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REVIEW

Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology

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Summary There is now an expanding volume of evidence to support the view that commensal organisms within the gut play a role in early programming and later responsivity of the stress system. The gut is inhabited by 10^{13}—10^{14} micro-organisms, which is ten times the number of cells in the human body and contains 150 times as many genes as our genome. It has long been recognised that gut pathogens such as *Escherichia coli*, if they enter the gut can activate the HPA. However, animals raised in a germ-free environment show exaggerated HPA responses to psychological stress, which normalises with monocolonisation by certain bacterial species including *Bifidobacterium infantis*. Moreover, increased evidence suggests that animals treated with probiotics have a blunted HPA response. Stress induces increased permeability of the gut allowing bacteria and bacterial antigens to cross the epithelial barrier and activate a mucosal immune response, which in turn alters the composition of the microbiome and leads to enhanced HPA drive. Increasing data from patients with irritable bowel syndrome and major depression indicate that in these syndromes alteration of the HPA may be induced by increased gut permeability. In the case of irritable bowel syndrome the increased permeability can respond to probiotic therapy. Detailed prospective studies in patients with mood disorders examining the gut microbiota, immune parameters and HPA activity are required to throw further light on this emerging area. It is however clear that the gut microbiota must be taken into account when considering the factors regulating the HPA.

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1. Introduction

The hypothalamic–pituitary-adrenal axis (HPA) and other aspects of the stress response are regulated by exposure both to psychological stressors and physical stressors such as infections (Dinan and Scott, 2005). Response of the HPA and the sympathoadrenal medullary system (SAM) to psychological stress is mediated by key neurotransmitter systems such as the serotonergic (5HT) and norepinephrine (NE) systems with endorphins playing an important inhibitory role (Dinan, 1996; Bhatnagar and Dallman, 1998). Infective agents activate the neuroendocrine system via pro-inflammatory cytokines, which exert influence on the hypothalamus especially on the parvicellular neurons of the paraventricular nucleus (Dinan, 2001; Rivest, 2010). Moreover, the HPA is tightly regulated to respond efficiently to gut pathogens such as *Escherichia coli*. In an elegant series of recent studies Zimomra et al. (2011) demonstrated that the initial activation of the HPA axis in response to *E. coli* infection is largely mediated by COX-induced prostanoid synthesis. This rise in circulating corticosterone correlates with the rise in circulating PGE2 and administration of indomethacin (non-selective COX inhibitor), abolished the early rise in plasma corticosterone. Furthermore, corticosterone levels reach a peak before maximum circulating ACTH, indicating that prostaglandins stimulate corticosterone release through an ACTH-independent mechanism. When prostaglandin production is completely inhibited, corticosterone levels increased 2 h following *E. coli* challenge, confirming that other factors stimulate HPA responses at this later time. A significant correlation between the rise of pro-inflammatory cytokines and corticosterone is observed. Administration of rat IL-6 antibodies attenuates the elevation in corticosterone 2 h following *E. coli* challenge. This data is consistent with previous work showing that PGE2 directly mediates corticosterone release from cultured rat adrenals (Mohn et al., 2005) and cortisol release from human adrenal H295R cells (Vakharia and Hinson, 2005) (Fig. 1).

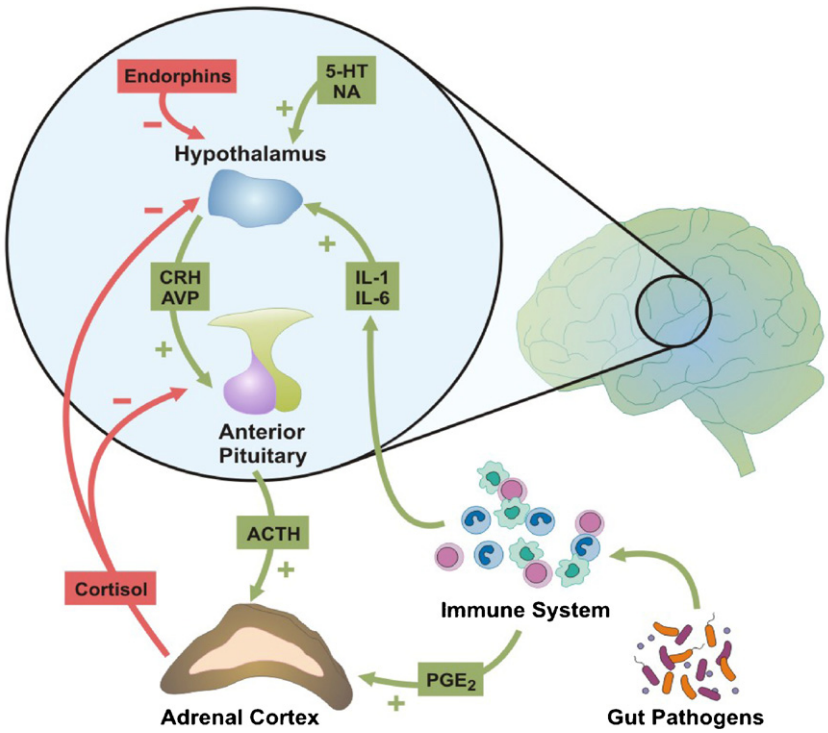

Figure 1 At a hypothalamic level classic neurotransmitters and cytokines regulate corticotrophin releasing hormone (CRH) and vasopressin (AVP) release into the portal vasculature. A series of negative feedback loops controls the forward drive. The adrenal cortex can be directly activated by PGE2 from the immune system stimulated by gut pathogens.

However, that non-pathogenic gut microbes might influence the HPA is a relatively novel concept. There is now an expanding volume of evidence to support the view that commensal organisms within the gut play a role in early programming and later responsivity of the stress system (Grenham et al., 2011). This network has been termed the brain–gut–microbiota and here we will review the data supporting the role of gut microbes in determining stress reactivity especially through the HPA.

2. Brain–gut axis

It is well established that the brain regulates gut activity but recent attention has focused on the reverse pathway and the manner in which gut microbes can influence the brain (Grenham et al., 2011). This brain–gut–microbiota axis includes the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system, the enteric nervous system (ENS) and of course the intestinal microbiota (Rhee et al., 2009; Cryan and O'Mahony, 2011). These components interact to form a complex reflex network with afferent fibres that project to integrative CNS structures and efferent projections to the smooth muscle. This bidirectional communication network enables signals from the brain to influence the motor, sensory and secretory modalities of the gastrointestinal tract and conversely, visceral messages from the gut can influence brain function, especially areas of the brain devoted to stress regulation, most notably the hypothalamus.

The vagus provides an important line of communication between the gut microbiota and the HPA. Hosoi et al. (2000) measured the expression of CRF mRNA in the hypothalamus and plasma levels of ACTH and corticosterone after vagal stimulation in rodents. CRF mRNA in the hypothalamus was increased 2 h after vagal stimulation and plasma levels of ACTH were markedly elevated. They also observed increases in plasma levels of corticosterone. Of clinical relevance is the fact that vagal nerve stimulation is associated with clinical antidepressant benefit (Nemeroff et al., 2006) coupled with normalisation of HPA parameters in patients with treatment refractory depression (O'Keane et al., 2005), indicating that the vagus not only impacts on HPA activity but also on the core pathophysiology of major depression.

The top-down and bottom-up perspective of information flow as well as the detailed structural integration and functioning of the various brain–gut–microbe axis components has been reviewed extensively elsewhere (Mayer, 2011). It is becoming clear that alterations in this communication play a fundamental role in a wide variety of disorders. Moreover, the possibility of regulating the gut microbiota is opening up as a tractable therapeutic target for a host of stress-related disorders.

3. ENS and CRF

The ENS is a complex neuronal network with multiple neurotransmitters and neuromodulators including 5-HT, acetylcholine and CRF. Although prominence has been given to the central origins of CRF-mediated changes in gastrointestinal function, the presence of the CRF ligand and receptors within the ENS indicate the likelihood that peripheral pathways also play a role in the local regulation of gut function during times of stress (O'Malley et al., 2010). Indeed, activation of gut CRFR1 contributes to stress-induced increases in colonic motility, defecation, permeability and visceral pain sensation (Larauche et al., 2009). Activation of peripheral CRFR2 inhibits gastric emptying, suppresses stimulated colonic motor function and prevents hypersensitivity to repeated colorectal distension. CRFR2 has also been proposed to have a role in stress-induced permeability dysfunction and the modulation of mucosal immune and inflammatory responses in the colon (Gareau et al., 2008). Furthermore, CRF can directly activate myenteric neurons to increase colonic motility and permeability and stimulate diarrhoea in rodents (Taché, 2004). As the ENS is comprised of sensory, motor and interneurons which are bidirectionally linked to the CNS via sympathetic and parasympathetic pathways, the expression of CRFR1 and CRFR2 on these neurons and nerve fibres places them in an ideal position to act as signalling peptides in the brain–gut axis (Tache et al., 1999).

The contrasting actions of CRFR1 and CRFR2 are further underlined by differential expression patterns. CRFR2 is prevalent in upper regions of the gut tract (Wu et al., 2008), whereas CRFR1 is more widespread in the colon. CRFR1 is strongly expressed in mucosal cells (O'Malley et al., 2010), where it may regulate stress-induced ion secretion and paracellular permeability leading to bacterial–host interactions and mucosal inflammation. CRFR2 has also been detected in the colonic mucosa and has similarly been proposed to have a role in stress-induced permeability dysfunction and the modulation of mucosal immune and inflammatory responses in the colon (Teitelbaum et al., 2008), but is also important in exerting anti-nociceptive effects on visceral pain (Million et al., 2005). Evidence is now mounting that stress results in the recruitment and activation of CRF receptors in the colon to induce the stress-related changes in gut function and that a heightened stress susceptibility results in altered expression of CRF receptors.

4. Content of gut microbiota

The gut is inhabited by 10^{13}–10^{14} micro-organisms, which is ten times the number of human cells in our bodies and contains 150 times as many genes as our genome (Qin et al., 2010). The estimated species number varies greatly but it is generally accepted that the adult microbiome consists of greater than 1000 species and more than 7000 strains (Ley et al., 2006). It is an environment dominated by bacteria, mainly strict anaerobes, but also including viruses, protozoa, archae and fungi. The microbiome is largely defined by 2 bacterial phylotypes, Bacteroidetes and Firmicutes with Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia phyla present in relatively low abundance (Xu et al., 2007).

Traditional culture-based analysis used to define the enteric flora, is only adequate for a minority of the gut microbiota that is amenable to cultivation (Xu et al., 2007; Eckburg et al., 2005). This methodological problem has been circumvented by the use of culture-independent techniques, a portfolio consisting of sequencing based methods, genetic fingerprinting, fluorescently labelled oligonucleotide probes

(FISH), quantitative PCR as well as metagenomic approaches (Archie and Theis, 2011). The realisation that the secretory and metabolic capability of the microbiome was likely as important as phylotype composition has also led to the use of metabolomic and metaproteomic approaches to improve our understanding of gut microbial—host interactions (O'Hara and Shanahan, 2006). Unfortunately, advances in culture methods have not kept pace with the rise of these alternative technologies and a dual-pronged line of attack may be required to complete the circle, a not inconsiderable logistical challenge.

While the gut shows enormous microbial diversity it is now clear that there are important developmental and longitudinal variations which impact on the functioning of the HPA and the wider stress axis. Colonisation of the infant gut commences at birth when delivery exposes the infant to a complex microflora and its initial microbiome has a maternal signature (Mändar and Mikelsaar, 1996; Mackie et al., 1999; Adlerberth and Wold, 2009). The microbiome of unweaned infants is simple with high inter-individual variability (Kurokawa et al., 2007; McCracken and Lorenz, 2001). The numbers and diversity of strict anaerobes increase as a result of diet and environment, and after 1 year of age a complex adult-like microbiome is evident. Despite a significant interpersonal variation in the enteric microbiota, there seems to be a balance that confers health benefits and an alteration in beneficial bacteria can negatively influence the wellbeing of the individual (Cryan and O'Mahony, 2011). Several factors may alter the microbiome such as infection, disease, diet and antibiotics, but as a general rule, it tends to revert to the stable diversity established in infancy once the threat of the initial distorting factor has subsided (Forsythe et al., 2010). Interestingly, it has been shown that the core microbiota of an aged individual is distinct from that of younger adults (Claesson et al., 2011) and that age related shifts in the composition of the intestinal microbiota are linked to adverse health effects in the elderly host (Woodmansey, 2007). The number of Bifidobacteria decreases with age (Claesson et al., 2011) and parallels changes in health status and decreased plasticity within the HPA. It is important to note that the HPA has been strongly implicated in susceptibility to the development of obesity and the metabolic syndrome (Dallman, 2010; Finger et al., 2011, 2012). In tandem increasing evidence points to a role of gut microbiota in obesity (Turnbaugh and Gordon, 2009; Davey et al., 2012). Whether HPA and gut microbiome interact directly in obesity remains an area for future investigation.

5. Microbiota and the HPA

The use of germ free (GF) animals (with no bacterial exposure) has provided one of the most significant insights into the role of the microbiota in regulating the development of the HPA. The germ free paradigm is based on the fact that the uterine environment is sterile during prenatal development (Adlerberth and Wold, 2009) and with surgical delivery replacing the normal vaginal delivery, the opportunity for postnatal colonisation of the gut is eliminated once animals are maintained in a sterile environment. Subsequent comparison with their conventionally colonised counterparts allows inferences to be drawn regarding the morphological and physiological parameters that may be under the influence of the developing microbiota. An alternative approach is the induction of dysbiosis of the enteric flora, either through administration of antibiotics or deliberate infection in preclinical studies (Bennet et al., 2002). Broad spectrum antibiotics in particular are known to perturb the microbiome by reducing biodiversity and delaying colonisation and are widely used as a method to intentionally alter the microbiome in a reproducible manner (Donskey et al., 2003).

The morphological consequences of growing up germ free were evidenced by the greatly enlarged cecum, reduced intestinal surface area, increased enterochromaffin cell area, smaller Peyer's Patches and smaller villous thickness in these animals compared to conventional controls (Abrams et al., 1963). It was not surprising, given these gross structural aberrations, that multiple facets of normal function including that of the stress response would also be affected.

Toll-like receptors (TLRs) are present on cells of the innate immune system and recognise characteristic molecules termed pathogen associated molecular patterns (Akira and Hemmi, 2003). These receptors are the gateway to an immune response. Pathogen recognition by a particular TLR results in a cascade of events leading to the activation of the NF-κB signalling system, production and release of cytokines and activation of the HPA. However in the absence of the resident enteric flora, key members of the TLR family have low or absent expression profiles in the gut, thus compromising appropriate immune and neuroendocrine responses to pathogenic threats (O'Hara and Shanahan, 2006). For example the TLR4 knockout mouse does not respond to Gram negative bacteria with an activation of the HPA (Gosselin and Rivest, 2008)

Seminal studies by Sudo et al. (2004) provide insight into the role of the intestinal microbiota in the development of the HPA axis. In germ free mice a mild restraint stress induces an exaggerated release of corticosterone and ACTH compared to the specific pathogen free (SPF) controls. The stress response in the GF mice is partially reversed by colonisation with faecal matter from SPF animals and fully reversed by monoassociation with *Bifidobacterium infantis* in a time dependant manner (Bailey and Coe, 1999). This study clearly demonstrated that the microbial content of the gut is critical to the development of an appropriate stress response later in life and also that there is a narrow window in early life where colonisation must occur to ensure normal development of the HPA axis.

The question emerges whether the gut flora can have an influence over neural circuits and behaviour associated with the stress response? Sudo et al. (2004) reported a decrease in brain derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal growth and survival, and expression of the NMDA receptor subunit 2a (NR2a) in the cortex and hippocampus of male GF animals compared to SPF controls. On the other hand, Neufeld et al. (2011) actually found an increase in hippocampal BDNF mRNA in female mice that was contrary to the protein decreases observed in the earlier study. We have recently also found decreases in hippocampal BDNF mRNA levels as well as a distinct changes in the serotonergic system in male but not female mice (Clarke et al., submitted for publication). This suggests that a regulation of microbiome—gut—brain axis may be sex dependent. Alterations in hippocampal NMDA and 5HT1A receptor

expression have been shown in a number of studies (Neufeld et al., 2011). Both of these receptors are known to influence CRH release from the hypothalamus and changes in expression may explain altered HPA function in germ free animals. This decreased anxiety in germ free animals has been reproduced in other laboratories in both the elevated plus maze and the light dark box (where germ free animals spent more time in the light compartment) (Heijtz et al., 2011).

It is long known that stress and HPA can influence the composition of gut microbiome (Tannock and Savage, 1974). However, the functional consequences of such changes are now only being understood. Maternal separation, an early life stressor which can result in long-term HPA changes (O'Mahony et al., 2011) has been shown to, cause a significant decrease in faecal lactobacilli on day 3 post separation, which returns to baseline by day 7 as assessed by enumeration of total and Gram-negative aerobic and facultative anaerobic bacterial species (Bailey and Coe, 1999). However, early life stress can also have long term effects on the microbiome. Analysis of the 16S rRNA diversity in adult rats exposed to maternal separation for 3 h per day from post natal days 2—12 revealed a significantly altered faecal microbiome when compared to the non-separated control animals (O'Mahony et al., 2009). A study using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP) demonstrated that the community structure of microbiota from mice exposed to a prolonged restraint stressor was significantly different than the community structure found in non-stressed control mice (Bailey et al., 2010). More recently using the same approach repeated social stressor has been shown to decrease the relative abundance of bacteria in the genus Bacteroides, while increasing the relative abundance of bacteria in the genus Clostridium in the cecum. The stressor also increased circulating levels of IL-6 and MCP-1, which were significantly correlated with stressor-induced changes to three bacterial genera (i.e., Coprococcus, Pseudobutyrivibrio, and Dorea). Interestingly, antibiotic exposure blocked the increase IL-6 and MCP-1. These data show that exposure to repeated stress affects gut bacterial populations in a cytokine dependent manner (Bailey et al., 2011).

6. Probiotics and the stress response

A probiotic is generally defined as a live micro-organism which when administered in adequate amounts confers a health benefit on the host (Quigley, 2008). Probiotics are emerging as potential therapeutics for stress-related gastrointestinal disorders such as irritable bowel syndrome. Overstated and exaggerated claims for the health benefits of probiotics have been made. In reality many of these claims are based on weak or non-existent data. More recently, probiotic administration studies support a role for the microbiota in anxiety like behaviours (Bercik et al., 2011a). Administration of *L. helveticus* R0052 and *B. longum* R0175 taken in combination produces anxiolytic-like activity in rats (Messaoudi et al., 2011). This finding must be viewed cautiously given the fact that many small molecules which apparently have anxiolytic impact in rodents lack efficacy when subjected to rigorous evaluation in man.

A recent study found that chronic treatment with the probiotic *Lactobacillus rhamnosus* over 28 days produced animals with lower levels of corticosterone and reduced depressive behaviours in the forced swim test in addition to a less anxious phenotype in the elevated plus maze (Bravo et al., 2011). Alterations in GABA the main inhibitory neurotransmitter system were also observed. *L. rhamnosus* treated animals showed alterations of GABAB1b mRNA in the brain with increased expression in cortical regions and decreased expression in the hippocampus, amygdala, and locus coeruleus as well as reduced GABAAα2 mRNA expression in the prefrontal cortex and amygdala and increased GABAAα2 in the hippocampus. The mechanism behind these changes was partially elucidated by studies in vagotimised animals. The neurochemical and behavioural effects of this bacterium, do not occur following vagotomy, indicating that the vagus is a key route of communication between probiotic bacteria and the brain. A role for the gut microbiota in pain perception has also been indicated, with, one study demonstrating that specific *Lactobacillus* strains could induce the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells and mimic the effects of morphine in promoting analgesia (Rousseaux et al., 2007). HPA parameters were not measured in the study.

Bercik et al. (2011a) have shown that infection-induced behavioural changes were associated with decreased hippocampal BDNF mRNA which could be reversed by a *B. longum* without affecting cytokine or tryptophan metabolism. Interestingly, these authors find that many effects of probiotics occur independent of vagus nerve activation (Bercik et al., 2010, 2011b).

Other potential mechanisms through which probiotics may influence the HPA and stress responsivity include neurotransmitter modulation. *B. infantis* 35624, for example, has been shown in Sprague-Dawley rats to induce an elevation in plasma tryptophan levels, a precursor to serotonin (5-HT) which is a key neurotransmitter within the brain—gut axis (Desbonnet et al., 2008). Since CNS tryptophan concentrations are largely dependent on peripheral availability and the enzymatic machinery responsible for the production of 5-HT is not saturated at normal tryptophan concentrations (Ruddick et al., 2006), the implication here is that the microbiota might play some role in the regulation of CNS as well as enteric nervous system 5-HT synthesis. This effect is potentially mediated by the impact of the microbiota on the expression of indoleamine-2,3-dioxygenase, a key enzyme in the physiologically dominant kynurenine pathway of tryptophan degradation (Forsythe et al., 2010) but of course multiple mechanisms are possible and indeed likely, given the strain specific effects that have been observed in many probiotic studies to date.

Diet plays an important role in relation to the composition of the microbiota and alterations in diet are known to change the microbial content of the gut. A clinical trial was performed on a population of 30 human subjects, who were classified in low and high anxiety traits (Martin et al., 2009). Biological fluids (urine and blood plasma) were collected during 3 test days at the beginning, midway and at the end of a 2 week study. NMR and mass spectroscopy based metabonomics were employed to study global changes in metabolism. Human subjects with higher anxiety traits showed a distinct metabolic profile indicative of a different

energy homeostasis (lactate, citrate, succinate, trans-aconitate, urea, proline), hormonal metabolism (adrenaline, DOPA, 3-methoxy-tyrosine) and gut microbial activity (methylamines, p-cresol sulphate, hippurate). Interestingly, a dietary intervention reduced the urinary excretion of both cortisol and catecholamines and partially normalised stress-related differences in energy metabolism (glycine, citrate, trans-aconitate, proline, β-alanine) and gut microbial activities (hippurate and p-cresol sulphate). The study provides strong evidence that diet can alter gut microbes, which in turn can impact on the neuroendocrine stress profile.

7. Antibiotics and stress

An alternative strategy employing antibiotic induced dysbiosis of the microbiome, using a cocktail consisting of neomycin, bacitracin and the antifungal agent primaricin, resulted in mice which displayed less anxiety-like behaviours in the both the step down box and the light/dark box test. Interestingly altered BDNF protein levels in the amygdala and hippocampus were reported and discontinuation of the antibiotics restored the normal behavioural phenotype of the animals (Bercik et al., 2011a). Similarly perturbation of the microbiota by means of an infectious agent such as *Citrobacter rodentium* has been shown to increase anxiety like behaviour in mice 7—8 h post-infection as measured in the hole board open field apparatus (Lyte et al., 2006) and to result in stress induced memory dysfunction 10 and 30 days post infection (Gareau et al., 2011). Memory dysfunction was prevented by daily administration of a probiotic cocktail and when GF mice were infected they developed memory dysfunction regardless of whether they were stressed or not.

8. Probiotics and glucocorticoids

Demonstrating changes in glucocorticoid levels in either rodents or man based on single serum samples is notoriously unreliable. In a maternal separation model Desbonnet et al. (2008) found behavioural changes with bifidobacteria treatment but no reduction in corticosterone while using a similar model Gareau et al. (2011) found that a lactobacillus reduced corticosterone levels and McKernan et al. (2010) found that *B. infantis* reduced corticosterone levels, though the reduction did not reach statistical significance.

In a recent clinical study, healthy volunteers were given *L. helveticus* R0052 and *B. longum* R0175 in combination or placebo in a double-blind, randomised parallel group study for 30 days and assessed with the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL) and 24 h urinary free cortisol (UFC) (Messaoudi et al., 2011). So far no in-depth analysis in man of HPA activity in response to a course of any putative probiotic has been undertaken either in healthy subjects or patient populations such depression. As a result we have no mechanistic understanding as to how a probiotic might result in reduced cortisol output. We do not know the impact of probiotics on CRH stimulated ACTH and cortisol release or on the dexamethasone\CRH response. It is possible that a reduction in cortisol is brought about by a decrease in pro-inflammatory cytokines, which activate the HPA or alternatively by an alteration of neurotransmitter inputs such as 5-HT. A clue may be provided by patients with irritable bowel syndrome who have an exaggerated ACTH and cortisol response to CRH infusion (Dinan et al., 2006) together with an altered microbiota (Grenham et al., 2011). O'Mahony et al. (2005) in a study of such patients showed that *B. infantis* altered the pro- to anti-inflammatory cytokine profile. This alteration may explain how probiotics benefit patients with this syndrome (Quigley, 2008) and normalise HPA responses.

9. Influence of stress on the microbiome

Although the bulk of research to date has focused on the impact of the microbiota on CNS function, there is also research to suggest that the brain can alter the microbiome. Signalling molecules released into the gut lumen from cells in the lamina propria that are under the control of the CNS can result in changes in gastrointestinal motility and secretion as well as intestinal permeability, thus altering the gastrointestinal environment in which the bacteria reside (Rhee et al., 2009). Under normal circumstances, non-pathogenic bacteria in the colon are restrained by the intestinal epithelium and it has long been established that conditions such as intestinal obstruction or hemorrhagic shock can alter gut permeability and thus translocation of bacteria across the epithelium. Accumulating evidence supports the view that psychological stress can also increase permeability of the gut allowing bacteria and bacterial antigens to cross the epithelial barrier and this can activate a mucosal immune response which in turn alters pro-inflammatory cytokines and perhaps activate the HPA. Acute stress was shown to cause an increase in colonic paracellular permeability (Kiliaan et al., 1998) which involved mast cells and overproduction of IFN-γ with decreased expression of ZO-2 and occludin mRNA. The psychological components of social stress were shown to facilitate the translocation of indigenous bacteria into the host (Demaude et al., 2006). Other studies have demonstrated that stress hormones promoted the growth of non-pathogenic isolates of *E. coli* as well as the pathogenic *E. coli* 0157:H7 strain via interactions with host catecholamines such as adrenaline and noradrenaline (Freestone et al., 2002). Different psychological stressors are known to alter the composition of the microbiome by modulating the composition of total biomass in infants (Kiliaan et al., 1998). Prenatal stressors have been shown to alter the microbiome in rhesus monkeys by reducing the overall numbers of bifidobacteria and lactobacilli (Bailey et al., 2004). Female monkeys were left undisturbed or were stressed during pregnancy using an acoustical startle paradigm for 6 weeks either early or late in their 24-week gestation. Several types of intestinal microflora were repeatedly enumerated by faecal culture while infants were reared normally by their mothers. Significant changes in microflora concentrations occurred during the first 6 months of life. The profile of total aerobes and facultative anaerobes was biphasic, with peak concentrations occurring between 2 and 16 weeks of age. The numbers of bifidobacteria and lactobacilli were low at 2 days after birth but rapidly increased to a peak between 8 and 16 weeks of age. Although similar temporal patterns were evident in all infants, prenatal stress reduced the overall numbers of bifidobacteria and lactobacilli. Moderate disturbance during

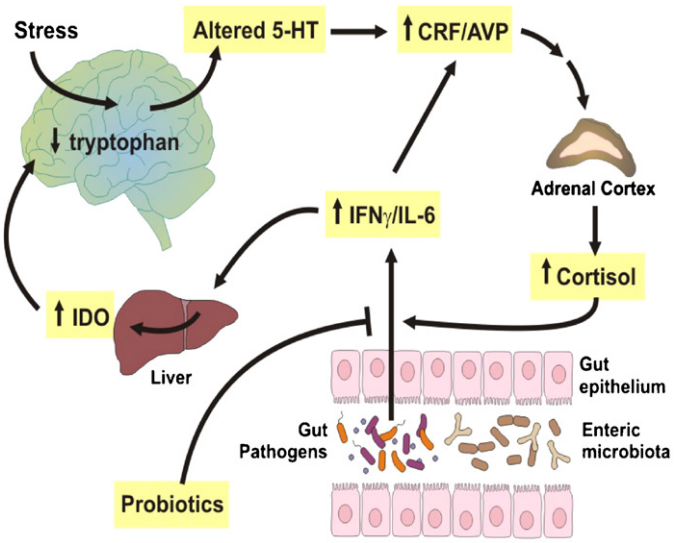

Figure 2 Stress can alter barrier function in the gut increasing gut 'leakiness' and leading to an increase in pro-inflammatory cytokines which in turn can alter indoleamine 2,3-dioxygenase (IDO) activity. This leads to altered tryptophan availability. Pro-inflammatory cytokines such as IL-1 and IL-6 together with 5-HT influence the release of CRF and AVP from the paraventricular nucleus of the hypothalamus. Certain probiotic bacteria can alter gut barrier function and via the vagus may impact on key central neurotransmitter systems.

pregnancy leading to activation of the HPA was sufficient to alter the intestinal microflora in the newborn infant. These alterations could result in enhanced susceptibility to infection and suggest a mechanism for some effects of maternal pregnancy conditions on infant health.

Through what mechanism can stress impact on gut permeability? In a pivotal study Clark (2005) showed that a rise in the pro-inflammatory cytokine interferon γ can exploit lipid raft-mediated translocation pathways to cross the epithelium, prior to cytokine-induced disruption of tight junctions. Meddings and Swain (2000) found that restraint stress resulted in increased intestinal permeability and an associated increase in corticosterone. The time course of the HPA response to the psychological stress was similar to that of an ulcerogeneic stress model. Ait-Belgnaoui et al. (in press) have found that prevention of gut leakiness by intestinal microbiota modulation leads to attenuated HPA response to an acute psychological stress in rats; the probiotic *L. farciminis* suppressed stress-induced hyperpermeability and endotoxemia and prevented HPA activation. To date in humans few studies have examined the microbiota in stress-related disorders with the obvious exception of irritable bowel syndrome (Codling et al., 2010) (Fig. 2).

Maes et al. (2008) suggest stress-induced intestinal mucosal dysfunction with an increased translocation from Gram negative bacteria plays an important role in the pathophysiology of depression and may account for HPA over-activity in some patients. The study examined the serum concentrations of IgM and IgA against LPS of the Gram-negative enterobacteria, *Hafnia alvei*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Pseudomonas putida*, *Citrobacter koseri*, and *Klebsielle pneumoniae* in patients with major depression and healthy controls. The prevalences and median values for serum IgM and IgA against LPS of enterobacteria were significantly greater in patients with major depression than in normal volunteers. These differences were significant to the extent that a diagnostic performance was obtained (the area under the ROC curve was greater than 90%). The symptom profiles of increased IgM and IgA levels were reported as fatigue, autonomic and gastrointestinal symptoms and a subjective feeling of infection. The results suggest that intestinal mucosal dysfunction characterised by an increased translocation of Gram-negative bacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. It is possible that the increased LPS translocation may mount an immune response and thus innate response system activation in some patients with major depression and specific "sickness behaviour" symptoms. This 'leaky-gut' effect of psychological stress may explain the HPA overactivity in some cases of depression. However, a detailed analysis of gut permeability and faecal microbiota in major depression needs to be undertaken before firm conclusions can be made.

10. Concluding remarks

It has long been recognised that pathogenic gut microbes can activate the HPA. We now know that such activation involves TLRs within the innate immune system and is subsequently mediated by vagal, spinal cord and humoral components. Recent studies support the view that the early programming and subsequent responsivity of the HPA is determined by a variety of factors including the gut microbiota. There is accumulating evidence that certain probiotics are capable of decreasing the behavioural and endocrine components of stress. Detailed prospective studies in patients with mood disorders examining the gut microbiota, immune parameters and HPA activity are required to throw further light on this emerging area. What is clear is that developmental studies and those involving stress-related disorders should include the gut microbiota as an important regulator of the HPA. Failure to do so can result in the introduction of a significant

confounding variable. If the animal data translate to man (and much of the HPA physiology in rodents does) then use of probiotic bacteria or antibiotics can influence stress studies of the HPA. Research in psychoneuroendocrinology needs to take this on board. It is tempting to speculate that therapeutic agents targeting the gut microflora may be useful treatments for stress-related psychiatric and gastrointestinal disorders (Dinan and Quigley, 2011).

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Conflict of interest

None declared.

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