Embargoed until Nov. 15, 12:00 p.m. EST
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ON THE LEADING EDGE OF STRESS:
NEW GENOMIC, OPTOGENETIC, AND EPIGENETIC FINDINGS

Research offers clues that may lead to more effective interventions for stress-related disorders

Washington — Research released today uses the latest genetic tools to explore how stress alters brain function, leading to anxiety, depression, and other stress-related mood disorders. The research was presented at Neuroscience 2011, the Society for Neuroscience's annual meeting and the world's largest source of emerging news about brain science and health.

Stress has many meanings; in neuroscience stress is generally defined as any kind of change that causes physical or psychological strain. Today's findings provide more clues as to how different kinds of stress alter genes and brain function — clues that may explain behavior and mood changes in stress-related disorders.

Specifically, the research released today shows that:

- Brain cells that produce serotonin may be critical for stress to affect mood and behavior. Researchers found that "silencing" serotonin cells blocked increased fear behaviors in stressed mice (Michael Baratta, PhD, abstract 719.07, see attached summary).
- An extra copy of a single gene, general transcription factor II-I (GTF2I), is linked to separation anxiety in both mice and humans (Lucy Osborne, PhD, abstract 901.28, see attached summary).
- The effects of prenatal stress can be passed across generations in male mice. The study suggests that epigenetics are to blame for disruptions in male brain development in mice that experienced prenatal stress and their offspring (Christopher Morgan, abstract 190.02, see attached summary).

Another recent finding discussed shows that:

- Female mice with a modification in the serotonin transporter gene are particularly vulnerable to prenatal stress. This finding suggests that vulnerability to emotional disorders is determined by a complex interaction of genes and environment, including prenatal stress (Sissi Jakob, MSc, see attached speaker's summary).

"Specific types of stress are a serious risk factor for many psychiatric and physical illnesses, including quite common ones such as depression and heart disease," said Klaus A. Miczek, PhD, of Tufts University, press conference moderator and an expert on social stressors and the brain. "Understanding the underlying mechanisms of stress will help identify novel targets for treating these illnesses, thus improving the health — and lives — of millions of people."

This research was supported by national funding agencies, such as the National Institutes of Health, as well as private and philanthropic organizations.

– more –

Related Presentations:

Symposium: **Sex, Stress, Immunity, and Neural Development**
Sunday, Nov. 13, 1:30–4 p.m., Ballroom A

Special Lecture: **Defining the Neuronal Circuitry of Fear**
Sunday, Nov.13, 10–11:10 a.m., Hall D
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Abstract 719.07 Summary

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Serotonin Increases Fearful Behavior Following Stressful Events

Mouse study sheds new light on how stress can lead to emotional disorders

New animal research helps explain how stress affects mood and behavior. The study shows brain cells that produce serotonin are involved in enhancing fearful memories following stress. The research was presented at Neuroscience 2011, the Society for Neuroscience's annual meeting and the world's largest source of emerging news about brain science and health.

The researchers, led by Michael Baratta, PhD, of the Massachusetts Institute of Technology, found that prior stress increased later fear responses in mice. However, when the researchers "silenced" serotonin-producing cells in a brain region called the dorsal raphe nucleus, the stress enhancement of fear was eliminated.

The researchers combined genetic and optical techniques, a strategy called "optogenetics," to turn off the serotonin-producing cells during fearful learning. They used a noninfectious virus to deliver a light-sensitive protein called archaehodopsin-3 (Arch) to serotonergic neurons in the mice. Cells that make Arch are silenced when illuminated with green light. The researchers were then able to turn off the dorsal raphe neurons as the animals moved about freely. This technique allowed the scientists to observe what happens when serotonin activity is turned off at specific times.

"Our findings suggest that serotonin plays a key role in how stress alters how an animal detects danger in its environment," said Baratta. "Future experiments aimed at how serotonin alters brain circuits supporting fear and anxiety should provide a better biological understanding of how stress can turn adaptive responses to maladaptive ones," he said.

This research was supported by the National Institutes of Health, National Science Foundation, Benesse Foundation, Jerry and Marge Burnett, Department of Defense, Massachusetts Institute of Technology, NARSAD, New York Stem Cell Foundation Robertson Investigator Award, Paul Allen Distinguished Investigator Award, Alfred P. Sloan Foundation, SfN Research Award for Innovation in Neuroscience, the Wallace H. Coulter Foundation, and the Army Research Office.

Scientific Presentation: Tuesday, Nov. 15, 3–4 p.m., Halls A–C

719.07, Effects of stress on aversive learning require temporally precise serotonergic signaling
M. V. BARATTA[1,2,3], S. B. KONDANDARAMAIAH[1,5], P. E. MONAHAN[1,2,3], K. KIM[1], A. YANG[1], C. R. FOREST[5], K. A. GOOSENS[2,3], E. S. BOYDEN[1,2,3,4]; [1]MIT Media Lab., [2]McGovern Inst. for Brain Res., [3]Brain and Cognitive Sci., [4]Biol. Engin., MIT, Cambridge, MA; [5]George W. Woodruff Sch. of Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA

TECHNICAL ABSTRACT: Serotonin (5-HT) is implicated in affective control, and dysregulation of serotonergic systems is associated with stress-related affective disorders such as anxiety and depression. Multiple lines of evidence suggest that the serotonergic dorsal raphe nucleus (DRN) is critical for mediating the impact of stress on aversive processing. Although emotionally-relevant sensorimotor events occur over durations spanning milliseconds to minutes, previously used methods for silencing 5-HT activity operate over much longer timescales (hours to permanently). Thus, the causal relationship between sensorimotor events during aversive processing and the relevant timescales over which 5-HT activity mediates the impact of stress is unclear.

To address these issues, we used temporally precise optogenetic silencing tools to examine the role of DRN 5-HT in stress-induced enhancement of auditory fear conditioning in which the tone-footshock contingency was reduced to 50%. This enabled silencing of DRN 5-HT activity on a timescale that was time-locked to the presentation of paired or unpaired tones and footshocks. Initial experiments indicated that immobilization stress led to enhanced conditional freezing during the tone test; this effect was observed only when stress preceded fear conditioning and not when it occurred after acquisition. Additional studies suggested that the stress-induced enhancement of fear learning depended on DRN activation as intra-DRN infusion of a nonselective corticotropin-releasing factor receptor antagonist prior to, but not immediately after, acquisition blocked stress enhancement of freezing. We then selectively targeted Arch, a green-yellow light-driven silencing opsin, to 5-HT neurons by delivering a Cre-inducible adeno-associated virus carrying a reversed and double-floxed transgene encoding Arch-GFP into the DRN of SERT-Cre mice. Loose cell-attached in vivo recordings of 5-HT neurons revealed that these neurons were rapidly silenced during the delivery of

green light (532 nm) to the DRN. Arch-mediated silencing of DRN 5-HT during unpaired tone presentations did not affect the ability of prior stress to enhance fear responding to the tone the next day. In contrast, when optical silencing of DRN 5-HT activity was restricted to tones paired with shocks, stress enhancement of the conditioned fear response was blocked. Silencing DRN 5-HT activity in either condition had no effect on freezing levels in Arch-GFP mice not exposed to stress. Our data suggest that DRN 5-HT mediates the effects of prior stressful experiences on aversive learning by augmenting the impact of specific, temporally delimited, sensory events.

Abstract 901.28 Summary

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Scientists Link Gene to Separation Anxiety in Mice and Humans
Findings may lead to more targeted treatments for anxiety disorders

New research at Neuroscience 2011, the Society for Neuroscience's annual meeting and the world's largest source of emerging news about brain science and health, identifies the genetic roots of separation anxiety.

To identify genes that cause anxiety, Lucy Osborne, PhD, and colleagues at the University of Toronto and the University of Louisville, measured separation anxiety in children with two rare genetic disorders. One group of children had 7q11.23 duplication syndrome (Dup7qll.23), a developmental disorder that results from extra copies of 26 genes on chromosome seven, including the protein GTF2I. People with this disorder tend to have social anxiety problems and specific phobias. The other children had Williams-Beuren syndrome (WBS), a disorder caused by a deletion of 7q11.23. Children with WBS have cognitive deficits, but are unusually social and lack social anxieties. Using standardized diagnostic interviews with the children's parents, the researchers found that 26 percent of the children with Dup7qll.23 had separation anxiety disorder, compared with less than 5 percent for both children in the general population and children with Williams-Beuren syndrome.

"Our research provides evidence of the first gene to cause separation anxiety," said Osborne. "These findings may lead to the development of more targeted therapies for anxiety disorders."

To specifically test the role of GTF2I in separation anxiety, the researchers bred mice with either additional or missing copies of the gene. When separated from their mothers, mouse pups with extra copies of the gene vocalized more; mouse pups with fewer copies did not. "These data were consistent with our results of elevated rates of separation anxiety disorder among children," said Osborne.

This research was supported by the National Institute of Neurological Disorders and Stroke, the National Institute of Child Health and Human Development, and the Canadian Institutes of Health Research.

Scientific Presentation: Wednesday, Nov. 16, 4–5 p.m., Halls A–C

901.28, Duplication of GTF2I results in separation anxiety in mice and humans
J. DIDA[1,2], C. B. MERVIS[6], E. LAM[2], N. CRAWFORD-ZELLI[6], N. CRAWFORD-ZELLI[6], E. YOUNG[2], D. HENDERSON[6], C. A. MORRIS[7], T. ONAY[8], J. WOODRUFF-BORDEN[6], J. YEOMANS[1,3], **L. R. OSBORNE**[4,5,2]; [1]Dept. of Psychology, [2]Inst. of Med. Sci., [3]Ctr. for Biol. Timing and Cognition, [4]Dept. of Med., [5]Dept. of Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [6]Dept. of Psychological and Brain Sci., Univ. of Louisville, Louisville, KY; [7]Dept. of Pediatrics, Univ. of Nevada Sch. of Med., Las Vegas, NV; [8]Samuel Lunenfeld Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada

TECHNICAL ABSTRACT: Deletion of a 1.5 million base pair segment of human chromosome 7q11.23 causes Williams-Beuren syndrome (WBS), a neurodevelopmental disorder characterized by intellectual disability, deficits in visuospatial construction, relative strength in concrete language, lack of stranger anxiety, social disinhibition, and non-social anxiety. Duplication of this region results in a contrasting syndrome, Dup7q11.23, associated with speech delay and/or disorder and social anxiety. GTF2I is one of 26 genes commonly deleted in WBS and duplicated in Dup7q11.23. Using Cre-loxP technology, we generated mice with either reduced or increased Gtf2i genomic copy number and corresponding changes in mRNA and protein expression, to examine the effect of Gtf2i dose on phenotype. P7 mouse pups with 3 (n=78) or 4 (n=37) genomic copies of Gtf2i showed an increase in separation-induced ultrasonic vocalizations (USVs) [157 and 192 USVs respectively on average during the first 2 minutes] compared to wild type littermates (n=119) [102 USVs] ($p<.005$; $p<.001$), whereas P7 pups with only a single copy of Gtf2i (n=23) showed reduced vocalizations [80 USVs] ($p<0.05$). This pattern suggests that Gtf2i has a dose-dependent effect on maternal separation anxiety in mice. To determine if a similar effect was present in humans, we measured separation anxiety in children with Dup7q11.23 (3 copies of GTF2I) and children with WBS (1 copy of GTF2I). Nineteen children [ages 4 - 13 years] with Dup7q11.23 and 214 age-matched children with WBS were assessed using the Anxiety Disorders Interview Schedule for DSM-IV-Parent Interview (ADIS-P). In addition, parental responses for 14 children with Dup7q11.23 aged 2 - 5 years and 189 age-matched children with WBS were compared on the separation anxiety question of the Child Behavior Checklist (CBCL) for Ages 11/2 -5. Based on the ADIS-P, 26% of children with Dup7q11.23 were diagnosed with separation anxiety disorder, compared with only 4.2% of those with WBS ($p = .000$). CBCL findings indicated that 28.6%of children with Dup7q11.23 but only 1.1% of children with WBS had unusual difficulty separating from their parents ($p = .000$). These results suggest that GTF2I plays a significant role in the contrasting separation anxiety phenotypes seen in children with Dup7q11.23 and WBS. This study links the copy number of a single gene from 7q11.23 to separation anxiety in both mice and humans and demonstrates the

utility of mouse models in dissecting disorders that include several genes in humans. The linking of GTF2I to separation anxiety provides the first evidence of single gene associated with separation anxiety and offers a molecular target for future therapies.

Abstract 190.02 Summary

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Effects of Prenatal Stress Passed Across Generations in Mice

Animal research shows prenatal stress affects "epigenome"

Sons of male mice exposed to prenatal stress show changes in the brain, according to new research. The study suggests the effects of prenatal stress are passed across generations due to alterations in the "epigenome," heritable changes that do not affect gene sequence. The findings were presented at Neuroscience 2011, the Society for Neuroscience's annual meeting and the world's largest source of emerging news about brain science and health.

"This finding suggests a mechanism through which prenatal stress may alter male brain development," said lead author Christopher Morgan, of the University of Pennsylvania.

Previous research showed male mice exposed prenatally to chronic maternal stress exhibited increased sensitivity to stress as adults, as well as reduced testosterone levels, smaller testes, and other signs of "dysmasculinization." In the new study, the researchers found these characteristics could also be found in the male offspring of these mice.

The researchers also found prenatal stress resulted in "feminized" levels of microRNAs in the brains of the male mice and their offspring. MicroRNAs are small molecules that regulate dozens of different genes, including, potentially, those involved in masculinizing the brain. Additionally, the researchers were able to create the same results, e.g. "feminized" levels of microRNAs, using a drug that modifies the epigenome. The drug is called a histone deacetylase (HDAC) inhibitor.

"We've now found that the HDAC inhibitor has a similar feminization effect on brain microRNA levels, suggesting that in normal mice, HDACs are involved in producing masculine levels of microRNA," said Morgan. "Together, these findings suggest that prenatal stress may affect male brain development by disrupting masculinization of the brain, and that microRNAs may play a previously unappreciated role in this critical process," he said.

This research was supported by the National Institutes of Health.

Scientific Presentation: Sunday, Nov. 13, 9–10 a.m., Halls A–C

190.02, Prenatal stress dysmasculinizes the perinatal brain miRNA environment
C. P. MORGAN[1,2], T. L. BALE[2]; [1]Philadelphia, PA; [2]Animal biology, Univ. of Pennsylvania, Philadelphia, PA

TECHNICAL ABSTRACT: Over the past 15 years epidemiological studies have repeatedly suggested that variation in the prenatal environment can affect later adult health outcomes. We are investigating the effects of maternal stress exposure during pregnancy on neurodevelopment, and have found that offspring of mice that experience chronic stress early during their pregnancy are stress-sensitive compared to those of unexposed animals. Changes in stress-sensitivity are common across neuropsychiatric disease. Interestingly vulnerability to the effects of prenatal stress was sex-dependent. Prenatal stress affects the adult stress-sensitivity of male, but not female, offspring. Increased stress-sensitivity was observed using both behavioral and physiological tests, and included elevated hypothalamic pituitary adrenal (HPA) axis stress hormones. There are well-established differences in stress-sensitivity between male and female mice, with females generally exerting a greater physiological response than males do to the same stressor. The increased stress-sensitivity exhibited by prenatally stressed males fits a more female-like pattern. Indeed, prenatally stressed males also appear to have a mildly feminized morphology, including smaller testes and reduced anogenital distance, a measure of early life testosterone exposure. Importantly testosterone released during a short window around the time of birth, known as the perinatal sensitive period, is responsible for permanently establishing much of the male-specific neurocircuitry. Previous researchers have shown that prenatal stress during the final week of gestation can disrupt brain masculinization in rats. Our finding that prenatal stress during the first week of gestation also appears to disrupt masculinization, a process that takes places two weeks after the stress is stopped, suggests that masculinization of the male brain may be particularly sensitive to changes in the intrauterine environment. To examine this critical period of gene regulation, we performed a screen of 96 genes in the brains of neonatal F2 males and found that they in fact had a more female-typical gene expression pattern. Subsequent analysis identified several small regulatory RNAs, known as micro-RNAs, whose expression also appeared feminized in these brains. Micro-RNAs have the ability to change the expression of up to a hundred different genes, thus the altered levels of just a few micro-RNAs could affect the expression of entire programs of genes, such as those involved in masculinizing the brain. To confirm that micro-RNAs are involved in masculinizing the brain, we administered an aromatase inhibitor, a drug that blocks the effects of testosterone in the brain, to mice the morning they were born and measured its effects on nearly 250 micro-RNAs. The aromatase inhibitor feminized the expression of micro-RNAs in male mice such that while we were able to separate untreated males from untreated females, based solely on the levels of these micro-RNAs, we were unable to

distinguish between untreated females and males treated with the drug. To identify mechanisms through which prenatal stress may effect brain masculinization, we tested the importance of histone deacetylases in controlling micro-RNA levels. Histone deacetylases (HDACs) can affect the expression of genes, including genes encoding micro-RNAs, by modifying histones, the proteins used to package DNA in the nucleus. HDAC inhibitors have previously been shown to be necessary for brain masculinization. Similar to the effect of the aromatase inhibitor, administration of an HDAC inhibitor to neonatal mice also feminized brain micro-RNA levels. Thus, testosterone may affect miRNA levels, at least in part, through regulating the action of HDACs. Together these findings suggest that prenatal stress may affect male neurodevelopment by disrupting masculinization of the brain and suggest that micro-RNAs may play a previously unappreciated role in this critical process.

Speaker's Summary

Speaker: Sissi Jakob, MSc 49-931-2017-7360
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Prenatal Stress and Adult Psychopathology: Role of the Serotonin Transporter and Epigenetic Programming
(792.15)
Poster Session: Mood Disorders: Animal Models: Epigenetic and Intracellular Signaling Mechanisms
Wednesday, Nov. 16, 10–11 a.m., Halls A–C

Stress during pregnancy may have severe consequences for offspring´s mental health later in life, especially if the child is genetically vulnerable to stress. Interestingly, epigenetic mechanisms have recently been shown to represent a molecular memory by which environmental adversity can program the DNA to change an individual's physiology and behavior throughout the lifespan. The major goal of our study was to identify new genes that represent targets for the epigenetic storage of prenatal stressful events. As such, our results might serve as a valuable knowledge base and source of discovery in the quest for a more effective treatment of psychiatric disorders.

A growing number of people suffer from disorders characterized by emotional dysregulation, such as depression and anxiety disorders. In many cases, chronic and/or excessive stress is the trigger for a disease episode. Interestingly, not all individuals who experience excessive stress are at risk. A combination of genetic vulnerability and an inadequately programmed stress response may, at least partially, explain this phenomenon.

For example, variations in the gene coding for the serotonin transporter (a protein which terminates the signals conveyed by serotonin) render individuals more susceptible to stress. There are two variants of this gene in the population that lead to inter-individual differences in serotonergic function. The serotonergic system plays a pivotal role in the regulation of cognition and emotion, which becomes even more obvious when considering that key players of this system, like the serotonin transporter, are targets of various drugs including antidepressants and anxiolytics. Furthermore, stress vulnerability is known to be programmed by an adverse environment early in life. In fact, fetal brain development is extremely sensitive to disturbances. For example, severe physical or psychological stress, such as experience of violence, death of a beloved person, divorce, earthquakes, terror attacks, or war, increases the pregnant woman's stress hormone levels, which pass the placenta, reach the fetus, and can influence proper programming of stress regulating systems.

This programming process is operated by epigenetic mechanisms which alter gene activation, e.g. through adding or removing chemical groups to or from the DNA (DNA methylation). It is hypothesized that the adaptation to a negative prenatal environment by epigenetic processes can be stored at distinct genes with persistence into adulthood which can affect the onset and course of psychiatric disorders.

To test this notion, we stressed pregnant female mice (genetically normal mice and mice with a modified serotonin transporter gene) during the last week of gestation by restraining them in small glass cylinders. Cognitive, anxiety-, and depression-related behavior of the offspring was examined when the offspring had reached adulthood. Additionally, in the female offspring, genome-wide gene activation and DNA methylation screenings of the hippocampus, a brain region known to be critically involved in emotion regulation, were performed.

Exposure of mice with a modified serotonin transporter gene to prenatal stress was associated with increased depression-like behavior, an effect which seemed to be more pronounced in female offspring. Further, within the female hippocampus, the activation of genes altered by the modified genotype, prenatal stress, and their interaction was partly regulated by DNA methylation. Genes programmed by DNA methylation were players of molecular pathways highly relevant in the etiology of psychiatric disorders like the mitogen-activated protein kinase (MAPK) signaling and neurotrophin signaling pathways.

Our results confirm that vulnerability to emotional disorders is determined by a complex interaction of genes and early life stress. Studying the underlying molecular mechanisms will help to identify novel targets for the treatment of disorders of emotion regulation.