Impact of stressor exposure on the interplay between commensal microbiota and host inflammation

Jeffrey D Galley¹ and Michael T Bailey^{1,2,*}

¹Division of Oral Biology; College of Dentistry; The Ohio State University; Columbus, OH USA; ²Institute for Behavioral Medicine Research; Wexner Medical Center; The Ohio State University; Columbus, OH USA

Keywords: psychological stress, dysbiosis, *Lactobacillus*, bacterial translocation, colitis, social defeat, anxiety, brain gut axis

Exposure to stressful stimuli results in the activation of multiple physiological processes aimed at maintaining homeostasis within the body. These physiological processes also have the capacity to influence the composition of microbial communities, and research now indicates that exposure to stressful stimuli leads to gut microbiota dysbiosis. While the relative abundance of many different bacterial types can be altered during stressor exposure, findings in nonhuman primates and laboratory rodents, as well as humans, indicate that bacteria in the genus *Lactobacillus* are consistently reduced in the gut during stress. The gut microbiota, including the lactobacilli, have many functions that enhance the health of the host. This review presents studies involving germfree and antibiotic treated mice, as well as mice given *Lactobacillus* spp. to prevent stressor-induced reductions in lactobacilli, to provide evidence that the microbiota contribute to stressor-induced immunomodulation, both in gut mucosa as well as in systemic compartments. This review will also discuss the evidence that commensal gut microbes have bidirectional effects on gastrointestinal physiology during stressor exposure.

Introduction

Humans, as well as most multi-celled organisms, are home to a menagerie of bacteria, viruses, and fungi. These bacteria that colonize a variety of niches throughout their host, including the oral cavity, gastrointestinal tract, and skin on human hosts, are termed the microbiota. The densest of these microbial niches is the gastrointestinal tract, and it is here that an estimated 10¹⁴ microbes exist in largely stable climax communities.¹ Most estimates suggest that the bacteria of the human host outnumber human cells by 10-fold.² As would be expected, this large density of microbes has the ability to impact host physiological functions, such as gut development and maturation, nutrient absorption and storage, vitamin synthesis, mucosal and systemic immune responses, as well as endocrine and neural activity.^{3–11} The extent

of these effects on the host, as well as the mechanisms by which they occur, are only beginning to be understood. However, it is recognized that there are bidirectional homeostatic interactions between the host and its microbiota; disrupting host physiology can alter commensal microbial populations and disrupting commensal microbial populations can alter host physiology. This review will discuss the impact of the physiological response to stress on gut microbiota and the importance for host responses with an emphasis placed on host inflammatory responses.

Stress, the Stress Response, and Impact on Gut Physiological Functioning

The external world is constantly changing, and maintaining internal homeostasis while adapting to an ever changing environment is essential for any living organism. Stress is a process in which a stimulus, termed the stressor, that can be physical, physiological, or psychological in nature, disrupts internal homeostasis. The body's response to the stressor involves a combined behavioral and physiological response which comprises the stress response. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic branch of the autonomic nervous system (SNS) are two of the primary neuroendocrine pathways involved in the stress response. These neuroendocrine pathways become activated when corticotrophin releasing hormone (CRH) is released from the hypothalamus in the brain during stressor exposure. Activation of the HPA axis results in the release of glucocorticoid hormones, such as cortisol in humans and corticosterone in rodents, whereas activation of the SNS results in the release of norepinephrine (NE) from sympathetic nerve terminals and the release of epinephrine (Epi) from the adrenal medulla. These hormones have a variety of effects throughout the body that are all aimed at helping the body respond to the insulting stressor.

It is well recognized that the central nervous system and the gastrointestinal tract are intimately connected, and stressor exposure can significantly impact gastrointestinal physiology through the effects of glucocorticoid hormones, as well as NE and Epi. For example, activation of the sympathetic nervous system has been found to be responsible for stressor-induced reduction in gastric acid secretion,¹² with activation of the parasympathetic

*Correspondence to: Michael T Bailey; Email: Michael.bailey@osumc.edu
Submitted: 21/08/2013; Revised: 20/02/2014; Accepted: 27/03/2014;
Published Online: 01/04/2014
<http://dx.doi.org/10.4161/gmic.28683>

nervous system enhancing gastric acid secretion.¹³ Likewise, stressor exposure can reduce gastric emptying and slow transit in the small intestine.^{14,15} Unlike the slower motility in the stomach and small intestine, stressor exposure tends to enhance motility in the colon.¹⁶ In addition to affecting these secretory and motor responses, the physiological stress response can also affect protective barriers in the intestines. Stress exposure can significantly alter mucous levels in the colon, as well as the levels of secretory immunoglobulin A.^{17,18}

Stressor-Induced Alterations of Community Structure

The complete set of factors that influence the ability of the host to select its gut microbiota is not well understood, but physiological activity in the gastrointestinal tract can significantly impact gut microbiota. For example, it is well recognized that the secretion of gastric acid helps to regulate microbial populations as evidenced by the development of achlorhydria, which leads to gut bacterial overgrowth.¹⁹ Gastrointestinal motility is also well recognized for its role in shaping gut bacterial communities. Regions in the gastrointestinal tract where motility is naturally slow, such as the ileum and the colon, contain the highest density of microbes, and experimentally reducing gut motility often leads to bacterial overgrowth.²⁰ Gastrointestinal mucous is also a known substrate for bacterial growth. Common commensal *Akkermansia muciniphila* can degrade mucins for energy.²¹ The neuroendocrine system may also have a more direct effect on gut microbes by producing neuroendocrine hormones capable of changing gut microbe populations. Studies demonstrate that many types of gut microbes directly respond to mammalian hormones resulting in enhanced bacterial growth and pathogenicity,^{22,23} such as the ability to adhere to mucosal surfaces.²⁴ As a result of the close association between stressor exposure and changes to gastrointestinal hormones and physiological functioning, it is perhaps not surprising that exposure to different types of stressors significantly alters gut microbiota community structure.

It has been known for over 35 years that altering an animal's environment in turn affects gut microbial populations. Tannock and Savage demonstrated in 1974 that depriving mice of food, water, and cage bedding was sufficient to significantly reduce the number of lactobacilli that could be cultured from the stomach.²⁵ To determine whether these previous findings could be due to the host stress response, the effects of psychological stressors on the gut microbiota were assessed by quantifying bacteria in coprocultures from infant monkeys after maternal separation. The levels of lactobacilli that could be cultured from the stool were significantly different the week following separation, with significantly lower levels of lactobacilli being found 3 days after the separation. Interestingly, the reduction in lactobacilli inversely correlated with stress-indicative behaviors. In general, monkeys who had the lowest levels of lactobacilli also had the highest levels of stress-indicative behaviors.²⁶

Stressor-induced reductions in the number of lactobacilli cultured from the stool have also been found in rhesus monkeys

exposed to a stressor prenatally²⁷ as well as in college students under the stress of final examinations.²⁸ These studies clearly showed that stressor exposure could change the number of specific microbes that could be cultured from the stool, but it was not until culture-independent methods were utilized that it was realized that stressor exposure could shift the structure of entire microbial communities. For example, by using denaturing gradient gel electrophoresis (DGGE) to characterize the intestinal microbiota, O'Mahoney et al. demonstrated that separating rat pups from their mothers for 3 hours a day for the first 12 days of life was sufficient to result in significant community-wide alterations in the microbiota when the rats reached maturity.²⁹

Studies from this laboratory have also utilized culture-independent methods to characterize the intestinal microbiota of stressor-exposed mice. Next generation, high throughput 454 FLX-Titanium pyrosequencing has been used to characterize the microbial community in the cecum of mice exposed to a prolonged restraint stressor³⁰ as well as in mice exposed to repeated social defeat.³¹ In both cases, stressor exposure was sufficient to cause significant community-wide alterations in microbial community structure. These changes are not permanent, and data indicate that by 15 hours after stressor exposure, stressor-induced alterations in microbiota community structure begin to revert toward normal profiles.³¹

Prolonged stressor exposure is often associated with alterations in feeding behavior and food preference. For example, humans often seek out comfort foods during stressful periods and laboratory animals may experience either hyperphagia or hypophagia during stressor exposure.³²⁻³⁴ This effect is particularly evident in laboratory mice exposed to prolonged restraint that will not eat or drink during stressor exposure even if food and water are provided (unpublished observation). This prolonged food and water deprivation has been found to impact the microbiota.³⁰ Other commonly used paradigms to assess the effects of stress on the microbiota, such as maternal deprivation, may also involve alterations to infant diet, since maternal separation is also stressful for the dam and stressor exposure has been shown to affect breast milk volume³⁵ as well as maternal and/or nursing behavior.³⁶ The effects of stressor exposure on diet may be even more problematic in human studies, with natural stressors, such as the stress of taking school exams, also being associated with alterations in diet.²⁸ Such interactions between stressor exposure and diet make it difficult to determine whether stressor effects on the microbiota occur independently of changes in diet.

To address this question, we determined whether social defeat during a single 2 hours exposure to a stressor paradigm called social disruption was sufficient to impact the composition of the colonic tissue-associated microbiota. During this stressor, mice have ad libitum access to food and water. However, the use of next generation, high throughput 454 FLX-Titanium pyrosequencing demonstrated that as little as 2 hours of social defeat was sufficient to significantly change the composition of the intestinal microbiota (Galley et al., ref. 67). This was evident in significant changes to the β , but not α , diversity of microbial communities associated with the colonic tissue, and demonstrates that stressor exposure can change the microbiota independent of

differences in diet. Of importance, reductions in the relative and absolute abundance of bacteria within the genus *Lactobacillus* were observed, which is in agreement with observations of stressor-induced reductions in lactobacilli shed from laboratory rodents, nonhuman primates, as well as humans assessed using culture-based methods. Thus, although there is tremendous individual variability in the composition of the intestinal microbiota, and many different bacterial types can be affected by stressor exposure, alterations in commensal lactobacilli have consistently been identified in different individuals, across different stressor paradigms, and in different host species.

Biological Functions of Microbiota during Stressor Exposure

Role of microbiota in stressor-induced immunomodulation

With an increasing number of studies indicating that exposure to stressful situations can impact the stability of the intestinal microbiota, it is now incumbent upon researchers in the field to determine whether these stressor-induced alterations also influence the biological functions of the microbiota. Because multiple studies demonstrated that stressor exposure consistently reduces the relative abundance of bacteria in the genus *Lactobacillus*, and because the lactobacilli are known to have immunomodulatory functions^{37,38,39}, studies from this laboratory have been focused on determining whether the lactobacilli are involved in stressor-induced immunomodulation.

Exposing mice to a prolonged restraint stressor during oral challenge with a colonic pathogen (namely *Citrobacter rodentium*) significantly increases colonic pathogen levels and resultant colonic pathology.³⁹ Stressor exposure also increases the deterioration of the colonic barrier. In our study, *C. rodentium*, which does not possess virulence mechanisms to invade across the epithelial barrier, could be cultured from systemic organs, such as the spleen, suggesting the pathogen was able to cross a deteriorated intestinal barrier in stressor-exposed mice. This systemic pathogen translocation was associated with increases in circulating cytokines, such as IL-6, and an increase in anxiety-like behavior.³⁹ Interestingly, feeding the mice *L. reuteri* enhanced intestinal barrier function. Pathogen translocation to the spleen, as well as circulating IL-6 levels, was significantly reduced in stressor-exposed mice fed the *L. reuteri*. In addition, the *L. reuteri* prevented the development of anxiety-like behavior in the mice exposed to the prolonged restraint stressor during oral challenge with *C. rodentium*.³⁹

The finding that *L. reuteri* can ameliorate some aspects of stressor exposure on infectious colitis, along with our unpublished observations indicating that stressor exposure reduces the relative abundance of colonic tissue-associated *L. reuteri*, has led us to propose a paradigm in which stressor-induced reduction in commensal microbes, such as *L. reuteri*, results in an internal microenvironment that is conducive to overproduction of inflammatory cytokines and chemokines. Preventing stressor-induced reductions in *L. reuteri* by feeding *L. reuteri* to the mice in turn prevents stressor-induced exacerbation of infectious colitis.

Given the importance of NFκB in both mucosal homeostasis and colonic inflammation, it is likely that the commensal microbes exert their effects by influencing the expression of colonic NFκB. Further studies are required to test the validity of this paradigm.

It is also possible that *L. reuteri* has a more indirect effect on colonic inflammation in stressor-exposed mice. Studies demonstrate that intestinal microbes can impact the activation of the HPA axis.⁴⁰ This could be of particular importance, because glucocorticoids produced by activation of the HPA axis potentially suppress inflammatory responses.⁴¹ Reduced glucocorticoid production during stressor exposure as a result of adrenal insufficiency leads to intestinal inflammation during stressor exposure.⁴² Thus, it is conceivable that *L. reuteri* does not directly impact host colonic inflammation, but rather stimulates host physiological responses that are known to have suppressive effects on the inflammatory response.

Although the majority of studies from this laboratory have focused on stressor-induced reductions in commensal lactobacilli, other microbial types are undoubtedly important. For example, it is recognized that stressor exposure can reduce the fecal counts of *Bifidobacterium* in rats and infant monkeys.^{27,43} While the biological importance of stressor-induced reductions in bifidobacteria is not yet understood, it is interesting to note that many bacterial species within the *Bifidobacterium* genus have anxiolytic and anti-depressive effects on the host when administered as a probiotic to stressor-exposed animals.^{44,45} Thus, it is a possibility that stressor-induced reductions in *Bifidobacterium* contributes to stressor-induced anxiety that in turn can exacerbate inflammatory responses. This hypothesis warrants further investigation.

It is also possible that bacterial species other than the lactobacilli and bifidobacteria contribute to the interplay between stress, gastrointestinal functioning, and inflammatory responses. For example, stressor exposure has been shown to increase symptom severity in IBD patients.⁴⁶ Because commensal microbes, such as *Faecalibacterium prausnitzii*, are reduced in IBD patients and can ameliorate symptom severity in mice with IBD-like colitis, an interesting hypothesis is that stressor exposure leads to reduced abundance of *F. prausnitzii* and, thus, the loss of an indigenous mechanism to limit colonic inflammation.⁴⁷⁻⁵⁰ To date, however, whether stressor exposure can reduce other commensal microbes, such as *F. prausnitzii* is unknown. Thus, a future goal of this laboratory is to further delineate which types of microbes can be affected by stressor exposure.

The intestinal barrier is likely an important contributor to the interplay between the microbiota and host physiology during stressor exposure. In addition to being disrupted during pathogen-induced colitis, barrier disruption is also evident in uninfected stressor-exposed mice.⁵¹ As a result, stressor exposure can lead to the translocation of intestinal microbes and their products, such as lipopolysaccharide and peptidoglycan, from the lumen of the intestines to the interior of the body. Once in circulation, these microbes and their products can induce peripheral inflammatory responses. This is important, because stressor exposure often results in increases in circulating cytokines. In our studies of social defeat, the relative abundance of several commensal

microbes was significantly correlated with circulating cytokine levels.³¹ Interestingly, treating mice with antibiotics to reduce the microbiota is sufficient to prevent stressor-induced increases in circulating cytokines.^{31,52} In addition to increasing circulating cytokines, stressor exposure often primes macrophages for enhanced reactivity to microbial pathogens. For example, repeated social defeat increases the ability of splenic macrophages to kill target microbes. The stressor-induced increase in splenic macrophage activity is dependent upon the intestinal microbiota; exposing germfree mice to repeated social defeat did not impact macrophage microbicidal activity. Reconstituting the mice with microbes, however, again allowed the stressor-induced increase in macrophage microbicidal activity to be manifest.⁵³ These data indicate that stressor-induced disruption of commensal microbiota community structure and the passage of microbes, and/or their products, from the lumen of the intestines to the interior of the body primes the immune system for enhanced reactivity against microbial pathogens.

Effects of the microbiota on the stress response and gastrointestinal physiology

Gut microbes can directly affect leukocyte activity. Thus, it is plausible that stressor-induced alterations in the composition of the gut microbiota contribute to stressor-induced immunomodulation through direct effects on immune system reactivity. However, it is also possible that the microbiota have more indirect effects by affecting the physiological response to stress. Pioneering studies by Sudo et al. showed that stressor-induced activation of the HPA axis was greater in germfree mice compared with conventional mice.⁴⁰ Interestingly, stressor-induced activation of the HPA axis could be changed in the germfree mice by colonizing them with bacteria. The magnitude and direction of the change, however, was dependent upon the age of the germfree mouse and the type of bacteria used for colonization. For example, 9-week old germfree mice had increased stressor-induced HPA axis activation compared with conventional mice. This was abolished by colonizing the germfree mice with probiotic *Bifidobacterium infantis*. Colonizing the mice with microbiota from specific pathogen free (SPF) mice also reduced stressor-induced HPA axis activity, but only if the germfree mice were colonized at 6 weeks of age. Germfree mice colonized with microbiota from SPF mice at 14 weeks of age still had a heightened HPA response to stress in comparison to conventional mice.⁴⁰ Of importance, colonizing the germfree mice with enteropathogenic *Escherichia coli* increased rather than decreased stressor-induced activation of the HPA axis. These data demonstrate that gut microbes can influence stressor-induced activation of the HPA axis, but whether activation of the HPA axis is increased or decreased is dependent upon specific microbes. Further work is needed to fully understand how commensal microbes can affect the physiological stress response.

In addition to affecting stressor-induced activation of the HPA axis, the microbiota can also impact gut physiological functioning. For example, germfree mice have slower intestinal transit time than do conventional mice.⁵⁴ Moreover, the interdigestive migrating myoelectric complex (MMC) in the jejunum and ileum was significantly reduced in germfree rats compared with conventional rats. Colonizing the germfree rats with bacteria resulted in an

increase of the interdigestive MMC almost equal to that found in conventional rats, suggesting the microbiota are necessary for normal intestinal motility.⁵⁵ It is not well understood how gut microbes can impact intestinal motility, but a recent study found that bacterial signaling to enteric neurons via lipopolysaccharide (LPS) binding to Toll-like receptor (TLR)-4 is a key process by which intestinal motility is accelerated by gut microbes.⁵⁶ Like germfree mice, mice deficient in TLR-4 signaling had reductions in intestinal motility compared with wild-type mice.⁵⁶

Microbiota-dependent, or microbial product-dependent, activation of host pattern recognition receptors is not the only way in which the microbiota can influence enteric nervous system activity. It is now recognized that gut microbes can produce mammalian neurotransmitters and hormones, including serotonin, that are involved in enteric nervous system regulation of colonic motility. In addition to producing serotonin, and other neuroendocrine mediators, the microbiota are also able to modulate host release of serotonin and affect the levels of serotonin producing enterochromaffin cells.⁵⁷ Thus, there are multiple mechanisms by which gut microbiota can impact enteric nervous system activity and colonic motility.

The ability of gut microbes to affect the enteric nervous system is also apparent in studies involving IBS and visceral hypersensitivity. Visceral hypersensitivity, which is thought to reflect the heightened pain awareness evident in human IBS patients, is also affected by the microbiota. Germfree rats gavaged with fecal samples collected from IBS patients showed evidence of visceral hypersensitivity and exaggerated pain responses.⁵⁸ Although still in its infancy, this research indicates that IBS-induced shifts in the microbiota contribute to increased pain sensitivity. This conclusion is consistent with conclusions from others that have shown that the development of inflammatory hypernociception is dependent upon the microbiota. Germfree animals fail to develop inflammatory hypernociception.⁵⁹

In order for microbes and/or their products to impact host physiological responses, the microbes must penetrate the host intestinal mucous barrier. Interestingly, the presence of the microbiota stimulates the production of the thick inner mucous layer. Only a thin mucous layer is evident in germfree mice, but colonizing germfree mice with commensal microbes or treating the mice with bacterial components, such as LPS, induces the production of a thicker mucous layer that is similar to the mucous layer found in conventional mice.⁶⁰ Not all microbes are able to induce mucous secretion. In fact, it has been shown that some commensal microbes, such as *Bacteroides thetaiotaomicron*, increased mucous production, but other commensal microbes, such as *F. prausnitzii*, prevented this change.⁶¹ These findings highlight the differential impacts various microbial groups can have on host physiological functioning.

Conclusion

While it is widely recognized that factors such as antibiotics, diet, and infection can lead to gut microbial dysbiosis, the association between psychological stress exposure and dysbiosis is

Figure 1. Multi-directional interactions between the physiological stress response, gastrointestinal functioning, immunomodulation, and the gut microbiota. In response to a psychological stressor, the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) are activated, resulting in a downstream cascade of physiological shifts to maintain homeostasis. Stressor-induced shifts in GI functioning or in immune system activity can alter the community structure of the resident GI microbiota. Changes in microbiota community structure can be deleterious to the host due to the involvement of the microbiota in GI functioning and immunomodulation and may result in feedback on host CNS and behavioral function via the brain-gut axis.

only now becoming realized. It should not be surprising, however, that stressor exposure leads to alterations in gut microbes, since it has long been recognized that the physiological stress response can affect gastrointestinal functioning like gastrointestinal motility and mucous secretion,^{62,63} as well as secretory components of mucosal immunity, like sIgA.⁶⁴ Such stressor-induced alterations in gut functioning undoubtedly impact gut microbes, and are likely involved in stressor-induced alterations in microbial community structure.

Gut microbes can in turn impact the activity of the same gut functions that influence gut microbiota community structure. Gut microbes can impact motility, mucous secretion, and secretory immunity, as well as stressor-induced neuroendocrine activity (such as HPA axis activity) and behavioral responses (such as anxiety-like behavior). When considered together, the bidirectional communication suggests that there is a positive feedback loop in which stressor exposure alters gut activity, ultimately leading to microbial dysbiosis, and the dysbiotic microbial populations in turn modify the stressor-induced neuroendocrine activity and behavioral responses (Fig. 1). The importance of this bidirectional feedback between the brain and the gut for overall health is only beginning to be understood, but evidence exists that these interactions are important in the immunomodulatory effects of stressor exposure.

The reviewed studies suggest that stressor exposure disrupts homeostatic interactions between commensal microbial populations and host inflammatory responses with changes in the microbiota enhancing colonic inflammatory responses, and enhanced colonic inflammation further changing microbial communities. These stressor-induced effects are postulated to be important for the exacerbation of pathogen-induced or idiopathic colonic inflammation,⁶⁵ and could help to explain why stressor exposure is associated with exacerbation of intestinal diseases. It is possible, however, that these host-microbe interactions are not always detrimental. For example, stressor-induced increases in innate immunity, which are thought to be microbiota-dependent, can be protective by enhancing the ability of phagocytes to clear infectious organisms.⁶⁶ Thus, future studies should focus on mechanisms by which the stress response influences adaptive, as well as maladaptive, host-microbe interactions.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

References

- Huffnagle GB. The microbiota and allergies/asthma. *PLoS Pathog* 2010; 6:e1000549; PMID:20523892; <http://dx.doi.org/10.1371/journal.ppat.1000549>
- Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; 307:1915-20; PMID:15790844; <http://dx.doi.org/10.1126/science.1104816>
- Reinhardt C, Bergentall M, Greiner TU, Schaffner F, Ostergren-Lundén G, Petersen LC, Ruf W, Bäckhed F. Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. *Nature* 2012; 483:627-31; PMID:22407318; <http://dx.doi.org/10.1038/nature10893>
- Tomas J, Wrzosek L, Bouznad N, Bouet S, Mayeur C, Noordine ML, Honvo-Houeto E, Langella P, Thomas M, Cherbuy C. Primocolonization is associated with colonic epithelial maturation during conventionalization. *FASEB J* 2013; 27:645-55; PMID:23118025; <http://dx.doi.org/10.1096/fj.12-216861>
- Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004; 101:15718-23; PMID:15505215; <http://dx.doi.org/10.1073/pnas.040706101>
- Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešić M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; 17:225-35; PMID:23395169; <http://dx.doi.org/10.1016/j.cmet.2013.01.003>
- Resta SC. Effects of probiotics and commensals on intestinal epithelial physiology: implications for nutrient handling. *J Physiol* 2009; 587:4169-74; PMID:19596893; <http://dx.doi.org/10.1113/jphysiol.2009.176370>
- Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, et al. The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology* 2011; 141:599-609, e1-3; PMID:21683077; <http://dx.doi.org/10.1053/j.gastro.2011.04.052>
- Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* 2011; 108:3047-52; PMID:21282636; <http://dx.doi.org/10.1073/pnas.1010529108>
- Hansen CH, Nielsen DS, Kverka M, Zakostelska Z, Klimesova K, Hudcovic T, Tlaskalova-Hogenova H, Hansen AK. Patterns of early gut colonization shape future immune responses of the host. *PLoS One* 2012; 7:e34043; PMID:22479515; <http://dx.doi.org/10.1371/journal.pone.0034043>
- El Aidy S, van Baaren P, Derrien M, Lindenberg-Kortleve DJ, Hooiveld G, Levenez F, Doré J, Dekker J, Samsom JN, Nieuwenhuis EE, et al. Temporal and spatial interplay of microbiota and intestinal mucosa drive establishment of immune homeostasis in conventionalized mice. *Mucosal Immunol* 2012; 5:567-79; PMID:22617837; <http://dx.doi.org/10.1038/mi.2012.32>
- Shichijo K, Ito M, Taniyama K, Sekine I. The role of sympathetic neurons for low susceptibility to stress in gastric lesions. *Life Sci* 1993; 53:261-7; PMID:8321086; [http://dx.doi.org/10.1016/0024-3205\(93\)90677-U](http://dx.doi.org/10.1016/0024-3205(93)90677-U)
- Yang H, Yuan PQ, Wang L, Taché Y. Activation of the parasympathetic region in the ventral medulla stimulates gastric acid secretion through vagal pathways in rats. *Neuroscience* 2000; 95:773-9; PMID:10670444; [http://dx.doi.org/10.1016/S0306-4522\(99\)00490-X](http://dx.doi.org/10.1016/S0306-4522(99)00490-X)

14. Nakade Y, Tsuchida D, Fukuda H, Iwa M, Pappas TN, Takahashi T. Restraint stress delays solid gastric emptying via a central CRF and peripheral sympathetic neuron in rats. *Am J Physiol Regul Integr Comp Physiol* 2005; 288:R427-32; PMID:15458973; <http://dx.doi.org/10.1152/ajpregu.00499.2004>
15. Wang SX, Wu WC. Effects of psychological stress on small intestinal motility and bacteria and mucosa in mice. *World J Gastroenterol* 2005; 11:2016-21; PMID:15800998
16. Nakade Y, Fukuda H, Iwa M, Tsukamoto K, Yanagi H, Yamamura T, Mantyh C, Pappas TN, Takahashi T. Restraint stress stimulates colonic motility via central corticotropin-releasing factor and peripheral 5-HT₃ receptors in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2007; 292:G1037-44; PMID:17158256; <http://dx.doi.org/10.1152/ajpgi.00419.2006>
17. Castagliuolo I, Lamont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C, Pothoulakis C. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol* 1996; 271:G884-92; PMID:8944704
18. Jarillo-Luna A, Rivera-Aguilar V, Garfias HR, Lara-Padilla E, Kormanovsky A, Campos-Rodríguez R. Effect of repeated restraint stress on the levels of intestinal IgA in mice. *Psychoneuroendocrinology* 2007; 32:681-92; PMID:17590521; <http://dx.doi.org/10.1016/j.psyneuen.2007.04.009>
19. Drasar BS, Shiner M, McLeod GM. Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 1969; 56:71-9; PMID:4885396
20. Nieuwenhuijs VB, Verheem A, van Duijvenbode-Beumer H, Visser MR, Verhoef J, Gooszen HG, Akkermans LM. The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats. *Ann Surg* 1998; 228:188-93; PMID:9712563; <http://dx.doi.org/10.1097/0000658-199808000-00007>
21. Derrien M, Vaughan EE, Plugge CM, de Vos WM. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* 2004; 54:1469-76; PMID:15388697; <http://dx.doi.org/10.1099/ijs.0.02873-0>
22. Bailey MT, Karaszewski JW, Lubach GR, Coe CL, Lyte M. In vivo adaptation of attenuated *Salmonella typhimurium* results in increased growth upon exposure to norepinephrine. *Physiol Behav* 1999; 67:359-64; PMID:10497953; [http://dx.doi.org/10.1016/S0031-9384\(99\)00087-6](http://dx.doi.org/10.1016/S0031-9384(99)00087-6)
23. Pullinger GD, Carnell SC, Sharaff FF, van Diemen PM, Dziva F, Morgan E, Lyte M, Freestone PP, Stevens MP. Norepinephrine augments *Salmonella enterica*-induced enteritis in a manner associated with increased net replication but independent of the putative adrenergic sensor kinases QseC and QseE. *Infect Immun* 2010; 78:372-80; PMID:19884332; <http://dx.doi.org/10.1128/IAI.01203-09>
24. Green BT, Lyte M, Chen C, Xie Y, Casey MA, Kulkarni-Narla A, Vulchanova L, Brown DR. Adrenergic modulation of *Escherichia coli* O157:H7 adherence to the colonic mucosa. *Am J Physiol Gastrointest Liver Physiol* 2004; 287:G1238-46; PMID:15534374; <http://dx.doi.org/10.1152/ajpgi.00471.2003>
25. Tannock GW, Savage DC. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. *Infect Immun* 1974; 9:591-8; PMID:4593471
26. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev Psychobiol* 1999; 35:146-55; PMID:10461128; [http://dx.doi.org/10.1002/\(SICI\)1098-2302\(199909\)35:2<146::AID-DEV7>3.0.CO;2-G](http://dx.doi.org/10.1002/(SICI)1098-2302(199909)35:2<146::AID-DEV7>3.0.CO;2-G)
27. Bailey MT, Lubach GR, Coe CL. Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J Pediatr Gastroenterol Nutr* 2004; 38:414-21; PMID:15085020; <http://dx.doi.org/10.1097/00005176-200404000-00009>
28. Knowles SR, Nelson EA, Palombo EA. Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. *Biol Psychol* 2008; 77:132-7; PMID:18023961; <http://dx.doi.org/10.1016/j.biopsycho.2007.09.010>
29. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EM, Cryan JF, Dinan TG. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* 2009; 65:263-7; PMID:18723164; <http://dx.doi.org/10.1016/j.biopsych.2008.06.026>
30. Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB, Lyte M. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. *Infect Immun* 2010; 78:1509-19; PMID:20145094; <http://dx.doi.org/10.1128/IAI.00862-09>
31. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011; 25:397-407; PMID:21040780; <http://dx.doi.org/10.1016/j.bbi.2010.10.023>
32. Roberts CJ, Campbell IC, Troop N. Increases in weight during chronic stress are partially associated with a switch in food choice towards increased carbohydrate and saturated fat intake. *Eur Eat Disord Rev* 2014; 22:77-82; PMID:24123563; <http://dx.doi.org/10.1002/erv.2264>
33. Sanghez V, Razzoli M, Carobbio S, Campbell M, McCallum J, Cero C, Ceresini G, Cabassi A, Govoni P, Franceschini P, et al. Psychosocial stress induces hyperphagia and exacerbates diet-induced insulin resistance and the manifestations of the Metabolic Syndrome. *Psychoneuroendocrinology* 2013; 38:2933-42; PMID:24060458; <http://dx.doi.org/10.1016/j.psyneuen.2013.07.022>
34. Krahn DD, Gosnell BA, Grace M, Levine AS. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res Bull* 1986; 17:285-9; PMID:3490298; [http://dx.doi.org/10.1016/0361-9230\(86\)90233-9](http://dx.doi.org/10.1016/0361-9230(86)90233-9)
35. Lau C, Simpson C. Animal models for the study of the effect of prolonged stress on lactation in rats. *Physiol Behav* 2004; 82:193-7; PMID:15276780; <http://dx.doi.org/10.1016/j.physbeh.2004.05.004>
36. Aguggia JP, Suárez MM, Rivarola MA. Early maternal separation: neurobehavioral consequences in mother rats. *Behav Brain Res* 2013; 248:25-31; PMID:23567892; <http://dx.doi.org/10.1016/j.bbr.2013.03.040>
37. Goyal N, Shukla G. Probiotic *Lactobacillus rhamnosus* GG modulates the mucosal immune response in *Giardia intestinalis*-infected BALB/c mice. *Dig Dis Sci* 2013; 58:1218-25; PMID:23263901; <http://dx.doi.org/10.1007/s10620-012-2503-y>
38. Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, Britton RA, Kalkum M, Versalovic J. Histamine derived from probiotic *Lactobacillus reuteri* suppresses TNF via modulation of PKA and ERK signaling. *PLoS One* 2012; 7:e31951; PMID:22384111; <http://dx.doi.org/10.1371/journal.pone.0031951>
39. Mackos AR, Eubank TD, Parry NM, Bailey MT. Probiotic *Lactobacillus reuteri* attenuates the stressor-enhanced severity of *Citrobacter rodentium* infection. *Infect Immun* 2013; 81:3253-63; PMID:23798531; <http://dx.doi.org/10.1128/IAI.00278-13>
40. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 2004; 558:263-75; PMID:15133062; <http://dx.doi.org/10.1113/jphysiol.2004.063388>
41. Padgett DA, Glaser R. How stress influences the immune response. *Trends Immunol* 2003; 24:444-8; PMID:12909458; [http://dx.doi.org/10.1016/S1471-4906\(03\)00173-X](http://dx.doi.org/10.1016/S1471-4906(03)00173-X)
42. Reber SO, Birkeneder L, Veenema AH, Obermeier F, Falk W, Straub RH, Neumann ID. Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. *Endocrinology* 2007; 148:670-82; PMID:17110427; <http://dx.doi.org/10.1210/en.2006-0983>
43. Barouei J, Moussavi M, Hodgson DM. Effect of maternal probiotic intervention on HPA axis, immunity and gut microbiota in a rat model of irritable bowel syndrome. *PLoS One* 2012; 7:e46051; PMID:23071537; <http://dx.doi.org/10.1371/journal.pone.0046051>
44. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* 2010; 170:1179-88; PMID:20696216; <http://dx.doi.org/10.1016/j.neuroscience.2010.08.005>
45. Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdí A, Bisson JF, Rougeot C, Pichelin M, Cazaubiel M, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr* 2011; 105:755-64; PMID:20974015; <http://dx.doi.org/10.1017/S0007114510004319>
46. Bernstein CN, Singh S, Graff LA, Walker JR, Miller N, Cheang M. A prospective population-based study of triggers of symptomatic flares in IBD. *Am J Gastroenterol* 2010; 105:1994-2002; PMID:20372115; <http://dx.doi.org/10.1038/ajg.2010.140>
47. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 2009; 15:1183-9; PMID:19235886; <http://dx.doi.org/10.1002/ibd.20903>
48. Machiels K, Joossens M, Sabino J, De Preter V, Arijis I, Eeckhaut V, Ballet V, Claes K, Van Immerseel F, Verbeke K, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* 2013; PMID:24021287; <http://dx.doi.org/10.1136/gutjnl-2013-304833>
49. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 2008; 105:16731-6; PMID:18936492; <http://dx.doi.org/10.1073/pnas.0804812105>
50. Martín R, Chain F, Miquel S, Lu J, Gratadoux JJ, Sokol H, Verdu EF, Bercik P, Bermúdez-Humarán LG, Langella P. The commensal bacterium *Faecalibacterium prausnitzii* is protective in DNBS-induced chronic moderate and severe colitis models. *Inflamm Bowel Dis* 2014; 20:417-30; <http://dx.doi.org/10.1097/01.MIB.0000440815.76627.64>; PMID:24418903
51. Söderholm JD, Yates DA, Gareau MG, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. *Am J Physiol Gastrointest Liver Physiol* 2002; 283:G1257-63; PMID:12388189

52. Maslanik T, Tannura K, Mahaffey L, Loughridge AB, Benninson L, Ursell L, Greenwood BN, Knight R, Fleshner M. Commensal bacteria and MAMPs are necessary for stress-induced increases in IL-1 β and IL-18 but not IL-6, IL-10 or MCP-1. *PLoS One* 2012; 7:e50636; PMID:23236381; <http://dx.doi.org/10.1371/journal.pone.0050636>
53. Allen RG, Lafuse WP, Galley JD, Ali MM, Ahmer BM, Bailey MT. The intestinal microbiota are necessary for stressor-induced enhancement of splenic macrophage microbicidal activity. *Brain Behav Immun* 2012; 26:371-82; PMID:22100833; <http://dx.doi.org/10.1016/j.bbi.2011.11.002>
54. Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. *Proc Soc Exp Biol Med* 1967; 126:301-4; PMID:6066182; <http://dx.doi.org/10.3181/00379727-126-32430>
55. Caenepeel P, Janssens J, Vantrappen G, Eyssen H, Coremans G. Interdigestive myoelectric complex in germ-free rats. *Dig Dis Sci* 1989; 34:1180-4; PMID:2752868; <http://dx.doi.org/10.1007/BF01537265>
56. Anitha M, Vijay-Kumar M, Sitaraman SV, Gewirtz AT, Srinivasan S. Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology* 2012; 143:1006-16, e4; PMID:22732731; <http://dx.doi.org/10.1053/j.gastro.2012.06.034>
57. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. *Gastroenterology* 1994; 107:1259-69; PMID:7926490; [http://dx.doi.org/10.1016/0016-5085\(94\)90526-6](http://dx.doi.org/10.1016/0016-5085(94)90526-6)
58. Crouzet L, Gaultier E, Del'Homme C, Cartier C, Delmas E, Dapoigny M, Fioramonti J, Bernalier-Donadille A. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil* 2013; 25:e272-82; PMID:23433203; <http://dx.doi.org/10.1111/nmo.12103>
59. Amaral FA, Sachs D, Costa VV, Fagundes CT, Cisalpino D, Cunha TM, Ferreira SH, Cunha FQ, Silva TA, Nicoli JR, et al. Commensal microbiota is fundamental for the development of inflammatory pain. *Proc Natl Acad Sci U S A* 2008; 105:2193-7; PMID:18268332; <http://dx.doi.org/10.1073/pnas.0711891105>
60. Petersson J, Schreiber O, Hansson GC, Gendler SJ, Velcich A, Lundberg JO, Roos S, Holm L, Phillipson M. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2011; 300:G327-33; PMID:21109593; <http://dx.doi.org/10.1152/ajpgi.00422.2010>
61. Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, Philippe C, Bridonneau C, Cherbuy C, Robbe-Masselot C, et al. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol* 2013; 11:61; PMID:23692866; <http://dx.doi.org/10.1186/1741-7007-11-61>
62. Guo S, Gao Q, Jiao Q, Hao W, Gao X, Cao JM. Gastric mucosal damage in water immersion stress: mechanism and prevention with GHRP-6. *World J Gastroenterol* 2012; 18:3145-55; PMID:22791951; <http://dx.doi.org/10.3748/wjg.v18.i24.3145>
63. Castagliuolo I, Lamont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C, Pothoulakis C. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am J Physiol* 1996; 271:G884-92; PMID:8944704
64. Reyna-Garfias H, Miliar A, Jarillo-Luna A, Rivera-Aguilar V, Pacheco-Yepe J, Baeza I, Campos-Rodríguez R. Repeated restraint stress increases IgA concentration in rat small intestine. *Brain Behav Immun* 2010; 24:110-8; PMID:19748568; <http://dx.doi.org/10.1016/j.bbi.2009.09.005>
65. Lewis K, Caldwell J, Phan V, Prescott D, Nazli A, Wang A, Soderholm JD, Perdue MH, Sherman PM, McKay DM. Decreased epithelial barrier function evoked by exposure to metabolic stress and nonpathogenic *E. coli* is enhanced by TNF α . *Am J Physiol Gastrointest Liver Physiol* 2008; 294:G669-78; PMID:18187519; <http://dx.doi.org/10.1152/ajpgi.00382.2007>
66. Allen RG, Lafuse WP, Powell ND, Webster Marketon JL, Stiner-Jones LM, Sheridan JF, Bailey MT. Stressor-induced increase in microbicidal activity of splenic macrophages is dependent upon peroxynitrite production. *Infect Immun* 2012; 80:3429-37; PMID:22825446; <http://dx.doi.org/10.1128/IAI.00714-12>
67. Galley JD, Nelson M, Yu ZT, Kumar P, Dowd SE, Lyte M, Bailey MT. Exposure to a social stressor reduces commensal colonic tissue-associated *Lactobacillus* spp. *BMC Microbiology* 2014; 14:189; PMID:25028050; <http://dx.doi.org/10.1186/1471-2180-14-189>