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Of Bowels, Brain and Behaviour: A Role for the Gut Microbiota in Psychiatric Comorbidities in Irritable Bowel Syndrome

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# Equal Contributions

Running Title: Microbiome-gut-brain axis signalling and psychiatric comorbidities in IBS

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Abstract

The gastrointestinal microbiota has emerged as a key regulator of gut-brain axis signalling with important implications for neurogastroenterology. There is continuous bidirectional communication between the gut and the brain facilitated by neuronal, endocrine, metabolic, and immune pathways. The microbiota influences these signalling pathways via several mechanisms. Studies have shown compositional and functional alterations in the gut microbiota in stress-related psychiatric disorders. Gut microbiota reconfigurations are also a feature of irritable bowel syndrome (IBS), a gut-brain axis disorder sharing high levels of psychiatric comorbidity including both anxiety and depression. It remains unclear how the gut microbiota alterations in IBS align with both core symptoms and these psychiatric comorbidities. In this review, we highlight common and disparate features of these microbial signatures as well as the associated gut-brain axis signalling pathways. Studies suggest that patients with either IBS, depression or anxiety, alone or comorbid, present with alterations in gut microbiota composition and harbour immune, endocrine, and serotonergic system alterations relevant to the common pathophysiology of these comorbid conditions. Research has illustrated the utility of faecal microbiota transplantation in animal models, expanding the evidence base for a potential causal role of disorder-specific gut microbiota compositions in symptom set expression. Moreover, an exciting study by Constante and colleagues in this issue highlights the possibility of counteracting this microbiota-associated aberrant behavioural phenotype with a probiotic yeast, *Saccharomyces boulardii CNCM I-745.* Such data highlights the potential for therapeutic targeting of the gut microbiota as a valuable strategy for the management of comorbid psychiatric symptoms in IBS.

Keywords

IBS, Comorbidity, Microbiota-Gut-Brain Axis, Depression, Anxiety.

Abbreviations

AhR; Aryl hydrocarbon receptor, ANS; Autonomic Nervous System, CNS; Central Nervous System, ENS; Enteric Nervous System, FMT; Faecal Microbiota Transplantation, HPA; Hypothalamic-Pituitary-Adrenal, IAA; Indole-3-acetic acid, IBS; Irritable Bowel Syndrome, IBS-D; Diarrhoea-predominant Irritable Bowel Syndrome, IPA; Indole-2,3-dioxygenase, , PFC; Prefrontal Cortex, S. bou; Saccharomyces boulardii, SCFA; Short-chain Fatty Acids, TLR; Toll-like Receptor.

1. Introduction

Irritable bowel syndrome (IBS), now regarded as a disorder of gut-brain axis interactions, is one of the most prevalent gastrointestinal disorders, with varying incidence rates around the globe, constituting 20-50% of the gastrointestinal workload [1,2]. IBS is characterized by abdominal pain and altered bowel movement without overt structural or biochemical abnormalities [3]. While the understanding of IBS has been improved in recent years concurrent with some effective therapeutic options becoming available, many IBS patients present with psychiatric comorbidities, a subset that is much more difficult to treat. This significant cohort includes approximately 44% and 25% of IBS patients presenting at gastroenterology clinics with comorbid anxiety and depression respectively [4]. Moreover, the co-occurrence of psychiatric comorbidities is associated with IBS symptom severity [5,6] , while some studies show the efficacy of specific antidepressants in reducing IBS symptomatology [7].

Psychiatric disorders, such as anxiety disorders (hereafter referred to as anxiety) and major depressive disorder (hereafter referred to as depression) are among the most prevalent mental health problems worldwide. It is estimated that 10% of the global population suffers from these disorders each year [8,9]. Although there has been extensive research into the pathophysiology of depression and anxiety, their diagnosis is still symptom based, with treatment options remaining suboptimal and stubbornly focused on targeting monoamine neurotransmitter pathways [10]. Independently, IBS, depression and anxiety are complex heterogenous disorders with an already difficult clinical management profile made more challenging when combined in comorbid gastrointestinal and psychiatric phenotypes [3,8,9] .

Research in the last decade or more points towards a role of the gut-brain axis in both IBS and psychiatric disorders [11-13]. The gut is in continuous bidirectional communication with the brain through neuronal, endocrine and immune signalling pathways. The important role the gut microbiota plays in regulating these routes of communication to influence brain function and behaviour has seen this axis renamed to reflect this and it is now termed the microbiota-gut-brain axis [14].

The clinical care of IBS patients with psychiatric comorbidity is complex with treatment failure common. Repositioning IBS as a disorder of gut-brain axis interactions, along with recognition of the important role played by the gut microbiota in symptom expression, has led to calls for integrated clinical management models that blend medical management with behavioural and dietary interventions [15]. Here, we outline why the success of this approach for this particular subset of comorbid IBS patients demands greater focus on the common ground, and the diverging routes, that might explain why particular microbiota configurations lead to distinct clinical representations of IBS. As of now, the mechanisms underpinning these comorbidities are not fully known. A recent study also highlighted the bidirectional nature of this comorbidity by showing that psychiatric symptoms are predictive for the development of IBS, while IBS is also predictive of depression and anxiety later in life [16]. Interestingly, most comorbid IBS patients develop gastrointestinal symptoms before psychiatric comorbidities [16]. After a summary of the communication pathways of the microbiota-gut-brain axis, we will analyse the latest literature on psychiatric comorbidities in IBS. This review will focus in particular on alterations in the gut microbiota reported in IBS, depression, and anxiety and in IBS with comorbid anxiety and depression. We will discuss how gut microbiota signatures associated with these disorders might impact on gut-brain axis signalling pathways and the therapeutic implications of these observations.

2. Signalling pathways of the microbiota-gut-brain axis

Understanding the role of the gut microbiota in IBS and its psychiatric comorbidities requires an appreciation of the signalling pathways of the microbiota-gut-brain axis. The main routes of communication are summarised in Figure 1 and include neuronal, immune and endocrine host signalling pathways as well as the microbial production or regulation of bioactive molecules such as neurotransmitters, their precursors and short-chain fatty acids (SCFAs).

-- Insert Figure 1 here –

Neuronal communication along the microbiota-gut-brain axis is mostly mediated by the autonomic nervous (ANS), with the enteric nervous system (ENS) arm regulating important mechanisms locally in the gastrointestinal tract. One of the most important routes of communication is the vagus nerve. The vagus nerve connects the brain to all visceral organs among others and relays information via 80% afferent and 20% efferent fibers [17-19]. A portion of afferent axonal endings are located in the mucosa of the GI tract. These afferents are thought to contain a wide array of receptors, making them able to detect signals such as gut hormones, neurotransmitters, and bacterial metabolites [14].

A major player in endocrine signalling of the microbiota-gut-brain axis is the hypothalamic-pituitary-adrenal (HPA) axis, the major stress axis of the body, whose activation results in the release of glucocorticoids. This endocrine signalling pathway can be restrained at brain-level by negative feedback of glucocorticoids acting on glucocorticoid receptors. Both IBS and psychiatric disorders show dysregulation of the HPA axis [20,21]. It is now appreciated that the microbiota plays a key role in the priming and regulation of this axis, shown initially by increased stress response in germ-free animals, which is reversed by colonisation with specific bacteria or a more complete microbiota [22,23]. In turn, it has long been known but recently reinforced in the preclinical literature that stress exposures can also modify gut microbiota composition and function [14,24].

The crosstalk between the microbiota and the hosts' immune system mostly takes place at the mucosa either by direct contact or through molecules secreted by the microbiota and is essential for priming and education of the immune system [25]. The communication is facilitated by microbe-associated molecular patterns, which are sensed by colonocytes and immune cells through pattern recognition receptors such as toll-like receptors (TLRs), triggering an immune response by the secretion of cytokines. The impact of the gut microbiota on the immune system extends to the brain, shown by changes in microglia morphology and gene expression profile in germ-free animals [26,27].

An important topic in the context of inflammation in the gut-brain axis is the integrity of the intestinal barrier. Changes in intestinal permeability creates a passage for bacteria and their products from the lumen to the ENS, immune cells and systemic circulation, which can evoke an immune response. Increased intestinal permeability is associated with low-grade inflammation, a neurobiological feature of both IBS and depression [28,29].

Another form of communication in the microbiota-gut-brain axis is via microbial metabolites, such as SCFAs and neurotransmitters. SCFAs are mostly used as an energy source by the host, for example butyrate is the primary energy source for colonocytes. The SCFAs not utilized by colonocytes enter the systemic circulation and other tissues including the brain [30]. SCFAs can activate a set of G-protein coupled receptors, FFAR2 and FFAR3 being the most investigated. They are found in tissues such as the colon, the heart, and immune cells. FFAR3 is also expressed in the peripheral nervous system in enteric plexi, the portal nerve and autonomic and sensory ganglia [31], which further implicates their involvement in gut-brain signalling [32].

The microbiota can produce a wide range of neuroactive molecules that have implications for behaviour, mood, and cognition. Many of these neurotransmitters (GABA, noradrenaline, serotonin) are involved in both gastrointestinal and brain function. One of the most important neurotransmitters in terms of the microbiota-gut-brain axis is serotonin. Serotonin is an important signalling molecule in both the CNS and the ENS and is produced from the precursor tryptophan, an essential amino acid [33]. The majority of serotonin is synthesized by enterochromaffin cells. However, most tryptophan is metabolised along the kynurenine pathway, whose end products have neuroactive properties and are NMDA receptor antagonists and agonists [34]. In contrast to serotonin, both tryptophan and kynurenine can cross the blood brain barrier and are further metabolised in the brain by glial cells [35].

The microbiota can directly modulate the levels of tryptophan and its metabolites by producing or utilising tryptophan themselves [36]. The third major pathway of tryptophan metabolism is microbial and results in indoles and its derivates, such as indole-3-acetic-acid (IAA), indole-3-

propionic acid (IPA) ligands of the aryl hydrocarbon receptor (AhR) [37]. AhR is a key regulator of the immune system, involved in the function of macrophages, dendritic cells and neutrophils [38]. For example, a lack of AhR ligand-producing bacteria is associated with increased intestinal inflammation [39].

Although the majority of serotonin is synthesised by the host, its production is strongly modulated by gut bacteria. Studies in germ-free animals showed that the levels of tryptophan, serotonin, and kynurenine are significantly different from conventional animals in the gut lumen, plasma, and the brain, both at baseline and following acute stress exposures [23,40-42]. One of the theories involving the role of tryptophan in affective disorders is that the more tryptophan is converted into its alternative metabolites, the less tryptophan can enter the brain via the circulation, decreasing central levels of serotonin [43].

3. Gut microbiota compositional alterations associated with disorders of the gut-brain axis

There is a growing body of evidence suggesting alterations in gut microbiota composition or function in psychiatric disorders [44], which has been associated with increased levels of inflammation [45]. It is generally thought that gastrointestinal and psychiatric disorders are associated with decreased alpha diversity (richness, evenness, and biodiversity of the microbiome) [46-48]. However, while some published articles show reduced alpha diversity in these disorders, other studies found no changes [49,50].

Table 1 summarises changes in relative abundance of specific bacteria associated with IBS, depression and anxiety, based on the findings in these systematic reviews, in comparison with the relatively few studies looking at IBS with comorbid anxiety and depression. Overall, these disorders present an altered gut microbiota signature but likely due to the heterogeneity of these disorders, conflicting results are common. However, two recent metanalyses identified the gut microbiota signatures most consistently found in depression, anxiety, and IBS [51,52].

These changes in microbial abundance were hypothesized to play functional roles in these disorders. For examples, the increased abundance of strains such as *Escherichia* in anxiety has been hypothesized to lead to increased secretion of exotoxins potentially inducing inflammatory processes impacting on the central nervous system [53]. In relation to IBS, it was hypothesized that the metabolic products of the strains *Lactobacillaceae* and *Bacteroides,* such as organic acids or toxins respectively, may contribute to the IBS pathology by causing bloating or inflammation peripherally [52].

Fewer studies have investigated the microbiota using the more informative shotgun metagenomic approach. One such study found that numerous species of the genus *Bifidobacterium* such as *B. adolescentis*, *B. longum*, *B. dentium* are increased in depressed patients [54]. This was unexpected because *Bifidobacterium* strains are commonly used as probiotics with preclinical evidence supporting their possible use for the treatment of psychiatric disorders [55], although whether a particular microbial member of the gut microbiota should be considered beneficial or harmful depends on context. The most recent study using metagenomic assessment identified 47 species with altered relative abundances in patients with depression compared to healthy controls. Most of the enriched species belonged to the genera *Bacteroides*, whereas the depleted species belonged to the genera *Blautia*, *Eubacterium* and *Clostridium* [53]. The largest study to date, which included a discovery and validation cohort, showed that *Coprococcus spp*. and *Dialister* are both depleted in depression [56]. In addition, the study by Valles-Colomer and colleagues [56] conducted a module-based analysis, profiling microbial pathways with neuroactive potential involved in microbiota-gut-brain axis communication. They showed that depression and quality of life were associated with GABA and DOPAC, a metabolite of dopamine. Interestingly, GABA has also been linked to visceral pain perception [57].

Some studies aimed to subdivide IBS patients with and without distinct microbial signatures. For example, IBS patients characterized by an increased Firmicutes:Bacteroidetes ratio show increased abundance of strains of SCFA-producing eubacteria as well as flagellin producing

bacteria [58], which are associated with increased visceral hypersensitivity and low-grade inflammation [59,60]. Interestingly, it was the patients showing a similar gut microbiota signature compared to healthy controls were linked to comorbid depression [58]. Similarly, a distinct gut microbiota signature was shown with increased IBS symptom severity. However, in this study, psychiatric comorbidities were associated with the gut microbiota signatures reported in severe cases of IBS [61].

Relatively few studies have directly assessed the gut microbiota signatures associated with psychiatric comorbidities in IBS. A recent study, analysing the therapeutic effect of FMT, showed that IBS patients and healthy controls show higher alpha diversity compared to IBS with comorbid depression. Similarly, comorbid IBS patients clustered differently from IBS patients and healthy controls in a beta-diversity analysis [62]. Research has also suggested that patients with IBS and depression show a similar gut microbiota imbalance characterized by either high levels of *Bacteriodes* or *Prevotella* [63]. Further analysis showed that comorbid patients show a similar enterotype to healthy controls, characterized by dominant genera including *Bacteroides*, *Faecalibacterium* and *Lachnospiraceae*. However, differences were shown in the composition of non-dominant bacteria. Of note is that the presence of depression at baseline was associated with lasting effect of FMT in IBS-related quality of life and fatigue in patients with non-constipated IBS [64].

There have not yet been extensive attempts to address the gut microbiota in IBS patients with comorbid anxiety. De Palma and colleagues identified indicator species, rather than taxonomic differences in relative abundances *per se*, of the genera Eggerthella, Blautia, Copcrococcus, Streptococcus and Clostridium, which were associated with the disease state of comorbid anxiety [65]. However, this was based on a small number of IBS subjects with and without anxiety, making definitive conclusions about a distinct comorbid-anxiety related gut microbiota signature difficult.

While it is hard to confidently compare results derived from single studies to that of meta-analyses, it does appear possible that comorbid patients cluster differently than patients with

one of the disorders alone. However, there is a greater need for studies including a clinical diagnosis of IBS patients with comorbid depression and anxiety rather than the more common approach of assessing high levels of depression and anxiety scores.

4. **Signalling pathways altered in gastrointestinal and psychiatric disorders.**

It has been theorised that the low-grade inflammation, such as increased cytokine levels [66] associated with depression, stems from increased intestinal permeability [67,68] which in turn results in increased contact of the immune system to bacteria. Similarly, anxiety is associated with a distinct inflammatory state [69,70]. Increased inflammatory signalling may dysregulate the HPA axis, which is associated with symptoms of anxiety and depression [71]. Bacteria showing a higher relative abundance in depression and anxiety, including *Eggerthella* and Enterobacterales, are associated with increased intestinal inflammation and permeability [72]. This low-grade inflammation can be further exacerbated by the loss of SCFA-producing bacteria, such as *Faecalibacterium,* which have anti-inflammatory properties [73].

IBS is similarly associated with low-grade intestinal and systemic inflammation [74]. Studies showing an increased production of pro-inflammatory cytokines in IBS patient derived PBMCs, indicate also an association with anxiety symptoms [75]. Low-grade intestinal inflammation characterized by increased eosinophil and mast cell numbers in the descending colon may drive the gastrointestinal pathology of IBS [76]. Mucosal inflammation driven by changes in microbiota composition and strains including *Prevotella* is associated with overall immune dysregulation [77]. In conjunction with this, it has been shown that IBS patients show altered tryptophan metabolism with a shift towards the kynurenine pathway [78]. This change has been linked to an altered proinflammatory state via activation of TLRs [79]. Kazemi et al. additionally showed an improved kynurenine/tryptophan ratio in the blood of the subjects using a probiotic mix containing *L. helveticus* and *B. longum* [80]. Psychiatric comorbidities in IBS can potentially be linked to increased neuroinflammation triggered by the systemic inflammation seen in these

disorders [66,69,74]. These changes are thought to also be in part modulated by changes in SCFA production [32]. In addition, microglia activation has been observed in animal models of stress-induced changes in the microbiota-gut-brain axis [81]. Changes of the gut microbiota signature in these disorders could potentially evoke similar changes relevant for the pathophysiology.

Affective disorders are believed to be mainly caused by dysregulation of neurotransmitters in the brain. For example, the majority of current medications for depression and anxiety act by increasing the level of monoamines in the synapses [8,9]. The level of these neurotransmitters in the brain is also strongly affected by the gut microbiome. Germ-free animals show altered neurotransmitter concentrations in the brain in addition to reduced anxiety-like behaviours [23,82] and these serotonergic system alterations are differentially modulated by acute stress [42]. Interestingly, one of the common therapeutic interventions for IBS are antidepressants. While tricyclic antidepressants (TCAs) are recognized to be an effective treatment in IBS, selective serotonin reuptake inhibitors are not as efficacious [83]. However, serotonin plays an important role in gastrointestinal motility whereby antagonism of the serotonin 5-HT3 receptor improves stool quality [84] and decreases motility in IBS-D patients [85]. 5-HT4 receptor agonist have also proven useful in relief of constipation [86]. Serotonin is also a modulator of visceral pain as 5HT-3 antagonism increased colonic compliance [87] and agonism of 5HT-4 reduced sensitivity to rectal distension [88]. The involvement of serotonin in mood disorders has also been extensively studied, particularly in depression, however, its precise neurobiological role in psychiatric disorders is likely of greater complexity than heretofore appreciated [89]. Overall, serotonin is a key signalling molecule in the gut-brain axis implicated in the core symptoms experienced by IBS and patients with psychiatric comorbidity.

IBS has frequently been associated with structural brain changes. For example, one study showed reduced volumes in multiple cortical and limbic structures in female IBS patients compared to healthy controls [90]. However, the majority of the differences were associated with early life trauma and not IBS alone *per se*, highlighting the importance of early-life stress in this disorder [90]. Other studies showed alterations in white matter of IBS patients between basal

ganglia, thalamus, and prefrontal cortex (PFC) [91]. Interestingly, when grouping IBS patients based on the microbiota profile, patients characterised by a reduced Firmicutes:Bacteriodetes ratio present alterations in the anterior insula, the motor cortex and the ventral PFC. Furthermore, increasing volume of the posterior insula, was associated with changes in SCFA metabolism and glutamate metabolism [92]. Similarly, patients with depression show structural brain alterations such as reduced hippocampal volume [9,93]. These alterations are accompanied by reduced expression of BDNF in the corresponding brain regions and in the serum [94,95]. Interestingly, reduced serum levels of BDNF have also been reported in comorbid IBS patients [96]. It has been shown that brain BDNF levels are modulated by the microbiota with germ-free animals showing reduced BDNF expression in the hippocampus [22,23]. However, mechanisms behind the regulation of BDNF by the microbiota are still unclear. Future studies should identify brain regions and circuits, such as the thalamus or prefrontal areas important for modulation of sensory information and emotions, common across these pathologies responsible for the symptom presentation.

5. Preclinical models for comorbid IBS

Part of the difficulty in gaining mechanistic insights in IBS with psychiatric comorbidity pertains to the limited availability of preclinical animal models of complex heterogenous behavioural phenotypes. Nevertheless, some options do go some way towards recapitulating a relevant constellation of gastrointestinal and psychiatric symptoms.

5.1. Maternal separation

Maternal separation is a well-established rodent model of early life stress and results in widespread changes across the microbiota-gut-brain axis [97]. The maternal separation paradigm does not just model the animal behavioural correlates of one specific disorder but rather recapitulates several aspects of stress-induced psychiatric disorders and produces robust and reproducible changes across the microbiota-gut-brain axis. These alterations

include perturbations in gut microbiota [97], which are detectable in adulthood, increases in anxiety- and depressive-like behaviours [98,99] as well as development of visceral hypersensitivity [100], a hallmark of IBS thought to explain the abdominal pain which is a dominant characteristic of this disorder [101].

In terms of maternal separation-induced alterations in signalling pathways of the microbiota-gut-brain axis, this early-life stress exposure has also been shown to alter central neurotransmitter levels, particularly monoamines such as serotonin and noradrenaline [102,103]. As serotonin plays an important role in gut to brain communication with respect to mood [104] and descending pain pathways [105], changes in levels may adversely affect gut function and communication with the CNS. Interestingly, maternal separation has also been shown to alter central serotonin transporter expression [106]. It has been seen that maternal separation also results in upregulation of TLR4 in the paraventricular nucleus of mice as well as visceral hypersensitivity which is blocked by inhibition of TLR4 signalling [107], supporting the notion of stress-induced dysregulation of gut-brain axis signalling.

Maternal separation has also has also been shown to cause reprogramming of the HPA axis, leading to profound effects on endocrine signalling whereby both baseline [97,108] and stress-induced [109] corticosterone levels are increased. Dysregulation of the HPA axis by maternal separation may be likened to clinical cases of IBS where stress reactivity and recovery is altered, and early life stress is a known risk factor [110,111].

5.2. Faecal microbiota transplantation

FMT studies in rodents currently provide the strongest evidence for an involvement of the gut microbiota, both in the expression of specific symptoms and the alterations in gut-brain axis signalling pathways of relevance to the pathophysiology of IBS and affective disorders. Multiple studies have used FMT to investigate gastrointestinal, behavioural and molecular alterations associated with IBS. Animals in receipt of a microbiota transplant from IBS patients with predominant constipation or patients with chronic constipation developed delayed GI transit

and alterations in intestinal contractions, which was accompanied by decreased levels of SCFAs [112,113]. Conversely, a study using faecal material from diarrhoea-predominant IBS patients (IBS-D) developed increased gastrointestinal transit. Furthermore, they showed associations between the gut microbiota, IBS and psychiatric comorbidities.

Studies showing a disturbed gut microbiota profile in patients with depression linked these alterations to disrupted tryptophan metabolism and intestinal low-grade inflammation [47]. This was achieved by FMT of depressed patients to rats, which induced a similar behavioural and molecular phenotype to the donors. Two studies using a similar approach linked the microbiota-induced depression in mice to alterations in the CREB signalling pathway in the olfactory bulb [114] and alterations of carbohydrate and amino acid metabolism [115]. The latest study transferring the microbiome of depressed patients into mice showed alterations in neurotransmitter levels in the brain and inflammatory markers in the serum [116].

Earlier studies indicated the rodent-to-rodent transfer of anxiety-like behaviours [117] and human-rat transfer of visceral hypersensitivity [118]. Taken together, FMT studies confirm the individual adoptive transfer of both the cardinal features of IBS (visceral hypersensitivity, motility) as well as the psychiatric comorbidity (depression and anxiety-like behaviours) [14]. Germ-free mice colonized with faecal microbiota of IBS-D patients with comorbid anxiety showed, in addition to gastrointestinal motility alterations, increased anxiety-like behaviour, [65] which was absent in mice receiving the donor material from patients with IBS only and associated with increased immune activation in the colon. This study confirms the simultaneous transfer of multiple phenotypes via the gut microbiota, positioning FMT studies as a useful preclinical approach to study IBS with psychiatric comorbidity.

In this issue, leading on from their previous study [65], Constante and colleagues [119] investigated the treatment of comorbid anxiety in IBS using FMT in germ-free mice treated with the probiotic *Saccharomyces boulardii* CNCM I-745 (*S. bou*). Treatment with *S. bou* improved anxiety-like behaviour, but not gastrointestinal motility alterations in mice. These results go a step beyond implicating this microbiota configuration in comorbid symptom expression by confirming that

385 an intervention targeting this microbiota can improve symptoms relevant to anxiety. The
386 microbiota profiles revealed differences between the mice transplanted with material from the
387 IBS patient and the healthy control, which were in part normalized by *S. bou* treatment. On the
388 molecular level, they showed a role of indoles (microbial metabolites of tryptophan) and
389 immune activation in IBS with comorbid anxiety. While no clear association was shown
390 between the gut microbiota compositional differences and alterations in indole levels, they
391 nicely linked the anxiolytic effect of *S. bou* to increased indole production. *S. bou* increased
392 both the levels of IAA in the faeces as well as the expression of bacterial genes relevant for
393 indole alkaloid synthesis, possibly by increasing the abundance of indole producing bacteria,
394 such as *Lactobacillus*. However, the associated increase in AhR activity failed to reach
395 significance posing the questions of if, and by which mechanisms, the increased indole
396 production induces the anxiolytic effects. Conversely, the authors reported increased
397 expression of the capsaicin receptor TRPV1 in colonic tissue of mice with comorbid IBS-
398 associated microbiota. This receptor, important for the modulation of nociception, is mainly
399 found on neurons of the peripheral nervous system. While TRPV1 expression was associated
400 with the anxiety-like behaviour, it was not modulated by *S. bou*. Altogether, this study reports
401 some interesting observations which are potentially relevant to comorbid IBS treatment.

402 As provocative and timely as the study is, the authors use a single donor for FMT into mice, in
403 contrast to recommendations for the use of multiple individual donors made recently by Walter
404 and colleagues [120]. The authors previous work showed the successful transplantation of
405 phenotypes via the use of multiple donors, providing strong evidence for the gut microbiota in
406 both IBS specifically and its comorbidities [65]. It is not clear from the current study whether the
407 beneficial effects of *S. bou* are applicable to a wider range of microbiome compositions of
408 different IBS patients or indeed how well it applies to different comorbidities such as
409 depression. Gut microbial signatures of different donors could be differentially affected by *S.*
410 *bou*, leading to different outcomes. It would also be interesting to see how effective *S. bou*
411 treatment is against IBS patients without psychiatric comorbidities and whether some of the

other cardinal features of IBS including visceral hypersensitivity were impacted. This raises the question of whether the mechanisms described are exclusively altered in comorbid patients or if they also generalise to other subgroups of IBS. The authors recommend that the first point of study in future clinical trials in IBS should be in the subpopulation with this psychiatric comorbidity. These considerations aside, this study brings important additional insights, expanding on the results reported in previous studies with mechanistic insights and highlighting the therapeutic possibilities of *S. bou*.

6. The gut microbiota: a novel target for treating psychiatric comorbidities in IBS

Currently, treatment options for IBS revolve around symptom control. Some of the more common medications in the treatment of IBS are antispasmodics or tricyclic antidepressants (TCAs) [121]. Antispasmodics, exerting their effects by relaxation of intestinal smooth muscle, are currently not recommended by the new clinical guideline by the American college of gastroenterology although only currently available in the United States were evaluated [122]. TCAs such as amitriptyline mainly improve visceral pain, possibly by acting on the norepinephrine-, dopamine- and acetylcholine system [123]. The dose of TCA used is often below that employed in the treatment of depression so the extent to which psychiatric comorbidities are potentially treated by gut-brain neuromodulatory agents is unclear [121]. The integration of psychological behavioural approaches into gastroenterology practice is now more routinely considered [124], building on the success of gut-focused hypnotherapy as an option in treatment-refractory IBS [125]. The use of food supplements and diet as treatment options has recently been evaluated in this journal [126]. These varied approaches reflect a willingness to target multiple levels of the gut-brain axis to deliver gastrointestinal symptom relief.

One important implication of the study from Constante and colleagues [119] is the potential for therapeutic targeting of the gut microbiota to alleviate the comorbid psychiatric symptoms. Does this mean that specific features of the comorbid gut microbiota lead independently to the

cardinal and behavioural features of IBS? The use of a single probiotic strain then, based on the results of this study, is unlikely to be sufficient to improve the global symptom profile in IBS. It has of course long been appreciated that the beneficial effects of specific probiotics are strain specific and a number of therapeutic options can be considered for targeting the gut microbiota to improve gut-brain axis signalling pathways [127].

6.1. Prebiotics and probiotics

Consideration of probiotics (defined as "live microorganisms which when administered in adequate amounts confers a health benefit on the host" [128] and prebiotics (defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' [129]) use for treatment of IBS symptoms and associated psychiatric comorbidities has increased in recent years (for review see [130]). Although the exact mechanisms of action of specific prebiotics and probiotics have not been fully elucidated, it has been seen that different prebiotic blends such as polydextrose, galactooligosaccharide and probiotics such as *Lactobacillus rhamnosus GG* ameliorated maternal separation-induced anxiety-like behaviour as well as altering hippocampal levels of stress-related genes [131]. Similarly, a prebiotic blend combined with milk fat globule membrane, the bioactive fraction of breastmilk, attenuated MS-induced visceral hypersensitivity and facilitated faster return to baseline of stress-induced corticosterone levels [132].

Evidence supporting the role of prebiotics and probiotics against IBS symptoms is not purely preclinical whereby IBS patients administered *B. longum subsp. longum 35624* (formerly *B. infantis 35624)* for 8 weeks reported a reduction in IBS symptomatology with respect to abdominal pain, bloating and bowel movement difficulty as well as normalisation of the anti-inflammatory: proinflammatory cytokine ratio [133]. Several other studies have assessed the efficacy of this treatment with varying degrees of success (for review see [130]). It can be seen above and from recent technical reviews and clinical guidelines that while some prebiotic and

probiotics have shown promise in the symptomatic treatment of IBS specifically in the context of a single trial, the jury remains out on making strong recommendations [134,135]. Additional and robust clinical studies are required to determine if we can achieve benefits for associated comorbid psychiatric conditions.

6.2. Therapeutic faecal microbiota transplantation

In the recent years, evaluation of the use of FMT from healthy donors as a treatment option for gastrointestinal disorders has increased. There are multiple studies showing the benefits of FMT as a treatment for IBS, further supporting the role of the microbiota in this disorder. The use of a single FMT in IBS patients improved the gastrointestinal symptoms in a subset of patients for a prolonged duration [136,137]. Furthermore, studies showed that the use of FMT additionally improved symptoms of affective disorders, providing evidence for a causal role of the microbiota in psychiatric comorbidities in IBS [62,138]. A double-blind, randomized, placebo-controlled study investigating the effect of FMT in IBS patients showed the effectiveness of FMT as a treatment option and determined that the presence of depression at baseline is predictive of successful treatment [64]. While these studies look promising for the treatment, FMT is currently not recommended as a treatment option, as evidence is still limited and large double-blind, placebo-controlled trials are required to determine the treatment efficacy [122,139]. There are a number of important factors to consider in the selection of suitable donors, including microbiota profile, in addition to FMT dose that may be critical to a successful FMT [140]. Interestingly, European guidelines on donor selection for the use of faecal microbiota transplantation in clinical practice does recommend exclusion of subjects with a history of psychiatric conditions [141].

7. Conclusion

Evidence continues to accumulate in support of the view that the strong link between gastrointestinal and psychiatric disorders is mediated by the microbiota-gut-brain axis. Individually these disorders share similar pathophysiological mechanisms, such as increased pro-inflammatory states or changes in monoamine levels. Many questions remain surrounding the nature of the clinical entity that sits at the intersection between IBS, depression and anxiety. It is plausible to conceptualise common dysfunctions in gut-brain axis signalling pathways that define this troublesome subset of patients. While this may be a preferable conclusion from a treatment perspective, the reality hinted at by Constante and colleagues [119] is more complex and may develop around a number of diverging targets.

-- Insert figure 2 here --

What this intriguing study does not answer is why specific microbiota configurations, compositional or functional, lead in some cases to IBS and in others IBS with psychiatric comorbidity. This is an important missing piece in the puzzle that requires increased research focus as the current evidence is insufficient to draw definitive conclusions. Animal models of IBS with psychiatric comorbidity hold promise to help disentangle the molecular mechanisms at play and to expand on the associations identified between the gut microbiota, pain pathways and indole production [119]. It will be important to tread carefully in this regard and not to assume that the signalling pathways implicated in the benefits of particular interventions automatically double as a neurobiological basis for psychiatric comorbidity in IBS. Improving our understanding of how the relevant signalling pathways for depression and anxiety overlap with, or deviate from, those important for the cardinal gastrointestinal features of IBS will be critical. Despite the complexity of these interactions, therapeutic targeting of the gut microbiota for the management of comorbid psychiatric symptoms in IBS may be a strategy worth the effort involved.

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Tables

Table 1: Gut signatures associated with IBS, depression, anxiety, and comorbid IBS.

↑ indicates increase, ↓ indicates decrease.

Taxonomic rank	IBS	Depression	Anxiety	Comorbid IBS and Depression
Phylum	*Firmicutes: Bacteroidetes* ↑[58]	Actinobacteria↑[51] Bacteroidetes↓[51]	Firmicutes↓[51]	
Order			Enterobacterales↑ [51]	
Family	*Lactobacillaceae*↑[52] *Enterobacteriaceae*↑[52]	*Prevotellaceae*↓[51]	*Enterobacteriaceae*↑[51] *Ruminococcaceae*↓[51]	
Genus	*Bacteroides*↑[52] *Bifidobacterium*↓[52] *Faecalibacterium*↓[52] *Eubacterium*↑[58]	*Faecalibacterium*↓[51] *Sutturella*↓[51] *Coprococcus*↓[51] *Eggerthella*↑[51]	*Escherichia/Shigella*↑[51] *Subdoligranulum*↓[51] *Dialister*↓[51]	*Bacteriodes* ↑[63] *Faecalibacterium* ↑[63] *Lachnospiraceae*↑[63]

Figure Legends

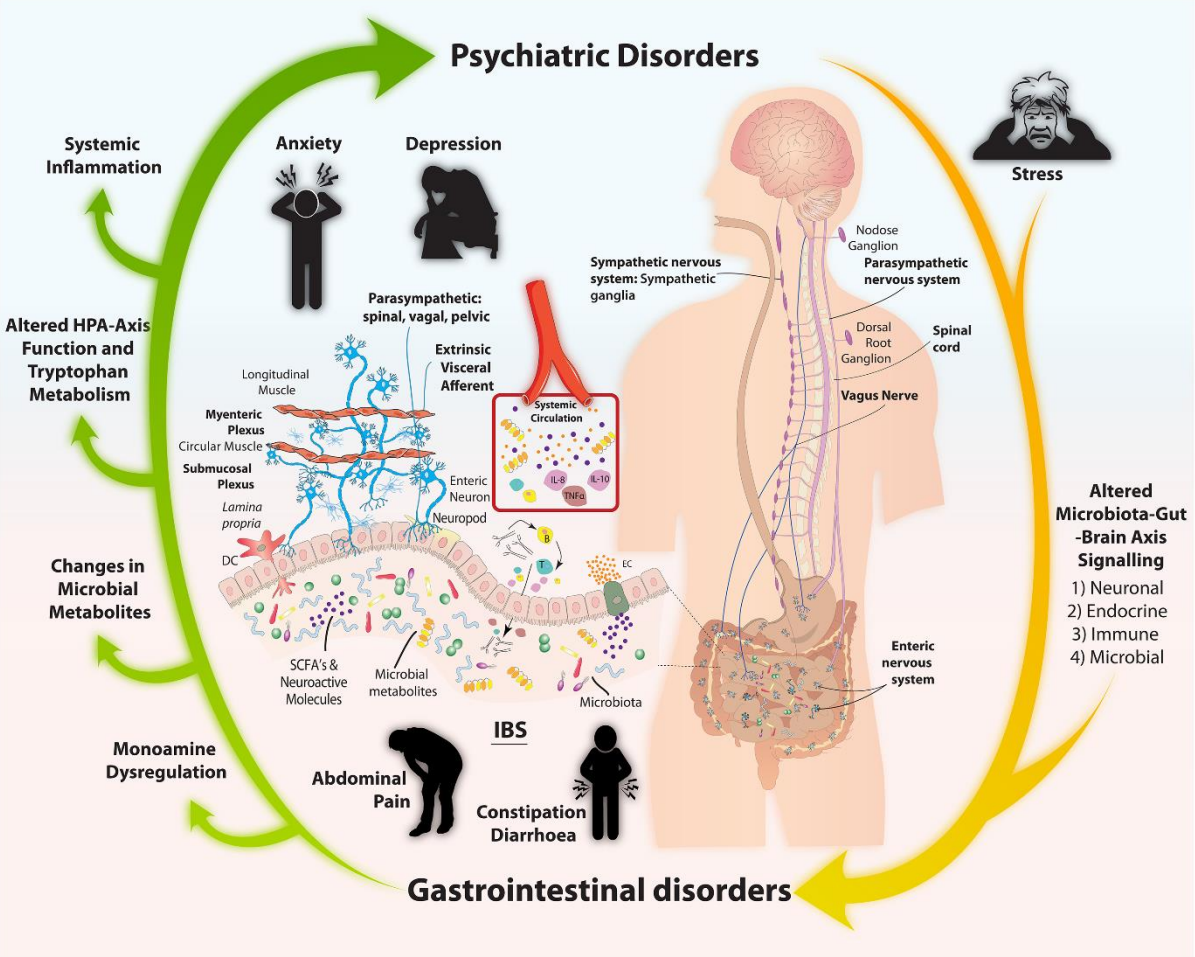
Figure 1. Summary of microbiota-gut-brain axis signalling pathways

There are a number of important routes of communication in the microbiota-gut-brain axis that may be relevant for the expression of gastrointestinal and psychiatric symptoms in IBS. It is well known that stress, a major predisposing factor for the development of both IBS and depression in later life, may also impact on gut microbiota composition and function. 1) Neuronal 2) Endocrine 3) Immune and 4) Microbial signalling pathways are also associated with specific symptom sets. Alterations in the composition and function of the microbiota have been reported in IBS, depression and anxiety. These alterations can, for example, result in dysregulation of monoamine signalling and alterations in microbial metabolites which may be related to systemic inflammation. It is also now appreciated that the prominent gastrointestinal features of IBS including constipation, diarrhoea and visceral pain may worsen the associated comorbid psychiatric symptoms such as anxiety and depression. It is still unclear if IBS with psychiatric comorbidity represents a distinct clinical entity that can be explained on the basis of converging gut-brain axis signalling pathways.

Figure 2: A microbial perspective on the intersection between IBS, depression and anxiety

There is currently a poor understanding of the nature of the clinical entity that sits at the intersection between IBS, depression and anxiety. One possibility is that a comorbid gut microbiota drives aberrant signalling along the gut-brain axis, leading to the manifestation of both gastrointestinal and behavioural symptom sets. Increased research efforts are required to understand why specific microbiota configurations lead in some cases to IBS and in others IBS with psychiatric comorbidity.

911 **Figure 1**



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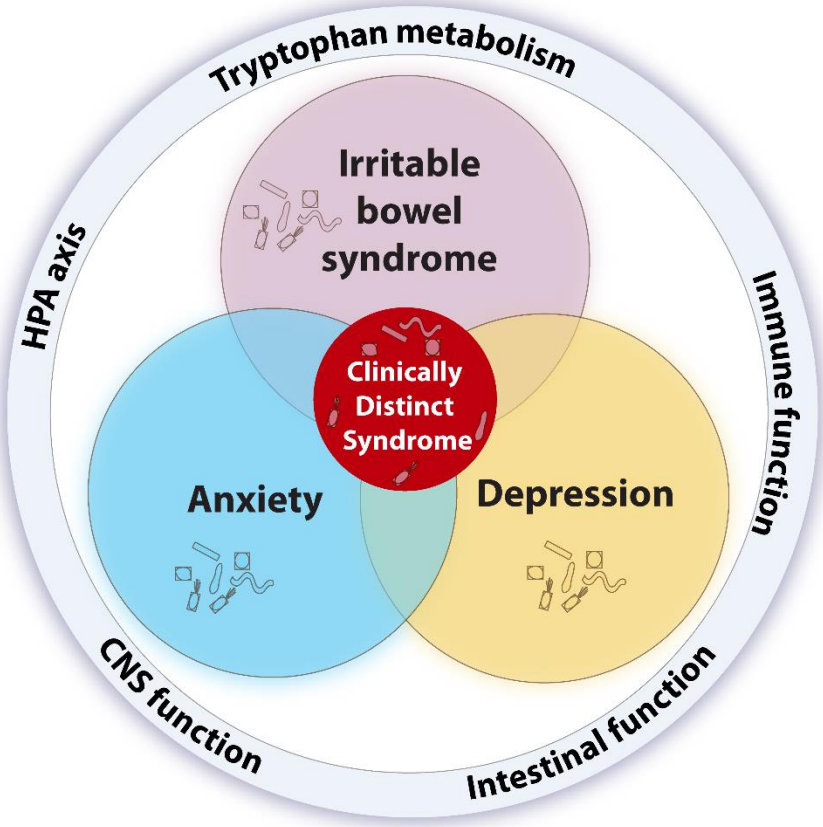
915 **Figure 2**



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