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NURTURE IMPACTS NATURE:
EXPERIENCES LEAVE GENETIC MARK ON BRAIN, BEHAVIOR

New studies show life events influence genes important for memory and drug use

SAN DIEGO — New human and animal research released today demonstrates how experiences impact genes that influence behavior and health. Today's studies, presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health, provide new insights into how experience might produce long-term brain changes in behaviors like drug addiction and memory formation.

The studies focus on an area of research called epigenetics, in which the environment and experiences can turn genes "on" or "off," while keeping underlying DNA intact. These changes affect normal brain processes, such as development or memory, and abnormal brain processes, such as depression, drug dependence, and other psychiatric disease — and can pass down to subsequent generations.

Today's new findings show that:

- Long-term heroin abusers show differences in small chemical modifications of their DNA and the histone proteins attached to it, compared to non-abusers. These differences could account for some of the changes in DNA/histone structures that develop during addiction, suggesting a potential biological difference driving long-term abuse versus overdose (Yasmin Hurd, abstract 257.2, see attached summary).
- Male rats exposed to cocaine may pass epigenetic changes on to their male offspring, thereby altering the next generation's response to the drug. Researchers found that male offspring in particular responded much less to the drug's influence (Matheiu Wimmer, PhD, abstract 449.19, see attached summary).
- Drug addiction can remodel mouse DNA and chromosomal material in predictable ways, leaving "signatures," or signs of the remodeling, over time. A better understanding of these signatures could be used to diagnose drug addiction in humans (Eric Nestler, PhD, abstract 59.02, see attached summary).

Other recent findings discussed show that:

- Researchers have identified a potentially new genetic mechanism, called piRNA, underlying long-term memory. Molecules of piRNA were previously thought to be restricted to egg and sperm cells (Eric Kandel, MD, see attached summary).
- Epigenetic DNA remodeling is important for forming memories. Blocking this process causes memory deficits and stunts brain cell structure, suggesting a mechanism for some types of intellectual disability (Marcelo Wood, PhD, see attached summary).

"DNA may shape who we are, but we also shape our own DNA," said press conference moderator Schahram Akbarian, of the Icahn School of Medicine at Mount Sinai, an expert in epigenetics. "These findings show how experiences like learning or drug exposure change the way genes are expressed, and could be incredibly important in developing treatments for addiction and for understanding processes like memory."

This research was supported by national funding agencies such as the National Institutes of Health, as well as private and philanthropic organizations. Find more information on epigenetics at *BrainFacts.org*.

– more –

Related Neuroscience 2013 Presentations:

Special Lecture: Transgenerational Epigenetics: Programming Behavior in a Dynamic Landscape
Sunday, Nov. 10, 1-2:10 p.m., Ballroom 20

Special Lecture: Plasticity in the Adult Brain: Neurogenesis and Neuroepigenetics
Tuesday, Nov. 12, 1-2:10 p.m., Ballroom 20

Abstract 257.2 Summary

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Brains of Heroin Users Change with Years of Addiction

Heroin abuse and overdose changes DNA "signatures" in the brain

Years of heroin abuse may change how genes are expressed and how the brain functions, according to new human research release today. This research was presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Our study addresses a critical gap in our knowledge about heroin addiction because we cannot often directly study the brains of addicted humans," said senior author Yasmin Hurd, PhD, of the Icahn School of Medicine at Mount Sinai in New York. "Our results provide important insights into how human brains change in response to long-term heroin use, and give us to knowledge to help treat this dangerous disease."

According to the World Health Organization, 9.5 million people abuse heroin around the world, which increases their risk of death by 20 to 30 times those of non-drug users.

The changes to brain function from heroin use are driven by epigenetics, a process by which environmental events modify the shape and packaging of DNA without changing the underlying DNA itself. Instead, the structure of the DNA becomes more "open" or "closed," allowing some genes to be expressed more or less often. This changes what proteins are produced and, as a result, can change how the brain functions.

To determine the epigenetic changes relating to opiate abuse, Hurd and colleagues examined the post mortem brains of heroin abusers, looking at which genes were being "used" to make functional molecules, and at what levels. The team focused on the striatum, an area of the brain that is closely involved in drug abuse. They found significant changes to how DNA was being "used," and this disturbance was correlated with the number of years of heroin addiction. Strikingly, there was an inverse correlation between "open" DNA and heroin overdose, suggesting that chronic drug abuse produces different changes than those resulting from drug overdose. This indicates that the behaviors that lead to overdose have a different neural basis than those that lead to long term abuse.

Research was supported with funds from the National Institutes of Health and National Institute of Drug Abuse.

Scientific Presentation: Sunday, Nov. 10, 4–5 p.m., Halls B-H

257.2, Impairments of chromatin remodeling and gene expression in the striatum of human heroin abusers
*G. EGERVARI[1], E. KELLER[2], **Y. L. HURD[1]**; [1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Forensic and Insurance Med., Semmelweis Univ., Budapest, Hungary

TECHNICAL ABSTRACT: Heroin abuse continues to have a detrimental impact on the individual and society in the United States and worldwide. Knowledge is, however, still lacking regarding neurobiological disturbances in human heroin abusers that could better guide treatment interventions. Abnormal gene transcription has been noted in opiate users, but no information currently exists regarding related epigenetic mechanisms, key regulators of transcription, in human drug users. As such, we studied the human striatum to characterize the state of epigenetic marks and genes related to synaptic plasticity, dysregulation of which is a core feature of addiction disorders.
We used a homogeneous postmortem collection of human heroin abusers to explore expression of genes in the striatum (microarray, Nanostring, quantitative real-time polymerase chain reaction) directly related to synaptic plasticity and glutamatergic neurotransmission, and to assess associated epigenetic mechanisms (Western blot, chromatin immunoprecipitation).
We observed marked perturbations of glutamatergic gene expression and epigenetic regulation in the striatum of human heroin abusers. In the nucleus accumbens, we found heroin-related transcriptional changes of chromatin remodeling enzymes and of genes involved in synaptic plasticity and glutamatergic neurotransmission. In the dorsal striatum, we found a significant increase in nuclear coactivator 1 (NCOA1) histone acetyltransferase and global histone H3 acetylation (AcH3) that correlated with years of heroin use, and showed negative correlations with heroin toxicology. In addition, we also observed a significant increase in AcH3 at the gene body and promoter region of selected glutamatergic genes. Other marks examined such as tri- or dimethylation of histone H3 lysine-9 (H3K9me3/2) related to transcriptional repression were not significantly altered.
Overall, the data to date suggest that epigenetic perturbations, particularly the hyperacetylation of histone H3, and thus the resulting more open chromatin configuration, might be intimately involved in the regulation of heroin-induced striatal synaptic plasticity. In addition, our findings indicate that molecular mechanisms are differentially affected by acute drug toxicity versus the chronic pathologic state of substance abuse.

Abstract 449.19 Summary

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Cocaine Use in Males May Alter Resistance in Offspring

New animal research suggests a father's cocaine use may desensitize male offspring to the drug. This effect may be caused by changes in the brain's epigenome, a system that determines how genes are translated into molecules in cells. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"Our study shows that cocaine abuse in a rodent can affect how its male offspring respond to cocaine," said lead author Mathieu Wimmer, PhD, from the University of Pennsylvania. "The results suggest that a history of cocaine abuse can make the next generation of animals more resistant to drugs."

Previous studies have shown that activities and experiences that animals are exposed to, such as stress or drugs, can make changes to how its DNA is expressed. To determine how paternal cocaine use might impact the next generation, Wimmer and colleagues allowed a group of rats to take cocaine for two months, and then mate with a female. Results showed the male offspring of the rats exposed to cocaine were less sensitive to the drug.

Cocaine causes a large increase in motor activity, and as the drug is given over a few days, the motor activity increases even further in a process called sensitization that models early stages of cocaine addiction. Male offspring of cocaine-exposed rats were resistant to this sensitization, suggesting that the father's exposure to cocaine had changed how the male pups responded, and that the next generation might be less likely to be addicted.

The difference was in an area of the brain called the nucleus accumbens, which is involved in drug addiction. In male rats whose fathers used cocaine, the neurons in the nucleus accumbens were less sensitive to cocaine. With the results of these studies, the researchers hope to conduct further research to understand how these behavioral changes are passed from father to son, and how this might play out in humans.

Research was supported with funds from the National Institutes of Health and the National Institute on Drug Abuse.

Scientific Presentation: Monday, Nov. 11, 3–4 p.m., Halls B-H

449.19, Cocaine-induced plasticity deficits in the male offspring of cocaine-experienced sires
***M. E. WIMMER**[1], F. M. VASSOLER[2], P. I. ORTINSKI[3], S. L. WHITE[3], H. D. SCHMIDT[3], G. SADRI-VAKILI[4], C. PIERCE[3]; [1]Ctr. for Neurobio. & Behavior, Univ. Pennsylvania, PHILADELPHIA, PA; [2]Biomed. Sci., Tufts Univ., North Grafton, MA; [3]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [4]Inst. for Neurodegenerative Dis., Mass Gen. Hosp., Charlestown, MA

TECHNICAL ABSTRACT: Cocaine abuse remains a major public health problem in the United States. A growing body of evidence suggests that environmental information, including that related to cocaine exposure, can be inherited epigenetically. We recently established a rat model to examine the influence of paternal cocaine self-administration on the behavior of the progeny. Here, we combined behavioral, electrophysiological and molecular approaches to examine cocaine-induced behavioral and neuronal plasticity in the offspring of cocaine-experienced sires (F1 CocSired) and controls (saline sired, F1 SalSired). Repeated exposure to cocaine produces behavioral sensitization, which is characterized by an augmented locomotor response to a subsequent psychostimulant challenge injection. Here, we show that male, but not female, CocSired rats show deficits in cocaine sensitization, indicating a resistance in the long-lasting behavioral responses consecutive to cocaine exposure. Expression of sensitization is accompanied by neuroadaptations in the nucleus accumbens (NAc), which is critically involved in the development of addiction. In particular, AMPA receptors (AMPARs) and their composition influence cocaine sensitization. We used whole-cell patch clamp recordings to examine the AMPAR component of stimulus-evoked excitatory post-synaptic currents (EPSCs) in NAc slices of F1 rats. EPSCs at negative potentials were larger than those measured at the symmetrical positive potentials in naïve CocSired animals compared to naïve SalSired rats. This increased rectification index suggests that some AMPAR are lacking the GluA2 subunit in naïve CocSired rat. Consistent with these results, GluA2 mRNA expression was reduced in naïve male CocSired rats compared to control, suggesting that epigenetic mechanisms contribute to the inheritance of this phenotype. GluA2-lacking AMPARs exhibit several unique properties such as permeability to calcium (Ca2+) and are critically involved in the expression of cocaine-induced behavioral sensitization. Following 10 days of cocaine self-administration, SalSired rats show an increase in rectification index, which was not present in CocSired rats. Taken together, these findings indicate that the offspring of cocaine-experienced rats show deficits in cocaine-induced behavioral and neuronal plasticity and that epigenetic mechanisms may contribute to this phenomenon.

Abstract 59.02 Summary

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Cocaine Addiction May Result from Epigenetic Changes in Brain Cells

Cocaine abuse changes gene expression, leaving lasting changes in brain function

Drug use changes brain cells in repeatable, predictable ways to bring about the behavioral symptoms of addiction in mice. The findings are a small yet significant step toward one day potentially using biomarkers in a person's blood to identify vulnerability to addiction. Knowledge of these cellular changes could also help improve diagnosis of drug use disorders to give people better access to treatment. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"It's well established that cocaine addiction has lasting effects on the brain," said senior author Eric Nestler, MD, PhD, from the Icahn School of Medicine at Mt. Sinai. "But studies in our lab begin to reveal just how extensive those changes are and how they might change brain function. This level of understanding could lead to better treatments for addiction."

Cocaine abuse is a chronic, relapsing disorder that changes brain function drastically, usurping brain reward systems. The World Health Organization estimates that 1 to 3 percent of the world population currently abuses cocaine, changing the way they, and their brain, function in daily life.

In stages, Nestler and his colleagues have been mapping out cellular changes caused by cocaine that can lead to addiction. In the study, the researchers exposed mice to cocaine and then examined a portion of the inner brain (the nucleus accumbens) that controls responses to addiction, reward, pleasure, aggression, fear, and impulsivity. They found that cocaine altered the structure of chromatin, a material in the cell nucleus that manipulates how DNA is coiled and influences how genes are expressed. These epigenetic changes can alter how a cell behaves and, in this case, potentially produced behavioral changes associated with addiction, such as working harder for a fix.

It turns out that cocaine abuse changes chromatin in repeatable, predictable ways, resulting in what Nestler calls a "chromatin signature." Eventually, Nestler hopes to use the chromatin signatures and other signals to identify biomarkers in the blood stream that indicate whether a person is addicted or may be vulnerable to addiction.

Research was supported with funds from the National Institute on Drug Abuse.

Scientific Presentation: Saturday, Nov. 9, 2–3 p.m., Halls B-H

59.02, Epigenetic regulation of cocaine action in mouse nucleus accumbens
L. SHEN[1], J. FENG[1], M. WILKINSON[1], *D. FERGUSON[1], V. VIALOU[1], X. LIU[1], I. MAZE[2], N. SHAO[1], J. KOO[1], P. KENNEDY[1], C. DIAS[1], Q. LAPLANT[1], M. CAHILL[1], **E. NESTLER[1]**; [1]Neurosci., Mount Sinai Sch. of Med., NYC, NY; [2]Rockefeller Univ., New York, NY

TECHNICAL ABSTRACT: Increasing evidence supports a role for altered gene expression in mediating the lasting effects of cocaine on the brain, and recent work has demonstrated the involvement of chromatin modifications in these alterations. We utilize next generation sequencing technology, RNA-seq and ChIP-seq, to obtain an unprecedented view of cocaine-induced changes in gene expression and associated adaptations in numerous modes of histone modification in mouse nucleus accumbens, a key brain reward region. We map at a genome-wide scale RNA polymerase II and several histone modifications including H3K4me1, H3K4me3, H3K9me2, H3K27me3, and H3K36me3 after chronic cocaine administration. We then superimpose these data with RNA-seq transcriptome alterations. We identify unique combinations of chromatin changes, or signatures, that accompany cocaine's regulation of gene expression, including the dramatic involvement of pre-mRNA alternative splicing in cocaine action. In addition, we identify one splicing factor A2BP1 (Rbfox1/Fox-1), which is enriched at genes that display certain chromatin signatures and contributes to drug-induced behavioral abnormalities. Together, this delineation of cocaine-induced epigenome dynamics in nucleus accumbens reveals several novel modes of drug regulation, thereby providing new insight into the biological basis of cocaine addiction. More broadly, the combinatorial chromatin and transcriptional approaches that we describe serve as an important resource that can be applied to other systems to reveal novel transcriptional and epigenetic mechanisms of neuronal regulation.

Speaker Summary (396)

Speaker: Eric Kandel, MD (212) 543-5204
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The Coordinated Regulation of Transcription in the Nucleus, and Translation at the Synapse, by microRNAs and piRNAs

Symposium: *Neuro-Epigenetics in Neural Development, Plasticity, and Brain Disorders*
Monday, Nov. 11, 2:10–2:45 p.m., Room 6B

Classic behavioral studies of memory storage in people and animals have defined two temporally distinct phases for memory storage: a short-term memory lasting minutes that can be elicited by one training trial, and a long-term memory lasting days or more that typically requires repeated training trials.

In earlier work, we delineated these two behavioral memory phases in studies of learned fear, an implicit form of memory, using the simple gill-withdrawal reflex of Aplysia. This work revealed that there is a cellular representation of the learning process. The substrate of learning is the synapse, and learning leads to changes in the strength of synaptic connections. These studies found that short-term memory is mediated by a transient synaptic facilitation of pre-existing connections due to covalent modification of pre-existing proteins, whereas long-term memory results from a persistent facilitation mediated by transcription and synaptic growth.

The critical transcriptional switch that converts short-term to long-term facilitation and long-term memory in Aplysia is mediated by the removal of the repressive step of CREB-2 and the activation of CREB-1. Because small RNAs are important in transcriptional control and post-transcriptional regulation of gene expression, we wondered whether they might also regulate this key transcriptional switch from short-term to long-term memory.

Together with our collaborators we profiled the small RNAs of Aplysia and identified 170 distinct miRNAs, nine of which were CNS-enriched, and several were rapidly down-regulated by transient exposure to serotonin. The most abundant and well-conserved brain-specific miRNA, miR-124, was exclusively present presynaptically in the sensory neuron, where it inhibits CREB-1 mRNA. Serotonin, the modulatory transmitter released during learning, inhibits miR-24 and leads to the translation of CREB-1s, the activator of long-term memory transcription.

In the course of profiling these small RNAs, we discovered a new class of small RNAs, piRNAs, which had previously been thought to be germ-cell specific. We found that these neuronal piRNAs have predominant nuclear localization and sensitivity to serotonin. In response to serotonin, an increase in the level of a particular piRNA, piRNA-F, led to methylation and silencing of the CREB-2 promoter.

Since CREB-2 is the major inhibitory constraint of memory that represses CREB-1, methylation of CREB-2 presumably leads to prolonged activation of CREB-1 and of long-term memory. The finding that serotonin bi-directionally regulates piRNAs and miRNAs — such that a rise in piRNA-F levels silences CREB-2, while a fall in miR-124 levels activates CREB-1 — provides a coordinated small RNA-mediated gene regulatory mechanism that acts on both the nucleus and on cytoplasmic mRNAs to allow CREB-1 to be active for over 24 hours. This thereby establishes stable long-term changes in the sensory neurons for the consolidation of long-term memory storage.

Our discovery of piRNAs in the brain was surprising because piRNAs were thought to be restricted to germ cells. The abundance of these neuronal piRNAs, and their responsiveness to neuromodulators, suggests a much broader role for piRNA than had previously been appreciated. In a larger sense, this data indicates a new mechanism for epigenetic regulation of gene expression underlying long-term memory storage.

Although epigenetic regulation was widely known to occur in the context of development and differentiation, the discovery of their putative relevance in learning related adult brain function is relatively recent. Furthermore, the mechanisms that may recruit epigenetic factors in a target-specific manner have remained elusive, and the first study to suggest a role for small-RNAs in guiding chromatin modification and transcriptional control was in fission yeast.

Placed in the larger context of these and other earlier studies, our study provides an activity-dependent, piRNA-mediated, mechanism for DNA methylation of specific loci that can translate transient signals into long-term synaptic changes underlying memory storage.

This work was supported by the Howard Hughes Medical Institute and the National Institutes of Health.

Speaker's Summary (508)

Speaker: Marcelo Wood, PhD (949) 824-2259
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Neuron-Specific Nucleosome Remodeling: A Missing Link in Our Understanding of Epigenetic Mechanisms Underlying Memory Processes

Tuesday, Nov. 12, 10:45–11 a.m., Room 5B

Our findings indicate a critical role for nucleosome remodeling in the formation of long-term memories and may help explain how cognitive impairments in certain intellectual disabilities (ID) disorders, including autism spectrum disorder (ASD), may arise from mutations in specific genes involved in nucleosome remodeling.

The loss of the ability to learn and remember information impacts nearly every aspect of our daily lives. Without it you would not know where you parked your car, where your kids go to school, or perhaps even your own name. At its core memory makes us each a unique individual. Consequently, disorders that impair memory and general cognition are particularly devastating for patients and their families. As a field, the study of learning and memory has known for quite some time that new memory formation requires the expression of specific genes. The products of those genes help stabilize the communication between neurons to encode memories. We examined the role of a novel gene expression regulatory mechanism for long-term memory formation called nucleosome remodeling, a form of epigenetic gene regulation. Defects in nucleosome remodeling mechanisms were recently linked to ID and ASD in humans. However, how defects in nucleosome remodeling mechanisms give rise to deficits in human cognition remained unclear.

To examine this question we created genetically modified mice that have a mutation in a component of a nucleosome remodeling complex only found within neurons. Mutant mice have severe deficits in long-term memory formation for several different memory tasks. Similar to deficits observed in humans with ASD, the mice also showed impairments in dendritic spine morphology. Spines form the connections between neurons, enabling communication necessary for memory encoding. The mice also have deficits in gene expression, the first step in the production of new proteins, following a learning event. Together our findings indicate for the first time a role for nucleosome remodeling in regulating gene expression that is required for long-term memory formation, and that disruption of this process may contribute to the cognitive deficits observed in humans with ID or ASD.

So what is nucleosome remodeling? It is a process that is critically important for gaining access to the DNA within a cell, a necessary prerequisite for gene expression. DNA is highly compacted in order to physically fit within the nucleus of a cell via a complex called chromatin. The repeating unit of chromatin is the nucleosome, which is a structure of DNA wrapped around histone proteins, similar to thread wrapped around a spool. Nucleosome remodeling is the process by which nucleosomes are repositioned in order to gain access to the DNA for gene expression.

Our work is the first to show a potential link between nucleosome remodeling and long-term memory formation and suggests that misregulation of gene expression via nucleosome remodeling may be a central mechanism to cognitive impairments observed in certain intellectual disability disorders.

Funding for this research was provided by the National Institute of Mental Health and National Institute on Drug Abuse.