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Press Room, Nov. 12-16: (619) 525-6230

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Parents' Experiences Could Have Inheritable Effects on Offspring's Mental Health

Changes in gene activity can be passed from one generation to the next

SAN DIEGO — The air we breathe, the food we eat, and the stress or happiness we feel could influence whether our offspring develop neurological disorders. Recent studies in animal models and human brain tissue document how a parent's environment may affect future offspring. The findings were presented at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

DNA sequence is not destiny. The external environment can contribute to how the information stored in DNA translates into traits like height and risk for disease. What's more, sometimes those contributions can be passed down from one generation to the next.

Transgenerational epigenetic inheritance refers to offspring inheriting specific patterns of gene activity generated by epigenetic changes unrelated to alterations in the underlying DNA sequence. Such epigenetic changes can switch genes on or off and determine what proteins are made, affecting the traits of offspring.

Epigenetic changes occur regularly and are naturally influenced by factors such as age and lifestyle. Scientists are beginning to understand how a parent's experiences — like stress, diet, and drug use — may contribute to the risk for various neurodevelopmental diseases and disorders in offspring.

Recent research shows that:

- Female mice fed a high-fat diet before mating bore offspring with dysregulated gut bacteria as well as autism-like behaviors (Shelly A. Buffington, abstract 31.17, see attached speaker summary).
- In an animal model, binge alcohol consumption during adolescence impacts the DNA of future offspring and predisposes them to mental health disorders later in life, even when those offspring are never exposed to alcohol (Andie Asimes, abstract 728.08, see attached speaker summary).
- Treatment with the antidepressant fluoxetine during pregnancy may protect offspring from some of the long-term, detrimental effects of maternal stress in mice (Veronika Kiryanova, abstract 772.11, see attached speaker summary).
- Targeting the epigenetic control of a specific neurotransmitter gene in an area of the brain responsible for regulating mood promotes a faster response to antidepressants in a mouse model of depression (Carla Nasca, abstract 71.01, see attached speaker summary).
- Nerve cells from human brain tissue accrue unique mutations in their DNA that are not found in other body cells, representing an ongoing record of the life history of each individual nerve cell (Mollie B. Woodworth, abstract 19.01, see attached speaker summary).

"This research contributes to our understanding of transgenerational epigenetic inheritance and how the behavior and environment of one generation can affect the mental health of future offspring," said press conference moderator Ian Weaver, PhD, of Dalhousie University. "The better we understand such mechanisms and factors, the more likely we will be able to prvent and treat inherited mental health disorders."

The research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about epigenetics at *BrainFacts.org*.

Related Neuroscience 2016 Presentation
Symposia: Neuroepigenetics
Tuesday, Nov. 15, 8:30-11 a.m., SDCC 6A

Speaker Summary 31.17

Speaker: Shelly Buffington, PhD (832) 314-3713
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Houston

Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring
Saturday, Nov. 12, 1-2 p.m., Halls B-H

We found that a maternal high-fat diet induces long-term dysbiosis of the gut microbiome in offspring and that these changes in gut microbial ecology are responsible for the autism-like social deficits that manifest in the offspring. Buffington et al., Cell (2016).

Autism spectrum disorders (ASD) can be caused by either genetic or environmental factors, or a combination of these. Human epidemiological studies have shown that maternal obesity during pregnancy increases the risk of ASD in offspring by 1.5-fold. That risk increases to two-fold when maternal obesity is accompanied by gestational diabetes. The Centers for Disease Control and Prevention report that the incidence of obesity in women of childbearing age (20-34 years) in the United States increased by 80% over the last twenty years. Taken together, these epidemiological data argue that the rising rate of maternal obesity represents a major national mental health problem.

How maternal diet-induced obesity affects brain development and ultimately behavior, however, remains unclear. Dietary composition is known to shape the gut microbiome. Indeed, high-fat maternal diets have been shown to alter the offspring microbiome in both human and non-human primates. A growing body of research suggests that gut microbiota dysbiosis can negatively affect brain function. Given that many children with ASD co-present with both behavioral and gastrointestinal issues, we hypothesized that the two are causally related and that maternal diet-induced changes in the gut microbiome can impact offspring neurodevelopment and behavior.

In this study, we placed female mice on either a regular or high-fat diet prior to pairing them with males to produce offspring. Over the eight-week feeding period, high-fat diet-fed females showed significant increases in fat mass, consistent with diet-induced obesity. All offspring, regardless of maternal diet, were placed on a regular diet at weaning. We characterized the microbiome and performed a battery of behavioral tests on the adult offspring. 16S ribosomal RNA gene sequencing revealed significant, species-level differences between the fecal microbiome of regular versus maternal high-fat diet (MHFD) offspring, including a decrease in microbial diversity in the MHFD gut. MHFD mice showed several ASD-like behaviors including elevated anxiety, decreased social interaction, and exaggerated stereotypic behaviors. Interestingly, co-housing, allowing for the free exchange of microbiota between regular and MHFD offspring, restored both the gut microbiome and social behavior of MHFD mice.

These results support the idea that one or more species important for normal social behavior were missing from the MHFD gut microbiome. Whole genome shotgun sequencing revealed multiple species underrepresented in the MHFD offspring; of these, Lactobacillus reuteri was the most reduced. Intriguingly, Lactobacillus reuteri had previously been shown to increase levels of the “social hormone” oxytocin in mice. Oxytocin plays an important role in modulating social behavior, not only in rodents, but also in humans. Treatment with Lactobacillus reuteri, reversed social behavioral deficits in MHFD offspring. Furthermore, Lactobacillus reuteri also increased oxytocin levels in the hypothalamus and restored social interaction-induced plasticity in the ventral tegmental area, a key reward area implicated in social behavior, in the MHFD offspring.

Our findings provide direct evidence that the gut microbiome is an important player in modulating brain function and behavior and that probiotics such as Lactobacillus reuteri may hold therapeutic potential for the treatment of behavioral symptoms associated with neurodevelopmental disorders, including ASD.

Research was supported with funds from the National Institute of Mental Health and the National Insitute of Neurological Disorders and Stroke.

Speaker Summary 728.08

Speaker: Annadorothea Asimes (708) 216-6372
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Binge Alcohol Consumption in Puberty Causes Altered DNA Methylation in Brain of Alcohol-Naïve Offspring
Wednesday, Nov. 16 , 11 a.m.-noon, Halls B-H

Our results show that a pattern of repeated binge alcohol consumption during adolescence can impact DNA programming in the brain of future generations.

The genes encoded in DNA provide the instructions for cells to make proteins, which ultimately control physical and behavioral traits. Molecular changes to DNA, such as DNA methylation, represent a type of modification that typically turns genes "off". Therefore, a consequence of gene "silencing", through the process of DNA methylation, can be disruption in normal brain function and/or behaviors. Our data suggest that preconception alcohol abuse can impact the DNA of future offspring, even when those offspring are never exposed to alcohol. These modifications to the offspring DNA could predispose them to develop mental health disorders later in life.

This study was recently submitted for publication in the journal Epigenetics and we believe that this research is the first to show a molecular pathway that teenage binge drinking of either parent can cause changes in the neurological health of subsequent generations. These results are the first of their kind concerning alcohol but agree with research into the inherited effects of preconception exposure to other recreationally used drugs such as marijuana (or THC).

In our experiments, we used animal models to study the brain of offspring after parents were exposed to repeated binge alcohol consumption during adolescence (i.e before conception). We found that exposure of the father to binge alcohol drinking led to just as many changes in the offspring as exposure of the mother, however these marks were different between the sexes. Interestingly, when both parents were exposed to binge pattern drinking, there were more DNA methylation marks in the offspring, but they were different than those caused by either parent drinking alone.

Future research will be focused on how binge drinking during adolescence causes these effects in the offspring brain. With more research into this pathway, we think we will uncover a mechanism that many drugs of abuse work through to impact future generations, and want to spread awareness of the long-term impacts of these risky teenage behaviors.

An episode of binge drinking for men is around 5 drinks in one sitting and 4 drinks for women, and is defined as raising the blood alcohol level over 0.08% (legal driving limit) within 2 hours. Underage drinkers in the United States account for over 20% of all alcohol consumption in the country, and more than 90% of this is consumed in binge patterns. These behaviors are not only dangerous to the brain development of the teenager exposed, but may also impact the brains of their children.

Research was supported with funds from National Institute on Alcohol Abuse and Alcoholism.

Speaker Summary 772.11

Speaker: Veronika Kiryanova, MS (403) 617-7672
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Effects of Maternal Stress and Fluoxetine on Outcomes of Offspring as Adults
Wednesday, Nov. 16, 3:30-3:45 p.m., SDCC 5B

Our research indicates that treatment with fluoxetine (brand name Prozac, Sarafem) during pregnancy may protect the pups, once they grow up to be adults, from some of the long-term, deleterious effects of maternal stress.

Maternal stress during gestation and after birth can affect development of infants and children, and increases a child's risk for developing affective and psychiatric disorders such as schizophrenia and autism. Stress can also precipitate depressive episodes, which may occur during pregnancy. Women of childbearing age are the population at highest risk for depression. To treat depression in pregnant women, doctors often prescribe drugs like the selective serotonin reuptake inhibitor (SSRI), fluoxetine. However, current human and animal studies regarding the safety of antidepressant drug effects on the unborn child are controversial, and no study has yet examined the long-term effects of prenatal exposure to antidepressants in humans.

Animal models prove extremely useful in studying the long-term effects of antidepressants. Animal researchers have been able to examine effects of developmental exposure to antidepressants in detail, however, very few animal studies examine effects of antidepressants in combination with maternal stress. This is surprising, because antidepressants are not prescribed to healthy pregnant women; they are most often prescribed to treat depression, which co-occurs with stress. The combination of maternal stress and antidepressant treatment will likely affect the offspring differently than each of these variables alone. Because there is no clarity about the safety of antidepressants for the baby, pregnant women at times may refuse antidepressant treatment for fear of potentially harming their baby. However, avoiding treatment may also be harmful to the mother and baby.

In the present study, we investigated the effects of maternal stress and maternal fluoxetine treatment during pregnancy, and early postnatal life, on behavioural outcomes of the offspring at adulthood. Adult offspring of mothers exposed to stress, of mothers treated with fluoxetine, of mothers exposed to neither, or both treatments were tested on their cognitive abilities, memory, aggression, anxiety, sensorimotor information processing, and exploratory and risk assessment behaviours. The function of the brain's circadian "clock" (a system that keeps track of, and synchronises the body to, the time of the day) was also examined: adult male offspring were tested on their ability to respond to environmental time cues, such as light, and non-environmental time cues, such as certain drugs.

Male offspring of stressed mothers are hyperactive, timid, and show altered circadian reactions to a number of time cues. We demonstrate that mouse timidness and inappropriate circadian responses are reversed by fluoxetine. Effects of fluoxetine alone include a decrease in anxiety and an increase in time required recover from jet-lag. Female offspring of stressed mothers are hyperactive and show an abnormal function of the brain system required to filter irrelevant information from the environment. Maternal treatment with fluoxetine appeared to have neither harmful nor beneficial effects on female offspring.

The next step of this research is to better understand the underlying mechanisms of our findings. We are currently analysing the changes in gene expression and neurotransmitter levels in the offspring brains. Results of this study support those of previous reports showing that maternal stress during early stages of development results in sustained, detrimental consequences. We demonstrate that some of the long-term, stress-related consequences can be reversed by fluoxetine treatment, when administered to the mother during pregnancy and the early postpartum period. This study helps us understand neural and behavioural changes resulting from early exposure to fluoxetine, stress, and their combination. This research study will help guide human research regarding the effects of antidepressants and maternal stress on neurobehavioural, neuropsychological, and other health-related outcomes.

Research was supported with funds from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada.

Speaker Summary 71.01

Speaker: Carla Nasca, PhD
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Sex Differences and Commonalities in the Epigenetic Modulation of mGlu2-Dependent Structural Plasticity of Key Limbic Brain Regions by Rapid and Slow-Acting Antidepressants

Saturday, Nov. 12, 1-2 p.m., Hall B-H

Speed of action, treatment resistance and sex-dependent effects are major issues for treating depression, a global health problem. Conventional antidepressants are effective only after 2-3 weeks and about 50% depressed people do not respond to them. Furthermore, depression affects women twice as often as men. In the US, depression affects over 32 million adults with an economic cost of about $200 billion/year. Epigenetics provides a new approach for the development of next-generation antidepressants that act rapidly (good profile of efficacy) with minimal side effects (good profile of tolerability). Our research demonstrates that targeting the epigenetic control of a glutamate gene, mGlu2, in a limbic mood-regulatory brain region, the ventral dentate gyrus (vDG), has the potential to promote faster antidepressant responses than classical antidepressants, which more slowly up-regulate reduced mGlu2. The mGlu2 receptors regulate the synaptic levels of glutamate, the most abundant excitatory neurotransmitter in the central nervous system. Excess glutamate suppresses DG neurogenesis and causes dendritic atrophy. These can be corrected by normalizing glutamate activity through the use of agents, like acetyl-L-carnitine (LAC) and ketamine.

Using a light-dark screening test to evaluate anxiety-like endophenotype in a knock-in mouse model (VMhets) that encodes the human variant brain-derived neurotrophic factor (BDNF), we identified a subset of susceptible male and female mice that, while showing reduced mGlu2 receptors in the vDG, have impairments in depressive-like behaviors. The method of chromatin immuneprecipitation (ChIP) confirmed the epigenetic control of mGlu2 via regulation of acetylation of histone H3K27 in the mGlu2 gene. The decrease in mGlu2 expression in the susceptible male and female VMhets is concomitant with structural atrophy in neurons in the vDG in both male and female VMhets. These vDG structural features and depressive-like traits are corrected by a short term treatment with the rapid-acting antidepressant candidate, LAC, in both sexes. At the same time, we find meaningful sex differences, namely, male-specific stress-induced structural atrophy of neurons in the medial amygdala (MeA), a limbic brain region important for social behavior. Mechanistically, increasing mGlu2 in vDG of male VMhets by virus-mediated overexpression, corrects the impairments in hippocampal and medial amygdala dependent behavioral tasks and the structural alterations in vDG and MeA neurons. The same positive outcome is achieved by increasing mGlu2 with LAC.

The findings from this research provide promise for a next-generation class of antidepressants to more rapdily treat the signs of structural plasticity that are associated with depression, not by “rolling back the clock” but rather by changing the trajectory of brain plasticity in more positive directions. These results add to those from previous studies that have also found that normalization of the glutamate overactivity is effective in promoting rapid antidepressant responses.

Research was supported with funds from the Hope for Depression Research Foundation and the American Foundation for Suicide Prevention.

Speaker Summary 19.01

Speaker: Mollie Woodworth, PhD mollie.woodworth@childrens.harvard.edu
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Boston

Somatic Mutation in Single Human Neurons Tracks Developmental and Transcriptional History
Saturday, Nov. 12, 1-15 p.m., SDCC 25A

Our research indicates that each individual neuron in the human brain contains more than 1,000 unique DNA mutations. This tells us that the human brain is not a homogeneous organ, but a mosaic patchwork of neurons, each of which contains its own unique genome.

Ongoing random mutation to DNA ensures that no two cells in an individual are genetically identical. These mutations can cause cancer in many tissues, and in the brain in particular, they can cause neurological diseases such as epilepsy and developmental brain malformations. Neurons in the brain are born during fetal development, and survive for the life of the individual, while their DNA is under constant assault by environmental mutagens such as oxygen free radicals and electromagnetic radiation. These forces can cause mutations throughout the life of a neuron, and, over time, increasing numbers of these mutations potentially contribute to normal aging and neurodegenerative disease.

This work represents the first time the genomes of single neurons from normal human have been examined for single DNA base changes. In the past, it has only been possible to identify mutations that occur in large groups of cells, but not mutations that occur only in a single cell. This study was published as the cover story of the journal *Science* on October 2, 2015.

In this study, we selected a total of thirty-six individual neurons from donated human brain tissue and read the sequence of their genomes. We compared the sequence of each neuron's DNA to the sequence of DNA from elsewhere in that individual's body, looking for changes that occurred in one or more neurons, but not elsewhere. These changes are called somatic mutations, and since they are present in some cells in the body, but not all, we know that they occurred at some point after the individual began to develop in the womb. We found that these mutations occurred most frequently in highly expressed neuronal genes, indicating that the genes most central to a neuron's identity are those most likely to be mutated. In addition, we were able to identify somatic mutations that occurred during fetal development and were shared between multiple descendent neurons. Because these shared mutations marked neuronal lineages, we were able to use them to create a neuronal family tree. We found neurons that were more closely related to cells in the heart than to three-quarters of their neuronal neighbors in the cerebral cortex of the brain.

Going forward, we plan to investigate neurological disorders of aging, such as Alzheimer's disease, to determine whether diseased neurons have more somatic mutations than healthy neurons. In the future, we might be able to slow the effects of aging or disease by slowing the rate of somatic mutations in the brain.

Somatic mutations in the brain represent a durable and ongoing record of the life history of a neuron, from development through adulthood. By reading the DNA sequence of a single neuron, we can read the neuron's history, and can identify other cells in its family tree.

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