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Studies Explore Relationship Between the Gut Microbiome and the Brain

Targeting gut bacteria may help treat stress, anxiety, and depression

CHICAGO — Research released today reveals new insights into the important two-way communication that takes place between the brain and the bacteria residing in the gut. The findings demonstrate how acute, chronic, and prenatal stress can alter the bacterial populations in the gut and how these changes might impact responses to physiological and psychological stress. The studies were presented at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The human body has 10 times more bacterial cells living within it than human cells. The gut microbiome, the collection of microorganisms living within the gastrointestinal tract, is vital for immune function and metabolism.

Today's new findings show that:

- A probiotic, a supplement containing live bacteria that provides health benefits, reduced physiological and psychological stress and improved memory in a small study of healthy men, suggesting a potential new approach to treating psychological disorders, such as generalized anxiety disorder and depression (Andrew Allen, abstract 162.04, see attached summary).
- Prenatal stress changes the vaginal microbiome of pregnant mice and the altered bacteria are transferred to the gut microbiome of male offspring during delivery, altering their response to stress in adulthood (Eldin Jasarevic, abstract 248.01, see attached summary).
- Mice lacking the serotonin transporter have different gut microbiome and different behavioral responses to an inflammatory substance when compared with wild-type mice, suggesting a role of serotonin in regulating the microbiome's response to stress (Briana Kille, abstract 162.12, see attached summary).
- Chronic stress alters the gut microbiome of mice, potentially making the effects of stress worse (Aaron Shoskes, abstract 162.13, see attached summary).

"Today's findings continue to highlight the importance of the interaction between the gut and the brain," said Robert Yolken, MD, of Johns Hopkins University, an expert in the role of infections in psychiatric diseases. "A better understanding of this link will inform new strategies for preventing and treating many psychological disorders."

This research was supported by national funding agencies such as the National Institutes of Health, as well as other private and philanthropic organizations. Find out more about the microbiome and the brain at BrainFacts.org.

Related Neuroscience 2015 Presentation:

Nanosymposium: Stress and Anxiety
Sunday, Oct. 18, 8-11:30 a.m., S102

Abstract 162.04 Summary

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Probiotic May Help Alleviate Stress-Related Conditions

The bacteria strain Bifidobacterium longum 1714 reduces stress, improves memory in study of healthy men

A probiotic, a supplement containing live bacteria that provide health benefits, may help reduce stress and improve cognition, according to a small study of healthy men released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Previously the researchers showed that a bacterial strain *Bifidobacterium longum 1714* (*B. longum 1714*) reduced stress, anxiety, and depressive-like behaviors and improved memory in mice. To see whether the strain would have similar effects in humans, the researchers had 22 healthy male volunteers take the probiotic strain daily for four weeks and a placebo for another four weeks.

At the start of the study and after each of the four-week conditions, researchers measured the participants' acute stress, memory, and brain activity. The participants also rated their daily stress on a questionnaire throughout the study. The researchers found that both perceived daily stress and physiological reaction to an acute stressor were reduced in the probiotic condition. Participants also performed better on a visual memory task after receiving the probiotic. These findings suggest *B. longum 1714* may prove to be a useful probiotic for alleviating stress-related conditions, although additional studies with more participants are needed.

"This research shows a single probiotic can alter central nervous system processes such as stress and memory in humans," said lead author Andrew Allen, PhD, of University College Cork in Ireland. "These findings could be taken forward into people with psychological disorders related to stress, such as generalized anxiety disorder or major depression."

Research was supported with funds from the Science Foundation Ireland, the Health Research Board of Ireland, and the European Community's Seventh Framework Programme.

Scientific Presentation: Sunday, Oct. 18, 11 a.m.-noon, Hall A

162.04, Towards psychobiotics for stress & cognition: Bifidobacterium Longum blocks stress-induced behavioural and physiology changes and modulates brain activity and neurocognitive performance in healthy human subjects

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TECHNICAL ABSTRACT: Introduction: The emerging concept of the gut microbiome as a key regulator of brain and behaviour represents a paradigm shift in neuroscience. Precise targeting of the microbiome-gut-brain axis with psychobiotics - live microorganisms with a potential mental health benefit - is a novel approach for the management of stress-related conditions. Preclinical studies have identified a strain of Bifidobacterium, *B. longum* NCIMB 41676, as a putative psychobiotic with an impact on stress-related behaviours, physiology and cognitive performance. This study thus investigated whether these preclinical effects could be translated to healthy human volunteers.

Methods: Healthy male volunteers (N = 22, Mean age = 25.5 +/- 1.2 years) completed the study. Participants ingested (2.16 +/- 0.20) X 10⁹ CFU of *B. longum* NCIMB 41676 or placebo daily for four weeks each in a repeated-measures design. Participants completed study visits at baseline, post-placebo and post-probiotic. Acute stress (subjective and cortisol output) was assessed using the socially evaluated cold pressor test, and daily stress was assessed online via a validated questionnaire (Cohen Perceived Stress Scale). Cognitive performance was assessed using the CANTAB platform and neurological activity via resting electroencephalography (EEG). Results: In response to acute stress, *B. longum* NCIMB 41676 led to a reduction in cumulative cortisol output as well as a blunted increase in subjective anxiety. Self-reported daily stress was also lowered during daily consumption of the probiotic. There was a subtle improvement over placebo in visuospatial memory performance in paired associate learning (PAL) in the *B. longum* NCIMB 41676 group. Central EEG theta power was lower following *B. longum* NCIMB 41676 consumption compared to placebo.

Conclusions: *B. longum* NCIMB 41676 is associated with attenuated responses to psychological and physiological stress and a modest improvement in cognitive performance, as well as with altered EEG output in healthy volunteers. These clear but subtle benefits are in line with the predicted impact from preclinical screening platforms and highlight the promise of precision-microbiome manipulation strategies. Further studies are warranted to evaluate the benefits of this putative psychobiotic in relevant stress-related conditions and to unravel the mechanisms underlying such effects.

Abstract 248.01 Summary

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Maternal Stress Alters Microbiome of Male Offspring in Rodents

Pregnant mice exposed to stress transfer altered microbiome to male pups during delivery

New animal research suggests that mothers transfer some of the effects of prenatal stress to their male offspring during delivery via their vaginal microbiome, according to a study released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. When mouse pups were exposed to bacteria from mothers that experienced early prenatal stress, the male mouse pups — but not the females — had an altered microbiome and increased stress responses as adults.

An infant's gut microbiome is initially populated with bacteria transferred from the mother's vaginal microbiome during delivery. The infant microbiome is critical for developing metabolism, immunity, and a healthy brain. Healthy brain development is also influenced by prenatal stress, which can increase risk for neurodevelopmental disorders such as autism and schizophrenia.

The researchers tested whether the effects of early prenatal stress might be transferred to the offspring in part via the mother's vaginal microbiome. The researchers delivered pups by cesarean section and then exposed them to microbes from other mothers that had either been stressed during pregnancy or not. Male offspring exposed to the microbiota of stressed mothers had an altered microbiome and increased responses to stress in adulthood when compared with those exposed to microbiota of control mothers. The results suggest that some of the effects of prenatal stress may be due to the transfer of specific bacteria during delivery, providing evidence that treatments to change the gut microbes might help reverse the effects of prenatal stress.

“I think that this research is critical because it's really pushing us into a new era of understanding the multifactorial nature of neurodevelopmental disorders,” said lead author Eldin Jasarevic, PhD, of the University of Pennsylvania. “If we address the issues in the gut and reverse some of these effects, can we alleviate some of the developmental issues that we see in the brain? We now have different ways to think about intervention for neurodevelopmental disorders that we haven't had before.”

Research was supported with funds from the Penn Vet Center for Host-Microbial Interactions at the University of Pennsylvania and the National Institute of Mental Health.

Scientific Presentation: Sunday, Oct.18, 1-2 p.m., Hall A

248.01. Fumbling the maternal-fetal microbial handoff: maternal stress reprogramming of the developing gut-brain axis

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TECHNICAL ABSTRACT: Prenatal stress is associated with an increased risk for neurodevelopmental disorders. In our established mouse model of early prenatal stress (EPS), long-term programming effects on offspring development have been demonstrated, including reprogramming of the hypothalamic-pituitary-adrenal (HPA) axis, dysregulation of HPA stress axis responsivity, cognitive dysfunction, and post-pubertal growth. Mounting evidence points to a likely influence of maternal stress experience on reprogramming of the gut-brain axis via the maternal vaginal microbiome. As the neonate acquires its founding population of gut microbes during passage through the birth canal, changes in the vaginal microbiome produced by stress during pregnancy alters the composition and function of the microbiota colonizing the neonate gut. Such programming exerts lasting effects on the neonate brain by metabolically altering the neonate gut environment, and ultimately contributing to aspects of our EPS phenotype. We have found that EPS changes in the microbiome composition are paralleled by plasma and gut metabolite profiles related to impaired energy metabolism and availability, and sex- and brain region-specific changes in amino acid transport by postnatal day 2 (PN2). This early life disruption in neonate microbiota composition is associated with long-term and sex-specific impact on microbial composition, metabolism, and gastrointestinal barrier deficits into adulthood as well. To provide a causal link of the stress-altered vaginal microbiota with reprogramming of the developing gut-brain axis, we used cesarean delivery (CD) of neonate mice colonized with vaginal microbiota from either control or EPS dams, and assessed microbial composition, the gut metabolome, and the developing hypothalamic transcriptome at PN2. We confirmed that colonizing CD offspring with maternal vaginal microbiota restored microbial diversity and abundance similar to vaginally delivered offspring, providing validation that vertical transmission of maternal vaginal microbiota is a critical source of microbial diversity in the neonate gut. To determine whether colonization by a stress-altered microbiota recapitulates key features of our EPS phenotype, gastrointestinal barrier function and HPA stress responsivity in these offspring was assessed in adulthood. Remarkably, aspects of the EPS phenotype are transferrable by colonization of a stress-altered vaginal microbiota, validating the importance of the vaginal microbiome in neurodevelopmental programming. Together, these studies provide valuable insight into the novel role of maternal stress in driving the developing gut-brain axis.

Abstract 162.12 Summary

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Serotonin Transporter May Influence Gut Microbiome, Behavior

Mice lacking the serotonin transporter have altered microbiomes and behavioral responses to inflammation

The serotonin transporter appears to affect the composition of the gut microbiome and behavioral response to inflammation, according to new animal research presented today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. The serotonin transporter regulates reuptake of serotonin, a neurotransmitter implicated in depression, and is the target of antidepressants such as Prozac. After receiving an injection that triggered inflammation, mice lacking the serotonin transporter had different populations of gut bacteria and displayed fewer sickness behaviors compared with wild-type mice.

Previous work by the researchers indicated that mice lacking the serotonin transporter (called SERT knockout mice) didn't exhibit normal sickness behaviors, such as fevers and lethargy, when given lipopolysaccharide, a component of bacteria that triggers an immune response. Because 90 percent of the serotonin in the body is found in the gut, the researchers wondered whether the microbiomes of SERT knockout mice might be altered and whether that could explain their atypical behavior.

In this study, lead author Briana Kille and colleagues at the University of Missouri compared wild-type and SERT knockout mice four hours after an injection with either lipopolysaccharide or saline, as a control. The researchers measured the amount of time the mice spent moving and the distance they traveled to gauge sickness behavior after the injection. Once again, following lipopolysaccharide injection, the SERT knockout mice displayed less sickness-like behavior compared with wild-type littermates. When comparing the animals' microbiomes, they found that the two groups of mice that were injected with lipopolysaccharide had different microbiomes than the saline-treated controls. However, the changes to the microbiomes of the SERT knockouts and wild-type mice injected with lipopolysaccharide were significantly different. The study provides evidence that the serotonin transporter can influence the composition of the bacteria in the gut, which in turn could affect behavior.

"Knowing more about how the serotonin transporter affects the microbiome gives us a better idea of where we should target therapies for things like depression and anxiety," Kille said.

Research was supported with funds from the Department of Veterinary Pathobiology at the University of Missouri.

Scientific Presentation: Sunday, Oct. 18, 11 a.m.-noon, Hall A

162.12. Gut microbiota and blood analyses underlying reduced sickness-like behavior in serotonin transporter knockout mice following inflammatory challenge
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TECHNICAL ABSTRACT: Serotonin transporter (SERT) knockout mice have been used extensively in neuropsychiatric research to better understand the role of serotonin signaling in affective disorders. Additionally, the model has illuminated a role for SERT in gut function and metabolic disorders. Given the recognized role of the gut microbiota in host metabolic function and the increasing interest of the microbiome in the gut-brain axis, we sought to characterize how the SERT model genetically constrains the enteric ecological niche in the gut, particularly given the abundance and importance of serotonin in gut physiology and homeostasis. Here we tested the hypothesis that littermates of different genotypes would have differing microbiota which could be perturbed acutely (within 4 hours) by an inflammatory challenge, an injection of lipopolysaccharide. Our experimental approach involved next-generation sequencing, complete blood counts, serum chemistry panels, serum cytokines, serum corticosterone, and behavioral testing. The three major results of this study are that 1) wild-type and SERT knockout littermates have different gut microbiota; 2) the microbiota is highly responsive to inflammatory events occurring in the host; and 3) SERT knockout mice have reduced sickness-like behavior in response to an inflammatory challenge. These data underscore the multi-systemic complexity in coordinating behavioral responses to stimuli.

Abstract 162.13 Summary

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Chronic Stress Alters the Microbiome in Mice

Six weeks of stress leads to widespread changes in gut bacteria that may make the stress worse

New animal research provides evidence that chronic stress decreases species of bacteria in the gut that help regulate the stress response, according to a study released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. When mice were put through six weeks of unpredictable stress, the microorganisms that colonize their gastrointestinal tracts changed, as certain phyla of bacteria increased in number while others decreased.

Previous research has shown that disrupting gut bacteria can cause anxiety and depressive behaviors in animals. In addition, an acute, one-time stressor can significantly change the microbiome. Until now, it was unknown what effects more chronic stress might have on the microbiome and how that might influence the animal's behavior.

To examine the effects of chronic stress, Shoskes and colleagues subjected mice to twice-daily unpredictable stress for six weeks. The stressors were mild to moderate, such as predatory odors in the cage or flashing lights during the sleep cycle. By the end of the six weeks the mice showed behavioral signs of being stressed or depressed. The stress led to significant changes in the composition of gut bacteria, including a significant decrease in the genus *Lactobacillus*. Other studies have shown *Lactobacillus* alleviates stress, so a decrease may make the brain even more vulnerable to the effects of stress. Combined with previous research, these results suggest targeting the gut bacteria (increasing *Lactobacillus*, for example) may help prevent problems associated with chronic stress.

“What our research suggests is there's definitely an effect of chronic stress feeding back onto the microbiome and maybe setting up some kind of negative feedback loop,” said lead author Aaron Shoskes of Des Moines University. “It's a first step toward potential targeted therapies at the microbiome for a myriad of different possible conditions, like anxiety and depression.”

Research was supported with funds from the Iowa Osteopathic Education and Research Foundation.

Scientific Presentation: Sunday, Oct. 18, 8-9 a.m., Hall A

162.13. Longitudinal effects of chronic stress on the murine gut microbiota

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TECHNICAL ABSTRACT: Emerging evidence supports a bidirectional communication axis between the brain, the gastrointestinal tract, and the microbiota colonizing the gut. Various physical and psychological stressors have been shown to influence the function of the GI tract and its resident microbiota. Furthermore, dysbiosis of GI tract colonization has been associated with different diseases, including depression. We investigated the relationship between chronic stress and the murine gut microbiota by comparing the taxonomic composition before and after chronic stress. Mice were subjected to chronic unpredictable stress (CUS) for six weeks. Fecal samples were collected at different time points before and after CUS. Bacterial genomic DNA was extracted and amplicons representing the V4 region of 16S rRNA genes were amplified and sequenced. We observed phylum level differences: mean abundance of *Bacteroidetes* increased and *Firmicutes* decreased over the chronic stress period. Decreases in abundance of *Bacilli* (a class within *Firmicutes*) and *Lactobacillus* (a genus in *Bacilli*) appeared to contribute to the abundance decrease at the phylum level. No significant difference in alpha diversity was noted, indicating CUS induced abundance changes are unlikely due to differences in the amount of diversity. Changes in the ratio of the phyla *Bacteroidetes* to *Firmicutes* have been found to be associated with metabolic disease and obesity. Our results suggest that genera within *Bacilli* may be targets of prebiotic or probiotic therapies to restore a microbiota associated with well-being. Further studies are currently underway to address whether other forms of chronic stress such as chronic pain, result in similar taxonomic changes in GI resident microbiota.