Embargoed until Nov. 13, 9 a.m. EST
Press Room, Nov. 11–15: (202) 249-4230

Contacts: Emily Ortman, (202) 962-4090
Kym Kilbourne, (202) 962-4060

Understanding the Harmful Effects of Stress on the Brain and Promising Approaches for Relief

Stress reduction can guard against mental illness and alleviate the consequences of poor sleep

WASHINGTON, DC — Stress can have numerous harmful effects on the mind and body, both immediately and over long periods of time. New research reveals mechanisms by which stress exacts its toll throughout the body, from the brain to the male reproductive system, and points to potential paths for reducing the negative effects of stress. The studies were presented at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Stress is a state of physical, mental, or emotional strain resulting from adverse or demanding circumstances. Although some level of stress is inevitable — and even beneficial — chronic or excessive stress can wreak havoc on physical and mental health.

Today's new findings show that:

- Stress experienced by fathers may alter gene expression in their sperm, potentially leading to less resilient offspring (Jennifer Chan, abstract 505.02, see attached summary).
- Childhood trauma can result in altered patterns of gene expression and elevated stress hormones, biomarkers that may help identify and treat young trauma victims (Brianna S. Mulligan, abstract 600.04, see attached summary).
- Controlling signaling in brain cells called astrocytes may be an effective way to prevent heightened fear and anxiety responses associated with post-traumatic stress disorder (Meghan E. Jones, abstract 695.15, see attached summary).
- Newborn neurons in the hippocampus, a brain region that processes memory, can decrease effects of stress and symptoms of mental illness in mice (Christoph Anacker, abstract 115.23, see attached summary).
- Microbes found in soil can help guard against stress, vulnerability, and illness in mice after inadequate sleep (Samuel Bowers, abstract 241.19, see attached summary).

"Taken as a whole, these studies illuminate our understanding of the many negative effects of stress on the brain, whether early in life or as adults, and spur optimism about our ability to address these impacts," said press conference moderator Bruce McEwen, PhD, of Rockefeller University, an expert in the body's response to stress.

This 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 the effects of stress on the brain on *BrainFacts.org*.

Related Neuroscience 2017 Presentation

Symposium: Social Origins of Developmental Risk for Mental and Physical Illnesses
Tuesday, November 14, 1:30–4:00 p.m., WCC Ballroom A

Abstract 505.02 Summary

Lead Author: Jennifer Chan
University of Pennsylvania (215) 498-4365
Philadelphia chanje@mail.med.upenn.edu

Dad's Lifetime Stress Impacts His Offspring's Health

Research in mice shows father's stress can affect offspring brain development through his epididymis

The stress that a father experiences can alter how his sperm matures in the epididymis, subsequently impacting his offspring's brain development, according to new animal research released today at Neuroscience 2017, 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 the stressors a mother experiences during pregnancy, such as poor diet or disease, can negatively affect her offspring. Some evidence suggests that alterations in how a fetus' genes are expressed —known as epigenetics — contribute to the intergenerational effects of stress. Only recently, however, have scientists begun to explore whether a father's stress can also affect his offspring by altering the content of important markers in his sperm.

Previously, researchers found that male mice experiencing chronic, mild stress in their environment sire offspring with a "blunted" hormonal response to stress, an effect that has been linked to some neuropsychiatric disorders, such as PTSD. The research team further isolated the mechanism of that diminished offspring stress response, changes in noncoding snippets of RNA that regulate gene expression, called microRNAs, in the stressed father's sperm.

Now, the research team is identifying how mild stress can alter sperm microRNA content to impact the offspring brain development and stress response. In the male reproductive tract, the *caput epididymis,* the site of sperm maturation, releases vesicles packed with microRNA that can fuse with sperm to change its cargo. The caput epididymis was sensitive to stress hormones such as corticosterone, the researchers found. The researchers developed a stress-in-the-dish model to study these cells where they administered the same stress hormones to caput epididymal cultured cells. These cells responded by changing the microRNA content in the vesicles they released similar to what happens in dad. The findings suggest that paternal stress may affect future generations by affecting cells in the epididymis critical for sperm maturation and content.

"Even mild environmental challenges can have lasting effects on future offspring health," said lead author Jennifer Chan, of the University of Pennsylvania. "An improved understanding of how a father's life exposures to stress can affect his offspring is essential in understanding disease risk and improving the detection and prevention for these disorders."

Research was supported with funds from the National Institute of Mental Health.

Scientific Presentation: Tuesday, Nov. 14, 9–10 a.m., WCC Halls A–C

15557. Mechanisms of paternal stress programming of neurodevelopment via dad's epididymis
J. CHAN, N. V. BHANU, B. A. GARCIA, T. L. BALE;
Univ. of Pennsylvania, Philadelphia, PA.

TECHNICAL ABSTRACT: Paternal exposures to environmental insults such as stress, diet, drugs or toxins have been linked with increased risk of neuropsychiatric disease in subsequent generations. Recent studies using rodent models have shown that paternal exposure to a variety of perturbations can impact offspring behavior and physiology, and have identified changes in histone modifications, DNA methylation, and/or small non-coding RNAs in sperm as potential mechanisms of transmission. However, the mechanism by which paternal environmental exposures reprogram sperm epigenetic marks to subsequently influence offspring development is not known. We have developed a paternal stress model in which chronically stressed males sire offspring with a significantly blunted stress response as adults. Sperm analyses identified a significant increase in 9 microRNA (miRs) following paternal stress exposure. Zygote microinjection of these miRs recapitulated the offspring stress phenotype, providing a functional role for sperm miRs. Our current studies examine the mechanism and timing by which chronic stress alters sperm miRs. Remarkably, studies in our mouse model reveal that males bred 3 months following stress exposure continue to produce offspring with altered stress reactivity, suggesting lasting effects of experience on intergenerational transmission. To determine where in the sperm development or maturation process stress-mediated changes occur, we compared reproductive tissue miR expression where transcriptional assessment revealed upregulation of the same stress-sensitive miRs found in sperm in the caput epididymis, suggesting that the caput is involved in programming of sperm miRs. Moreover, levels of glucocorticoid receptor (GR) were specifically increased in the caput months after stress end, suggesting GR may play a role in long-term programming. Interestingly, extracellular vesicles (EV) from the caput have been suggested to fuse and alter RNA expression in maturing sperm. To determine if stress alters caput EV miR expression, we developed an *in vitro* model of chronic corticosterone treatment using caput epithelial cells. We show that our model mimics aspects of chronic stress programming *in vivo*, including increases in GR levels long-term. Remarkably, we show that chronic corticosterone treatment alters caput EV miR expression, suggesting a mechanism by which the caput may communicate stress programming to sperm. These studies suggest that paternal experiences can have lasting changes on the germline and future offspring brain development, and offer an exciting novel mechanism by which the environment can dynamically regulate sperm epigenetic marks.

Abstract 600.04 Summary

Co-Author: Brianna S. Mulligan
University of New Mexico Health Sciences Center (847) 807-9397
Albuquerque, N.M. bmulligan@salud.unm.edu

Researchers Identify Epigenetic Markers of Childhood Trauma

Biomarkers provide potential for flagging victims, intervening early in childhood post-traumatic stress disorder

Biomarkers in hair and saliva could be used to identify children who have recently experienced trauma and potentially flag young victims of child abuse, according to new research released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Pediatric trauma is the leading cause of death for children aged 1–14 in the United States, accounting for nearly 40 percent of all childhood fatalities. When children survive traumatic events, known as adverse childhood experiences (ACEs), many suffer long-term health and behavioral issues, including post-traumatic stress disorder (PTSD). Early detection of trauma is crucial to preventing abuse-related deaths, as well as for addressing early the health and behavioral consequences of ACEs.

Researchers investigated the impact of ACEs on children aged 4–8, all of whom were receiving trauma-focused cognitive behavioral therapy. Over six to eight months, the researchers collected hair samples and saliva from the children and compared two biological measures with children who had not experienced trauma. The first measure, obtained from hair, was levels of the stress hormone cortisol. The second measure, taken from saliva, was DNA methylation — a reversible biochemical process through which a methyl group is added to a DNA sequence, turning a gene "off" or "on."

Compared to children who had not experienced trauma, children with recent ACEs had elevated levels of cortisol and exhibited significantly altered DNA methylation patterns. These abnormal DNA methylation signatures might help researchers identify children who have experienced trauma, the researchers noted.

"Since DNA methylation is reversible, identifying the timing of alterations in this marker will guide early interventions and may inform new treatments for PTSD," said co-author Brianna Mulligan, of the University of New Mexico. The researchers' now hope to make it easy for others to perform methylation tests pertaining to childhood trauma through the development of a simple, low-cost screening platform.

Research was supported with funds from the National Institute of Mental Health and the Harvey Family Endowment.

Scientific Presentation: Tuesday, Nov. 14, 4–5 p.m., WCC Halls A–C

13559. Differential epigenetic patterns in children with documented trauma.
B. S. MULLIGAN, E. L. BEARER,
University of New Mexico, Albuquerque, NM.

TECHNICAL ABSTRACT: Pediatric trauma is prevalent, a leading cause of death in children in the USA (accounting for 34-39% of all deaths in ages 1 to 14 years), and is associated with development of post-traumatic stress disorder (PTSD). Why adverse childhood experiences (ACEs), such as traumatic injury, have lifelong impact on both mental and physical health is not understood. Our hypothesis is that ACEs trigger alterations in stress hormones and epigenetic changes (DNA methylation, DNAme) that persist over the lifespan. To test this we investigated cortisol levels from hair samples and DNA methylation from saliva collected from children, ages 4-8, referred for trauma-focused cognitive behavior therapy (TF-CBT). These children were followed at four time points, over the 6 to 8 months of therapy. We measured cortisol levels in 3cm of hair and DNA methylation at 850,000 sites in saliva at entry and one month after therapy. All but one of the children displayed altered cortisol levels: some were elevated and dropped towards normal at conclusion; one child was normal and rose significantly during TF-CBT. We found a global DNAme difference between the child whose cortisol rose and the other children. Since anxiety is known to cause dry mouth in children, we explored whether this global change may be due to cell composition of the saliva by obtaining methylation patterns for keratinocytes and whole blood and performing a reference-based cell deconvolution in RnBeads. We found that the global DNAme patterns were attributable to cell type composition of the saliva: children with ACE-related changes had saliva samples composed of 68.653% (+/- 3.343%) keratinocytes (K), with the remaining being whole blood. Those that did not report trauma showed 36.452%(+/- 5.735%) K composition. This variation in saliva cell composition of traumatized children was replicated with children from a different study, who also displayed high K composition (69.854% +/- 6.534%). Significant epigenetic differences between children who recently had trauma and those that did not remained after cell composition correction. These changes were primarily in genes involved in immune response and regulation of DNA methylation, while some were altered for genes over-expressed in the brain. To what extent duration and intensity of trauma effect these changes, or what mechanism may drive an individual's "resiliency" is unclear; we intend to explore these questions further by investigating the relative impact of injury on the methylome.

Abstract 695.15 Summary

Lead Author: Meghan E. Jones
University of North Carolina at Chapel Hill (919) 962-4149
Chapel Hill, N.C. meghanje@live.unc.edu

Astrocytes May Influence Fear and Anxiety Responses After Stressful Event

Researchers attenuated stress responses in rats by intervening in astrocyte function

Astrocytes, specialized glial cells that are vital to brain function and health, may play a key role in the development of symptoms similar to those of post-traumatic stress disorder, a discovery could lead to better treatments for PTSD, according to new animal research released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Astrocytes were previously thought to be the brain's support cells, providing a kind of "glue" between neurons, but a growing body of research suggests astrocytes play many other important roles in the brain, including in its immune response. PTSD, which causes persistent feelings of stress and anxiety long after a traumatic experience has occurred, has recently been found to affect the immune system.

To better understand how stress-related disorders affect the immune system, researchers subjected one group of rats to repeated stress — namely, a series of disorienting foot shocks — while another group of rats was not exposed to any stressor. Analysis of the rodents' brain tissue revealed that stress-exposed rats had elevated levels of a molecule called interleukin-1β (IL-1) in astrocytes in the hippocampus, a brain area critical to learning and memory.

Interleukin-1β belongs to a group of molecules called cytokines, which regulate the brain's response to illness. When the researchers blocked the cellular receptor for IL-1, the mice did not develop increased fear and anxiety after the foot shocks. In a separate experiment, the researchers used a genetic manipulation to directly control astrocyte function in this brain region and prevent the rodents from developing increased fear and anxiety after exposure to stress.

"We are the first to show that an astrocyte-specific manipulation influenced the of development stress-enhanced fear learning," said lead author Meghan Jones, of the University of North Carolina. This finding could ultimately provide new insights and potential treatments for PTSD, she added.

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

Scientific Presentation: Wednesday, Nov. 15, 10–11 a.m., WCC Halls A–C

16170. Neuroimmune signaling in stress-enhanced fear learning, an animal model of post-traumatic stress disorder
M. E. JONES, J. E. PANICCIA, C. LEBONVILLE, D. T. LYSLE,
University of North Carolina, Chapel Hill, NC.

TECHNICAL ABSTRACT: Neuroimmune signaling is important in learning and memory processes. Further, converging evidence suggests that neural immune interactions, including astrocyte activity, are altered following stress exposure. For example, our laboratory has shown that the severe stressor in stress-enhanced fear learning (SEFL), an animal model of PTSD, induces a time-dependent increase in interleukin-1β (IL-1β) protein and mRNA and that blocking IL-1 signaling prevents the development of SEFL. Here, we employed triple labeling fluorescence immunohistochemistry to determine the cellular source of IL-1β and recent advances in chemogenetic technology to begin to explore the role of astrocytes, a key immune cell type in the central nervous system, in stress-enhanced fear learning. We used glial DREADDs (designer receptors exclusively activated by designer drugs) to test whether Gq and/or G i signaling specifically in hippocampal astrocytes influences the development of SEFL. In Experiment 1, rats were exposed to Context A of the typical SEFL paradigm (15 2mA scrambled foot shocks over 90 minutes on a 6 minute variable interval schedule) and sacrificed via transcardial perfusion 48 hours later. Brains were extracted and tissue was processed for immunohistochemistry with primary antibodies against IL-1β and cell type specific markers for astrocytes, GFAP, neurons, NeuN, and microglia, Iba-1. Alexa fluor -conjugated secondary antibodies were used for visualization. Confocal microscopy and Bitplane Imaris colocalization analyses revealed that 92% of hippocampal IL-1β is expressed by astrocytes, while only 3% and 5% of hippocampal IL-1β is expressed in microglia and neurons, respectively. In Experiment 2, rats were infused with AAV8-GFAP-hm4di-mcherry or AAV5-GFAP-hm3dq-IRES- mCitrine directly into the dorsal hippocampus (DH) and allowed three weeks to recover from surgery and for the virus to express. Rats were then exposed to the typical SEFL paradigm and administered Clozapine-n-oxide (CNO) (3 mg/kg, s.c.) immediately, 24h and 48h after removal from Context A. Interestingly, while activating astroglial G i signaling significantly attenuated the development of SEFL, activating astroglial G q signaling did not influence SEFL. In summary, Experiment 1 quantitatively showed that astrocytes are the predominant cellular source of stress-induced IL-1β in the DH. Strikingly, our preliminary data in Experiment 2 showed that activating astroglial Gi signaling, but not astroglial G q signaling attenuated SEFL, a PTSD-like phenotype. Further studies in our laboratory are testing whether any effects of glial GPCR signaling occur through an IL-1β-dependent mechanism.

Abstract 115.23 Summary

Lead Author: Christoph Anacker, PhD
Columbia University (646) 774-7339
New York ca2635@cumc.columbia.edu

Newly Formed Hippocampal Cells Reduce Stress Responses in Mice

Research may provide new strategy for decreasing vulnerability to stress and mental illness

Newly generated cells in the hippocampus, which is involved in regulating memory processing and mood, reduce stress and symptoms of mental illness in mice, according to new research released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Unlike skin or hair cells, most brain cells do not divide to replace dead or damaged cells. Instead, humans and mice are largely stuck with the neurons they formed during early development. There is an exception to this rule, however: Throughout the lifespan, a small population of neurons is constantly renewed in the hippocampus.

To investigate whether these newly generated hippocampal cells play a role in reducing vulnerability to stress and mental illness, researchers compared two sets of mice: one with a genetic modification that led them to develop a profusion of new hippocampal cells and another that produced a normal quantity of newborn neurons. Over the course of 10 days, both groups of mice encountered larger, more aggressive mice, a situation designed to increase fear and stress. Using fluorescent molecules to track the activity of the hippocampus, the team found that mice with more new cells had a reduced response to the stressful situation and acted less anxious.

"Our research indicates that new brain cells that are generated during adult life can protect from mental illness by dampening the brain's responses to stress," said lead author Christoph Anacker, of Columbia University.

The next phase of this research will test pharmacological strategies that can stimulate the development of new hippocampal cells, both in mice and in humans.

Research was supported with funds from Hope for Depression Research Foundation, the National Institute of Mental Health, the German Research Foundation, and NYSTEM.

Scientific Presentation: Sunday, Nov. 12, 10–11 a.m., WCC Halls A–C

12088. Adult hippocampal neurogenesis buffers ventral dentate gyrus responses to chronic stress.
C. ANACKER, G. STEVENS, A. MILLETTE, R. SHORES, R. HEN;
Columbia University, New York, NY.

TECHNICAL ABSTRACT: Adult hippocampal neurogenesis has been proposed to confer resilience to chronic stress and to modulate dentate gyrus activity. However, it is unknown how adult-born neurons regulate information processing in the dentate gyrus granule cell network. Here, we used in vivo Ca2+-imaging with head-mounted miniature microscopes (Inscopix, CA) in the dentate gyrus of freely moving mice to investigate how young adult-born neurons regulate the response of mature granule cells during chronic psychosocial stress. The intracellular Ca2+ indicator, GCamp6f, was virally-expressed in mature granule cells of the ventral dentate gyrus. Ca2+ activity was imaged in wild-type mice with normal levels of neurogenesis and in transgenic mice with a 2±0.2 fold increase in doublecortin-positive young neurons, due to a deletion of the pro-apoptotic gene Bax from adult neural stem cells and their progeny. We imaged 30-100 granule cells per mouse throughout 10 days of social defeat stress and during subsequent tests of anxiety-like behavior.On the first day of social defeat, granule cells of the ventral dentate gyrus do not respond to an attack by a dominant aggressor mouse. However, Ca2+ firing rates are overall higher in wild-type mice with normal levels of neurogenesis (0.01±0.001, n=548 cells) than in mice with increased neurogenesis (0.008±0.001, n=540 cells, p=0.03). On the last day of the social defeat stress procedure (day 10), wild-type mice show increased Ca2+ activity in response to an attack (0.017±0.001, n=548 cells). This effect is reduced in mice with more neurogenesis (0.013±0.0006, n=540 cells, p=0.02). After chronic social defeat, mice with increased neurogenesis are resilient to the stress and interact longer with a novel mouse in a social interaction test than wild-type mice (by 35±4%; n=10 mice; p<0.01). These resilient mice with more neurogenesis also exhibit lower granule cell Ca2+ firing rates during social interaction than wild-type mice (by 23±1%; p=0.05). In the open field test, mice with increased neurogenesis spend more time in the brightly lit anxiogenic center of the open field than wild-type mice (40±5% sec; n=10 mice, p<0.05). Ca2+ firing rates are higher during center exploration than during exploration of the less anxiogenic periphery (periphery rate: 0.010±0.0003; center rate: 0.014±0.0007, n=548 cells, p=0.002). However, mice with increased neurogenesis exhibit lower center rates than wild-type mice (0.008±0.0008, n=540 cells, p<0.0001). Our findings demonstrate that hippocampal neurogenesis inhibits the response of ventral dentate gyrus granule cells to chronic stress and to anxiogenic conditions.

Abstract 241.19 Summary

Co-Author: Samuel Bowers
Northwestern University (720) 938-1525
Evanston, Ill. samuel.bowers@northwestern.edu

Soil Bacteria May Increase Resiliency to Stress After Sleep Disruption

Non-harmful microbes guard against stress and illness in mice after sleep loss

A common microbe found in soil may mitigate the harmful effects of sleep disruption, according to new animal research released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Sleep disruption increases vulnerability to stress, which in turn increases the risk for mental and physical health problems. Military personnel, medical staff, and others with jobs that require them to respond to acute emergencies are particularly prone to the combined effects of stress and sleep loss, studies have shown.

Previous research has demonstrated that harmless bacteria such as *Mycobacterium vaccae* (NCTC11659), found in soil, can have positive health effects. To investigate whether the bacteria can buffer against the effects of sleep loss, researchers at Northwestern University injected mice with the bacteria, subsequently exposed them to several days of inadequate sleep, and then placed them in a highly stressful situation in which they encountered larger and more aggressive mice. Compared to similarly sleep-deprived, stressed mice that did not receive the bacteria injection, the mice dosed with *Mycobacterium vaccae* performed significantly better on a learning and memory test. Indeed, they performed just as well as control mice that were neither sleep-deprived nor stressed.

"The trillions of microbes in us and on us, known collectively as the microbiota, play an integral role in maintaining physical and mental health," said co-author Samuel Bowers, of Northwestern University. "Our research supports a relatively new biomedical framework whereby the microbiota are therapeutic targets for prevention and treatment of neurobiological disorders."

Research was supported with funds from the Office of Naval Research.

Scientific Presentation: Sunday, Nov. 12, 3–4 p.m., WCC Halls A–C

12815. Mycobacterium vaccae enhances sleep and counteracts effects of stress and sleep disruption in mice
S. LAMBERT[1], **S. J. BOWERS[1]**, C. J. OLKER[1], E. SONG[1], K. P. WRIGHT[2], M. FLESHNER[2], C. A. LOWRY[2], M. VITATERNA[1], F. W. TUREK[1];
1: Northwestern University, Evanston, IL. 2: University of Colorado, Boulder, CO.

TECHNICAL ABSTRACT: Warfighters, emergency technicians, and medical staff are regularly exposed to acute stressors and long work hours that disrupt regular sleep. There is growing evidence that sleep disruption can potentiate the negative effects of acute stress, including impairments in cognition. There is a need, therefore, to investigate countermeasures that combat this "double-hit" of acute stress and sleep disruption. Immunization with heat-killed Mycobacterium vaccae, an environmental bacterium and known immunomodulator, has been shown to promote stress resilience in mice. However, its effects in the context of sleep disruption have not been studied. In this experiment, we investigated M.vaccae as a countermeasure to the double-hit. Mice (C57BL/6N) were surgically implanted with EEG/EMG recording devices then given three weekly injections of heat-killed M.vaccae preparations, followed by five days of 20-hour sleep disruption with a four-hour sleep opportunity. Immediately following sleep disruption, animals were exposed to a one-hour episode of social defeat, allowed 24 hours of recovery sleep, then tested in a hippocampal memory task. Mice that received social defeat alone or social defeat plus sleep disruption showed impaired learning and reduced habituation to the testing chamber. M.vaccae treated animals, however, did not display these memory deficits. Furthermore, M.vaccae treated animals that received social defeat alone or social defeat plus sleep disruption showed increased total sleep, particularly increased REM. These results indicate that immunization with M.vaccae curtails the synergistic effects of the double hit to cognition. Our findings suggest that host-microbial immunity merits investigation as therapeutic targets for stress-related pathologies.