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EPIGENETICS RESEARCH: NEW FINDINGS SHOW HOW LIFE EXPERIENCE INFLUENCES GENES IMPORTANT FOR OBESITY, MEMORY, MENTAL ILLNESS

New findings also find possible therapeutic benefit of changing DNA structure

Washington, DC — New research released today demonstrates how environmental cues — from early childhood neglect to high-fat diets — affect genes important to brain health and development, sometimes into future generations. Moreover, scientists have found that using a drug to modify DNA structure may result in improved cognition for people with memory disorders. The findings were reported at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Building on research conducted over the past decade to decode the human genome, new findings show:

- The stress of early abuse and neglect may mark emotional centers of the brain for life by disrupting the brain's ability to turn genes on. This offers a potential explanation for why adults who were abused when young have higher rates of behavioral problems, mental illness, and substance abuse (Tania Roth, abstract 281.6, see attached summary).
- High-fat diets during pregnancy can predispose two generations of offspring to larger bodies, shedding light on whether obesity can be inherited (Gregory Dunn, abstract 783.4, see attached summary).
- A drug known to keep strands of DNA untangled may improve both short- and long-term memory, suggesting new treatments for people with diseases that affect memory, such as Alzheimer's disease (Liza Leventhal, abstract 831.20, see attached summary).

Other research findings being discussed at the meeting show:

- Changes in genes that shape memory may contribute to mental disorders, from PTSD to schizophrenia to depression (see attached speaker's summary).
- The persistent effect of drug abuse on the packaging of DNA in nerve cells — and thus on an individual's behavioral habits — offers a new model for treating addiction (see attached speaker's summary).

"Brain structure and function are determined by both genes and environment throughout life," said press conference moderator Li-Huei Tsai, PhD, of the Picower Institute for Learning and Memory at MIT, who developed an innovative mouse model for the study of Alzheimer's disease. "This is one of the major advances in brain research over the last decade. As we continue to learn the secrets of the genome, epigenetic developments provide crucial information to scientists working to study the cause, onset, and treatments for brain disease."

Related Presentation:

Minisymposium: **Epigenetics in the Nervous System**
Saturday, November 15, 1:30–4 p.m., Washington Convention Center, Room 202B

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Abstract 281.6 Summary

Lead author: Tania Roth, PhD **(205) 934-6433**
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Abusive Environment in Early Life 'Marks' DNA in the Brain

Study may explain how childhood mistreatment affects adult behaviors

New animal research shows that parental abuse and neglect have long-lasting effects on genes important to brain health and development. The findings may explain why adult victims of child abuse show high rates of behavioral problems and increased vulnerability for substance abuse and mental illness. The research was presented at Neuroscience 2008, the Society for Neuroscience annual meeting and the world's largest source of emerging news about brain science and health.

"Child abuse and neglect have effects on the structure and chemistry of the brain," said Tania Roth, PhD, at the University of Alabama at Birmingham, who led the study. "These damages are not just evident early in development, but last into adulthood."

Roth and colleagues investigated how early-life stress affected lasting brain function, namely whether abuse or neglect from a stressed caregiver "marked" brain DNA with a chemical modification called methylation. The addition of these epigenetic marks disrupts the brain's ability to turn on genes.

The researchers found that exposing newborn rats to a stressed-out adult caregiver affected DNA methylation, all the way into adulthood. Brain-derived neurotrophic factor, or BDNF, is important in the development of new brain cells and the support of existing ones; researchers have shown that early-life stress affects the extent to which the BDNF gene is turned on. In the current study, Roth and colleagues found that in the amygdala, a brain region involved in emotions and fear, rats exposed as infants to stressed caregivers had lasting epigenetic marks on BDNF DNA.

These findings may reveal the mechanism by which mistreatment in early life affects adult behavior. "This now opens the door for future studies to explore the significance of these epigenetic changes on adolescent and adult emotional well-being," Roth said, "and importantly, to explore the efficacy of drugs aimed at reversing such epigenetic marks and addressing the behavioral deficits resulting from early mistreatment."

The research was supported by the U.S. National Institute of Mental Health, NARSAD, and the McKnight Brain Research Foundation.

Scientific Presentation: Sunday, November 16, 2–3 p.m., Washington Convention Center, Hall A-C

281.6, Early-life adversity and its impact on DNA Methylation patterns in the amygdala
*T. ROTH, J. SWEATT; Dept Neurobiol, Univ. Alabama Birmingham, Birmingham, AL
TECHNICAL ABSTRACT: Neural mechanisms responsible for the enduring effects of childhood maltreatment on mental health remain undefined. On a molecular level, one such mechanism may be aberrant programming of DNA methylation, an epigenetic mechanism that represses gene expression. Indeed, aberrant DNA methylation continues to be highlighted for its role in the etiology and expression of several mental disorders. In this study, we addressed whether early-life adversity in the form of caregiver abuse and neglect disrupts DNA methylation in the adolescent and adult amygdala. To model abuse and neglect, rat neonates were exposed to a stressed caregiver 30 min daily during the first postnatal week. Littermate controls were exposed to either a non-stressed caregiver or remained in the home cage. Results indicate that the quality of early postnatal experiences profoundly influences DNA methylation patterns and gene expression in the developing and adult amygdala. Such alterations may provide a framework for enduring effects of early stressors on mental health.

Abstract 783.4 Summary

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Diet During Pregnancy Programs Body Size for Animal Offspring

Research addresses inheritance of obesity

High-fat diets during pregnancy increase the body size of subsequent generations, according to new animal research. The findings, released at Neuroscience 2008, the Society for Neuroscience annual meeting and the world's largest source of emerging news about brain science and health, shed new light on the hereditary nature of obesity.

To determine whether gestational diet — what mothers consume during pregnancy — influences obesity risk in developing offspring, Tracy Bale, PhD, and her colleagues at the University of Pennsylvania fed pregnant mice high-fat or normal diets. Regardless of their diets as adults, mice born to mothers fed high-fat diets weighed more than those born to mothers fed normal diets.

However, the researchers found that the offspring exposed to high-fat diets *in utero* weighed more because they were longer, not because they had extra fat deposits. In fact, these mice had smaller fat deposits than mice exposed to normal diets *in utero*. These findings suggest that maternal diet influences offspring body size, though the researchers suspect that different types and amounts of fat may affect different aspects of body size.

Large body size was passed to the next generation as well. Both male and female mice exposed to high-fat diets *in utero* were able to pass the trait for large body size to their offspring, although this second generation had never been exposed to the high-fat diet.

These data suggest that environmental factors like gestational diet program body size in a way that is heritable by subsequent generations. Diet has been shown to induce epigenetic modifications — that is, biochemical changes to DNA — that can be passed from one generation to the next.

According to Bale, future research will focus on identifying the specific genes in the brain that are epigenetically modified by high-fat diets. "For now, insights into the realities of epigenetic inheritance will hopefully encourage us all to make decisions conducive to our own health and well-being — not only for our own benefit, but for that of our children, and for that of our children's children," said Bale.

The research was supported by the University of Pennsylvania Health Research Formula Fund.

Scientific Presentation: Wednesday, November 19, 11 a.m.–noon, Washington Convention Center, Hall A-C

783.4, Gene-specific DNA methylation during gestation as a mechanism in predisposing the fetus toward obesity
G. A. DUNN[1], K. CARLIN[1], *T. L. BALE[2]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Animal Biol, Univ. Pennsylvania, Philadelphia, PA
TECHNICAL ABSTRACT: The rates of obesity and associated metabolic complications such as type II diabetes, hypertension, and cardiovascular disease have risen to epidemic proportions in the last twenty years. We are conducting mechanistic examinations of the long-term effects of gestation within a mother consuming a high fat diet on offspring obesity risk. C57Bl/6:129 females are given a high fat diet throughout pregnancy and suckling (HF group) while control females consume a chow diet for the duration of this period (Chow group). We hypothesized that HF offspring would exhibit increased body mass characterized by enhanced adiposity, metabolic complications, and hyperphagia. Mechanistically, we anticipated long-term perturbations in expression of the orexigenic and anorexigenic hormones neuropeptide Y (NPY) and proopiomelanocortin (POMC) in the arcuate nucleus of the hypothalamus (ARC) that may be the result of prenatal epigenetic events.
HF pups display greater body length at embryonic day 14 and increased body weight beginning at three weeks extending through adulthood regardless of diet. HF offspring show a trend of hyperphagia on both chow and high fat diet as adults, and comparisons within individual animals suggests an increased ratio of high fat to chow consumption within the HF group. In accordance with these phenotypes suggesting perturbations

in feeding behavior at the level of the hypothalamus, in situ hybridization analysis at three weeks revealed decreased expression of POMC but not NPY within ARC. Interestingly, bisulfite sequencing of the POMC promoter region in micropunches from ARC reveals extensive heterogeneity in methylation state likely due to cell specific epigenetic regulation. Studies attempting to elucidate a potential mechanism of obesity transmission through parallel analysis of POMC methylation state and mRNA transcription in a pure population of POMC neurons are currently underway. We have found that gestation within an obese mother predisposes offspring toward obesity and is correlated with alterations in POMC expression possibly explained by differential methylation. Ongoing studies will attempt to further determine the mechanisms of the in utero programming of the obesity phenotype.

Abstract 831.20 Summary

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New Drug Improves Learning and Memory in Animal Study

Findings suggest treatments that modify DNA structure may benefit people with memory disorders

Blocking enzymes that help wind DNA into compact spools improves learning and memory, according to new animal research released at Neuroscience 2008, the Society for Neuroscience annual meeting and the world's largest source of emerging news about brain science and health. The findings suggest new treatments for people with diseases that affect memory, such as Alzheimer's disease.

"Our research indicates that blocking the activity of a class of enzymes called histone deacetylases is a promising new approach for the treatment of memory disorders," said Michael Ahlijanian, PhD, of EnVivo Pharmaceuticals, a co-author of the study.

The histone deacetylase inhibitor EVP-0334 helped mice remember objects for short and long periods of time. EVP-0334 also helped mice learn and remember the location of a hidden platform in a pool of milky water.

EVP-0334 works by keeping strands of DNA untangled. To save cellular space, long strands of DNA are wound around small protein spools called histones. When the histones are marked by chemicals called acetyl groups, the DNA can unwind and be accessed by other enzymes that turn genes on. However, without the acetyl groups, the DNA cannot be unwound, and genes remain silent, or off. EVP-0334 blocks naturally occurring histone deacetylase enzymes from removing acetyl groups, thereby maintaining a looser structure of DNA that is more open to gene expression. Over the past few years, an increasing number of experimental studies have shown that histone deacetylase inhibitors are effective in improving memory in animal models of memory disorders.

"Our data suggest that EVP-0334 significantly enhances both short- and long-term memory in mice," said Ahlijanian. "EVP-0334 may have therapeutic potential to treat the cognitive deficits observed in a broad range of human neurological and psychiatric disorders."

Scientific Presentation: Wednesday, November 19, 2–3 p.m., Washington Convention Center, Hall A-C

831.20, The histone deacetylase inhibitor EVP-0334 is pro-cognitive in mice
*L. LEVENTHAL, A. TRAN, I. GALLAGER, F. P. ALBAYYA, D. SPAULDING, D. COSTA, S. NOLAN, M. K. AHLIJANIAN, H. PATZKE, K. E. ROGERS; EnVivo Pharmaceuticals, Watertown, MA
TECHNICAL ABSTRACT: Abnormal gene transcription has been proposed to be an underlying mechanism in the pathophysiology of both neurological and psychiatric disorders including: Huntington's disease, Parkinson's disease, Fragile X syndrome, depression and anxiety. Histone modifications regulate gene expression through multiple mechanisms. Further, histone acetylation in the nervous system in part mediates the enzymatic activity and signal transduction pathways that regulate post-translational modification. Therefore, histone deacetylase (HDAC) inhibition is an attractive target for regulating aberrant gene transcription in a wide range of diseases and it follows that a potent HDAC inhibitor could be a novel therapeutic approach for treating CNS disorders. EVP-0334 has previously been shown to be a spectrum HDAC inhibitor potent in enzymatic and cellular assays. Furthermore, following oral administration, EVP-0334 is highly brain penetrant and induces increased central histone acetylation which is critical for potential CNS therapeutic efficacy (see back-to-back presentation at this meeting). The present series of studies evaluated the in vivo activity of EVP-0334 in mice. In the novel object recognition assay, EVP-0334 significantly increased exploration of the novel object at both 1.5 and 24 hours after administration compared to vehicle-treated mice at doses that increase histone acetylation in the striatum. Furthermore, EVP-0334 improved acquisition of learning in a mouse Morris water maze assay. Currently, EVP-0334 is being evaluated in additional cognition assays. Overall, our data suggest that EVP-0334 significantly enhances both short- and long-term memory in mice. In conclusion, EVP-0334 may have therapeutic potential to treat the cognitive deficits observed in a broad range of neurological and psychiatric disorders.

Speaker's Summary

Speaker: Farah Lubin, PhD **(205) 975-5196**
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Linking the Epigenetic Code of Gene Regulation to Fear Memory Formation (4.3)
Minisymposium: Epigenetics in the Nervous System
Saturday, November 15, 1:55–2:15 p.m., Washington Convention Center, Room 202B

It is well-established that mental illness, including schizophrenia (which affects 1.1% of U.S. population), depression (6.7%) and bipolar disorder (2.6%), is a prevalent public health problem. Numerous factors have been linked to the development of mental disorders. In many cases, there is no single accepted or consistent cause of mental disorders. This emphasizes the vital need for strategies that both prevent mental disorders and treat those who have already been diagnosed.

A common belief is that mental disorders often result from genetic vulnerabilities combined with environmental factors. Scientists have found through genetic studies that genes play an important role in the development of mental disorders. Thus, it is believed that abnormal gene regulation is responsible for changes in behavior. One such gene is brain-derived neurotrophic factor (*bdnf*). Indeed, BDNF is known to play an important role in behavior, and aberrant regulation of this gene has been implicated in the etiology of mental illnesses. Epigenetics is a newly proposed mechanism for activation of gene expression in the central nervous system. Epigenetics can encompass several processes that serve to chemically mark DNA and its associated proteins, such as histones, without change to the DNA sequence. Typically, the addition or removal of these marks to DNA disrupts or enables gene expression, respectively. In this study, we explored the possibility that normal memory processing involves the epigenetic marking of DNA that encode the *bdnf* gene.

In relation to mental disorders such as schizophrenia and depression, fear processing is especially relevant, as many patients manifest deficits in the processing and attribution of negative emotional states. Given the importance of the BDNF protein in mammalian learning and mental illness, we investigated whether DNA methylation, an epigenetic mark of DNA, regulates *bdnf* gene expression within the hippocampus. To model fear processing, we used a rodent contextual fear conditioning model wherein a novel context (training chamber) is paired with a mild footshock. After this training, a long-term memory for this association is formed. Memories that are formed are susceptible to disruption immediately after training due to a necessity to be consolidated (stored) after memory acquisition. During memory consolidation, new genes are expressed and become new proteins, and inhibition of this process specifically blocks long-term memory. Exposing the animal to the training chamber again triggers memory retrieval of the associative memory and the animal shows conditioned fear responses such as freezing.

Our results indicate that regulation of *bdnf* gene expression in hippocampus during fear memory processing is associated with specific changes in *bdnf* DNA methylation. Zebularine is a drug that is currently used for the treatment of cancer because of its ability to reverse the chemical modifications made to DNA. Interestingly, we found that Zebularine treatment significantly altered *bdnf* DNA methylation; triggered changes in *bdnf* gene expression; and interfered with normal fear learning (freezing). These findings indicate that altered DNA methylation is sufficient to drive *bdnf* gene expression.

It is well-appreciated that activation of the glutamate receptor, N-methyl-D-aspartic acid (NMDA), is important for the acquisition and consolidation of memories. We discovered that blocking NMDA receptor activation prevented memory-associated alterations in *bdnf* DNA methylation, resulting in a lack

of *bdnf* gene expression in hippocampus and a deficit in long-term memory formation. This study is the first to suggest epigenetic modification of the *bdnf* DNA as a mechanism for specific gene readout during memory processing. As aberrant *bdnf* gene expression continues to be implicated in psychiatric disorders associated with cognitive dysfunction (schizophrenia, depression, and bipolar disorder), our results provide support for the hypothesis that manipulating these epigenetic marks may be a viable therapeutic mechanism to restore cognitive function in these disorders.

Speaker's Summary

Speaker: William Renthal **(214) 648-1108**
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Epigenetic Mechanisms in Drug Addiction (4.6)
Minisymposium: Epigenetics in the Nervous System
Saturday, November 15, 2:55–3:15 p.m., Washington Convention Center, Room 202B

People who repeatedly use drugs of abuse often become addicted, a state characterized by compulsive drug seeking and taking despite severe consequences. While current treatment options have improved over the last decade, relapse rates remain high for most addictive drugs. Thus, addiction research has focused on understanding how people become addicted after recreational drug use in hopes of reversing these processes clinically. Moreover, research is also actively studying why people relapse even after long periods of drug abstinence, which could provide novel therapeutic targets that reduce an individual's likelihood to use drugs again.

Drugs of abuse are known to cause substantial changes in gene expression in the brain, many of which have been associated with addictive behaviors that can persist for months after drug use. Our studies reveal a new mechanism by which addictive drugs cause these long-lasting changes in gene expression that may ultimately lead to addiction or relapse. We have found that repeated exposure to addictive drugs alters the way DNA is packaged inside neurons. In each cell, DNA normally exists in a complex with proteins in a highly condensed structure called chromatin. Enzymes that regulate how strongly DNA can interact with these proteins (called histones) can alter the structure of chromatin and control the activity of genes, a process called chromatin remodeling. Enzymes that loosen chromatin structure will often increase gene expression, while enzymes that further condense chromatin usually reduce gene expression. For example, histone deacetylases modify histones such that they more tightly interact with DNA and decrease gene activity.

Our findings indicate that repeated cocaine exposure inhibits the function of histone deacetylases in a way that facilitates specific gene activation and addictive behaviors in rodents. Moreover, we have begun to map precisely how chronic cocaine exposure changes chromatin structure at each gene in the genome. In addition to providing novel insight into the basic gene-regulatory mechanisms co-opted by cocaine, our data describe several new cell signaling pathways which are regulated by cocaine.

These data provide a wealth of new information about how chronic cocaine exposure alters chromatin structure and gene expression in the brain and, most importantly, suggest a fundamentally new approach for developing improved therapeutic interventions for drug addiction.