The Microbiota-Gut-Brain axis in gastrointestinal disorders: Stressed bugs, stressed brain or both?

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ABSTRACT

The gut-brain axis is the bi-directional communication between the gut and the brain which occurs through multiple pathways that include hormonal, neural and immune mediators. The signals along this axis can originate in the gut, the brain, or both, with the objective of maintaining normal gut function and appropriate behavior. In recent years, the study of gut microbiota has become one of the most important areas in biomedical research. Attention has focused on the role of gut microbiota in determining normal gut physiology and immunity, and more recently on its role as modulator of host behavior (“microbiota-gut-brain axis”). We therefore review the literature on the role of gut microbiota in gut homeostasis and link it with mechanisms that could influence behavior. We discuss the association of dysbiosis with disease with particular focus on functional bowel disorders and its relation to psychological stress. This is of particular interest as exposure to stressors has long been known to increase susceptibility to and severity of gastrointestinal diseases.
Introduction

The central nervous system (CNS) and the gastrointestinal (GI) tract are in constant bidirectional communication through neural pathways, such as the vagus nerve, and by humoral and cellular mediators that include the immune system and the hypothalamus-pituitary-adrenal (HPA) axis. The gut is colonized with a complex community of bacteria (microbiota), which helps to shape the immune system, metabolic function, and behavior in health and disease throughout life. The microbiota is a relatively new player in the gut-brain axis, fulfilling key roles in its communication (Bailey & Coe, 1999; Bercik et al., 2011a; Heijtz et al., 2011; Neufeld et al., 2011; Matsumoto et al., 2013), which has led to the term “microbiota-gut-brain axis” (Rhee et al., 2009; Collins et al., 2012). Alterations in gut microbiota (dysbiosis) can arise as a consequence of gastrointestinal disease or of its treatment. All major chronic disorders of the gut, namely inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and celiac disease, are associated with dysbiosis (Nadal et al., 2007; Collado et al., 2009; De Palma et al., 2010). Although an overall decrease in diversity and richness of the microbiota seems to be common findings across studies, no specific dysbiotic signature has emerged between studies. This may be in part due to differences in sampling (small intestinal, colonic, fecal), as well as analytical techniques employed (culture, DGGE, Illumina, 454 sequencing, MALDI-TOF) (Lagier et al., 2012). However, there is now increasing evidence that dysbiosis modulates peripheral and central nervous system function leading to alterations in brain signalling and behavior (Bercik et al., 2011a; Collins et al., 2013; Mulle et al., 2013). This observation is important in view of the fact that stress and depression, common co-morbidities in GI disorders, influence in turn the natural course of these illnesses (Collins, 2001; Wu, 2012). The microbiota-gut-brain axis has been the subject of numerous reviews in recent years (Rhee et al., 2009; Mayer, 2011; Bercik
et al., 2012; Collins et al., 2012; Collins et al., 2013; Mayer et al., 2014). The influence of psychosocial and environmental stressors on the pathogenesis of gastrointestinal diseases has long been recognized. Recently, the mechanisms through which stress may affect various physiologic functions of the gastrointestinal tract have been reviewed (Konturek et al., 2011). We will discuss the recent progress on specific mechanisms of interaction between gut microbiota and brain, with focus on the effect of psychological stress.

**Gut microbiota and its host: A mutualistic relationship**

A unique combination of different populations of organisms inhabits our gut, mainly bacteria but also archaea, viruses and protozoa, roughly reaching $10^{14}$ cells, outnumbering the human cells in our bodies by a factor of ten (Sekirov et al., 2010). While bacterial profiling and its understanding has become easier during the last decade, the analysis of the mycobiome and the virome is still in its infancy (Minot et al., 2011; Cui et al., 2013; Minot et al., 2013). The human intestinal tract is essentially sterile at birth when it is immediately colonized. The gut microbiota evolves during early life until unique, subject-specific (fingerprint) adult-like community arises, which is relatively stable throughout life (Rajilic-Stojanovic et al., 2012). Out of the more than 50 phyla described in the literature, only few are found in the human GI tract, dominated by two phyla in particular (Firmicutes, Bacteroidetes), together with members of Actinobacteria, Verrucomicrobia, Proteobacteria Fusobacteria and Cyanobacteria phyla (Sommer & Backhed, 2013). These autochthonous phyla colonize the gastrointestinal tract and are present in a majority of individuals. The concept of ‘enterotypes’ has recently been proposed, and according to this humans can be subdivided into *Bacteroides*, *Prevotella* or *Ruminococcus* types (Zoetendal et al., 2008; Arumugam et al., 2011). However, this categorization has recently become a matter of debate and the term ‘enteroorganisms’ has been proposed instead, to describe bacterial communities with prevalence of *Bacteroides* or *Prevotella* (Jeffery et al., 2012). Microbes in the human gut undergo selective pressure from
the host as well as from microbial competitors and once the ecosystems reaches homeostasis, some species will occur in high and many in low abundance (Backhed, 2011; Nicholson et al., 2012). Even though the gut microbiota still differs greatly between subjects in membership and community structure, it still appears largely functionally equivalent and necessary for the proper development of the host. Mammals have co-evolved to exist with its gut microbiota largely in a mutualistic relationship: these organisms participate in the conversion of non-digestible carbohydrates (dietary fiber) to short-chain fatty acids (SCFAs), in bile acid metabolism, provide a barrier against pathogenic bacteria, and modulate the innate and adaptive immune systems (Nicholson et al., 2012). In turn, the host provides a unique nutrient rich niche at constant temperature (Sommer & Backhed, 2013). Studies using germ-free animals have highlighted the importance of the gut microbiota in the maintenance of homeostasis. Germ-free animals have physiologic and metabolic abnormalities compared to conventional animals and an imbalanced immune system (Slack et al., 2009; Hapfelmeier et al., 2010; Geuking et al., 2011; Kunić et al., 2011; Hansen et al., 2012; Macpherson et al., 2012; Olszak et al., 2012). In addition, germ free animals exhibit abnormal gastrointestinal motility (Abrams & Bishop, 1967; Gustafsson et al., 1970; Wostmann, 1981), increased expression of genes encoding transporters throughout the gut (Backhed, 2011), and altered perception of inflammatory pain (Amaral et al., 2008). Moreover, germ-free mice have impaired capacity to harvest energy from the diet (Wostmann, 1981) and are protected against diet-induced obesity (Backhed et al., 2007; Rabot et al., 2010). It is not surprising then that alterations in the normal gut microbiota composition (dysbiosis) are associated with a variety of GI disorders, such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), and celiac disease (Nadal et al., 2007; Collado et al., 2009; De Palma et al., 2010). Future work will have to determine whether a microbial signature for dysbiosis is associated with specific disease states. Nevertheless, sufficient data support the concept that
changes in the microbiota may arise in adulthood as a consequence of disease, long term
dietary habits, antibiotics and medications. These changes may be short term or long term
depending on the duration of the trigger that induced it and of particular characteristics of the
host. On the other hand, factors that impact on the normal colonization process during early
life, such as psychological stress, may exert long term effects on both microbiota composition
that will impact susceptibility to disease.

Microbiota-gut-brain axis

It is well known that the gut and the brain are in bidirectional communication. The concept of
gut-brain axis originated from the field of GI endocrinology and the discovery of hormonal
regulation of digestion (Track, 1980). Since then it has evolved to include the maintenance of
homeostasis of several systems, including GI function, appetite, and weight control (Collins
& Bercik, 2009). Thus, it is only logical to consider and include the gut microbiota as an
important modulator of this system, and consequently the term “microbiota-gut-brain axis”
has emerged (Figure 1) (Bercik et al., 2009; Bercik et al., 2011a). The known beneficial
effects of laxatives and oral antibiotics in patients with hepatic encephalopathy is perhaps of
the earliest pieces of evidence for a role of gut bacteria in brain function (Victor & Quigley,
2014). Antibiotics have also been anecdotally reported to induce acute psychosis that
resolved after withdrawal of the drug (Sternbach & State, 1997; Mehdi, 2010). More recently,
abnormal microbiota composition has been associated with autism (Bolte, 1998; Finegold et
al., 2010; Yap et al., 2010; Wang et al., 2011; Williams et al., 2011; Finegold et al., 2012;
Wang et al., 2012; Williams et al., 2012; De et al., 2013; Kang et al., 2013; Wang et al.,
2013); treatment with antibiotics in patients with late onset autism seems to partially improve
their symptoms (Sandler et al., 2000; Finegold et al., 2012). The bacterium Bacteroides
fragilis, a Gram-negative anaerobic bacterium that inhabits the lower GI tract of most
mammals (Ley et al., 2008), has been shown to ameliorate anxiety-like behavior,
sensorimotor, communicative and repetitive behavior, but not sociability and social preference, in an animal model of autism, possibly through modulation of gut microbiota composition and serum metabolomic profile (Hsiao et al., 2013). An association between major depressive disorder (MDD) and altered gut metabolism has also been proposed (Ledochowski et al., 1998a; Ledochowski et al., 1998b; Ledochowski et al., 2000; Ledochowski et al., 2001; Ochoa-Reparaz et al., 2011). It is difficult to interpret whether this is a chicken or egg situation, whether brain and behavioral alterations precede gut dysfunction and dysbiosis, or whether gut dysfunction and dysbiosis precede brain and behavioral changes. It has been reported that chronic depression is associated with altered microbial profiles and colonic motility in mice (Park et al., 2013). However it has been also reported that chronic gastrointestinal inflammation can induce anxiety-like behavior and alter central nervous system biochemistry (Bercik et al., 2010) (Bercik et al., 2011a). Therefore, it is likely that both situations co-exist in a self-perpetuating loop, and that the initial trigger can arise centrally or in the periphery. Additional research is needed to solve this intriguing concept, and an interaction between clinical and basic research using gnotobiotic technology will likely help provide mechanistic insight.

**Stress and microbiota-gut-brain axis**

Stress is defined as an organism’s total response to environmental demands or pressures. Several different types of stressors can be distinguished, such as acute or chronic, some of which may occur only once, while others are repetitive and can be anticipated. However, stress can be unpredictable and uncontrollable, mild or severe, and occurring in or out of context (Lucassen et al., 2014). Moreover, the perception of stress is variable between individuals, and so is the persistence of its consequences (Lucassen et al., 2014). Exposure to stressors has long been known to increase susceptibility to disease, including GI disorders,
and contributes to many disabilities worldwide and as such represents a severe economic burden.

Chronic and acute stress models are widely employed in GI research, since stress has been identified as a risk factor or modulator of the expression of several gastrointestinal disorders (Collins, 2001; Soderholm & Perdue, 2001; Konturek et al., 2011). Tannock and Savage demonstrated 40 years ago, that environmental and dietary stress markedly altered the gut microbiota in mice, affecting factors regulating the localization and population levels of microorganisms along the GI tract (Tannock & Savage, 1974), possibly favouring the establishment of pathogenic bacterial species (Tannock & Smith, 1972; Tannock & Savage, 1974). More recently, Bailey et al. demonstrated that exposure to a social disruption stressor affects the gut microbiota, and circulating levels of cytokines, particularly IL-6 and MCP-1 (Bailey et al., 2011). In fact, social stress has been reported to increase the risk of inflammation-related diseases, promoting pro-inflammatory gene expression and monocyte differentiation (Powell et al., 2013). Thus, stressor-induced changes in the microbiota may enhance the ability of enteric pathogens (i.e. Citrobacter rodentium) to colonize the intestine (Bailey et al., 2010). Accordingly, it has been reported that acute and repeated stress impact levels of intestinal secretory IgA, impacting intestinal homeostasis and likely resulting in inflammation (Campos-Rodriguez et al., 2013). Therefore, altered levels of intestinal secretory IgA might cause shifts in commensals and possibly results in dysbiosis.

Psychological and physical stressors activate the HPA axis, resulting in the release of corticotropin-releasing hormone (CRH), the principal regulator of the HPA axis, that is synthesized and secreted by hypophysiotropic neurons localized in the medial parvocellular subdivision of the paraventricular nucleus (PVN) (Smith & Vale, 2006). CRH induces the release of adrenocorticotropic hormone (ACTH) into the systemic circulation that will in turn stimulate glucocorticoid synthesis in the adrenal cortex. Glucocorticoids, such as
corticosterone or cortisol in humans, are the downstream effectors of the HPA axis, and their biological effects are usually adaptive (Smith & Vale, 2006). Together with glucocorticoids, catecholamines (norepinephrine and epinephrine) are also released into the circulatory system after psychological and physical stressors (Lyte et al., 2011), and it is well known that glucocorticoids can potentiate some of the actions of catecholamines (Sapolsky et al., 2000). The gastrointestinal tract has long been known to be sensitive to stress and stress mediators, including catecholamines, but the notion that stress, and stress mediators, can influence the composition and function of the gut microbiota is relatively a new concept (Lyte et al., 2011).

In fact, stress can influence bacterial infection outcome as enteric bacteria can respond to the release of stress-related neurochemical mediators by the host (Lyte et al., 2011). Moreover, it has been recently hypothesized that bacteria act essentially as neuroactive compound delivery vehicles affecting host physiology through the provision of neurochemicals. Specifically, the presence of a stress-related neuroendocrine hormone family of catecholamines has been demonstrated in bacteria (Lyte, 2011).

Today’s conceptual framework of the most common entities in gastroenterology, functional gastrointestinal disorders (FGIDs), such as IBS and functional dyspepsia, involves the interaction of psychological factors and altered gut physiology via the gut-brain axis, where brain and gut symptoms are reciprocally influencing each other’s expression. Psychological, sexual and/or physical abuse in early life has been suggested to play an important role in the pathogenesis of FGIDs (Heitkemper et al., 2011; Wu, 2012; van Tilburg et al., 2013). This is a time of particular vulnerability, when neurological plasticity as well as establishment of a relatively stable gut microbiota occurs.

Maternal separation (MS) in rodents has been widely used as a model of early life stress that induces long-lasting hyperactivity of the HPA-axis (Ladd et al., 2000; Barreau et al., 2004b; Daniels et al., 2004; Lippmann et al., 2007; Aisa et al., 2008; Gareau et al., 2008; Oines et
al., 2012), anxiety-like behavior (Varghese et al., 2006; Lippmann et al., 2007; Desbonnet et al., 2010; O’Mahony et al., 2011; Abelaira et al., 2013; Li et al., 2013), visceral hypersensitivity (Eutamene et al., 2007; O’Mahony et al., 2011; Moloney et al., 2012; Felice et al., 2014), and altered cholinergic activity in the gut (Gareau et al., 2007b; O’Malley et al., 2010) accompanied by increased intestinal permeability (Soderholm et al., 2002; Barreau et al., 2004a; Garcia-Rodenas et al., 2006; Eutamene et al., 2007; Gareau et al., 2007b; Oines et al., 2012). Maternally separated rats show also increased neuronal activation in response to physical stressor, such as colorectal distension (Felice et al., 2014), likely due to central sensitization to noxious visceral stimuli (Chung et al., 2007), similarly to what has been reported for IBS patients (Tillisch & Labus, 2011; Tillisch et al., 2011; Larsson et al., 2012). Indeed, this model results in a dysfunctional gut-brain axis, mimicking many of the features found in IBS patients; thus, has been largely employed to study the mechanisms behind the dysfunctional communication between the gut and the brain in IBS (Barreau et al., 2007; Gareau et al., 2008; O’Mahony et al., 2009; O’Mahony et al., 2011). Similarly to IBS (Ringel & Maharshak, 2013), in animal models these alterations at physiological and behavioral levels are often accompanied by altered gut colonization (Garcia-Rodenas et al., 2006; O’Mahony et al., 2009; Barouei et al., 2012), and the use of probiotics appears to improve the detrimental effects of stress (Garcia-Rodenas et al., 2006; Eutamene & Bueno, 2007; Eutamene et al., 2007; Gareau et al., 2007a; Desbonnet et al., 2010; Distrutti et al., 2013). Our preliminary data shows that gut microbiota is essential for the expression of anxiety-like behavior and behavioral despair in mice, since MS germ-free mice do not show different behavior when compared with control germ-free mice (De Palma et al., 2012). However, we found that germ-free MS mice have increased levels of basal serum corticosterone and altered cholinergic nerve function (De Palma et al., 2012), similar to previous studies in conventional specific pathogen free animals (SPF) (Gareau et al., 2006; Gareau et al., 2007a; Gareau et al.,
Acetylcholine is the main excitatory neurotransmitter in the mammalian enteric nervous system and plays an important role in the control of gut motility (Olsson & Holmgren, 2011). Park et al. demonstrated that central administration of CRH induces changes in colonic motility in mice, accompanied by altered behavior in the open field test (Park et al., 2013). Thus, changes in HPA axis may contribute to the development of diverse pathologies, in this case it altered autonomic control of gut motility (Park et al., 2013). We obtained similar results in germ-free mice subjected to MS, demonstrating that alterations at the level of HPA-axis activity disrupt colonic homeostasis and in turn alter gut environment, in a microbiota independent fashion. MS also induces changes in the morphology of the colon of conventional SPF MS rats, with an increase in the numbers of goblet cells in the crypts of the proximal colon with subsequent increase in mucus secretion, and a thinner mucosal layer (O’Malley et al., 2010). It is therefore plausible that changes to the physiology (Soderholm et al., 2002; Gareau et al., 2007b; O’Malley et al., 2010; De Palma et al., 2012) and morphology (O’Malley et al., 2010) of the gut of MS animals explain the reported changes in gut microbiota composition of MS animals versus controls (O’Mahony et al., 2009). Altogether these findings suggest that stress, acute or chronic, modulates the gut environment to select a dysbiotic microbiota, which in turn can induce anxiety and depressive behavior. However, the exact pathways and mediators of this effect are still to be elucidated. Commensal bacteria might modulate brain biochemistry and behavior through the production of specific metabolites (Lyte, 2011; Barrett et al., 2012a; Barrett et al., 2012b; Hsiao et al., 2013). It has been previously shown that commensal bacteria can modulate behavior through the vagus nerve (Bercik et al., 2011b; Bravo et al., 2011), affecting neurotransmitter metabolism (Asano et al., 2012), or through alternative pathways, yet to be defined (Bercik et al., 2010; Bercik et al., 2011a).
However, it is plausible to postulate that in the future manipulation of gut microbiota, through probiotics or symbiotics, might be a valuable adjuvant to traditional medicine in the treatment of IBS patients with comorbid anxiety or depression.
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The microbiota-gut-brain axis comprises the bidirectional communication, through multiple pathways, between the gut and the brain. Under stress, alterations at the level of the central nervous system can influence gut neuro-motor and secretory function, immunity and microbiota composition. In turn, dysbiosis may contribute to perpetuate dysfunction and inflammation, further disrupting gut-brain communication. Some of these effects may be mediated by direct host microbial-interactions at the level of the intestinal epithelium, production of bacterial metabolites (catecholamines, gamma-aminobutyric acid, etc). The sequence of events can occur in a top-bottom or bottom-top fashion, but once initiated can perpetuate and exacerbate maladaptive responses that promote a state of disease.

We acknowledge dreamdesign and cooldesign (FreeDigitalPhotos.net) for the image of the gut and brain, respectively.

Figure 1.

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