

Stress and intestinal disease

Researchers investigate complexities of the brain-gut axis to unravel stress-related disorders in animals and humans

By Adam Moeser

Stress-related gastrointestinal disorders are among the most economically and socially burdensome illnesses in the United States. Maladies such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and gastro-esophageal reflux disease (GERD) are all known to be triggered by life stressors. The role stress plays in initiating intestinal problems in veterinary patients is also recognized.



Research assistant Jennifer Huang delivers nutrient solution containing glucose to a tissue bathing solution. Tissues mounted on Ussing chambers can be kept healthy for as long as six hours.

Stress has long been known to adversely affect gastrointestinal function. The first published reports date back to 1833 when a physician, William Beaumont, observed a patient with a permanent gastric fistula caused by a gunshot wound. Beaumont noted when his patient became angry or agitated, his stomach secretions would change resulting in impaired digestion. Despite these early accounts, our knowledge of how stress causes disturbances in gastrointestinal function remains unclear.

My laboratory within the Center for Comparative Medicine and Translational Research (CCMTR) is investigating how the intestine defends us against certain diseases and how the disease-fighting mechanisms within the intestine can be impaired by psychological stress. Our research in the emerging field of stress-related intestinal disorders have “one medicine” implications for both humans and animals. Regardless of species, disease requires interactions between the pathogen, host, and environment—a concept referred to as the disease triangle. Intestinal disease research has focused on the interactions between pathogen and host

but, thanks to the innate intestinal defense barriers of the host, the pathogenic agent alone is often incapable of causing disease. When these defense barriers are broken down, such as when an individual is undergoing stress—an environmental factor—disease results. The relationship between stress and intestinal disease appears simple, but the mechanisms are complex, involving signaling pathways from the central nervous system to the gut, which then alters gut defense properties. The signaling pathways between brain and gut are termed the brain-gut axis.

In order to understand how stress impairs the intestine’s ability to defend us against pathogens and other potentially harmful factors residing within the intestinal lumen, it is necessary to have a basic understanding of intestinal defense mechanisms. The surface area of the intestinal tract represents the largest interface between the external environment and body. The lining of the intestine is composed of a single layer of epithelial cells called enterocytes. This lining of enterocytes or epithelium plays a critical but paradoxical role in intestinal health and function. The intestinal epithelium facilitates digestion and uptake of luminal nutrients and the absorption of large volumes of water on a daily basis. At the same time, the intestinal epithelium provides a restrictive barrier that prevents pathogens, acids, antigens, and toxins that are present in the lumen from crossing the epithelium and gaining access to body and the blood system. This important function is referred to as the barrier function of the intestinal epithelium.

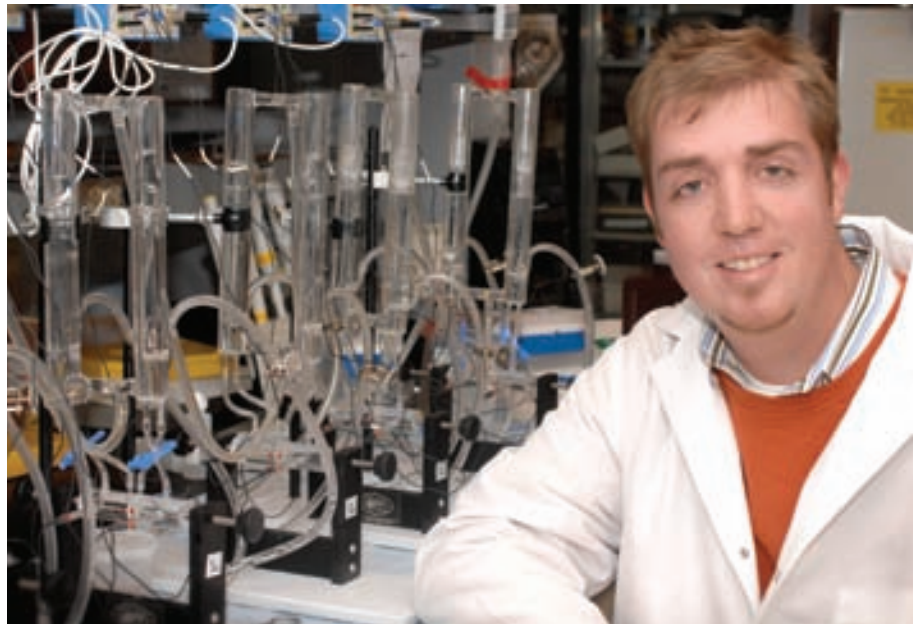
Intestinal barrier function can be grouped into three major components: extrinsic, intrinsic, and immunologic barriers. The extrinsic barrier includes protective compounds secreted by the epithelium, such as mucus and bicarbonate, which buffer acidic conditions in the lumen. Antimicrobial peptides secreted by specialized intestinal cells called Paneth cells and a well-developed commensal gut microbial population are also part of the extrinsic barrier. The intrinsic barrier, which has been the focus our lab's CC-MTR research, is important for regulating the permeability of the epithelium. In health, the intestinal epithelium is relatively impermeable. During stress or infection, the epithelium becomes permeable or "leaky" resulting in absorption of harmful luminal factors into the body. This critical defense property is regulated by a group of specialized proteins expressed in between intestinal epithelial cells called tight junctions. The immunologic barrier includes lymphoid tissue aggregates such as the Peyer's patches and follicle associated epithelium (FAE) that are responsible for intestinal immunity and for the continuous uptake of luminal antigens and presentation to the immune system establishing immunologic tolerance. Also included in the immunologic barrier are cellular components of the innate immune system such as neutrophils and mast cells which reside in tissues beneath the epithelium called the lamina propria. These cells can be rapidly recruited during infection to defend us against the pathogen. It is important to note that these intestinal barrier components are under neuroendocrine control and therefore can be altered by stress.

The focus of my laboratory is to study how psychological stress causes breakdown of the gastrointestinal barrier and predisposes animals to enteric disease. Most of our studies have utilized the pig as a model to study early life stress on gut barrier development. We have focused on the weaning period, prob-

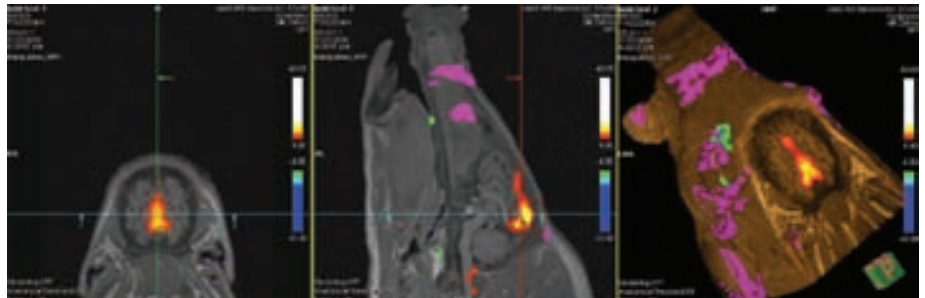
ably the most significant early life stress experienced by the commercially-raised pig. Under natural conditions, weaning is a gradual process that occurs at three months and represents the shift from the piglets' reliance on sow's milk to other food sources. In modern swine production systems, weaning age has been reduced to improve productivity. According to the United States Department of Agriculture (USDA), weaning age has decreased from an average of 28.8 days to 19.3 days between 1990 and 2000. The average weaning age in the U.S. currently is 18 days. Therefore, at a critical phase of physiologic development, the piglet is faced with multiple weaning stressors including separation from the sow and most littermates, loss of expected food source, and transport to a new environ-

ment with unfamiliar pigs. Consequently, the post-weaning period is associated with intestinal disease. In the European Union (EU), recent legislation has delayed the minimum weaning age to 28 days. This amendment was driven by consumer awareness and concerns regarding animal welfare. Another example of consumer preferences impacting animal husbandry is the emergence of organic pig farming in U.S. and EU markets. Organic farming guidelines set the minimum weaning age at 40 days.

Our initial studies concerned the impact of early life stress on intestinal barrier health and we measured intestinal permeability in early weaned piglets compared with unweaned littermates. Intestinal permeability can be measured in the lab by mounting intestinal tis-



Dr. Moeser with Ussing chambers that measure electro-physiologic properties of intestinal tissues from animals that have undergone periods of stress.

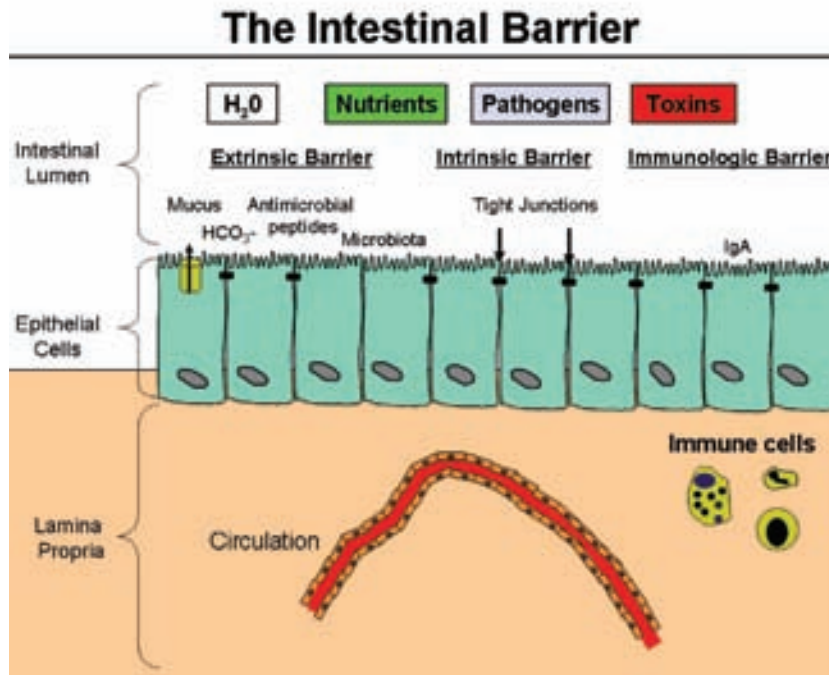


Functional magnetic resonance image of the brain of an early weaned pig demonstrating increased activity in response to stimulation.

sues on a device called an Ussing chamber. These studies revealed that early weaning caused the intestines of piglets to become more permeable indicating a compromised intestinal barrier. We then conducted time-course studies to see if early weaned piglets could eventually recover from this intestinal insult. We observed that barrier disturbances in early weaned pigs persisted as far as nine weeks after weaning suggesting that stress occurring early in life results in permanent defects in the gastrointestinal barrier. We also observed that when early weaned pigs faced a

mild social stress later in life, they were more sensitive to the stress as measured by high blood levels of stress mediators (corticotrophin-releasing factor [CRF] and cortisol) and increases in intestinal permeability compared with late weaned, control pigs. This is similar to the emerging paradigm of stress-related disorders in humans in which previous history of early life stress (childhood trauma, abuse) predisposes individuals to stress-related intestinal disorders in later life.

In investigating the pathophysiological mechanisms of stress-induced intestinal dysfunction in the early weaned pig, we identified a critical role of the intestinal mast cell in breakdown of intestinal barrier function. Mast cells—immune cells residing beneath the epithelium—contain a variety of bioactive mediators packaged as granules within the cell. These mediators—which include tryptases, histamine, prostaglandins, and cytokines—are well-known for their role in allergic and inflammatory diseases. Upon activation, mast cells release their mediators into surrounding



The intestinal barrier is comprised of several defense properties that serve to prevent harmful microorganisms within the intestine from gaining access to the body. The barrier must also facilitate the absorption of nutrients and water.

tissues in a process called degranulation, which can then have a profound influence on intestinal function by causing increases in permeability and secretion and disturbances in intestinal motility. These mast cell functions help rid the host of a pathogenic agent, especially parasites. Chronic activation of mast cells, however, is detrimental. In initial studies we found



Beth Overman (foreground), a doctoral student, and research assistant Jennifer Huang prepare intestinal tissues for study with the Ussing chambers.

that early weaned pig tissues had elevated markers of mast cell activation. Furthermore, we were able to prevent intestinal barrier disturbances in early weaned pigs by using cromolyn, a drug that blocks

mast cell activity prior to weaning. Therefore, treatments that focus on the mast cell may be viable options for stress-related intestinal disorders.

Our lab also has initiated studies to understand the mechanisms that link psychological stress and intestinal disease. We have determined that intestinal mast cells express receptors for the stress peptide CRF. CRF is released by both the hypothalamus as well as intestinal tissues in response to stress and is thought to be the

critical link between the brain and the gut. Previously published studies in our lab demonstrated that drugs that block intestinal CRF receptors prevent disturbances in intestinal function triggered by early weaning. My lab is currently collaborating with Dr. Soman Abraham, a mast cell expert from the Department of Pathology at Duke University Medical School, to investigate molecular mechanisms of CRF-mast cell interactions in the stressed intestine.

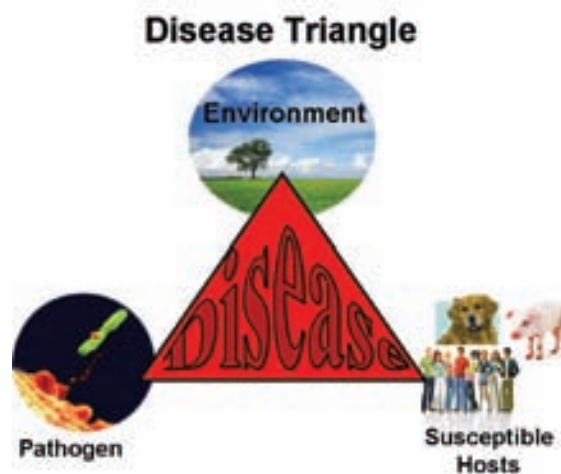
By understanding how stress causes intestinal disorders, we will know better the pathogenesis of important veterinary diseases. Whether it relates to food animals or companion animals, stress is an important component of intestinal disease that can result in economic losses and reductions in quality of life for these patients. In the pig, enteric disorders can often be linked to a previous production stress such as weaning, transport, and temperature changes of overheating or chilling. Based on our findings, we now are better able to design treatments and employ prevention

strategies to improve intestinal health in animals. In collaboration with Dr. Anthony Blikslager, a CVM gastrointestinal physiologist and equine surgeon, my lab is addressing alternative methods that may alleviate the burden of weaning stress on gut health and piglet well-being. This work is supported by a \$294,000 USDA National Research Initiative-funded grant. We have demonstrated that incrementally increasing weaning age can have dramatic improvements in gut health in pigs. For example, by increasing weaning age from 18 to 23 days, we prevented adverse changes in intestinal health that was observed in pigs weaned at 18 days. Although industry production targets encourage producers to wean pigs early, we believe that enhanced pig health achieved by modest increases in weaning age will be an economically favorable option for producers and the swine industry. This research has received interest from the European Union which has resulted in an invitation to speak at the University of Nottingham next year.

This research has important implications for human medicine. Pigs—with a complex brain structure and function as well as a central and intestinal nervous system that compares favorably with humans—are highly relevant models for studying brain-gut axis disorders in people. Pigs are omnivores with a gastrointestinal tract that is similar to that of humans. Additionally, pigs possess important developmental similarities to humans especially with regards to postnatal gut development and the immune system. Selecting appropriate animal models for human disease is important because it is imperative that therapeutic treatments are based on results from animals with gastrointestinal tracts that are extensively similar to that of humans. Consequently, the National Institutes of Health, National Center for Research Resources has established a National Swine Resource and Research

Center in Columbia, Missouri.

The changes in stress and intestinal physiology triggered by early weaning in the pig are similar to those observed with IBS, a common stress-related human intestinal disorder. IBS affects approximately 15% of the U.S. population, resulting in annual health care costs exceeding \$4,000 per patient. Early life stress maybe a predisposing factor for development of IBS later in life. The clinical symptoms of IBS include abdominal pain that is accompanied by either diarrhea (diarrhea predominant IBS, IBS-D) or constipation (constipation predominant IBS, IBS-C). These symptoms are thought to be caused by increased intestinal permeability.



The disease process is the same for all species.

Similar to IBS patients, early weaned pigs exhibit increased intestinal permeability and have heightened stress responses to social stress, resulting in diarrhea. The hallmark symptom of IBS is abdominal pain thought to be due to enhanced sensitization of nerves in the intestine as a result of stress. Sensitized intestinal nerves transmit signals to pain centers in the brain resulting in abdominal pain.

In animal models, abdominal discomfort can be difficult to assess. However, we have teamed up with CVM veterinary clinicians Dr. Duncan Lascelles (an animal pain specialist), Dr. Tony Pease (a radiologist), and Dr. Cliff Swanson (an anesthesiologist) along with Dr. Doug Drossman, a human IBS specialist

from UNC-Chapel Hill, to assess gut hypersensitivity in early weaned pigs. The team used functional Magnetic Resonance Imaging (fMRI) to measure pain signals and threshold values in response to a colorectal distention pressure test in anesthetized pigs. This same test is used in humans to determine the degree of gut sensitization as measured by activation of pain centers in the brain. IBS patients are much more sensitive to this procedure resulting in enhanced fMRI signaling in pain processing regions within the brain. Similar to human IBS, fMRI studies in early weaned pigs revealed increased brain activity in response to colorectal distention, an indication of gut hypersensitivity not shown in late weaned pigs.

The findings of this model were presented in the Distinguished Abstract Plenary at the Digestive Diseases Week conference in Los Angeles. Digestive Diseases Week is sponsored by the American Gastroenterological Association and is one of the largest medical conferences worldwide with more than 16,000 physicians and basic scientists attending annually. This research has also led to a NIH T32 post-doctoral fellowship award from the University of North Carolina-Chapel Hill, Center for Gastrointestinal Biology and Disease.

The long-term goal of my laboratory is to develop a more basic understanding of how stress influences gut health and function. Our work should have important implications in the understanding of stress-related gut disorders in both veterinary species and humans and should help facilitate the design of novel preventative and treatment strategies for patients suffering from these disorders.

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