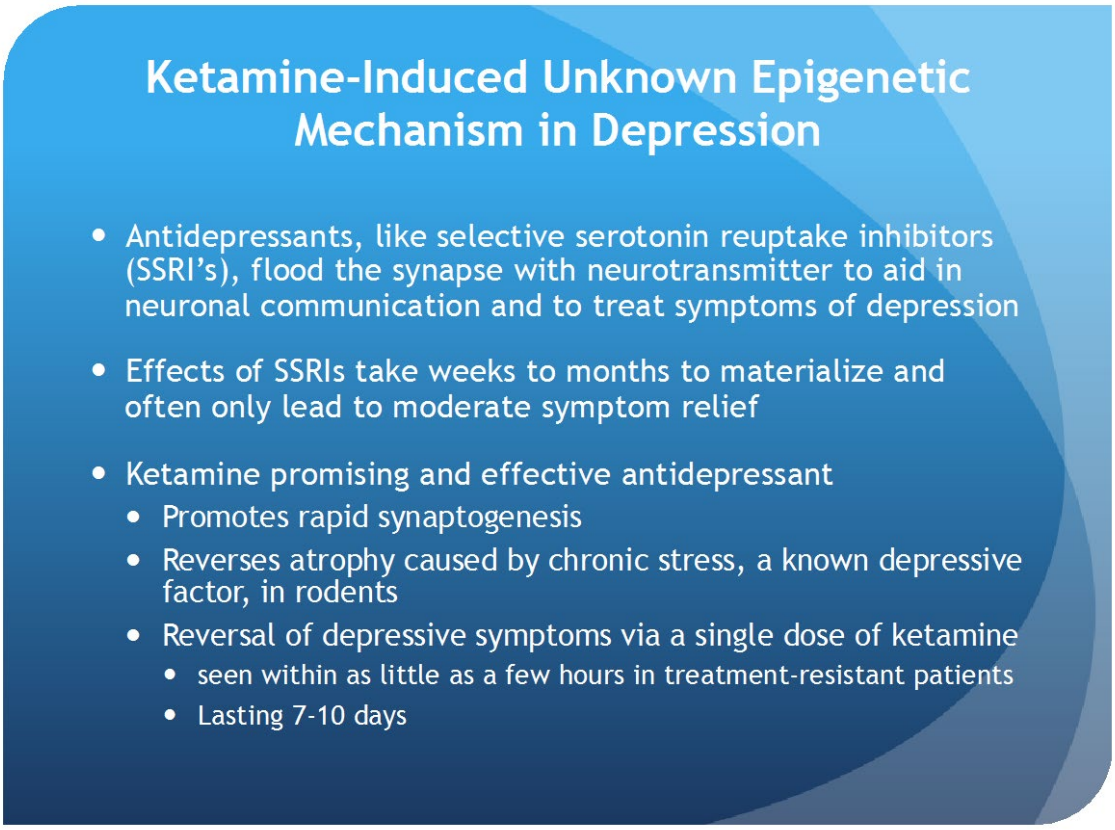

Linkage Themes between Epigenetics and Anesthesia
N-methyl D-aspartate (NMDA)
Ketamine (synthetic NMDA)
Glutamate
Pain
Malignant hyperthermia (MH)
Learning and memory
Depression
Reprogramming of infant brain by surgery/inflammation
Carcinogenicity of anesthetics

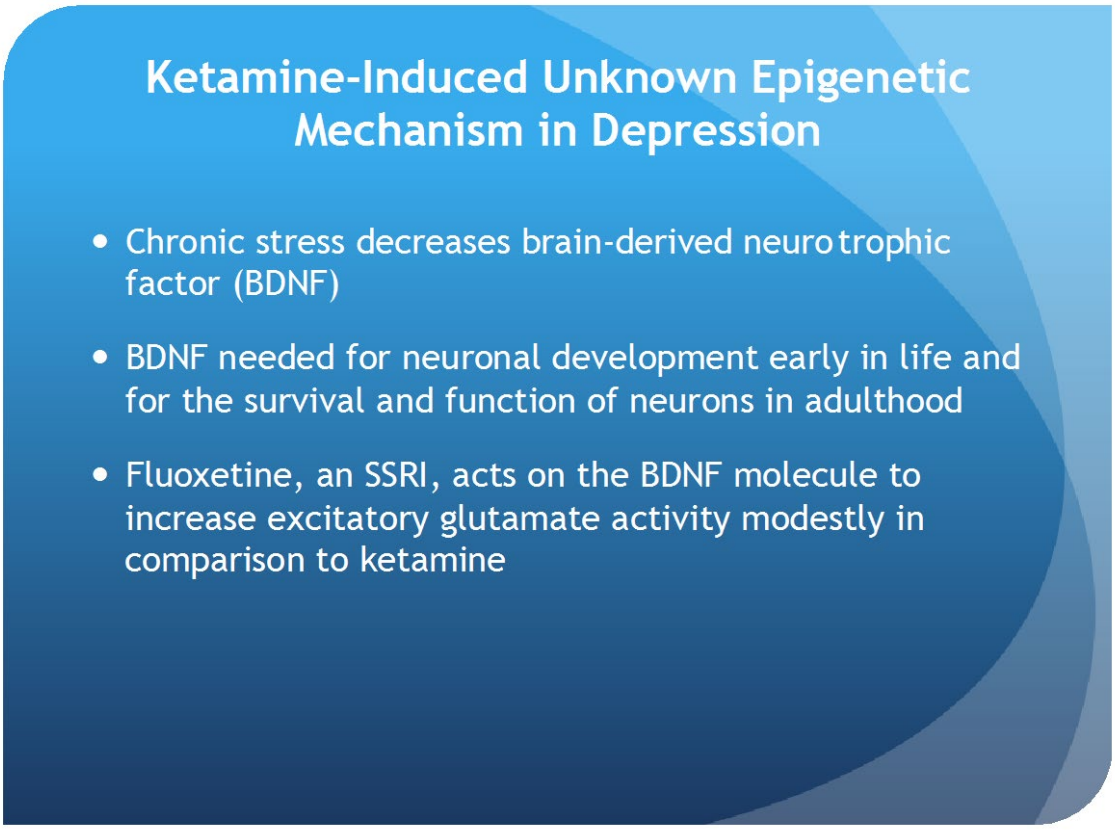

Conceptual Model
DEPRESSION
GENETICS
ANESTHESIA
CARCINOGENICITY
N-METHYL D-ASPARTATE
INFANT BRAIN RE-PROGRAMMING
EPIGENETICS
KETAMINE
ENVIRONMENT
GLUTAMATE
LEARNING/ MEMORY
PAIN
MALIGNANT HYPERTHERMA

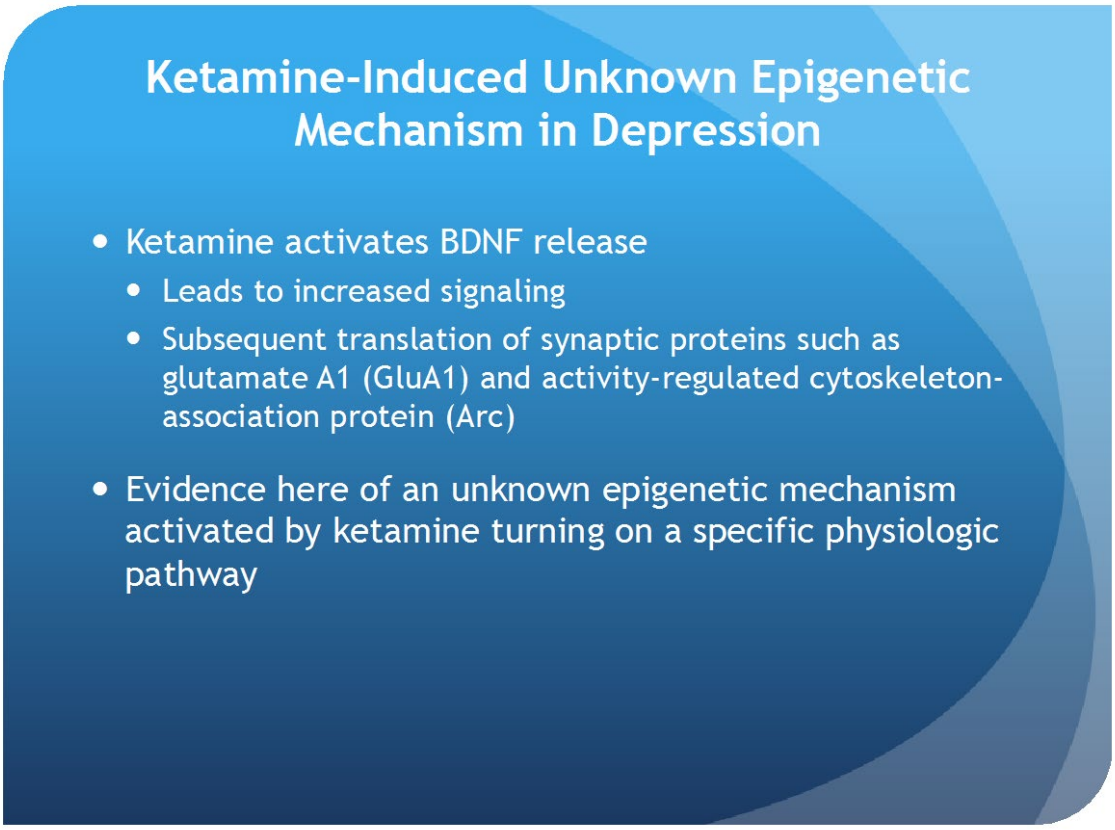

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
• Ketamine (synthetic NMDA) acts on the NMDA receptor (NMDAR) as an agonist
• Ketamine used to induce general anesthesia and to treat pain
• Glutamate and aspartate are excitatory neurotransmitters
• They engage the NMDA receptor (NMDAR)
• So does, D-serine (possibly through phosphorylation), which acts as a co-agonist thereby increasing neurotransmitter excitation

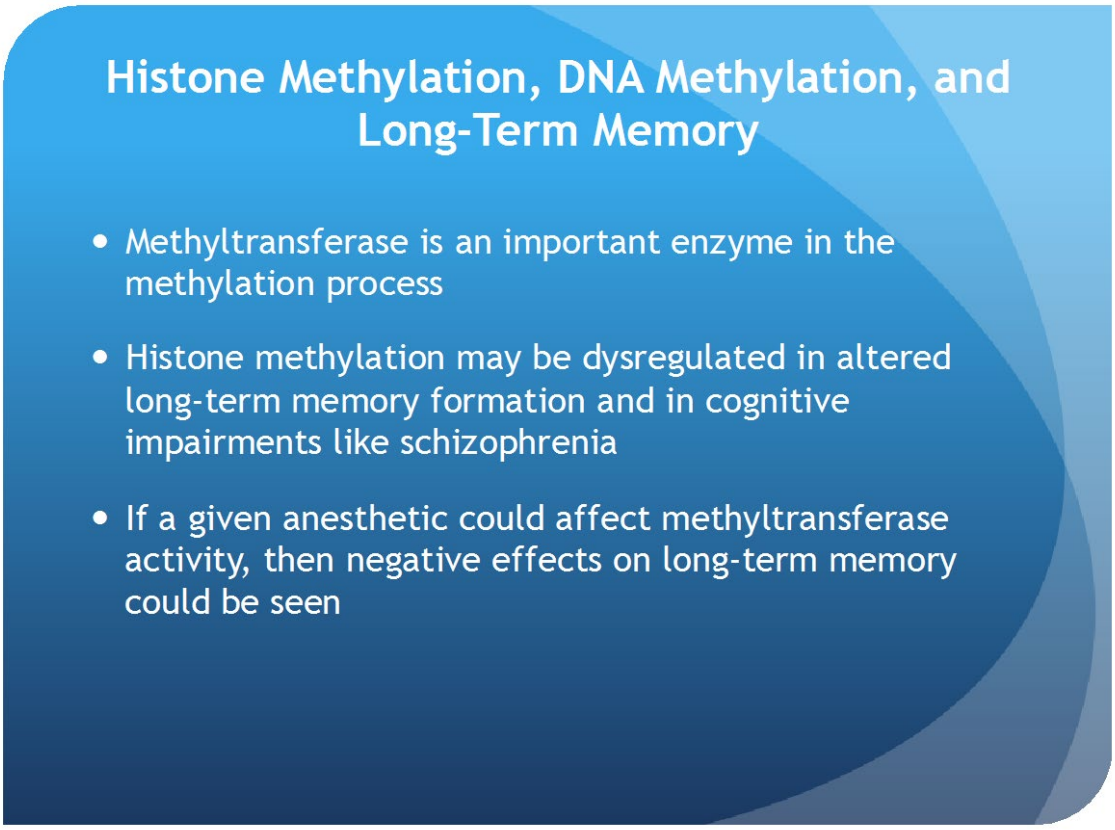

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
NMDAR involved in cellular membrane depolarization
rapidly increases permeability to magnesium and calcium
linked to synaptic plasticity of neurons
affects how organisms learn and memory development

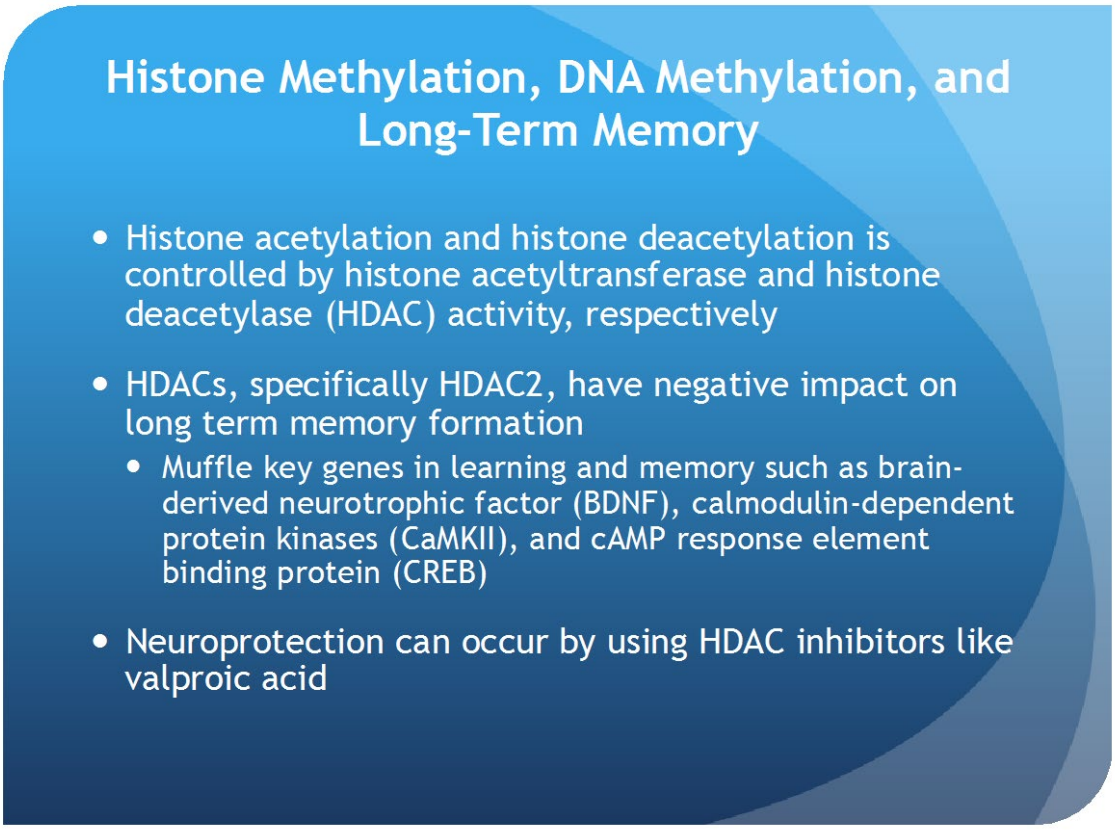

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
Depression characterized by:
pleasure loss
decreased cognition and memory
sleeping/eating/ambulation/sexual disturbances
In depression we see:
atrophy of the neuron
decreased neuron # in the brain's cortex and limbic regions
slowed neurotransmission between/across neurons

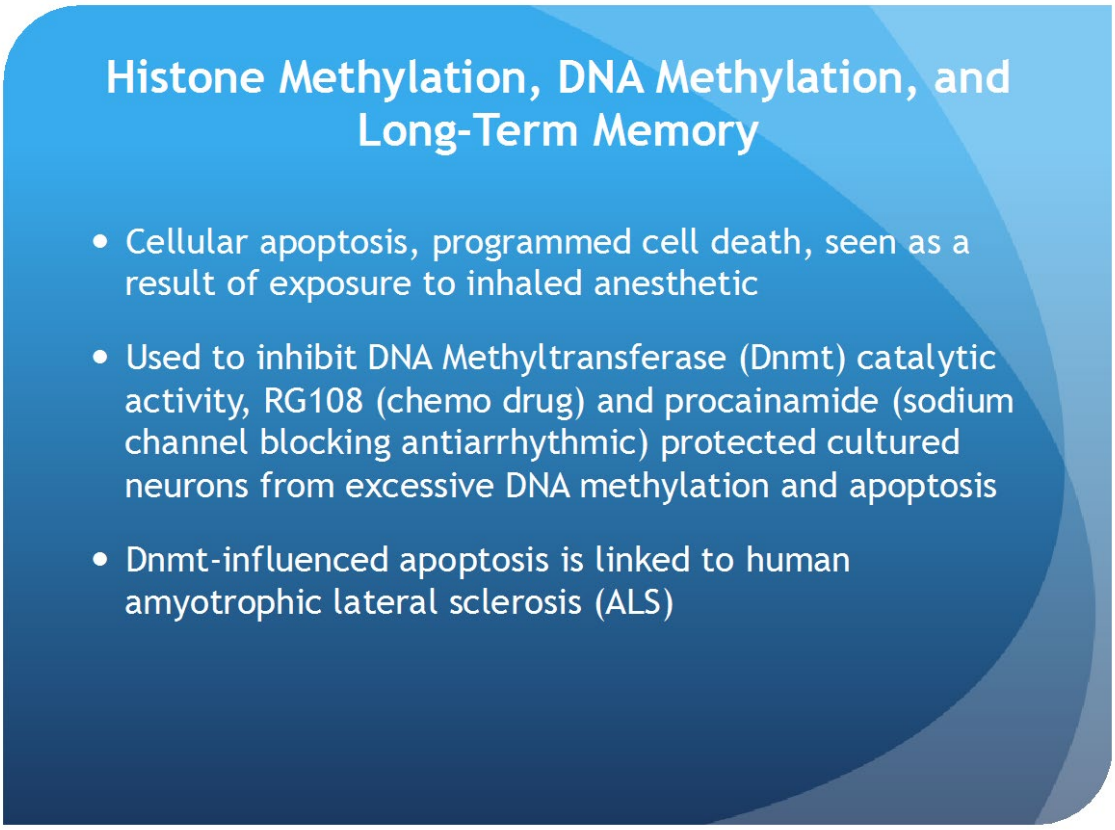

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
Antidepressants, like selective serotonin reuptake inhibitors (SSRI's), flood the synapse with neurotransmitter to aid in neuronal communication and to treat symptoms of depression
Effects of SSRIs take weeks to months to materialize and often only lead to moderate symptom relief
Ketamine promising and effective antidepressant
Promotes rapid synaptogenesis
Reverses atrophy caused by chronic stress, a known depressive factor, in rodents
Reversal of depressive symptoms via a single dose of ketamine
seen within as little as a few hours in treatment-resistant patients
Lasting 7-10 days

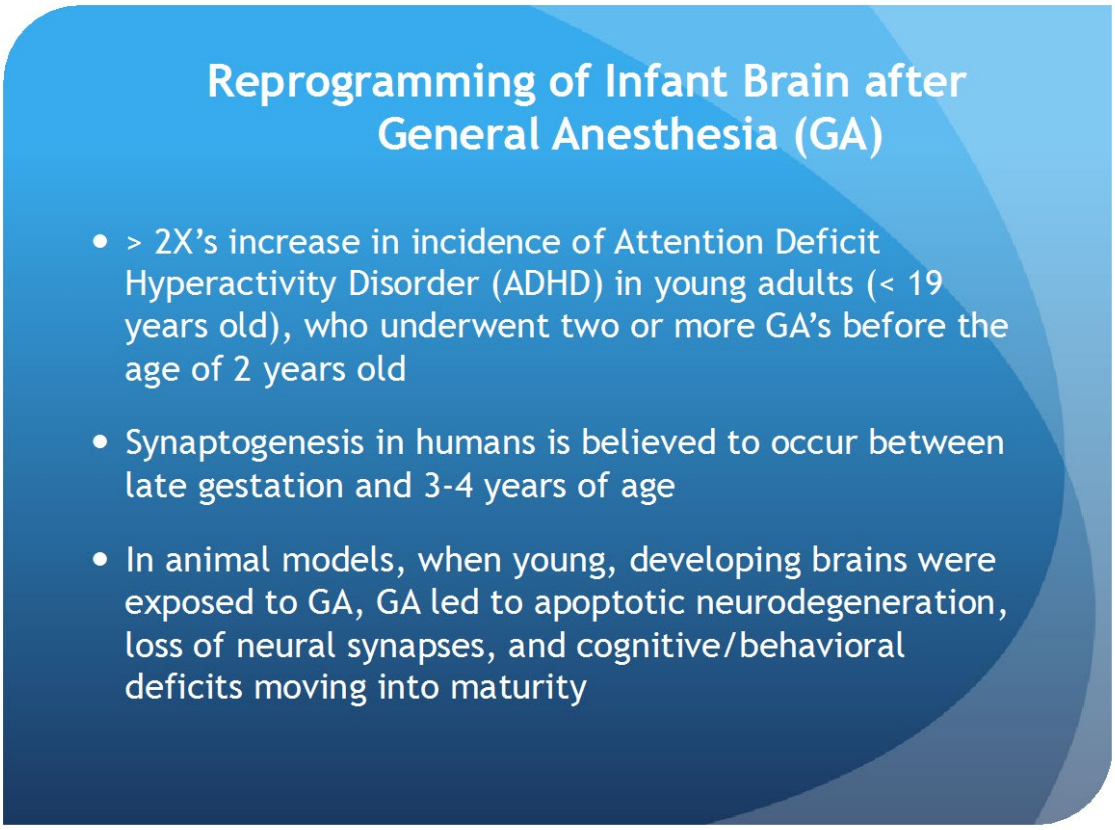

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
Chronic stress decreases brain-derived neuro trophic factor (BDNF)
BDNF needed for neuronal development early in life and for the survival and function of neurons in adulthood
Fluoxetine, an SSRI, acts on the BDNF molecule to increase excitatory glutamate activity modestly in comparison to ketamine

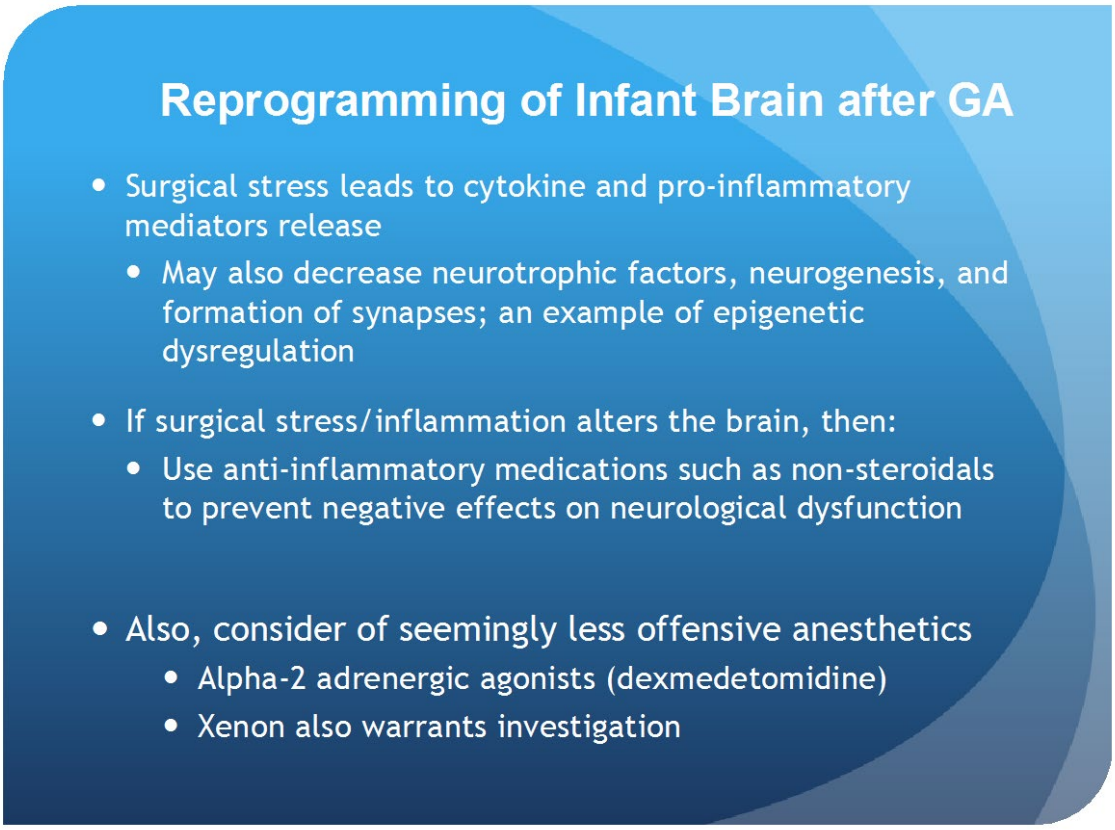

Ketamine-Induced Unknown Epigenetic Mechanism in Depression
Ketamine activates BDNF release
Leads to increased signaling
Subsequent translation of synaptic proteins such as glutamate A1 (GluA1) and activity-regulated cytoskeleton-association protein (Arc)
Evidence here of an unknown epigenetic mechanism activated by ketamine turning on a specific physiologic pathway

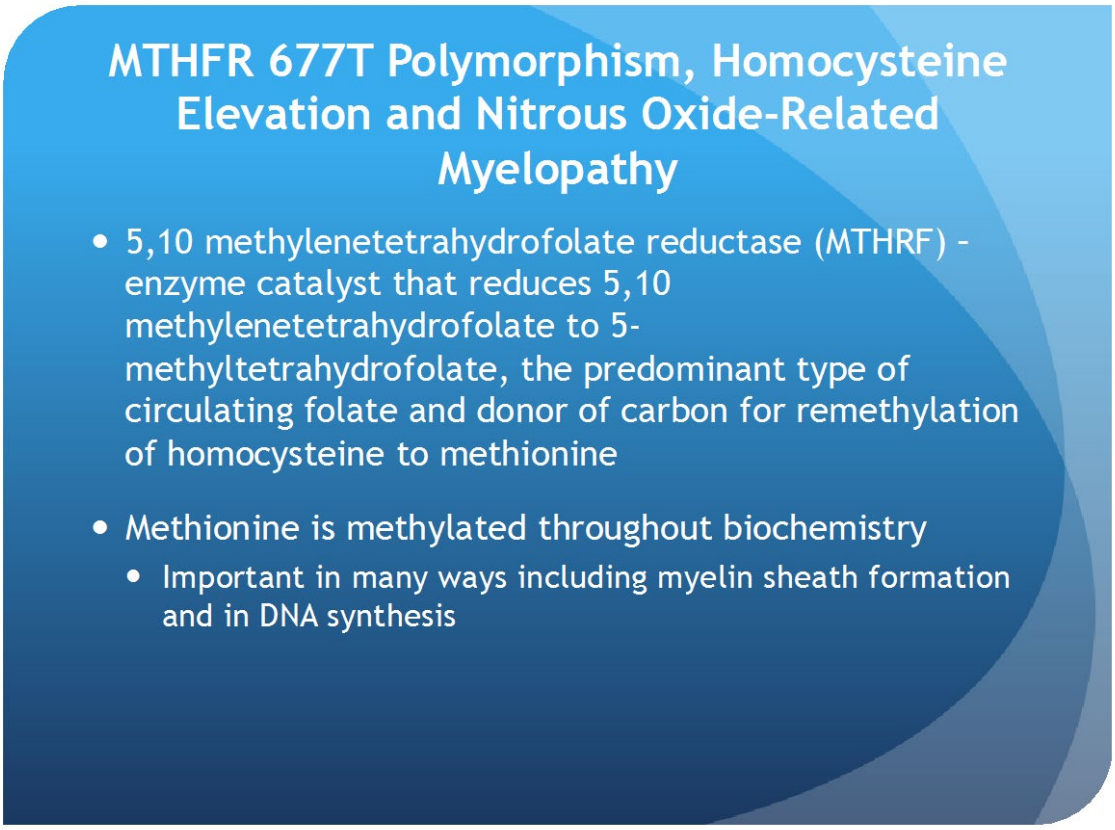

Histone Methylation, DNA Methylation, and Long-Term Memory
Methyltransferase is an important enzyme in the methylation process
Histone methylation may be dysregulated in altered long-term memory formation and in cognitive impairments like schizophrenia
If a given anesthetic could affect methyltransferase activity, then negative effects on long-term memory could be seen

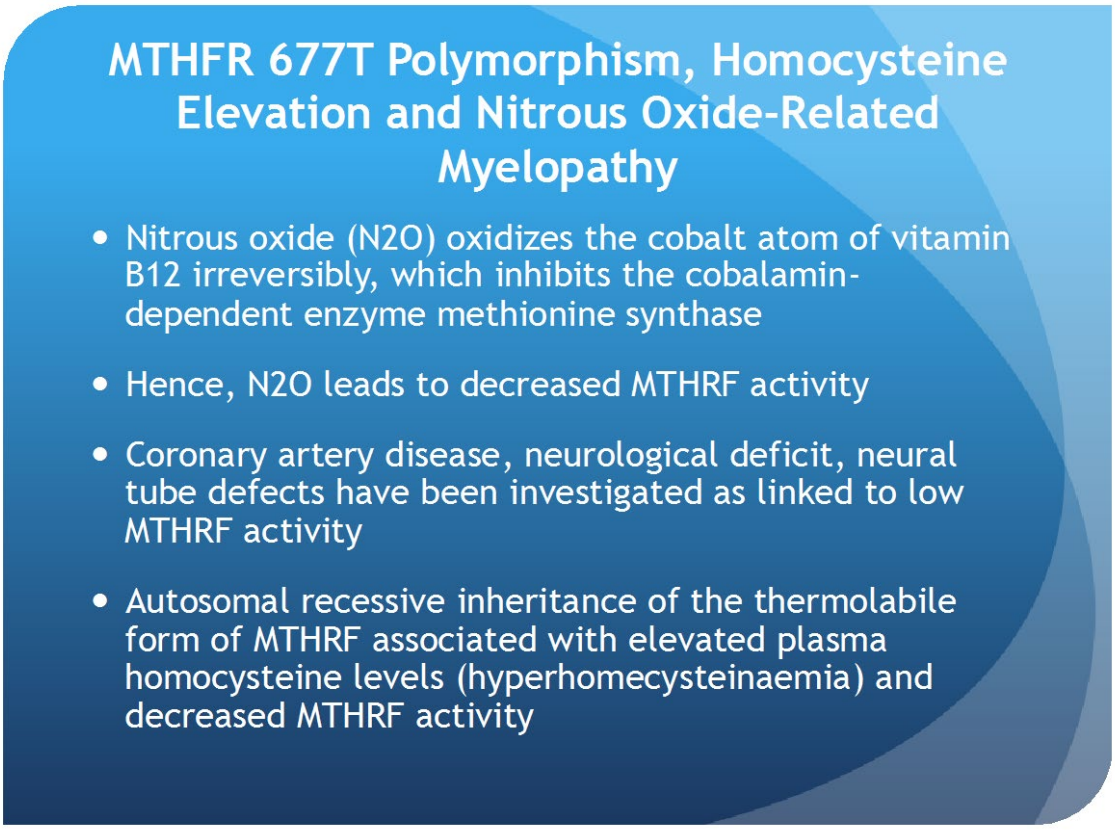

Histone Methylation, DNA Methylation, and Long-Term Memory
Histone acetylation and histone deacetylation is controlled by histone acetyltransferase and histone deacetylase (HDAC) activity, respectively
HDACs, specifically HDAC2, have negative impact on long term memory formation
Muffle key genes in learning and memory such as brain-derived neurotrophic factor (BDNF), calmodulin-dependent protein kinases (CaMKII), and cAMP response element binding protein (CREB)
Neuroprotection can occur by using HDAC inhibitors like valproic acid

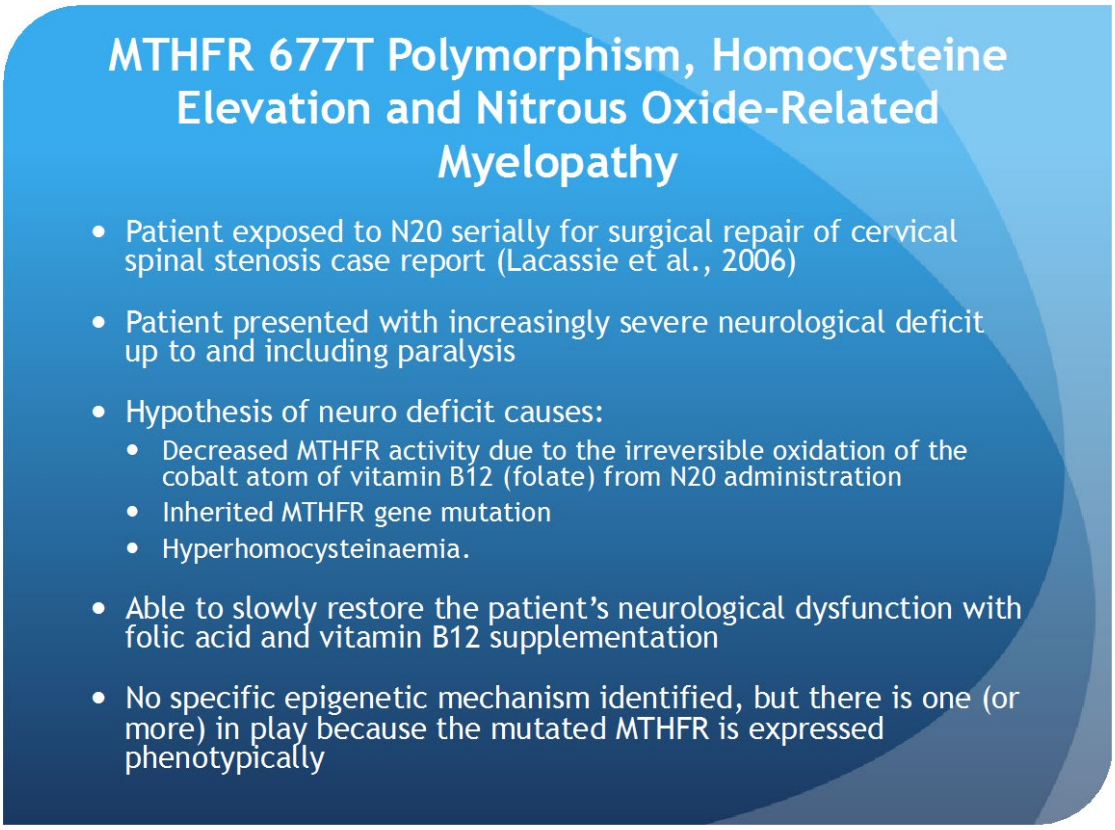

Histone Methylation, DNA Methylation, and Long-Term Memory
• Cellular apoptosis, programmed cell death, seen as a result of exposure to inhaled anesthetic
• Used to inhibit DNA Methyltransferase (Dnmt) catalytic activity, RG108 (chemo drug) and procainamide (sodium channel blocking antiarrhythmic) protected cultured neurons from excessive DNA methylation and apoptosis
• Dnmt-influenced apoptosis is linked to human amyotrophic lateral sclerosis (ALS)

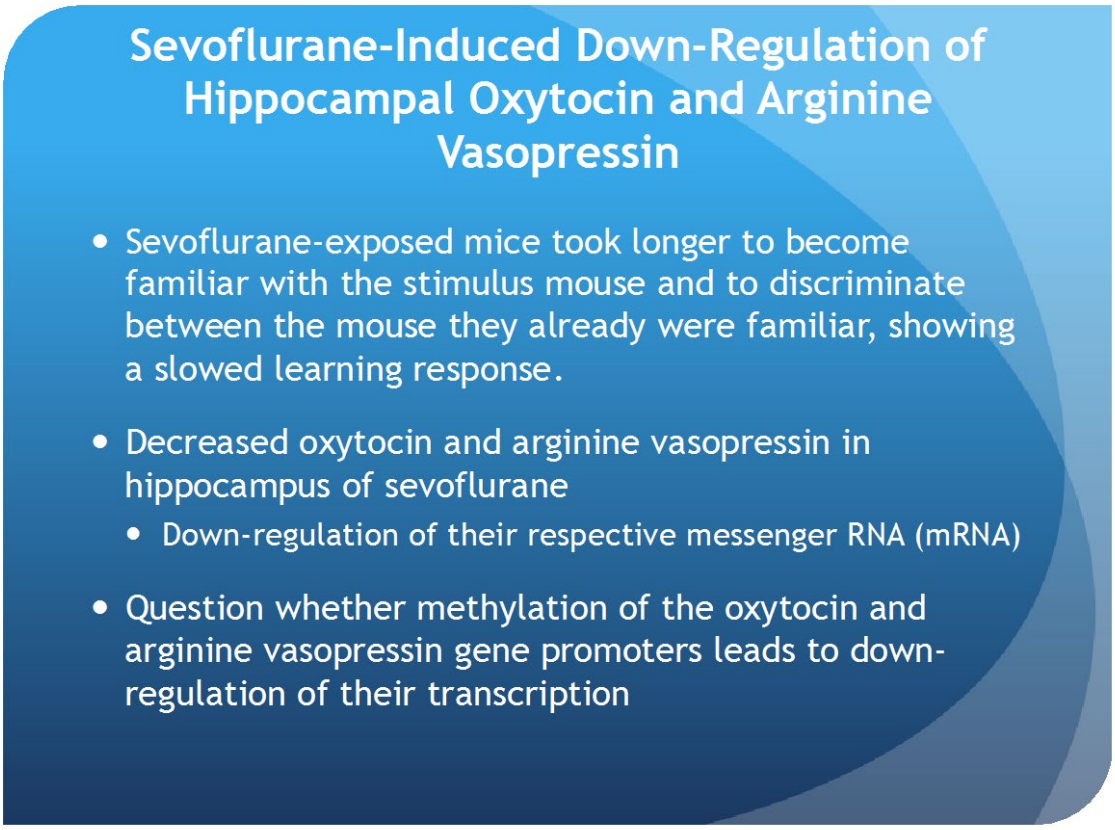

Reprogramming of Infant Brain after General Anesthesia (GA)
• > 2X's increase in incidence of Attention Deficit Hyperactivity Disorder (ADHD) in young adults (< 19 years old), who underwent two or more GA's before the age of 2 years old
• Synaptogenesis in humans is believed to occur between late gestation and 3-4 years of age
• In animal models, when young, developing brains were exposed to GA, GA led to apoptotic neurodegeneration, loss of neural synapses, and cognitive/behavioral deficits moving into maturity

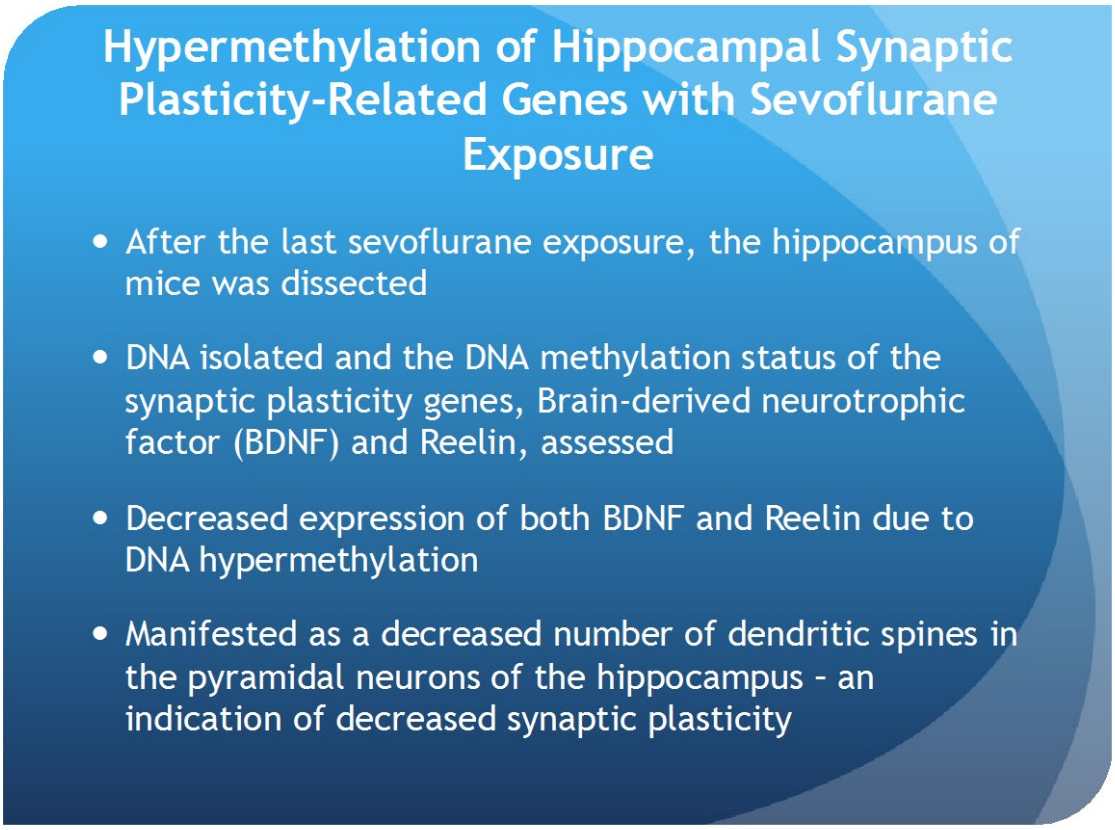

Reprogramming of Infant Brain after GA
Surgical stress leads to cytokine and pro-inflammatory mediators release
May also decrease neurotrophic factors, neurogenesis, and formation of synapses; an example of epigenetic dysregulation
If surgical stress/inflammation alters the brain, then:
Use anti-inflammatory medications such as non-steroidals to prevent negative effects on neurological dysfunction
Also, consider of seemingly less offensive anesthetics
Alpha-2 adrenergic agonists (dexmedetomidine)
Xenon also warrants investigation

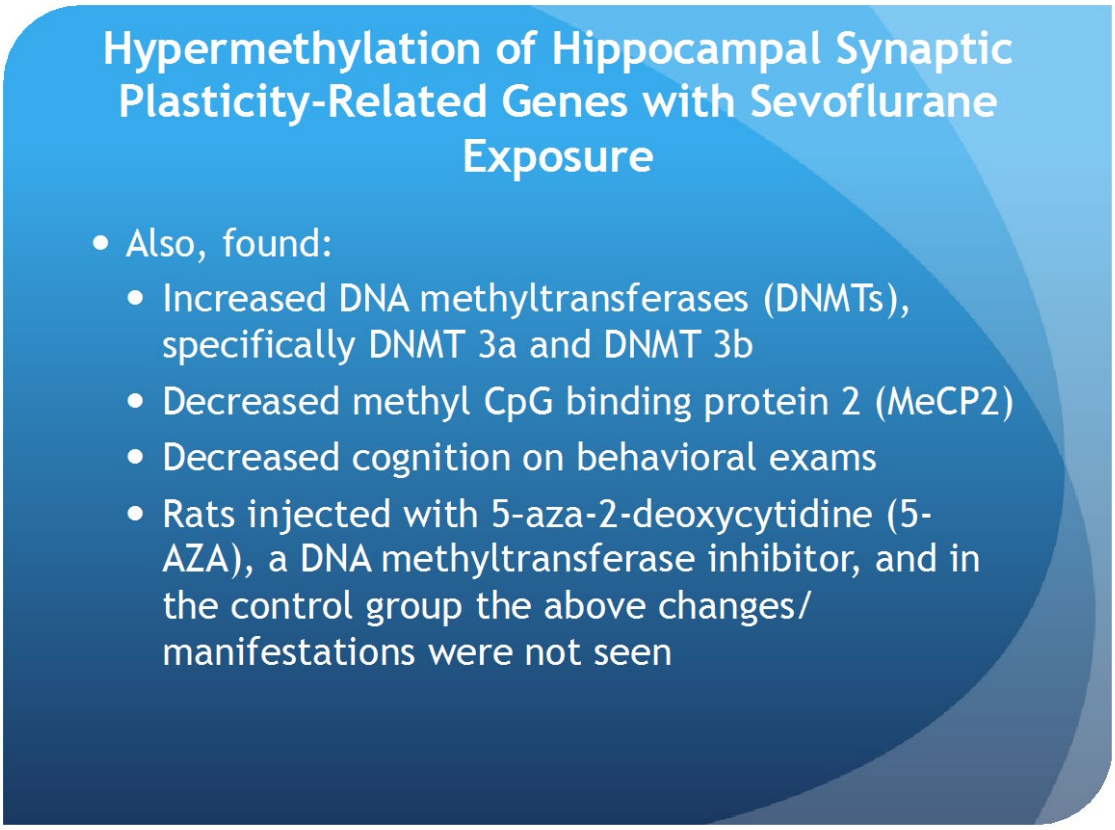

MTHFR 677T Polymorphism, Homocysteine Elevation and Nitrous Oxide-Related Myelopathy
• 5,10 methylenetetrahydrofolate reductase (MTHRF) - enzyme catalyst that reduces 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant type of circulating folate and donor of carbon for remethylation of homocysteine to methionine
• Methionine is methylated throughout biochemistry
• Important in many ways including myelin sheath formation and in DNA synthesis

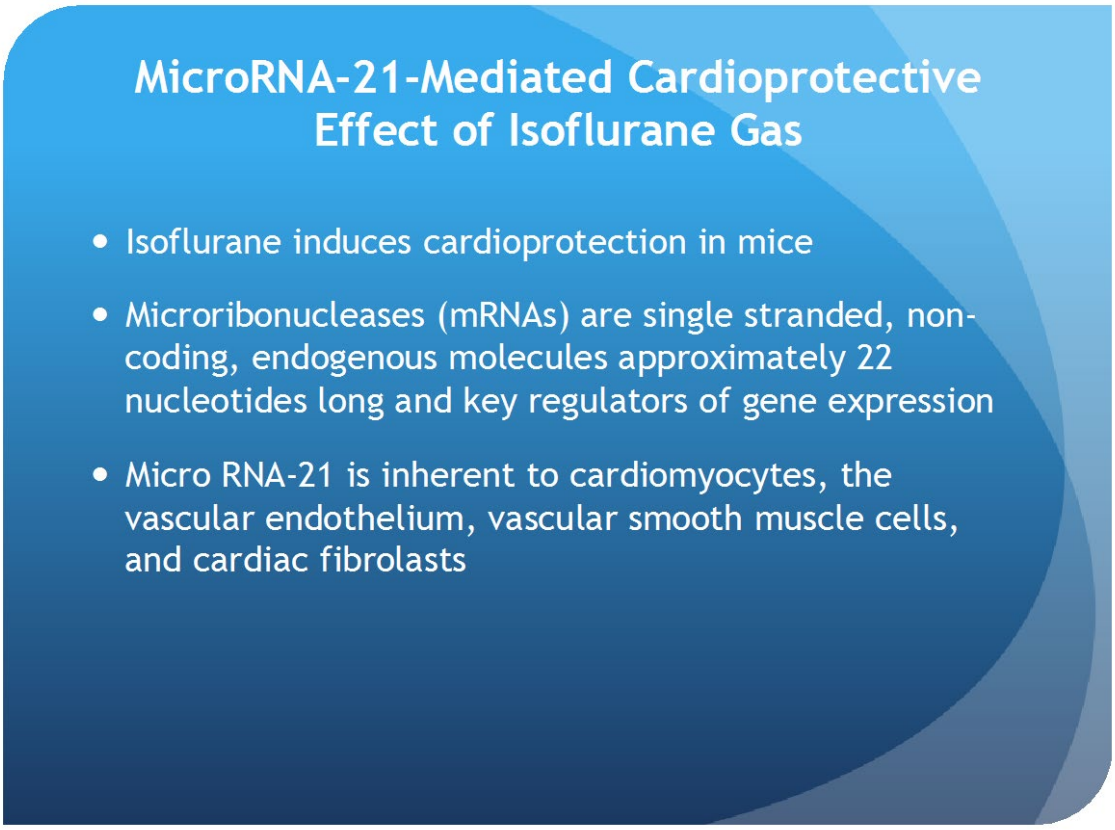

MTHFR 677T Polymorphism, Homocysteine Elevation and Nitrous Oxide-Related Myelopathy
• Nitrous oxide (N2O) oxidizes the cobalt atom of vitamin B12 irreversibly, which inhibits the cobalamin-dependent enzyme methionine synthase
• Hence, N2O leads to decreased MTHRF activity
• Coronary artery disease, neurological deficit, neural tube defects have been investigated as linked to low MTHRF activity
• Autosomal recessive inheritance of the thermolabile form of MTHRF associated with elevated plasma homocysteine levels (hyperhomecysteinaemia) and decreased MTHRF activity

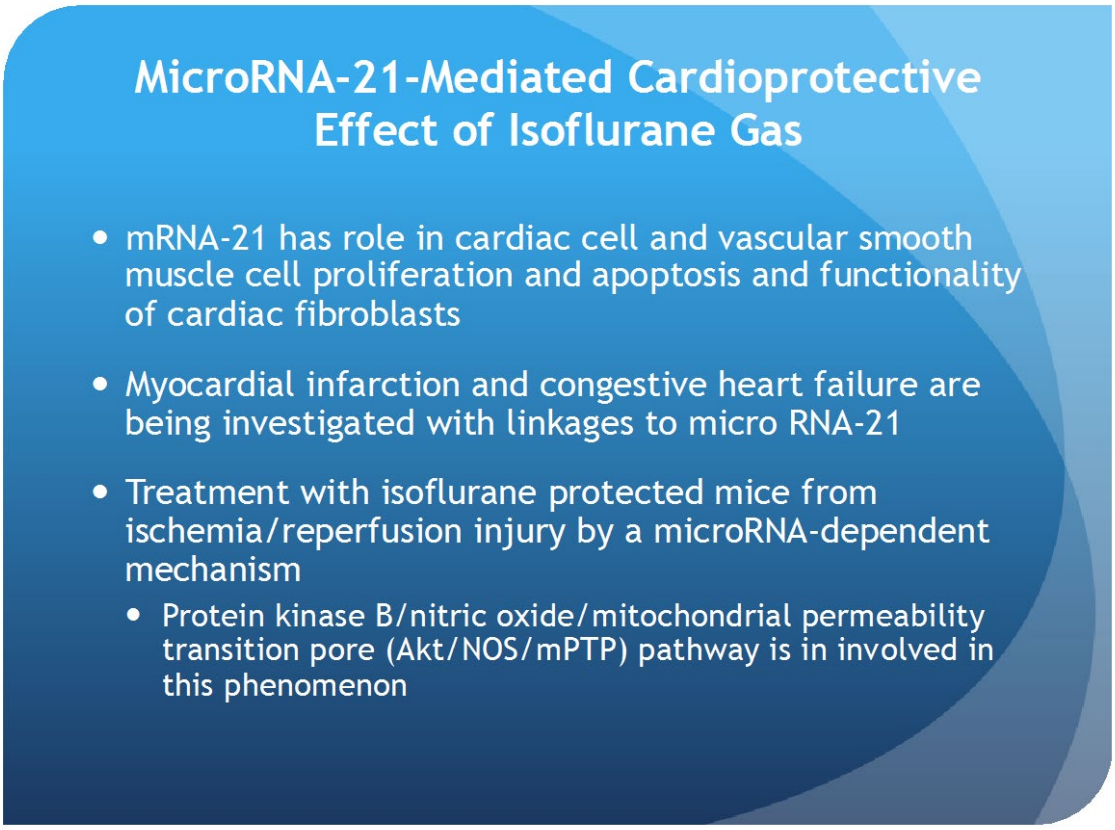

MTHFR 677T Polymorphism, Homocysteine Elevation and Nitrous Oxide-Related Myelopathy
Patient exposed to N20 serially for surgical repair of cervical spinal stenosis case report (Lacassie et al., 2006)
Patient presented with increasingly severe neurological deficit up to and including paralysis
Hypothesis of neuro deficit causes:
Decreased MTHFR activity due to the irreversible oxidation of the cobalt atom of vitamin B12 (folate) from N20 administration
Inherited MTHFR gene mutation
Hyperhomocysteinaemia.
Able to slowly restore the patient's neurological dysfunction with folic acid and vitamin B12 supplementation
No specific epigenetic mechanism identified, but there is one (or more) in play because the mutated MTHFR is expressed phenotypically

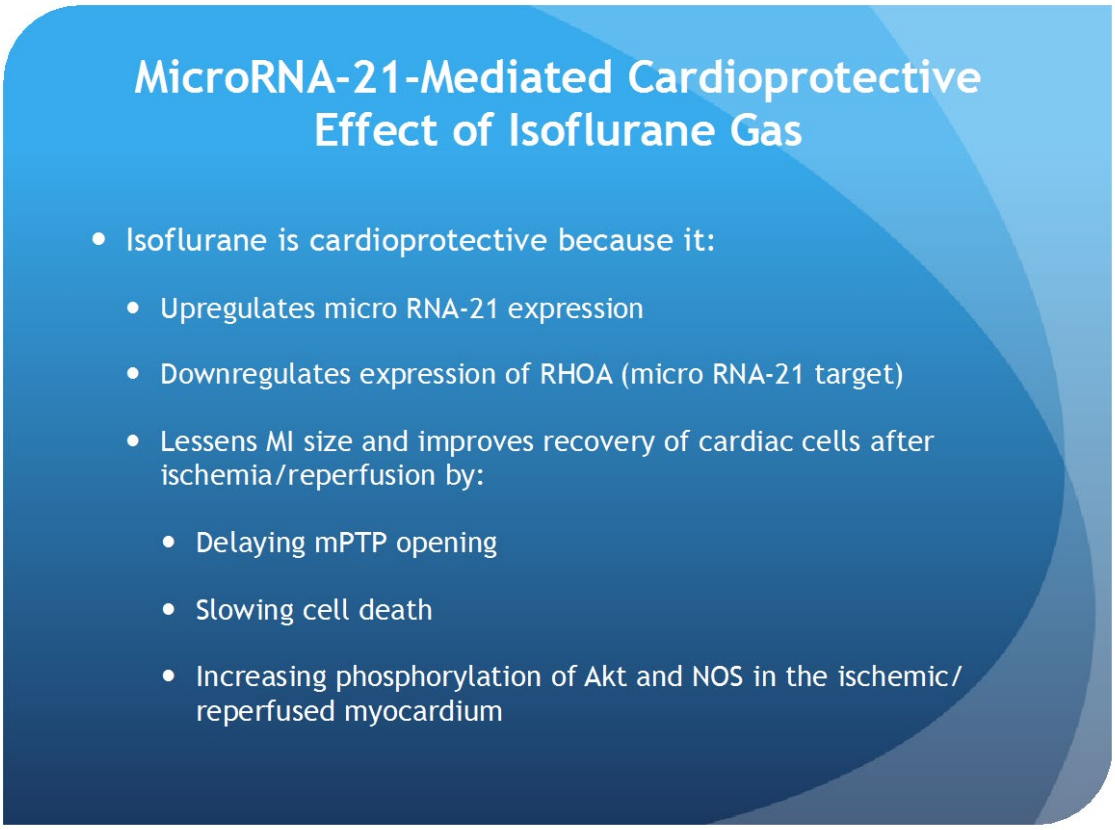

Sevoflurane-Induced Down-Regulation of Hippocampal Oxytocin and Arginine Vasopressin
Sevoflurane-exposed mice took longer to become familiar with the stimulus mouse and to discriminate between the mouse they already were familiar, showing a slowed learning response.
Decreased oxytocin and arginine vasopressin in hippocampus of sevoflurane
Down-regulation of their respective messenger RNA (mRNA)
Question whether methylation of the oxytocin and arginine vasopressin gene promoters leads to down-regulation of their transcription

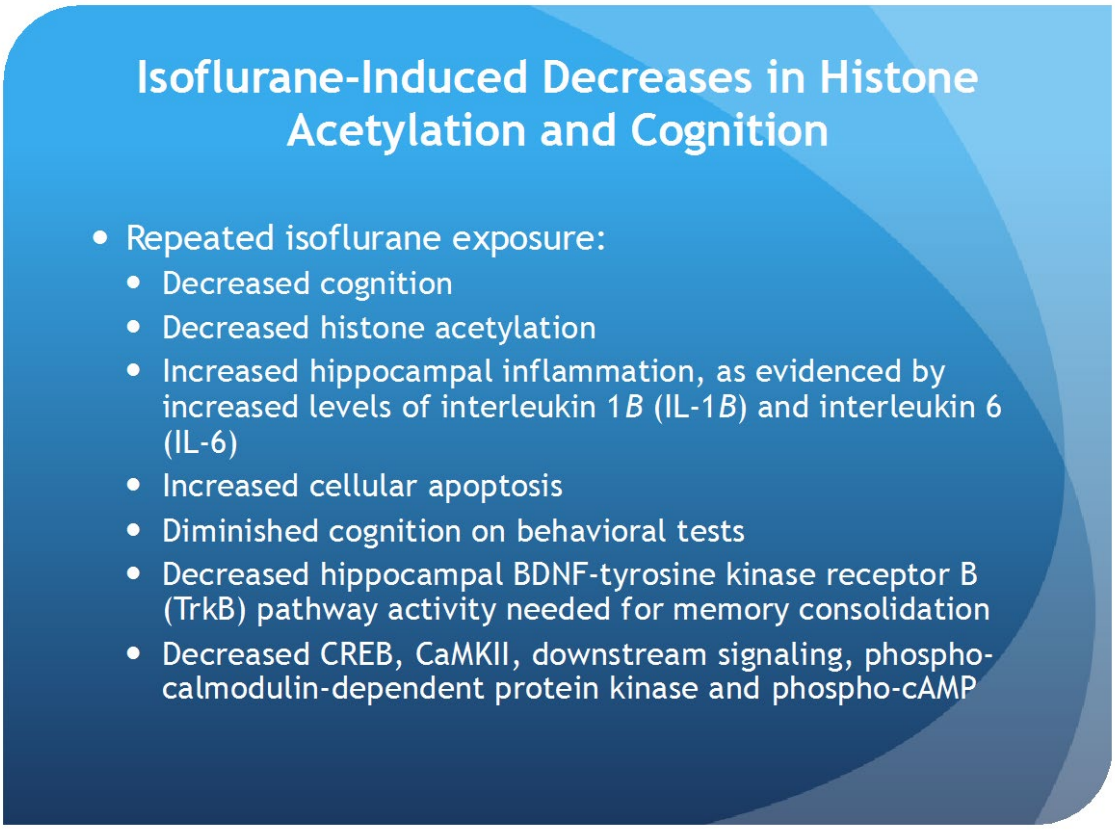

Hypermethylation of Hippocampal Synaptic Plasticity-Related Genes with Sevoflurane Exposure
After the last sevoflurane exposure, the hippocampus of mice was dissected
DNA isolated and the DNA methylation status of the synaptic plasticity genes, Brain-derived neurotrophic factor (BDNF) and Reelin, assessed
Decreased expression of both BDNF and Reelin due to DNA hypermethylation
Manifested as a decreased number of dendritic spines in the pyramidal neurons of the hippocampus - an indication of decreased synaptic plasticity

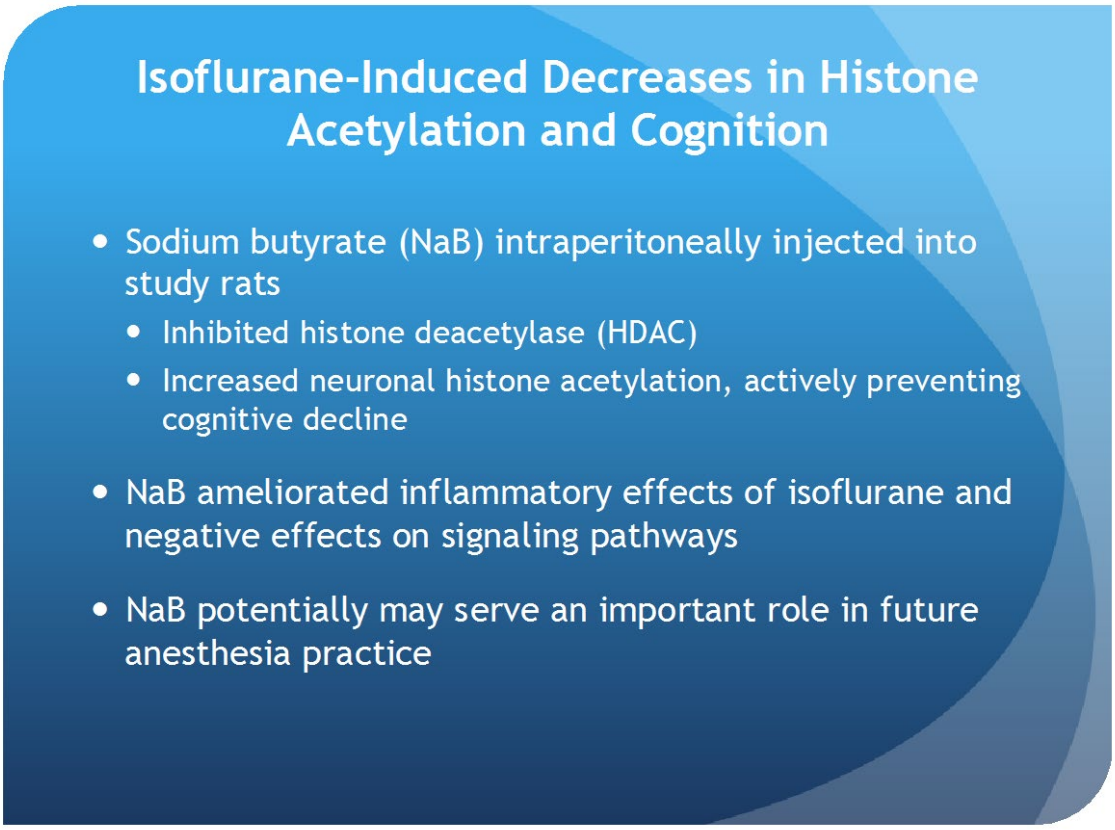

Hypermethylation of Hippocampal Synaptic Plasticity-Related Genes with Sevoflurane Exposure
Also, found:
Increased DNA methyltransferases (DNMTs), specifically DNMT 3a and DNMT 3b
Decreased methyl CpG binding protein 2 (MeCP2)
Decreased cognition on behavioral exams
Rats injected with 5-aza-2-deoxycytidine (5-AZA), a DNA methyltransferase inhibitor, and in the control group the above changes/ manifestations were not seen

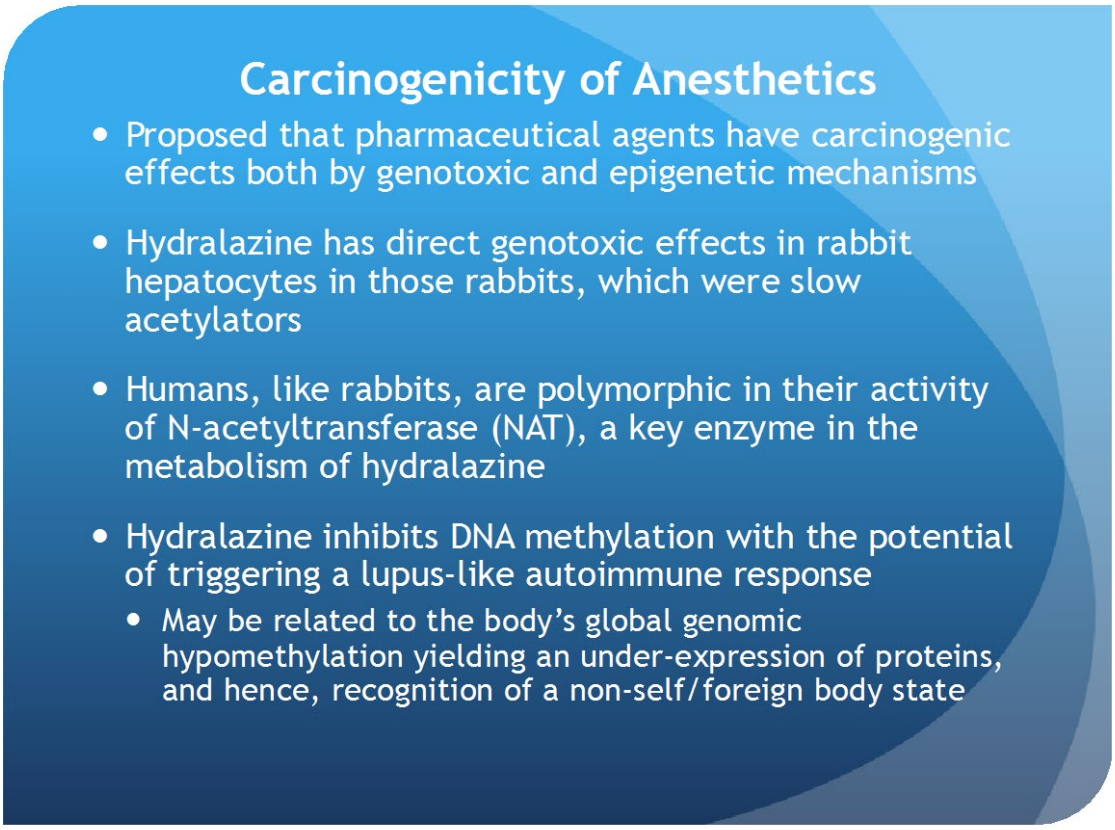

MicroRNA-21-Mediated Cardioprotective Effect of Isoflurane Gas
Isoflurane induces cardioprotection in mice
Microribonucleases (mRNAs) are single stranded, non-coding, endogenous molecules approximately 22 nucleotides long and key regulators of gene expression
Micro RNA-21 is inherent to cardiomyocytes, the vascular endothelium, vascular smooth muscle cells, and cardiac fibrolasts

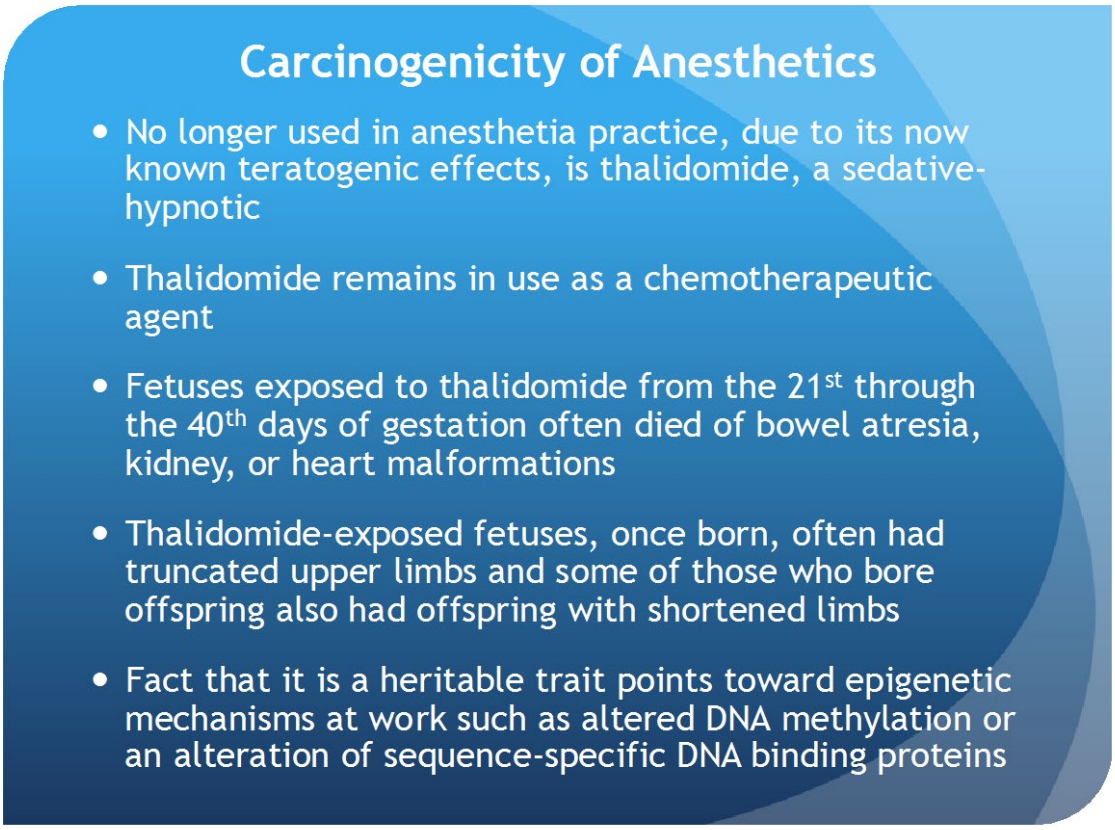

MicroRNA-21-Mediated Cardioprotective Effect of Isoflurane Gas
mRNA-21 has role in cardiac cell and vascular smooth muscle cell proliferation and apoptosis and functionality of cardiac fibroblasts
Myocardial infarction and congestive heart failure are being investigated with linkages to micro RNA-21
Treatment with isoflurane protected mice from ischemia/reperfusion injury by a microRNA-dependent mechanism
Protein kinase B/nitric oxide/mitochondrial permeability transition pore (Akt/NOS/mPTP) pathway is in involved in this phenomenon

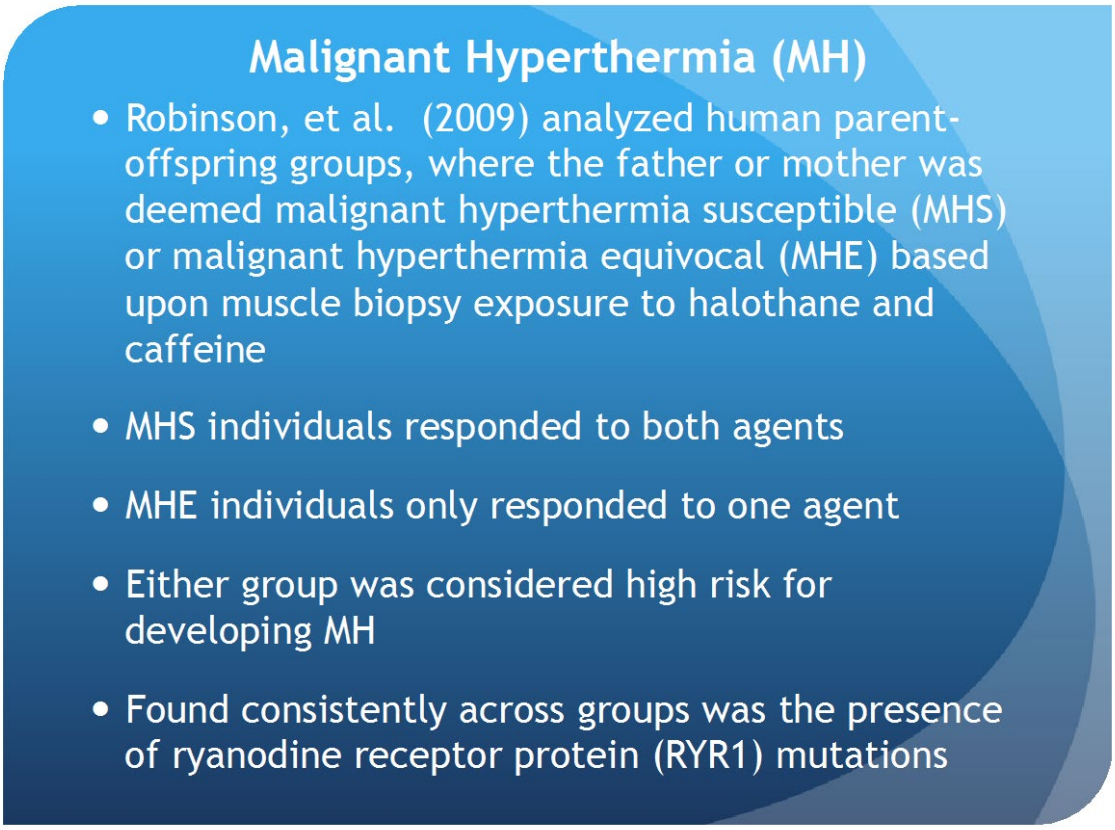

MicroRNA-21-Mediated Cardioprotective Effect of Isoflurane Gas
Isoflurane is cardioprotective because it:
Upregulates micro RNA-21 expression
Downregulates expression of RHOA (micro RNA-21 target)
Lessens MI size and improves recovery of cardiac cells after ischemia/reperfusion by:
Delaying mPTP opening
Slowing cell death
Increasing phosphorylation of Akt and NOS in the ischemic/ reperfused myocardium

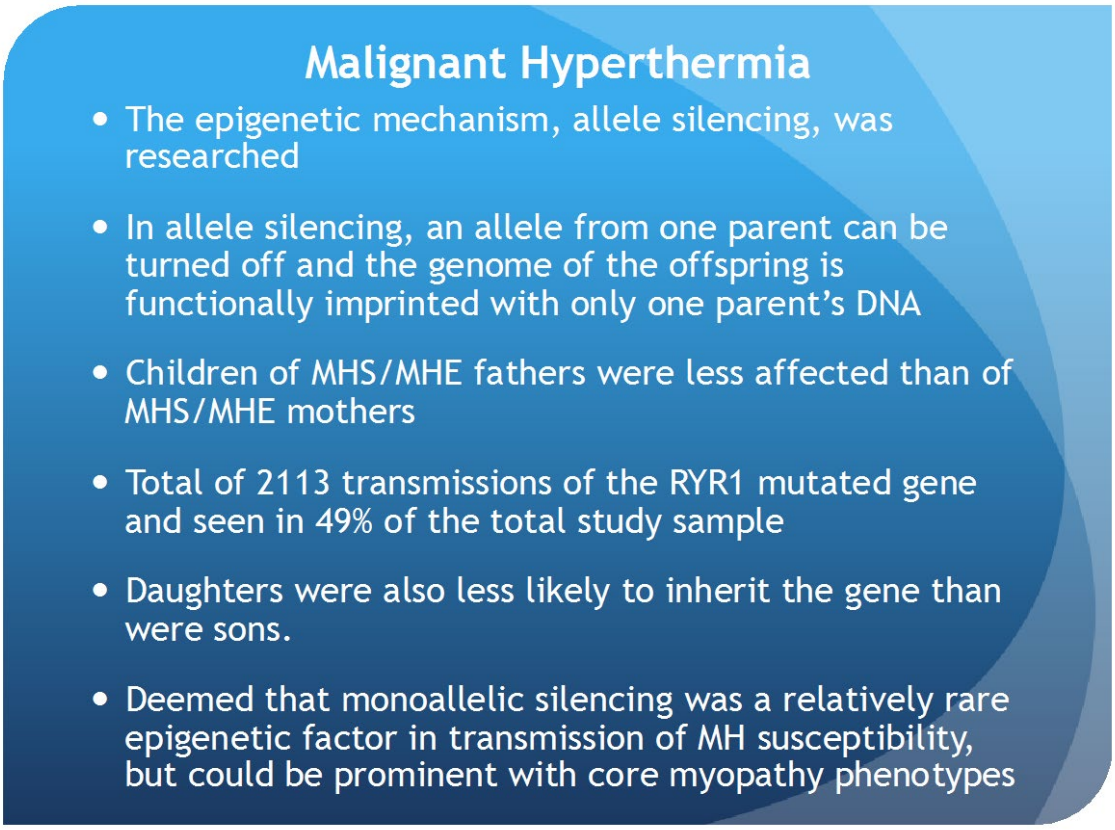

Isoflurane-Induced Decreases in Histone Acetylation and Cognition
Repeated isoflurane exposure:
Decreased cognition
Decreased histone acetylation
Increased hippocampal inflammation, as evidenced by increased levels of interleukin 1B (IL-1B) and interleukin 6 (IL-6)
Increased cellular apoptosis
Diminished cognition on behavioral tests
Decreased hippocampal BDNF-tyrosine kinase receptor B (TrkB) pathway activity needed for memory consolidation
Decreased CREB, CaMKII, downstream signaling, phospho-calmodulin-dependent protein kinase and phospho-cAMP

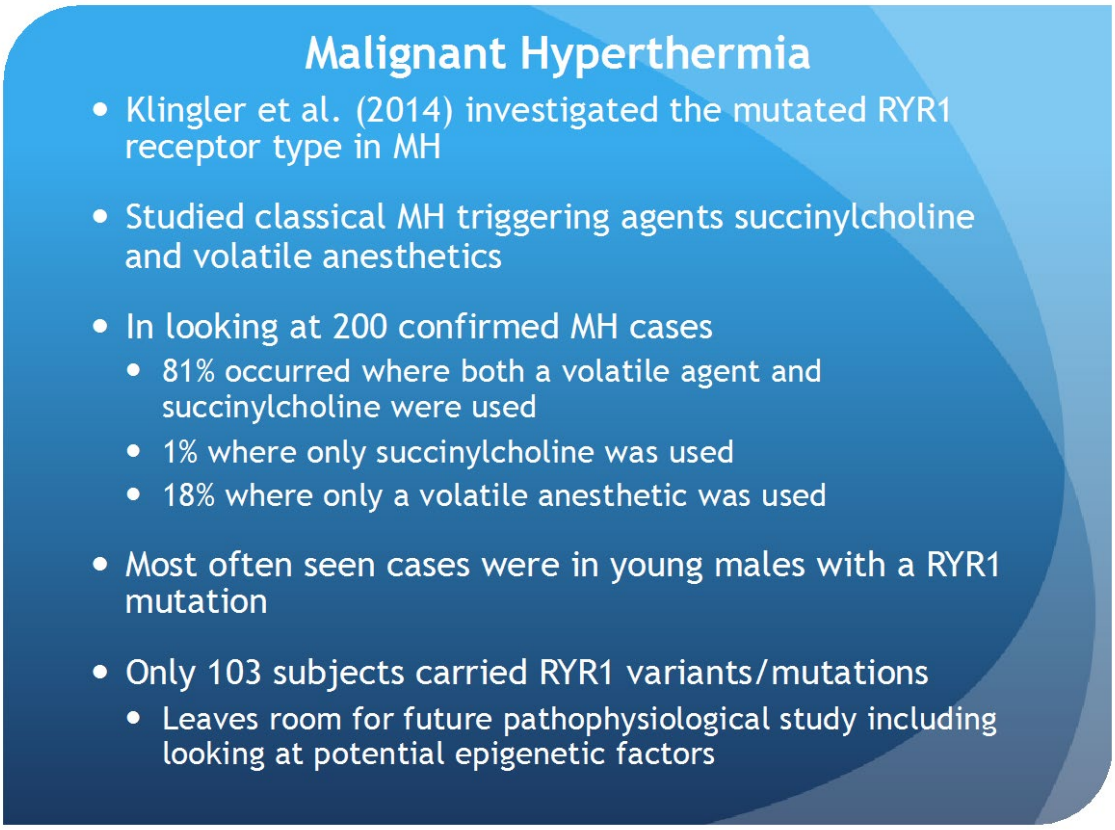

Isoflurane-Induced Decreases in Histone Acetylation and Cognition
Sodium butyrate (NaB) intraperitoneally injected into study rats
Inhibited histone deacetylase (HDAC)
Increased neuronal histone acetylation, actively preventing cognitive decline
NaB ameliorated inflammatory effects of isoflurane and negative effects on signaling pathways
NaB potentially may serve an important role in future anesthesia practice

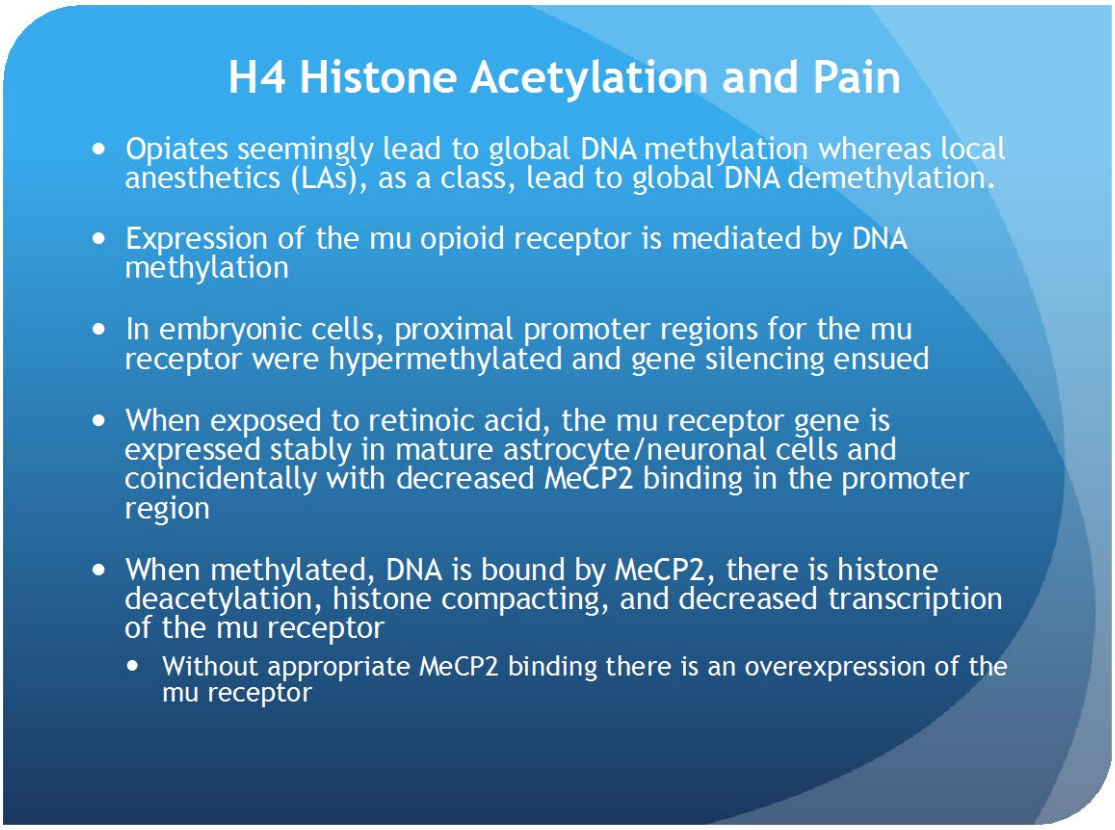

Carcinogenicity of Anesthetics
• Proposed that pharmaceutical agents have carcinogenic effects both by genotoxic and epigenetic mechanisms
• Hydralazine has direct genotoxic effects in rabbit hepatocytes in those rabbits, which were slow acetylators
• Humans, like rabbits, are polymorphic in their activity of N-acetyltransferase (NAT), a key enzyme in the metabolism of hydralazine
• Hydralazine inhibits DNA methylation with the potential of triggering a lupus-like autoimmune response
• May be related to the body's global genomic hypomethylation yielding an under-expression of proteins, and hence, recognition of a non-self/foreign body state


Carcinogenicity of Anesthetics
No longer used in anesthetia practice, due to its now known teratogenic effects, is thalidomide, a sedative-hypnotic
Thalidomide remains in use as a chemotherapeutic agent
Fetuses exposed to thalidomide from the 21st through the 40th days of gestation often died of bowel atresia, kidney, or heart malformations
Thalidomide-exposed fetuses, once born, often had truncated upper limbs and some of those who bore offspring also had offspring with shortened limbs
Fact that it is a heritable trait points toward epigenetic mechanisms at work such as altered DNA methylation or an alteration of sequence-specific DNA binding proteins


Malignant Hyperthermia (MH)
• Robinson, et al. (2009) analyzed human parent-offspring groups, where the father or mother was deemed malignant hyperthermia susceptible (MHS) or malignant hyperthermia equivocal (MHE) based upon muscle biopsy exposure to halothane and caffeine
• MHS individuals responded to both agents
• MHE individuals only responded to one agent
• Either group was considered high risk for developing MH
• Found consistently across groups was the presence of ryanodine receptor protein (RYR1) mutations

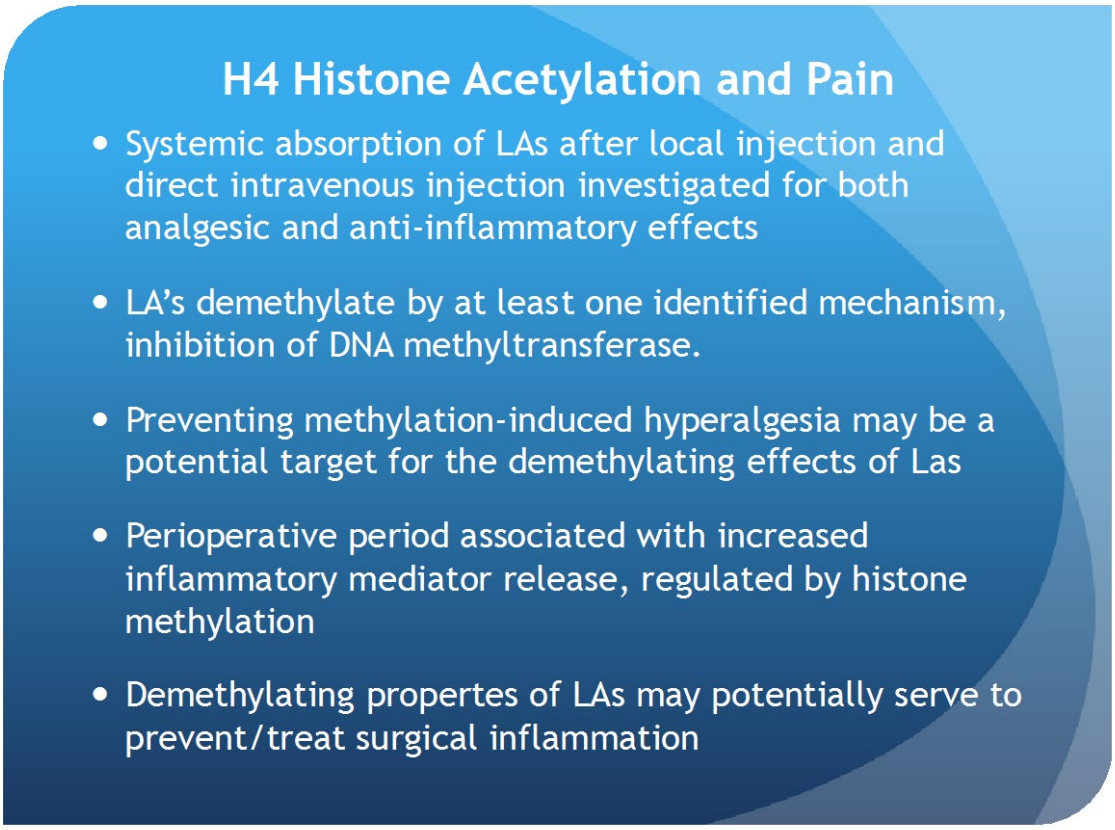

Malignant Hyperthermia
The epigenetic mechanism, allele silencing, was researched
In allele silencing, an allele from one parent can be turned off and the genome of the offspring is functionally imprinted with only one parent's DNA
Children of MHS/MHE fathers were less affected than of MHS/MHE mothers
Total of 2113 transmissions of the RYR1 mutated gene and seen in 49% of the total study sample
Daughters were also less likely to inherit the gene than were sons.
Deemed that monoallelic silencing was a relatively rare epigenetic factor in transmission of MH susceptibility, but could be prominent with core myopathy phenotypes

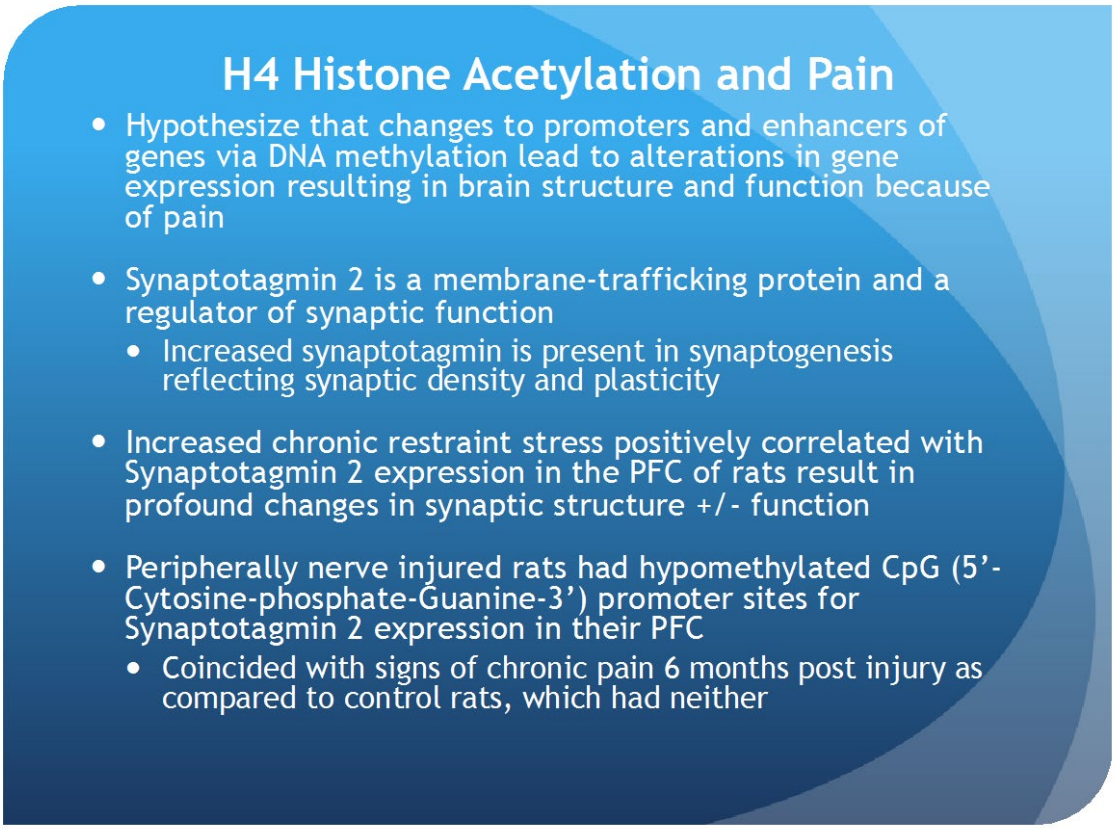

Malignant Hyperthermia
Klingler et al. (2014) investigated the mutated RYR1 receptor type in MH
Studied classical MH triggering agents succinylcholine and volatile anesthetics
In looking at 200 confirmed MH cases
81% occurred where both a volatile agent and succinylcholine were used
1% where only succinylcholine was used
18% where only a volatile anesthetic was used
Most often seen cases were in young males with a RYR1 mutation
Only 103 subjects carried RYR1 variants/mutations
Leaves room for future pathophysiological study including looking at potential epigenetic factors

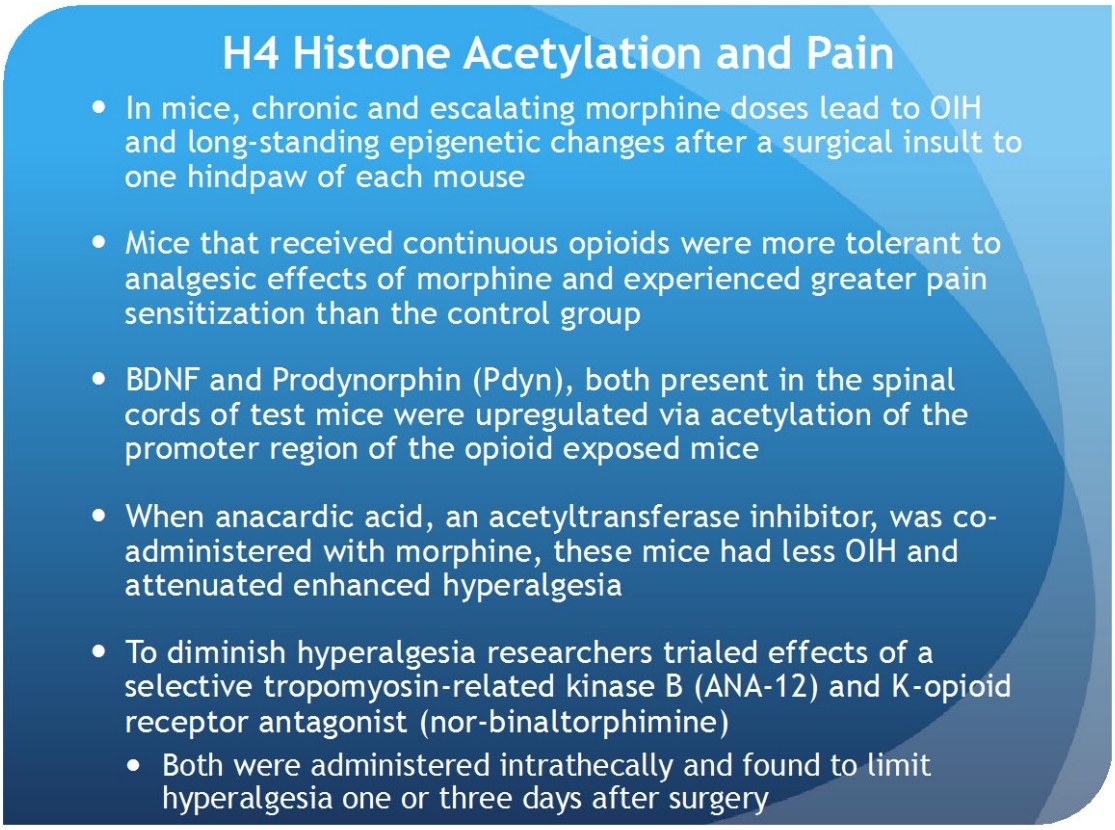

H4 Histone Acetylation and Pain
Opiates seemingly lead to global DNA methylation whereas local anesthetics (LAs), as a class, lead to global DNA demethylation.
Expression of the mu opioid receptor is mediated by DNA methylation
In embryonic cells, proximal promoter regions for the mu receptor were hypermethylated and gene silencing ensued
When exposed to retinoic acid, the mu receptor gene is expressed stably in mature astrocyte/neuronal cells and coincidentally with decreased MeCP2 binding in the promoter region
When methylated, DNA is bound by MeCP2, there is histone deacetylation, histone compacting, and decreased transcription of the mu receptor
Without appropriate MeCP2 binding there is an overexpression of the mu receptor

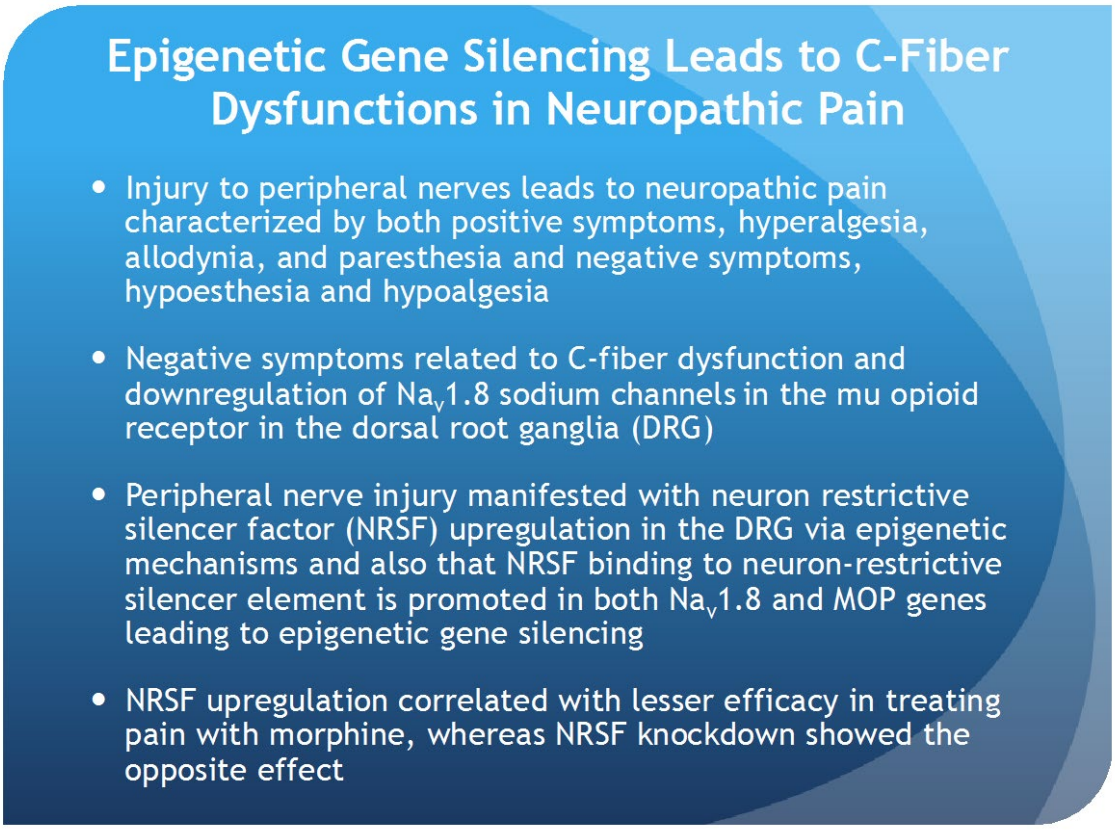
H4 Histone Acetylation and Pain

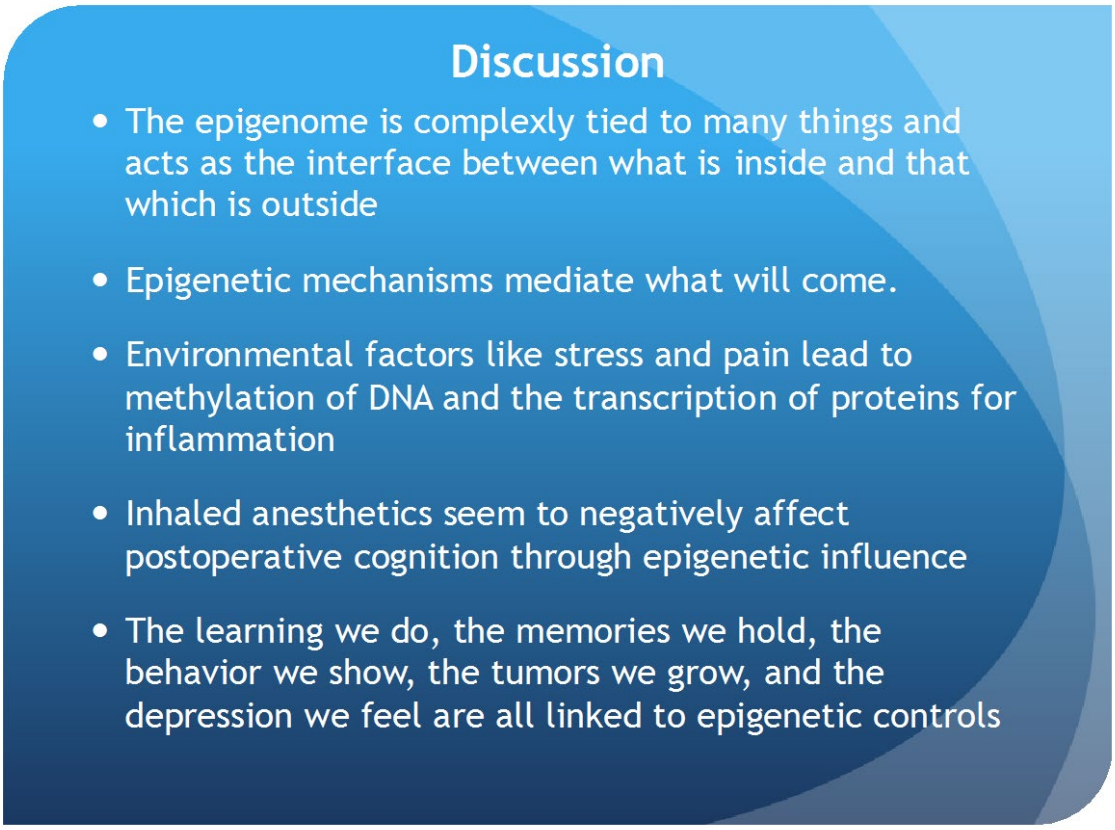
- Negative effects of opiates are nausea, vomiting, respiratory depression, slowed bowel peristalsis, and opioid-induced hyperalgesia (OIH), an excessive sensitivity to pain
- Development of OIH varies with the type of opioid used
- OIH seems worst with Remifentanil and least with buprenorphine, a partial mu receptor agonist and K receptor agonist
- OIH prevented by Ketamine
- Inhibiting histone acetyltransferase when morphine, the model opiate, is present in mice led to less signs of OIH, whereas inhibiting histone deacetylase worsens OIH

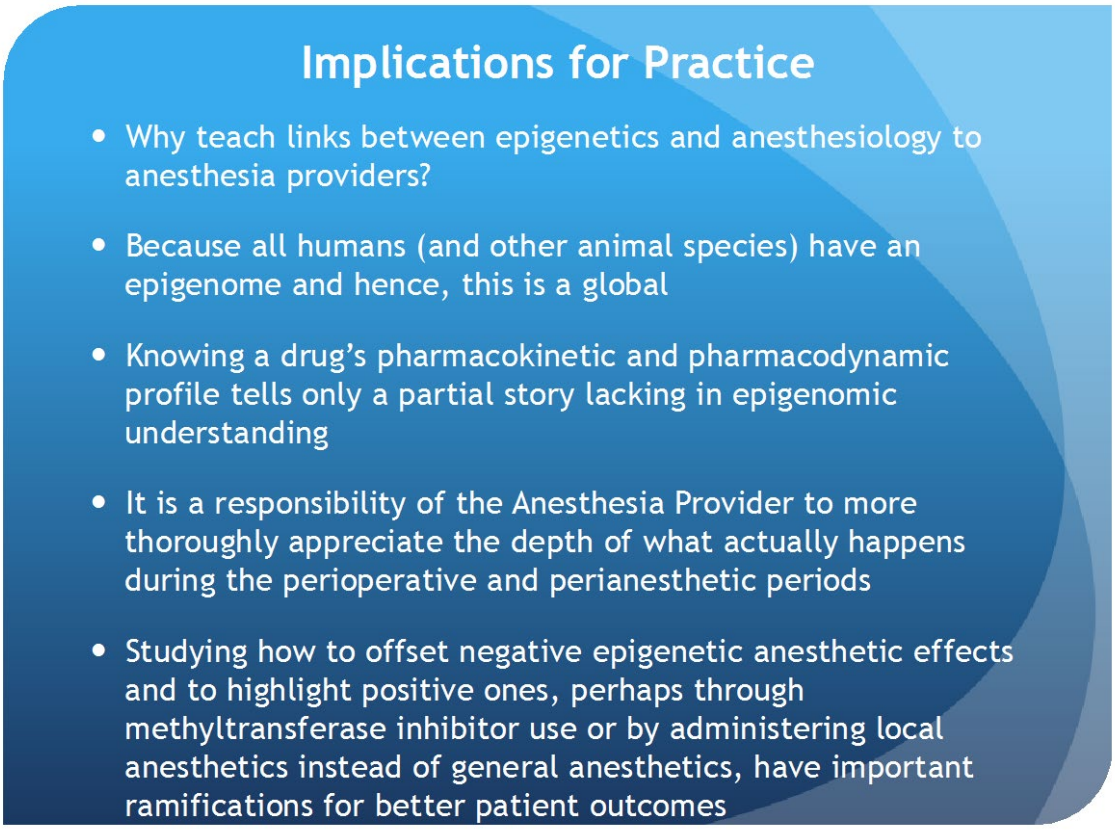

H4 Histone Acetylation and Pain
• Systemic absorption of LAs after local injection and direct intravenous injection investigated for both analgesic and anti-inflammatory effects
• LA's demethylate by at least one identified mechanism, inhibition of DNA methyltransferase.
• Preventing methylation-induced hyperalgesia may be a potential target for the demethylating effects of Las
• Perioperative period associated with increased inflammatory mediator release, regulated by histone methylation
• Demethylating propertes of LAs may potentially serve to prevent/treat surgical inflammation


H4 Histone Acetylation and Pain
Hypothesize that changes to promoters and enhancers of genes via DNA methylation lead to alterations in gene expression resulting in brain structure and function because of pain
Synaptotagmin 2 is a membrane-trafficking protein and a regulator of synaptic function
Increased synaptotagmin is present in synaptogenesis reflecting synaptic density and plasticity
Increased chronic restraint stress positively correlated with Synaptotagmin 2 expression in the PFC of rats result in profound changes in synaptic structure +/- function
Peripherally nerve injured rats had hypomethylated CpG (5'-Cytosine-phosphate-Guanine-3') promoter sites for Synaptotagmin 2 expression in their PFC
Coincided with signs of chronic pain 6 months post injury as compared to control rats, which had neither


H4 Histone Acetylation and Pain
In mice, chronic and escalating morphine doses lead to OIH and long-standing epigenetic changes after a surgical insult to one hindpaw of each mouse
Mice that received continuous opioids were more tolerant to analgesic effects of morphine and experienced greater pain sensitization than the control group
BDNF and Prodynorphin (Pdyn), both present in the spinal cords of test mice were upregulated via acetylation of the promoter region of the opioid exposed mice
When anacardic acid, an acetyltransferase inhibitor, was co-administered with morphine, these mice had less OIH and attenuated enhanced hyperalgesia
To diminish hyperalgesia researchers trialed effects of a selective tropomyosin-related kinase B (ANA-12) and K-opioid receptor antagonist (nor-binaltorphimine)
Both were administered intrathecally and found to limit hyperalgesia one or three days after surgery


Epigenetic Gene Silencing Leads to C-Fiber Dysfunctions in Neuropathic Pain
• Injury to peripheral nerves leads to neuropathic pain characterized by both positive symptoms, hyperalgesia, allodynia, and paresthesia and negative symptoms, hypoesthesia and hypoalgesia
• Negative symptoms related to C-fiber dysfunction and downregulation of $Na_v1.8$ sodium channels in the mu opioid receptor in the dorsal root ganglia (DRG)
• Peripheral nerve injury manifested with neuron restrictive silencer factor (NRSF) upregulation in the DRG via epigenetic mechanisms and also that NRSF binding to neuron-restrictive silencer element is promoted in both $Na_v1.8$ and MOP genes leading to epigenetic gene silencing
• NRSF upregulation correlated with lesser efficacy in treating pain with morphine, whereas NRSF knockdown showed the opposite effect


Discussion
The epigenome is complexly tied to many things and acts as the interface between what is inside and that which is outside
Epigenetic mechanisms mediate what will come.
Environmental factors like stress and pain lead to methylation of DNA and the transcription of proteins for inflammation
Inhaled anesthetics seem to negatively affect postoperative cognition through epigenetic influence
The learning we do, the memories we hold, the behavior we show, the tumors we grow, and the depression we feel are all linked to epigenetic controls


Implications for Practice
Why teach links between epigenetics and anesthesiology to anesthesia providers?
Because all humans (and other animal species) have an epigenome and hence, this is a global
Knowing a drug's pharmacokinetic and pharmacodynamic profile tells only a partial story lacking in epigenomic understanding
It is a responsibility of the Anesthesia Provider to more thoroughly appreciate the depth of what actually happens during the perioperative and perianesthetic periods
Studying how to offset negative epigenetic anesthetic effects and to highlight positive ones, perhaps through methyltransferase inhibitor use or by administering local anesthetics instead of general anesthetics, have important ramifications for better patient outcomes

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