J Physiol 592.14 (2014) pp 2989–2997

The Journal of Physiology

TOPICAL REVIEW

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 bidirectional 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 behaviour. 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 behaviour ('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 behaviour. We discuss the association of dysbiosis with disease, with particular focus on functional bowel disorders and their relationship to psychological stress. This is of particular interest because exposure to stressors has long been known to increase susceptibility to and severity of gastrointestinal diseases.

(Received 5 March 2014; accepted after revision 17 April 2014; first published online 22 April 2014)
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Introduction

The central nervous system 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 hypothalamic–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 behaviour 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.* 2011*a*; 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, irritable bowel syndrome and coeliac 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 a common finding across studies, no specific dysbiotic signature has emerged between studies. This may be due, in part, to differences in sampling (small intestinal, colonic, faecal), as well as analytical techniques employed (culture, Denaturing

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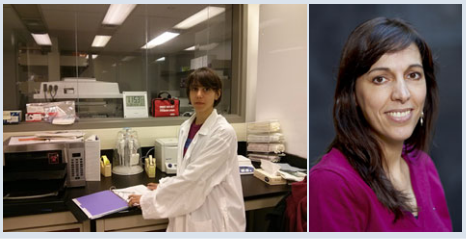

DOI: 10.1113/jphysiol.2014.273995

Gradient Gel Electrophoresis, DGGE, Illumina, 454 sequencing, Matrix-assisted laser desorption ionization time-of-flight mass spectrometer, 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 behaviour (Bercik *et al.* 2011*a*; 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, in turn influence 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, 2014; Bercik *et al.* 2012; Collins *et al.* 2012, 2013). 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 physiological functions of the GI 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 approximating 10^{14} cells, outnumbering the human cells in our bodies by a factor of 10 (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, 2013; Cui *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 a unique, subject-specific (fingerprint) adult-like community arises, which is relatively stable throughout life (Rajilić-Stojanović *et al.* 2013). 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 & Bäckhed, 2013). These autochthonous phyla colonize the GI 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 'enterogradients' 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 (Bäckhed, 2011; Nicholson *et al.* 2012).

Even though the gut microbiota differs greatly between subjects in membership and community structure, it still appears on the whole to be functionally equivalent and necessary for the proper development of the host. Mammals have co-evolved to exist with their gut microbiota largely in a mutualistic relationship; these organisms participate in the conversion of non-digestible carbohydrates (dietary fibre) to short-chain fatty acids, participate 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 & Bäckhed, 2013).

Studies using germ-free animals have highlighted the importance of the gut microbiota in the maintenance of homeostasis. Germ-free animals have physiological and metabolic abnormalities compared with conventional animals, as well as an imbalanced immune system (Slack *et al.* 2009; Hapfelmeier *et al.* 2010; Geuking *et al.* 2011; Kunii *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 (Bäckhed, 2011) and altered perception of inflammatory pain (Amaral *et al.* 2008). Moreover, germ-free mice have an impaired capacity to harvest energy from the diet (Wostmann, 1981) and are protected against diet-induced obesity (Bäckhed *et al.* 2007; Rabot *et al.* 2010).

It is therefore not surprising that alterations in the composition of the normal gut microbiota (dysbiosis) are associated with a variety of GI disorders, such as inflammatory bowel diseases, irritable bowel syndrome and coeliac 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 them and the particular characteristics of the host. In contrast, factors that impact on the normal colonization process during early life, such as psychological stress, may exert long-term effects on the composition of the microbiota 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 the 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 (Fig. 1; Bercik *et al.* 2009, 2011*a*).

The known beneficial effects of laxatives and oral antibiotics in patients with hepatic encephalopathy is perhaps one of the earliest pieces of evidence for a role of gut bacteria in brain function (Victor & Quigley, 2014). Antibiotics have also anecdotally been reported to induce acute psychosis that resolved after withdrawal of the drug (Sternbach & State, 1997; Mehdi, 2010). More recently, an abnormal composition of the microbiota has been associated with autism (Bolte, 1998; Finegold *et al.* 2010, 2012; Yap *et al.* 2010; Wang *et al.* 2011, 2012, 2013; Williams *et al.* 2011, 2012; De Angelis *et al.* 2013; Kang *et al.* 2013); treatment with antibiotics in patients with late-onset autism seems to improve their symptoms (Sandler *et al.* 2000; Finegold *et al.* 2012). *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. The underlying mechanisms may involve modulation of gut microbiota composition and serum metabolomic profile (Hsiao *et al.* 2013). An association between major depressive disorder and altered gut metabolism has also been proposed (Ledochowski *et al.* 1998*a*,*b*, 2000, 2001; Ochoa-Repáraz *et al.* 2011).

It is difficult to interpret whether this is a chicken or egg situation, whether brain and behavioural alterations precede gut dysfunction and dysbiosis, or whether gut dysfunction and dysbiosis precede brain and behavioural 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 behaviour and alter central nervous system biochemistry (Bercik *et al.* 2010, 2011*a*). Therefore, it is likely that both situations coexist 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 probably help to provide mechanistic insight.

Stress and the microbiota–gut–brain axis

Stress is defined as an organism's total response to environmental demands or pressures. Several different

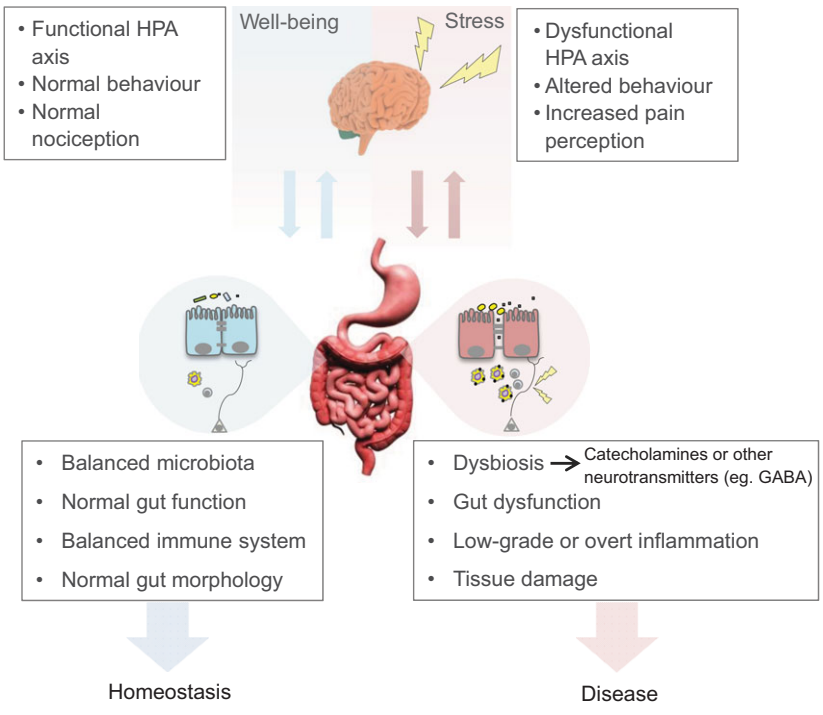

Figure 1. The microbiota–gut–brain axis comprises the bidirectional communication, through multiple pathways, between the gut and the brain
During stress, alterations at the level of the central nervous system can influence gut neuromotor and secretory function, immunity and microbiota composition. In 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 (cathecolamines, GABA, etc). The sequence of events can occur in a top-to-bottom or bottom-to-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.

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 occur 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. Stress contributes to many disabilities worldwide and, as such, represents a severe economic burden.

Chronic and acute stress models are widely employed in GI research, because stress has been identified as a risk factor or modulator of the expression of several GI disorders (Collins, 2001; Söderholm & 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 that regulate the localization and population levels of micro-organisms 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.* (2011) demonstrated that exposure to a social disruption stressor affects the gut microbiota and circulating levels of cytokines, particularly interleukin-6 and monocyte chemotactic protein-1. 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 (such as *Citrobacter rodentium*) to colonize the intestine (Bailey *et al.* 2010). Accordingly, it has been reported that acute and repeated stress affect levels of intestinal secretory IgA, impacting intestinal homeostasis and probably resulting in inflammation (Campos-Rodríguez *et al.* 2013). Altered levels of intestinal secretory IgA might cause shifts in commensals and possibly result in dysbiosis.

Psychological and physical stressors activate the HPA axis, resulting in the release of corticotrophin-releasing hormone, the principal regulator of the HPA axis, which is synthesized and secreted by hypophysiotrophic neurons localized in the medial parvocellular subdivision of the paraventricular nucleus (Smith & Vale, 2006). Corticotrophin-releasing hormone induces the release of adrenocorticotrophic hormone into the systemic circulation, which 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 (noradrenaline and adrenaline) 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 a relatively new concept (Lyte *et al.* 2011). In fact, stress can influence the outcome of bacterial infection, because enteric bacteria can respond to the release of stress-related neurochemical mediators by the host (Lyte *et al.* 2011). Moreover, it has been hypothesized recently 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, the functional gastrointestinal disorders, such as irritable bowel syndrome 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 functional gastrointestinal disorders (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 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.* 2004*b*; Daniels *et al.* 2004; Lippmann *et al.* 2007; Aisa *et al.* 2008; Gareau *et al.* 2008; Oines *et al.* 2012), anxiety-like behaviour (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.* 2007*b*; O'Malley *et al.* 2010) accompanied by increased intestinal permeability (Söderholm *et al.* 2002; Barreau *et al.* 2004*a*; García-Ródenas *et al.* 2006; Eutamene *et al.* 2007; Gareau *et al.* 2007*b*; Oines *et al.* 2012).

Maternally separated rats show also increased neuronal activation in response to a physical stressor, such as colorectal distension (Felice *et al.* 2014), probably due to central sensitization to noxious visceral stimuli (Chung *et al.* 2007), similar to what has been reported for irritable bowel syndrome 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 irritable bowel syndrome

patients; therefore, it has been widely employed to study the mechanisms behind the dysfunctional communication between the gut and the brain in irritable bowel syndrome (Barreau *et al.* 2007; Gareau *et al.* 2008; O'Mahony *et al.* 2009, 2011). Similar to irritable bowel syndrome (Ringel & Maharshak, 2013), in animal models these alterations at physiological and behavioural levels are often accompanied by altered gut colonization (García-Ródenas *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 (García-Ródenas *et al.* 2006; Eutamene & Bueno, 2007; Eutamene *et al.* 2007; Gareau *et al.* 2007*a*; Desbonnet *et al.* 2010; Distrutti *et al.* 2013).

Our preliminary data show that gut microbiota is essential for the expression of anxiety-like behaviour and behavioural despair in mice, because maternally separated germ-free mice do not show different behaviour when compared with control germ-free mice (De Palma *et al.* 2012). However, we found that germ-free maternally separated 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 (Gareau *et al.* 2006, 2007*a*,*b*; O'Malley *et al.* 2011), indicating that these alterations occur independently of the presence of gut microbiota.

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.* (2013) demonstrated that central administration of corticotrophin-releasing hormone induces changes in colonic motility in mice, accompanied by altered behaviour in the open field test. Thus, change in the 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 maternal separation, demonstrating that alterations at the level of HPA axis activity disrupt colonic homeostasis and, in turn, alter the gut environment, in a microbiota-independent fashion.

Maternal separation also induces changes in the morphology of the colon of conventional specific pathogen-free maternally separated rats, with an increase in the numbers of goblet cells in the crypts of the proximal colon and a subsequent increase in secretion of mucus, with a thinner mucosal layer (O'Malley *et al.* 2010). It is therefore plausible that changes to the physiology (Söderholm *et al.* 2002; Gareau *et al.* 2007*b*; O'Malley *et al.* 2010; De Palma *et al.* 2012) and morphology (O'Malley *et al.* 2010) of the gut of maternally separated animals explain the reported changes in gut microbiota composition of maternally separated animals *versus* control animals (O'Mahony *et al.* 2009).

Altogether, these findings suggest that stress, whether acute or chronic, modulates the gut environment to select a dysbiotic microbiota, which in turn can induce anxiety and depression; however, the exact pathways and mediators of this effect are yet to be elucidated. Commensal bacteria might modulate brain biochemistry and behaviour through the production of specific metabolites (Lyte, 2011; Barrett *et al.* 2012*a*,*b*; Hsiao *et al.* 2013). It has been shown previously that commensal bacteria can modulate behaviour through the vagus nerve (Bercik *et al.* 2011*b*; Bravo *et al.* 2011), affecting neurotransmitter metabolism (Asano *et al.* 2012), or through alternative pathways, yet to be defined (Bercik *et al.* 2010, 2011*a*).

It is plausible to postulate that in the future the manipulation of gut microbiota, through probiotics or symbiotics, might be a valuable adjuvant to traditional medicine in the treatment of irritable bowel syndrome patients with co-morbid anxiety or depression.

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Additional information

Competing interests

None declared.