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REVIEW ARTICLE

Microbes and the gut-brain axis

P. BERCIK, S. M. COLLINS & E. F. VERDU

Farcombe Family Digestive Health Research Institute, McMaster University, Hamilton, Canada

Abstract

Background *The 'gut-brain' or 'brain-gut axis', depending on whether we emphasize bottom-up or top-bottom pathways, is a bi-directional communication system, comprised of neural pathways, such as the enteric nervous system (ENS), vagus, sympathetic and spinal nerves, and humoral pathways, which include cytokines, hormones, and neuropeptides as signaling molecules. Recent evidence, mainly arising from animal models, supports a role of microbes as signaling components in the gut-brain axis.* ***Aims*** *The purpose of this review is to summarize our current knowledge regarding the role of microbes, including commensals, probiotics and gastrointestinal pathogens, in bottom-up pathways of communication in the gut-brain axis. Although this has clear implications for psychiatric co-morbidity in functional and inflammatory conditions of the gut, the focus of this review will be to discuss the current evidence for a role of bacteria (commensals, probiotics, and pathogens) as key modulators of gut-brain communication.* ***Results & Conclusions*** *The strongest evidence for a role of microbes as signaling components in the gut-brain axis currently arises from animal studies and indicate that mechanisms of communication are likely to be multiple. There is need for the concepts generated in animal models to be translated to the human in the future.*

INTRODUCTION

Clinicians and researchers have long recognized the link between gastrointestinal function and the central nervous system (CNS). Although the original description of a gut-brain axis related to the modulation of cholecystokinin secretion by bombesin,[1] the concept has since then been extended to describe any interaction between the gastrointestinal tract and the CNS. Recently, results in animal models have generated great interest into the role of intestinal microbes as key players in gut-brain communication (Fig. 1). The neural aspects and the role of centrally-driven pathways in gut-brain axis communication have recently been reviewed in detail by Mayer *et al.*[2] and O'Mahony *et al.*[3]

Address for Correspondence
Elena F. Verdu, 1280 main Street West, HSc 3N8, Hamilton, ON, L8S 4K1, Canada.
Tel: 905 525-9140x20051; fax: 905 522-3454;
e-mail: verdue@mcmaster.ca
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Intestinal microbiota and gut homeostasis

The intestinal microbiota involves a wide diversity of microbial species[4] and can be considered a postnatal acquired organ that performs different functions for the host. Intestinal microbes have developed a mutualistic relationship with its host and play a crucial role in the development of innate and adaptive immune responses,[5,6] influence physiological systems throughout life by modulating gut motility, intestinal barrier homeostasis,[7,8] absorption of nutrients and the distribution of somatic and visceral fat.[9,10]

The intestinal microbiota consists of a community of bacteria that colonize the gastrointestinal tract after birth and persist throughout adult life, and 'transient' bacteria, such as probiotic bacteria, which are temporarily acquired during ingestion of certain foods. The composition of the intestinal microbiota is established during the first few years of life and is likely shaped by multiple factors including maternal vertical transmission, genetic make up of the individual, diet, medications such as antibiotics, gastrointestinal infections and stress[11–15] (Fig. 2). Until recently composition of this microbial community was considered unique for each individual and relatively stable over time.[16,17] However, using deep sequencing of stool samples from

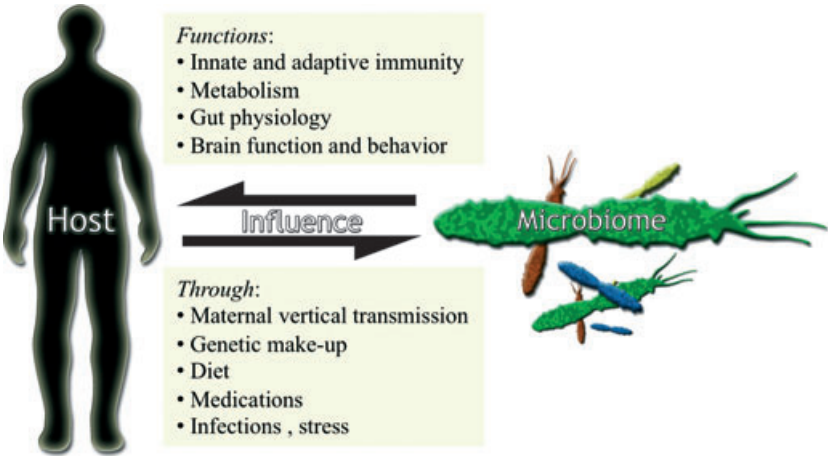

Figure 1 The intestinal microbiome: a complex and dynamic ecosystem that establishes bi-directional interactions with the host.

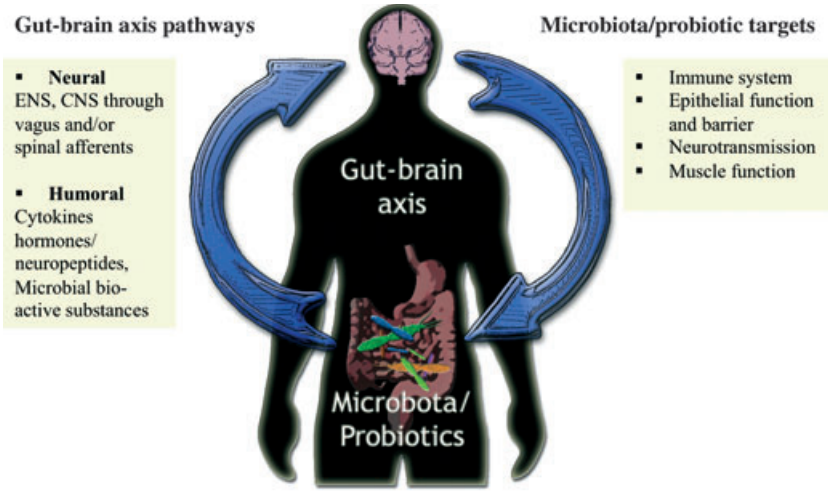

Figure 2 The gut-brain axis. Pathways of communication and probiotic targets.

several hundred individuals, the European MetaHit consortium study has shown that human microbiota profiles can be grouped in three major bacterial enterotypes dominated by *Bacteroides, Prevotella* and *Ruminococcus*, respectively.[18] Existence of distinct enterotypes strongly associated with long-term diets has been confirmed by Wu *et al.*, linking protein and animal fat with *Bacteroides* and consumption of carbohydrates with Prevotella.[19] This indicates that despite existence of large number of bacterial strains in the human intestine there are only a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet and drug intake.

Although the intestinal microbiota is considered relatively stable, some studies have shown a marked variation in the complexity and stability of *Bifidobacteria* and *Lactobacillus* populations over a 12-month period[20] and significant alterations in microbiota composition due to environmental factors.[21,22] Treatment with antibiotics is known to affect the intestinal microbiota and these changes may be long lasting. A study investigating the effect of a short course of amoxicillin showed that the microbial ecology of the intestinal tract was severely altered for up to 6 months after the end of antibiotic administration.[23] Similarly, 1-week course of clindamycin caused changes in the *Bacteroides* community, and this persisted for up to 2 years.[24,25] Diet is also likely to have a profound effect on gut bacterial composition. Different bacterial phenotypic profiles were found in subjects consuming either a Western style or an agrarian diet on a chronic

basis.[26] Although it would be logical to expect that acute changes in diet would alter the gut microbiota, recent short-term experiments in healthy volunteers using either high fat of high carbohydrate diets have not shown any marked changes in bacterial enterotypes.[19] It is thus possible that a stable microbiota is a hallmark of health, as unstable microbial profiles have been reported in patients with irritable bowel syndrome and inflammatory bowel disease.[27–30]

Intestinal microbiota and the gut brain axis

Indirect evidence for an effect of microbiota on CNS: clinical studies The concept that gut bacteria are a driving force for immune maturation and gut function in the host is well accepted. The notion that bacteria could also influence brain function and behavior is seemingly implausible, but clinicians use on a routine basis laxatives and oral antibiotics to treat patients with altered mental status due to hepatic encephalopathy.[31] Several clinical studies have also described altered composition of gut microbiota in patients with autism[32] and suggested at least short-term beneficial effect of antibiotic treatment,[33,34] although there is no randomized clinical trial available to date. There are also multiple reports of patients developing psychosis after administration of different antibiotics.[35] To our knowledge, there have been no studies that characterize the gut microbiota associated with depression or anxiety, but earlier studies demonstrated that depression in females is associated with increased fermentation of carbohydrates, indirectly implicating changes in the composition or metabolic activity of the gut microbiota.[36,37]

Direct evidence for an effect of commensals and pathogens on CNS: animal studies At this point, the brunt of evidence linking microbes with behavior and brain biochemistry comes from animal studies. Pivotal experiments performed by Lyte *et al.* have shown that mice display altered, anxiety-like behavior during the early phase of acute infection with *Campylobacter jejuni*.[38] This abnormal behavior occurred within several hours after introduction of the intestinal pathogen into the GI tract, before any significant immune response was mounted suggesting that this was not a consequence of cytokine-induced sickness behavior. Subsequent studies showed that presence of *C. jejuni* or *Citrobacter rodentium* triggers activity of vagal ascending pathways, such as nucleus tractus solitarius (NTS) and the lateral parabrachial nucleus,[39–41] and a specific activation pattern in multiple brain regions previously implicated in anxiety-like behavior.[42] This clearly illustrates that that the neural system can detect an acute change in the gut and selectively identifies the presence of a pathogen in the gut lumen.

Studies using chronic *H. pylori* infection in mice have shown that this pathogen alters gastric physiology, namely delayed gastric emptying and visceral sensitivity, with up-regulation of SP and CGRP-containing nerves in the stomach and the spinal cord.[43,44] Furthermore, chronic *H. pylori* infection leads to abnormal feeding behavior, characterized by frequent feeding bouts but with less food consumed per feeding bout than controls, which is reminiscent of early satiety observed in patients with functional dyspepsia.[44] The abnormal feeding pattern was accompanied by down-regulation of regulatory peptide Pro-opio-melanocortin (POMC) in the arcuate nucleus and up-regulation of the pro-inflammatory cytokine TNF-α in the median eminence (ME) of the hypothalamus. The ME is a part of the circumventricular organ, area of the brain where blood-brain barrier is relatively leaky enabling metabolites/molecules from the systemic circulation to enter the CNS. Interestingly, altered behavior and biochemical abnormalities persisted for at least two months postbacterial eradication suggesting that changes induced by chronic infection in the CNS may be long lasting or permanent.

To establish a link between commensal bacteria and the CNS, several experimental approaches can be undertaken. One is to compare germ-free with animals colonized with specific pathogen flora (SPF). Sudo *et al.* demonstrated an abnormal HPA axis with elevated ACTH and corticosterone levels in response to restraint stress in germ-free mice, which normalized after colonization with commensal bacteria.[45] Furthermore, germ-free mice had lower brain derived neurotrophic factor (BDNF) levels in the cortex and hippocampus. Several recent studies have compared behavior and brain biochemistry in germ-free and SPF mice. Overall, using standard behavioral tests, such as elevated plus maze, open field and light/dark preference tests, germ-free mice displayed higher exploratory and lower anxiety-like behavior than SPF mice.[46,47] Heijtz *et al.* showed that compared to germ-free mice, SPF mice had higher central expression of neurotrophins, such as nerve growth factor (NGF) and BDNF.[46] Furthermore, there was differential expression of multiple genes involved in the secondary messenger pathways and synaptic long-term potentiation in the hippocampus, frontal cortex and striatum. Similarly, Neufeld *et al.* demonstrated increased expression of NMDA receptor subunit NR2B in the central amygdala and serotonin receptor 1A (5-HT 1A) expression in the hippocampus in SPF mice compared to germ-free

mice.[47] The pronounced differences between germ-free mice and mice colonized with complex microbiota may relate to the ability of gut bacteria to affect multiple aspects of host metabolism, immunity and physiology. Colonization with a single commensal bacterium, *B. thetaiotaomicron* was shown to change expression of a vast array of genes in the intestine encoding for metabolism, intestinal permeability, angiogenesis but also for glutamate uptake, GABA production and neurotransmitter release.[48]

A different approach to investigate the role of microbiota in gut-brain axis is to perturb a previously 'stable' microbiota in adult healthy mice by oral administration of non-absorbable antimicrobials. Combination of neomycin, bacitracin and pimaricin induced changes in colonic microbiota composition (gut dysbiosis) in SPF mice, with a marked increase in *Firmicutes*, mainly *Lactobacilli* spp, and decrease in γ-proteobacteria. This was accompanied by an increase in mouse exploratory behavior and altered BDNF levels in hippocampus and amygdala[49] (Fig. 3A). The same antimicrobial treatment failed to induce behavior abnormalities in germ-free conditions or in mice treated with antimicrobials intraperitoneally. The antimicrobial regime employed in this study did not induce measurable changes in gut inflammation or change levels of intestinal serotonin (5-HT), noradrenalin (NA) or dopamine. Interestingly, studies using subdiaphragmatic vagotomy or chemical sympathectomy before antimicrobials suggest that vagal and sympathetic pathways are not involved in gut-brain communication in this experimentally-induced dysbiosis model of altered behavior.

Behavior has a genetic component, and it is known that mouse strains differ in their behavioral phenotype.[50,51] There is also a difference in microbiota composition among mouse strains, and the 'SPF' status does not indicate uniformity of the microbiota, only the fact that mice have been screened for the most common murine pathogens. BALB/c and NIH Swiss mice stand on opposite ends of the behavior phenotype: BALB/c mice are timid and less exploratory while NIH Swiss mice display a high exploratory drive. The BALB/c and NIH Swiss mice were reared under germ-free conditions and then colonized with SPF microbiota from either NIH Swiss or BALB/c mice. Germ-free mice colonized with microbiota from the same strain exhibited similar behavior as the SPF mice. However, mice colonized with microbiota from the other strain, exhibited a behavior profile similar to the donor[49] (Fig. 3B). This was not accompanied by any measurable change in systemic or gut immune activation or levels of intestinal 5-HT, NA or dopamine. A change in central neurotrophins was observed one week postcolonization. We can therefore speculate that host behavioral phenotype is also influenced by microbial factors.

The effect of microbiota may extend into memory and cognition, as recently suggested in a study comparing germ-free and SPF mice.[52] An Earlier study by Li *et al.* examined the effects of long-term dietary manipulation on memory.[53] Mice assigned to chow with high content of ground beef for 3 months displayed higher diversity of gut microbiota compared to mice on standard rodent chow. This was associated with improved working and reference memory, as well

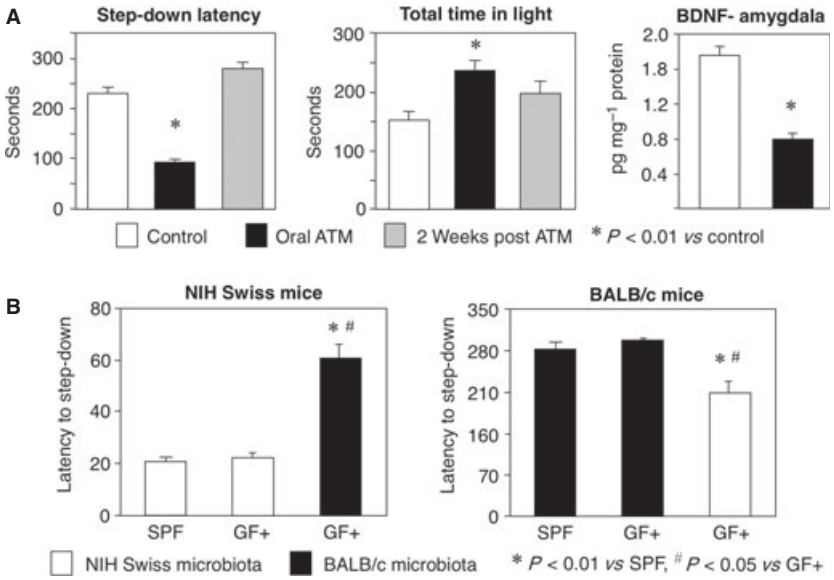

Figure 3 Effects of commensal bacteria on behavior. (A) Exploratory behavior of adult BALB/c mice increased transiently in parallel with gut dysbiosis induced by oral antimicrobial (ATM) treatment. This was accompanied by higher brain derived neurotrophic factor (BDNF) levels in the hippocampus. (B) Colonization of germ-free NIH Swiss and BALB/c mice with either NIH Swiss or BALB/c microbiota promoted or inhibited their exploratory behavior, respectively. Conventional, specific pathogen free (SPF) mice of both strains were used as controls. Adapted from Bercik *et al.*, Gastroenterology, 2011 (reproduced with permission from Gastroenterology).

as slower speed in seeking food and anxiety-like behavior in mice.[53]

Effects of probiotic bacteria on CNS and enteric nervous system function Psychiatric co-morbidities, such as anxiety and depression are common in patients with chronic bowel disorders, including IBS and inflammatory bowel disease.[54–58] Both of these disorders are also associated with abnormal intestinal microbiota profiles.[27–30] In this respect, chronic infection with a non-invasive parasite and mild chemically-induced colitis were shown to be associated with anxiety/depression-like behavior and decreased levels of hippocampal BDNF expression[59,60] (Fig. 4). Interestingly, both abnormalities were normalized by the treatment with the probiotic *B. longum NC3001* but not with *L. rhamnosus* NCC4007. *Bifidobacterium longum* did not improve gut inflammation or circulating cytokines, however, its anxiolytic effect was absent in mice with previous vagotomy, suggesting that its action was neurally mediated. This was further confirmed by *ex vivo* studies, in which electroresponsiveness of enteric neurons was assessed after perfusion with *B. longum* supernatant. Compared to controls, *B. longum* treated neurons fired less action potentials in response to supra-threshold depolarizing current.[60] The results suggest these signals may be initiated at the levels of the enteric nervous system (ENS). The beneficial effect of probiotic bacteria may extend to healthy individuals. A study by Desbonnet *et al.*[61] showed that administration of *Bifidobacterium infantis* to healthy Sprague-Dawley rats reduced concentrations of serotonin and dopamine metabolites in the frontal and the amygdaloid cortex, respectively. The authors suggested that this bacterium might have an anxiolytic potential, although no difference in behavior was found in that study. Subsequent experiments with the same bacterium using maternal separation model demonstrated beneficial effect on altered behavior together with normalization of noradrenaline concentrations in the brainstem.[62] Bravo *et al.* have recently demonstrated that administration of the probiotic *L. rhamnosus* JB1 promoted exploratory behavior and attenuated despair-like behavior, as assessed by elevated plus maze and forced swim test, respectively, in healthy BALB/c mice. This was accompanied by a region-dependent alterations in GABA(B1b) and GABA(Aα2) mRNA in the brain,[63] which was vagally dependent, as subdiaphragmatic vagotomy abolished both changes in brain biochemistry and behavior. Thus, animal studies support the notion that commensal bacteria and specific probiotics can influence brain chemistry and the function of the CNS, perhaps by modulating the ENS. This has implications for pain perception. Visceral pain perception is regu-

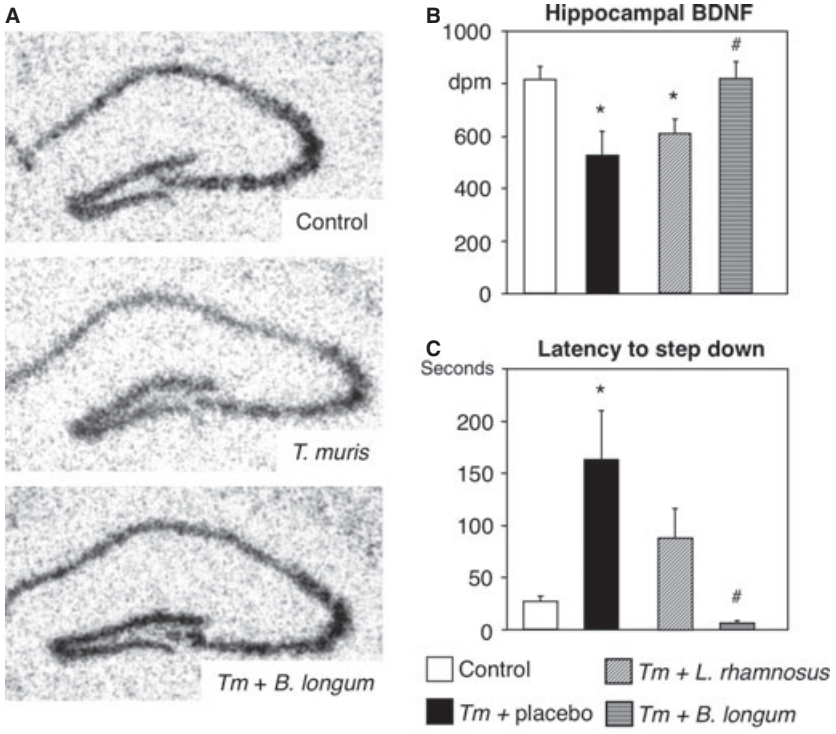

Figure 4 Effects of single probiotic on central brain derived neurotrophic factor (BDNF) and anxiety-like behavior. (A) Representative micrographs of BDNF mRNA expression in the hippocampus using in situ hybridization. BDNF was normalized in mice with chronic T. muris colitis treated for 2 weeks with Bifidobacterium longum (B). (C) Anxiety-like behavior normalized in mice with T. muris colitis after treatment with B. longum, but not with Lactobacillus rhamnosus. Adapted from Bercik *et al.*, Gastroenterology, 2011 (reproduced with permission from Gastroenterology).

lated by complex mechanisms, including peripheral sensitization of sensory nerves as well as central processing, which is modulated by concurrent anxiety and depression. Both of these processes could be affected by intestinal microbiota. There are multiple clinical trials that have reported improvement of pain after treatment with different probiotic bacteria (Table 1) but the mechanisms of action remain unknown. In one clinical trial, treatment with *Bifidobacterium infantis* normalized the increased ratio of serum pro-inflammatory cytokines suggesting an anti-inflammatory effect may mediate improvement in pain perception in IBS patients.[64]

Animal models have provided evidence on possible neural and metabolic pathways affected by commensals and probiotics. The first report on the role of bacteria on visceral perception comes from experiments where microbiota from healthy NIH Swiss mice was deliberately perturbed by administration of non-absorbable antibiotics. This treatment resulted in gut dysbiosis characterized by increased levels of *Enterobacteriae* and decrease in *Lactobacilli* and *Bacteroides,* and low-grade gut inflammation. Mice also developed increased visceral perception in response to colorectal balloon distension accompanied by up-regulation of SP in the myenteric plexus.[65] Interestingly, visceral hyperalgesia and enteric SP levels normalized after treatment with *Lactobacillus paracasei* NCC2461. The same probiotic was shown to also reverse rectal hyperalgesia in maternally deprived rats.[66] The effect of probiotics on pain mechanisms extends beyond an effect on enteric nerves as *Lactobacillus farciminis* was shown to ameliorate stress-induced visceral hyperalgesia and affect neural activation patterns assessed by c-fos staining in the sacral spine, paraventricular nucleus of hypothalamus and medial amygdaloid nucleus.[67]

Several probiotic bacteria have been tested for their ability to decrease pain perception during colorectal distension. *Bifidobacterium infantis* 35624 was shown to be effective in reducing visceral pain in both visceral normosensitive (Sprague-Dawley) and visceral hypersensitive (Wistar-Kyoto) rats.[68] *Lactobacillus reuteri* is a well- characterized strain with respect to its antinociceptive effect: its administration decreased response to colorectal distension in rats[69] and prevented hyperexcitability of colonic DRG neurons induced by noxious stimuli.[70] A subsequent study showed that *L. reuteri* increases excitability and number of action potentials per depolarizing pulse, decreases calcium-dependent potassium channel opening and decreases the slow after hyperpolarization in sensory AH neurons.[71] It is important to note that the effect of a given probiotic on visceral pain is likely site specific, as a recent study has shown that *L. reuteri* but not *Lactobacillus plantarum* alleviates the response to gastric distension in rats, although both of them have previously been shown to be effective anti-nociceptive agents in colorectal distension.[72]

The mechanisms of action of specific probiotics on visceral pain perception and ENS modulation are likely to be multiple and strain specific. The D-alanine content of lipoteichoic acid was found to be crucial for *Lactobacillus plantarum*-mediated protection from visceral pain perception in rats.[73] Another probiotic, *Lactobacillus acidophilus* NCFM induced the expression of opioid and cannabinoid receptors in intestinal epithelial cells, and modulated analgesic functions in the gut of healthy rats.[74]

Table 1 Randomized control trials showing beneficial effect of probiotics on abdominal pain

Reference	Probiotic treatment
Niedzielin *et al.*, Eur J Gastroenterol Hepatol 2001	*Lactobacillus plantarum* 299V
O'Mahony *et al.*, Gastroenterology 2005	*Bifidobacterium infantis* 35624
Bausserman *et al.*, J Pediatr 2005	*Lactobacillus* GG
Whorwell *et al.*, J Gastroenterol 2006	*Bifidobacterium infantis* 35624
Gawronska *et al.*, Alim Pharmacol Ther 2007	*Lactobacillus* GG
Sinn *et al.*, Dig Dis Sci 2008	*Lactobacillus acidophilus* SDC 2012
Drouault-Holowacz *et al.*, Gastroenterol Clin Biol 2008	*B. longum* LA101, *L. acidophilus* LA102, *L. lactis* LA103, *S. thermophilus* LA104
Enck *et al.*, Neurogastroeterol Motility 2008	*E. coli* DSM 17252, *Enterococcus faecalis* DSM 16440
Andriulli *et al.*, J Clin Gastroenterol 2008	*L. paracasei* B 21060, *L. paracasei* B21070, *L. gasseri* B21090
Williams *et al.*, Alim Pharmacol Ther 2009	*L. acidophilus* CUL60, *L. acidophilus* CUL21, *B. lactis* CUL34, *B. bifidum* CUL20
Enck *et al.*, Z Gastroenterol 2009	*E.coli* DSM17252
Kalman *et al.*, BMC Gastroenterol 2009	*Bacillus coagulans* GBI-30
Guandalini *et al.*, J Pediatr Gastroenterol Nutr 2010	VSL#3 preparation
Romano *et al.*, J Paediatr Child Health 2010	*L. reuteri* DSM 17938
Francavilla *et al.*, Pediatrics 2010	*Lactobacillus* GG
Guglielmetti *et al.*, Aliment Pharmacol Ther 2011	*B. bifidum* MIMBb75
Guerra *et al.*, World J Gastroenterol 2011	*B. longum (clinical isolate)*
Tarrerias *et al.*, Dig Dis 2011	*Lactobacillus* LB

VSL#3®: *B. breve, B. longum, B. infantis, L. acidophilus, L.plantarum, L. paracasei, L. bulgaricus, S. thermophilus.*

Challenges of studying the role of microbiota in the gut-brain axis

Although here is accumulating evidence for bacteria playing an important role in gut-brain axis, our knowledge is limited by our ability to identity the key players in this complex bacterial community. Most of the evidence comes from animal experiments where the microbiota is less diverse than in humans and can be controlled experimentally (e.g. gnotobiotic conditions). In the last decade, significant advancement has been provided by the availabiltity of molecular techniques, such as deep sequencing. These techniques enable us to determine bacteria at the species level. However, the task to identify a microorganism responsible for effects on the CNS is challenging. First, the key bacterium could be underrepresented in the community and could be missed when scanning for total microbiota composition. Also, results are influenced by the technique employed in the analysis of microbiota (454 pyrosequencing, Illumina platform, microarrays). The choice of technique can also make the comparison between individual studies difficult. Second, even if we identify the key microorganism(s) it may be difficult to culture and to determine its characteristics. Last, it is possible that the action of microbiota on the CNS is not related to a specific bacterium or group of bacteria, but rather to their metabolic activity. This metabolic profile may be influenced by environmental factors such as diet, stress or inflammation. Thus, a metabolomic approach would be more likely to yield answers than identification of single bacteria. Despite these methodological set-backs, our understanding of microbial-host interactions is rapidly advancing and this will likely translate into our ability to diagnose and treat patients with both gut and brain disorders.

CONCLUSIONS

While clinical observation and psychiatric co-morbidity in various chronic intestinal disorders support a role of the intestinal microbiota in gut-brain axis communication, the strongest evidence for a role of microbes as signaling components in the gut-brain axis comes form from animal studies using perturbation of the microbiota by antimicrobials and gnotobiotic models. Mechanisms of communication are likely to be multiple and involve neural, humoral and inflammatory pathways, depending on host and environmental factors. Animal studies will be crucial to continue to provide mechanistic insight and proof-of concept studies. However, there is need for the concepts generated in animal models to be translated to the human in the future.

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