
Gut microbiome and depression: what we know and what we need to know

Abstract: Gut microbiome diversity has been strongly associated with mood-relating behaviours, including major depressive disorder (MDD). This association stems from the recently characterised bi-directional communication system between the gut and the brain, mediated by neuroimmune, neuroendocrine and sensory neural pathways. While the link between gut microbiome and depression is well supported by research, a major question needing to be addressed is the causality in the connection between the two, which will support the understanding of the role that the gut microbiota play in depression. In this article, we address this question by examining a theoretical ‘chronology’, reviewing the evidence supporting two possible sequences of events. First, we discuss that alterations in the gut microbiota populations of specific species might contribute to depression, and secondly, that depressive states might induce modification of specific gut microbiota species and eventually contribute to more severe depression. The feasibility of both sequences is supported by pre-clinical trials. For instance, research in rodents has shown an onset of depressive behaviour following faecal transplantations from patients with MDD. On the other hand, mental induction of stress and depressive behaviour in rodents resulted in reduced gut microbiota richness and diversity. Synthesis of these chronology dynamics raises important research directions to further understand the role that gut microbiota play in mood-relating behaviours, which holds substantial potential clinical outcomes for persons who experience MDD or related depressive disorders.

Keywords: anxiety; depressive disorder; gut-brain axis; gut microbiota; stress.

Introduction

Depression is the leading cause of ill health and disability worldwide, with more than 300 million people being depressed currently, an increase of more than 18% between 2005 and 2015 (WHO, 2017). It also has greater adverse effects on personal health (Moussavi et al., 2007) and higher costs of care than other chronic diseases (Langa et al., 2004), and carries a similar risk for mortality from all causes as smoking does, even when related health factors such as blood pressure, alcohol intake, cholesterol and social status are taken into account (Mykletun et al., 2009). Recent meta-analytic data indicate that people with depression have a relative risk of mortality from all causes that is 1.86 times that for non-depressed individuals and that there are 2.74 million deaths annually from depression (Walker et al., 2015). It has been estimated that failing to recognise depression and provide access to treatment costs US$1 trillion globally each year from losses to households, employers and governments (WHO, 2017). However, despite these costs, standard pharmacological and psychological treatments for depression are effective in only about 74% of cases, even when combined (Rush et al., 2006).

These data regarding prevalence, effects and treatment underscore the need to investigate models of depression that encompass a wider range of possible ‘causal’ factors than simply neurotransmitter depletion in an effort to identify more efficacious treatment approaches. Although depression is primarily a disease of the brain and effective treatment requires that neurological factors are understood (Ross et al., 2015), the brain does not exist in isolation, but is embedded within the overall physiology of the individual. In addition, depressive behaviour as defined by the standard symptomatology includes several somatic indicators, such as sleeping difficulties, weight loss/gain and psychomotor agitation/retardation as well as the more easily recognised ‘mental health’ factors of concentration difficulties, feeling sad, anhedonia and thoughts of death (APA, 2013), supporting the case for considering organic factors in depression. Therefore, investigation of a multiplicity of physiological factors and pathways that might contribute to the state of the brain during depression has the potential to provide a more comprehensive (and perhaps efficacious) basis on which
to mount treatment models that seek to describe associations between various body systems and mental states. Some of the possible physiological pathways that might contribute to changes in brain function that are associated with depression include the immune system (Dantzer, 2009), the hypothalamus-pituitary-adrenal (HPA) axis (Dantzer, 2009) and the presence of preceding illness (Katon et al., 2007). Another potentially valuable pathway that has received some recent attention is the gut-brain axis (Alper and Ceylan, 2015), which is the focus of this review.

The gut microbiota and depression

A good deal of data have established that depression is associated with altered gut microbiota composition, generally in the form of reduced richness and diversity (Kelly et al., 2016; Zheng et al., 2016). The gut microbiota of adults is dominated primarily by members of the Bacteroidetes and Firmicutes phyla, representing approximately 90% of the adult microbiota (Tremaroli and Backhed, 2012). A comparison of the gut microbiota of patients diagnosed with major depressive disorder (MDD) and healthy individuals, as well as studies regarding the gut microbiota of rodent models following stressor exposure, has revealed significant alterations in the abundance of different genera within Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria phyla. Interestingly, while there is a general consensus between the different studies, for some genera there are conflicting reports, suggesting that there may be confounding factors in some of those relationships. Figure 1 illustrates the changes that have been identified in microbial diversity between patients with MDD and healthy individuals as well as changes to mice gut microbiota following stressor exposure. Detailed information of these modifications can be found in Table 1, describing the phylogenetic hierarchy of the different genera.

Figure 1: Alterations in microbial diversity observed in depressed patients and animal models following stressor exposure. Illustration of microbial diversity shift induced by external stressors, based on data presented in Table 1. Phylogenetic structure representation is outlined in the figure, including phyla names and genera names.
Table 1: Changes in gut microbial diversity observed in depressed patients and animal models following stressor exposure.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Model organism</th>
<th>Population shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td>Actinobacteria</td>
<td>Coriobacteriales</td>
<td>Coriobacteriaceae</td>
<td>Eggerthella</td>
<td>Human</td>
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<tr>
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<td>Actinobacteria</td>
<td>Coriobacteriales</td>
<td>Coriobacteriaceae</td>
<td>Unidentified genera</td>
<td>Mice</td>
<td>Increase (Bangsgaard et al., 2012)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Deltaproteobacteria</td>
<td>Desulfovibrionales</td>
<td>Desulfovibrionaceae</td>
<td>Desulfovibrio</td>
<td>Mice</td>
<td>Increase (Aoki-Yoshida et al., 2016)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Rhodobacteriales</td>
<td>Hyphomonoaceae</td>
<td>Ponticoccus</td>
<td>Mice</td>
<td>Increase (Galley et al., 2014)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Gamm proteobacteria</td>
<td>Enterobacteriales</td>
<td>Enterobacteriaceae</td>
<td>Unidentified genera</td>
<td>Human</td>
<td>Increase (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Rikenellaceae</td>
<td>Alistipes</td>
<td>Mice, Human</td>
<td>Increase (Bangsgaard et al., 2012; Jiang et al., 2015)</td>
</tr>
<tr>
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<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Porphyromonadaceae</td>
<td>Unidentified genera</td>
<td>Human</td>
<td>Increase (Jiang et al., 2015)</td>
</tr>
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<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Porphyromonadaceae</td>
<td>Odoribacter</td>
<td>Mice</td>
<td>Increase (Bangsgaard et al., 2012)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Porphyromonadaceae</td>
<td>Parabacteroides</td>
<td>Human</td>
<td>Increase (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Bacteroidaceae</td>
<td>Unidentified genera</td>
<td>Mice</td>
<td>Decrease (Bailey et al., 2011; Galley et al., 2014)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Bacteroidaceae</td>
<td>Bacteroides</td>
<td>Human</td>
<td>Decrease (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Prevotellaceae</td>
<td>Unidentified genera</td>
<td>Human</td>
<td>Decrease (Jiang et al., 2015; Kelly et al., 2016)</td>
</tr>
<tr>
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<td>Bacteroidia</td>
<td>Bacteroidales</td>
<td>Prevotellaceae</td>
<td>Paraprevotella</td>
<td>Human</td>
<td>Increase (Kelly et al., 2016)</td>
</tr>
<tr>
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<td>Bacteroidales</td>
<td>Prevotellaceae</td>
<td>Prevotella</td>
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<td>Decrease (Jiang et al., 2015; Kelly et al., 2016)</td>
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<td>Bacteroidales</td>
<td>Lachnospiraceae</td>
<td>Unidentified genera</td>
<td>Mice</td>
<td>Increase (Aoki-Yoshida et al., 2016)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Pseudobutyribrio</td>
<td>Mice</td>
<td>Decrease (Bailey et al., 2011)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Coprococcus</td>
<td>Mice</td>
<td>Decrease (Bailey et al., 2011)</td>
</tr>
<tr>
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<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Roseburia</td>
<td>Mice</td>
<td>Increase (Bailey et al., 2011)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Dorea</td>
<td>Mice</td>
<td>Decrease (Bailey et al., 2011)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Anaerofilum</td>
<td>Human</td>
<td>Increase (Kelly et al., 2016)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Lachnospiraceae</td>
<td>Blautia</td>
<td>Human</td>
<td>Increase (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Peptostreptococcaceae</td>
<td>Clostridium</td>
<td>Mice</td>
<td>Increase (Bailey et al., 2011)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Ruminococcaceae</td>
<td>Ruminococcus</td>
<td>Human</td>
<td>Decrease (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Ruminococcaceae</td>
<td>Oscillospira</td>
<td>Mice</td>
<td>Decrease (Bharwani et al., 2016)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Clostridiaceae</td>
<td>Faecalibacterium</td>
<td>Human</td>
<td>Decrease (Jiang et al., 2015)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridia</td>
<td>Thermoaerobacterales</td>
<td>Thermoadenaerobacteraceae</td>
<td>Gelria</td>
<td>Human</td>
<td>Increase (Kelly et al., 2016)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Bacilli</td>
<td>Lactobacillales</td>
<td>Enterococcaceae</td>
<td>Enterococcus</td>
<td>Mice</td>
<td>Increase (Bharwani et al., 2016)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Bacilli</td>
<td>Lactobacillales</td>
<td>Lactobacillaceae</td>
<td>Unidentified genera</td>
<td>Mice</td>
<td>Decrease (Galley et al., 2014)</td>
</tr>
</tbody>
</table>
and whether the microbial population of the genus was increased or decreased in rodent models or patients with MDD. Tables 2 and 3 provide more information regarding the nature of the clinical (Table 2) and preclinical (Table 3) experiments used to obtain these data.

### How do gut microbiota affect mood state?

Changes in the overall gut microbiota are relevant to mood state because gut microbiota interact with the brain via neuroimmune, neuroendocrine and neural pathways. While hypothalamic communication with the gut via the HPA axis is a significant component in the gut-brain axis, communication in this axis is hypothesised to be bidirectional, with the gut able to signal back to the brain (Mayer, 2011; Collins et al., 2012; Cryan and Dinan, 2012). The best evidence currently available shows that the primary conduit for this signalling is via the nervous system, in the form of the afferent vagus nerve. The vagus nerve is important in relaying signals from the brain to the viscera, becoming more active as the parasympathetic nervous system is activated, stimulating ‘rest-and-digest’ functions. However, approximately 80% of vagus nerve fibres are afferent, relaying sensory information from the viscera, including the digestive tract, to the brain for integration and appropriate responses to maintain homeostasis (Foley and DuBois, 1937; Berthoud and Neuhuber, 2000). Detailed mechanisms facilitating communication in the gut-brain axis will be discussed in detail, as they fit with our proposed hypotheses of how this communication changes in depressive states.

Gut-brain communication may also be indirect, mediated through different metabolites. For example, gut microbiota may have an influence upon brain states by the modulation of neuroactive substances such as serotonin, noradrenalin, dopamine and glutamate and gamma-aminobutyric acid (GABA), all of which (except GABA) are excitatory in their effects upon the post-synaptic neuron (GABA is inhibitory and, with glutamate, forms a ‘balance’ process for brain synaptic activity) (Fendt et al., 2007). There is some evidence that decreased levels of serotonin (Reimold et al., 2008), noradrenalin (Delgado and Morena, 2006) and dopamine (Willner, 1983), plus malfunction of the glutamate-GABA systems (Choudary et al., 2005), are associated with depression, based upon treatment studies in which the levels of these neurotransmitters have been either increased or decreased artificially, with some accompanying changes in depressive...
symptoms being observed (Foley and DuBois, 1937). Hence, gut microbiota may potentially contribute to the levels of these neurotransmitters in the brain as well as in the gut (Mittal et al., 2017). These neurotransmitter-modulating biota might also be influenced by gut health, microbial diversity and the relative activity of these organisms (Mittal et al., 2017). Moreover, gut microbiota can alter brain functioning in an indirect manner through changes in inflammatory states and immune status (Dinan and Cryan, 2013, 2016). Thus, a focus upon the gut-brain communication pathways is of interest and relevance when considering possible ‘causal’ pathways to depression.

### Chronology of depression

One of the major questions needing to be addressed in this pathway from gut microbiota to depression is the causal-ity in the connection between the two, which will elucidate the role that gut microbiota play in depression. This may be implied by the chronology of events connecting depression and gut microbiota changes. That is, there are three possible sequences of events that might occur between the gut microbiota and depression. First, reductions in the gut microbiota populations of specific species might precede reductions of neurotransmitter levels in the brain and hence contribute to depression. Second, depressive states might induce modification of specific gut microbiota species and eventually contribute to more severe depression. Third, these changes to neurotransmitter levels in the brain and gut might occur simultaneously, and any relationship between them could be merely coincidental. Clearly, the first and second hypotheses have clinical implications for treatment of depression and/or gut microbiota, but the third does not. Therefore, this review will focus on pathways 1 and 2.

To gather experimental evidence supporting the connection between gut microbiome composition and depression, a review of the literature was undertaken. A Google Scholar search was conducted using the search terms ‘gut’, ‘brain’, ‘communication’, ‘microbiota’, ‘microbiome’ with at least one of the following words: ‘depression’, ‘anxiety’, ‘immune’, ‘neuroendocrine’.  

### Table 2: Clinical studies comparing gut microbiota of depressed and healthy subjects.

<table>
<thead>
<tr>
<th>Study</th>
<th>Definition of depression</th>
<th>Diagnostic criteria</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kelly et al., 2016</td>
<td>MDD</td>
<td>Clinical diagnosis based on DSM-IV criteria for MDD, using MINI, plus HAM-D score &gt;17</td>
<td>34 Patients with MDD</td>
</tr>
<tr>
<td>Jiang et al., 2015</td>
<td>Active MDD and responded MDD</td>
<td>Clinical diagnosis based on DSM-IV using SCID</td>
<td>46 Patients with MDD</td>
</tr>
<tr>
<td>Naseribafrouei et al., 2014</td>
<td></td>
<td>Clinical diagnosis based on ICD-10, plus ‘mild to severe depression’ using Montgomery-Asberg Depression Rating Scale (MADRS)</td>
<td>37 Depressed</td>
</tr>
</tbody>
</table>

### Table 3: Preclinical studies comparing gut microbiota of mice following stressor exposure.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of stressor</th>
<th>Behaviour analysis</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangsgaard et al., 2012</td>
<td>Grid floor-induced stress</td>
<td>Tripletest (elevated plus maze, light/dark box and open field combined) Tail suspension test Burrowing</td>
<td>n = 14 Female BALB/c mice per group</td>
</tr>
<tr>
<td>Aoki-Yoshida et al., 2016</td>
<td>Subchronic and mild social defeat stress</td>
<td>Social interaction test Elevated plus maze test None Three-chambered sociability test Aggressor interaction test</td>
<td>n = 6 Male C57BL/6JmsSlc mice per group</td>
</tr>
<tr>
<td>Galley et al., 2014</td>
<td>Social disruption (2 h exposure)</td>
<td>None</td>
<td>n = 5 Male C57BL/6 mice per group</td>
</tr>
<tr>
<td>Bharwani et al., 2016</td>
<td>Chronic social defeat</td>
<td>None</td>
<td>n = 9 Male C57BL/6 mice per group</td>
</tr>
<tr>
<td>Bailey et al., 2010</td>
<td>Prolonged restraint stress</td>
<td>None</td>
<td>Male CD-1 mice</td>
</tr>
<tr>
<td>Bailey et al., 2011</td>
<td>Social disruption (6 daily 2 h cycles of stressor exposure)</td>
<td>None</td>
<td>n = 3 for treatment, n = 8 control</td>
</tr>
</tbody>
</table>

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These search terms were to be included anywhere within the article. The first study to point out a direct connection between gut microbiota and the brain was published in 2004 (Sudo et al., 2004); therefore, our search criteria were limited to articles published between 2004 and April 2017. Considering the excellent reviews published on the topic of gut and brain bi-directional communication (e.g. Dinan and Cryan, 2013; Dinan et al., 2015), a description of those communication pathways was deemed unnecessarily repetitive here. Instead, this review focused on the scientific evidence connecting depression and the gut microbiota vis-à-vis the chronology of changes in gut microbiota leading to changes in brain state versus changes in brain state leading to changes in gut microbiota. This focus was adopted in order to address the question of causality between these two hypothetically related physiological structures and their association with depression. Two directional hypotheses were therefore generated for testing on the basis of the literature. The first of these hypotheses is that changes in brain depressive state influence gut microbiota state.

Hypothesis 1: depressive state modulates gut microbiota

In 1998, Drossman published a description of the biopsychosocial model of mental states, showing a link between mental state and gastrointestinal diseases, and suggesting an integration between psychological and physiological information when diagnosing and treating depression (Drossman, 1998). Therefore, this section of the review is focused on experimental findings relating to the role that a psychological state of depression or anxiety has upon the gut microbiome. Demonstration of these effects necessitates the use of animal models to establish a cause and effect relationship; however, this approach has two main limitations. First, findings achieved using rodent animals have limited extrapolation capacity to humans (Shilov et al., 1971; Smirnov and Lizko, 1987), although a comparison between human and mice gut microbiota composition has found the two to be quantitatively different yet qualitatively alike (Krych et al., 2013). Second, while the induction of depressive-like behaviour in animals using stressor exposure is well established (Golden et al., 2011; Hennessy et al., 2011), it is still unknown whether the behavioural changes are a cause or an effect of changes in gut microbiota diversity. Nonetheless, with the inclusions of experimental controls, we can draw a cause and effect relation between stressor exposure and gut microbiota modulation.

Physiological implications of depressive-like behaviour in animal models

One of the established experimental models used to induce depressive-like behaviour with comorbid anxiety in rodents is via surgical removal of the olfactory bulb (olfactory bulbectomy [OB]), a practise shown to alter function of the prefrontal cortex (PFC) (Harkin et al., 2003; Song and Leonard, 2005), a change also observed in humans with depression in a region where negative emotions are thought to be generated (Koenigs and Grafman, 2009). OB mice demonstrate a significant reduction in the latency to the step-down test, prolonged immobility in a tail suspension test and hyperlocomotion and reduced exploratory activity that were consistent with a profile of depressive and anxiety-like behaviour (Harkin et al., 2003; Song and Leonard, 2005). Examination of OB mice revealed elevated corticotropin-releasing hormone (CRH) expression, indicative of increased HPA axis activity, which in turn increased colonic motility (Park et al., 2013) and altered the colonic gut microbiota profile. As the PFC has projections into the hypothalamus (Koenigs and Grafman, 2009), there is likely to be a pathway activated from the PFC or the hypothalamus, increasing HPA axis activity during this stress. Surprisingly, the authors did not observe changes in cytokine expression in OB mice. It is well known that bi-directional communication exists between the HPA axis and the immune system (Otmishi et al., 2008). A number of peripheral cytokines are known to activate the HPA axis, and in turn, the effects of the immune system can be altered through the secretion of one of the major HPA hormones, cortisol (Marques-Deak et al., 2005; Otmishi et al., 2008). Small elevations in cytokines and other inflammatory markers have been observed in patients with depression, and behavioural consequences of depression were noted following cytokine administration (Raedler, 2011; Raison and Miller, 2011). Considering the fact that cytokines levels were not changed in OB mice, the authors assume that the behavioural profile of OB mice is independent of peripheral inflammatory or immunological processes. Alternatively, the authors acknowledge the fact that cytokine secretion may have been impaired due to experimental conditions. Overall, the authors (Park et al., 2013) postulate that the observed changes in gut microbiome diversity were an effect of the changes in colon physiology rather than being the cause of those physiological changes. This supports the hypothesis that depressive state may induce changes to the gut microbiota through an increase of colonic activity.

A different, non-surgical, approach used to induce depressive behaviour in mice is by applying uncontrollable
social stress. Models using this approach include the sub-chronic mild social defeat stress model (sCSDS) (Goto et al., 2014), the chronic social defeat stress model (CSDS) (Golden et al., 2011) and the social disruption model (SDR) (Bailey et al., 2011). These models are based on the ‘resident intruder’ paradigm or inter-male aggression, where mice are repeatedly subjected to bouts of social defeat by a larger and more aggressive mouse (Berton et al., 2006; Golden et al., 2011). This social defeat results in the development of depressive-like symptoms, which have been alleviated with antidepressant medication (Berton et al., 2006; Golden et al., 2011). Mice exposed to 10 days of sCSDS displayed changes in microbiota diversity as well as differences in caecal and faecal metabolites between sCSDS mice and a control group of mice that did not experience social defeat (Aoki-Yoshida et al., 2016). While the authors did not report significant changes in microbial richness between the two populations, operational taxonomy unit (OTU) analysis revealed significant increases in OTUs belonging to families Desulfovibrionaceae, Rikenellaceae and Lachnospiraceae and a decrease in OTUs belonging to genera Allobaculum and Mucispirillum (Figure 1, Table 1). Correlational analysis of caecal OTUs and caecal metabolites identified a potential relationship between the two. For example, in the family Lachnospiraceae, five different OTUs that were increased in sCSDS mice were significantly correlated with metabolites that were more abundant in these mice. It has been reported that a noted metabolite that is significantly decreased in sCSDS mice is 5-aminovaleric acid (5-AV), a microbial-produced metabolite that is involved in the modulation of the GABA metabolic pathway and is implicated as a GABAb receptor antagonist (Dhaher et al., 2014). These findings suggest that suppressed intestinal concentration of 5-AV may have a negative effect on tissue homeostasis regulated by GABA receptors, thus linking sCSDS and brain activity.

The most elevated metabolite in the caecum of sCSDS mice has been identified as cholic acid (Aoki-Yoshida et al., 2016), a principal bile acid produced by the liver; this infers that an intestinal ecosystem change is induced by sCSDS. This inference was supported by gene expression studies of the ileum that showed an increased expression of genes involved in bile acid absorption (Aoki-Yoshida et al., 2016). Transcriptomic analyses also revealed the downregulation of genes involved in immune responses such as response to other organisms, defence responses and cytokine production (Aoki-Yoshida et al., 2016). While this study did not present temporal data documenting the changes in immune system regulation in relation to changes in gut microbiota diversity, the authors postulated that the downregulation of the immune system disturbed the balance of the gut microbiota, leading to the changes observed in the microbiome profile of sCSDS mice (Aoki-Yoshida et al., 2016) (Figure 1, Table 1).

Similar observations were made by Bharwani et al. (2016), who observed an altered immunoregulatory response in CSDS mice that included an increase in dendritic cell activation and transiently elevated levels of IL-10+ CD4+ T regulatory cells. These alterations led to a general trend of reduced diversity and reduced microbial richness in CSDS mice (Figure 1, Table 1). In contrast to Aoki-Yoshida et al. (2016), Bharwani et al. (2016) identified decreased abundance of OTUs belonging to the family Lachnospiraceae in CSDS mice as well as decreased OTUs belonging to the genus Oscillospira and increased abundance in genera Gelria and Lactobacillus. In silico metabolite prediction, based on OTUs genetic composition, predicted the metabolomic profile of CSDS mice gut microbiota to exhibit lower prevalence of pathways involved in the synthesis and metabolism of neurotransmitter precursors and short-chain fatty acids (Aoki-Yoshida et al., 2016). However, further research is needed to verify this prediction. The role of the immune system in modulating gut microbiome diversity is also seen in SDR mice that display enhanced innate immune activity and increased peripheral cytokines (Bailey et al., 2011). This rise in immune activity has been shown to reduce the population of genera from the Bacteroidetes and Firmicutes phyla following only 2 h of SDR exposure (Galley et al., 2014) (Figure 1, Table 1), although it has not been established that this effect is long lasting.

There is a substantial body of studies using different stress models that predict the same paradigm of stress modulation of immunoregulation and gut microbial diversity. These include stressors induced by maternal separation (Bailey and Coe, 1999; O’Mahony et al., 2009), prolonged restraint stressors (Bailey et al., 2010) and grid floor-induced stress (Bangsgaard et al., 2012). As stress, anxiety and depression are considered to be interrelated phenomena, the microbial shift observed in these studies is also considered as relevant for this review and is outlined in Figure 1 and Table 1. In short, the microbial shift includes an increase in the population of bacteria belonging to the family Coriobacteriaceae of phyla Actinobacteria (Bangsgaard et al., 2012), an increase in the population of genera Alistipes and Odoribacter of phyla Bacteroidetes (Bangsgaard et al., 2012) and a decrease in the population of identified genera classified to the same phyla (Bailey et al., 2010). Taken together, these studies demonstrate a shift in gut microbiota in response to stress, albeit there is some variability depending on the model used and the
experimental conditions under which the phenomenon is observed.

**Analysis of gut microbiota diversity**

Figure 1 provides a visual representation of the changes observed in microbial population in response to stress. It is important to recognise the limitations of the technologies used to obtain these data, predominately using 16S rRNA sequencing, with the main limitation being the ability to classify bacteria mostly down to the genus level and not the species or strain level. It is well known that there could be major differences between even two strains of the same species, let alone two species of the same genus. For example, both bacterial strains K-12 and EDL933 belong to the species *Escherichia coli*; however, the former is a non-pathogenic, commonly used laboratory strain and the latter is a pathogen (Conway and Cohen, 2015). New technologies for metagenomics offer a full genome sequencing which may provide a better characterisation of microbial identity and metabolic activity.

**Depressive state modulates gut microbiota diversity**

While its extent is yet to be fully described, it is clear from the above that induction of stress causes a significant shift in the gut microbiota diversity. There is general agreement that this diversity shift does not include the introduction of new genera or the complete elimination of certain bacterial genera (Bangsgaard et al., 2012; Galley et al., 2014; Jiang et al., 2015; Aoki-Yoshida et al., 2016; Bharwani et al., 2016; Kelly et al., 2016). Instead, the changes are limited to increases or decreases of pre-existing microbial population. It is also important to note that the alterations in microbial diversity across the bacterial phylogenetic hierarchy are not limited to a specific genus or even a specific phylum.

Overall, the evidence reviewed here shows similar trends in microbial population shift across studies, including an increase in genera from the *Actinobacteria* and *Proteobacteria* phyla and a mixed trend in the phyla *Bacteroidetes* and *Firmicutes* across the different families in both phyla. The information displayed in Figure 1 also shows some conflicting evidence where some studies reported an increase of a specific microbial group while others reported a decrease in the same group population in response to stress. Interestingly, the observations made using rodent models showed similarity to the observations made in humans diagnosed with MDD (Naseribafrouei et al., 2014; Jiang et al., 2015; Aizawa et al., 2016; Kelly et al., 2016; Zheng et al., 2016). Table 1 displays the microbial shift characterised in the gut microbiota of patients with MDD in comparison to control individuals. While the observations are quite different at the genera level, they display the same trend at the phyla and class level.

In conclusion, the data reviewed here demonstrate that stress and depression may precede gut microbiota alterations. These studies suggest that depressive state may precede modulation of HPA axis and cortisol secretion, which alters cytokine production and immune activity. These then cause changes to the gut microbiota habitat, which in turn lead to altered microbial population (Figure 2). Further research is needed to understand the exact nature of the microbial population shift as well as the metabolic and physiological implications of this change, but it is clear that depressive brain states can influence gut microbiota states.

**Hypothesis 2: gut microbiota modulate depressive state**

This hypothesis contends that the gut microbiome is capable of affecting brain function. Primarily, this is hypothesised to occur via activation of vagal afferent fibres or by production of humoral chemicals that ultimately enter the brain. Through the production of the agents stimulating these pathways, the gut microbiome may thus affect mental health.

**Gut microbial transplantation**

Perhaps the most conclusive evidence for gut induction of depressive state would be the transplantation of microbial population from depressed subjects into healthy subjects so that the latter become depressed themselves. This finding was reported by Zheng et al. (2016) and Kelly et al. (2016), who performed faecal microbiota transplantation from depressed patients into a germ-free mouse or a microbiota-depleted rat model, respectively. Both showed behavioural changes that correlated with human depression and anxiety (as measured by maze exploration and open field tests, sucrose preference testing, etc.), and physiological features that are characteristic of depression following this procedure. Interestingly, the transplanted rats, which were treated for 12 weeks (with twice weekly ‘top-up’ doses), also showed an increase in plasma kynurenine and in kynurenine/tryptophan ratio, a change reported in the depressed donor group (although
total tryptophan was not significantly altered) as well as an increase in acetate and total short-chain fatty acids, as measured in their faeces (Kelly et al., 2016). This use of animal models and faecal transplant provides conceptual support for the hypothesis that changes in the gut microbiome can induce depression through a gut-brain communication route. As far as manipulating the microbiota as a treatment, there are currently limited data available, but faecal microbiota transplant shows promise based on its use in treating recurrent *Clostridium difficile* infection (Bakken et al., 2011; Brandt et al., 2012; Youngster et al., 2014). The central effects of such treatment remain to be investigated.

**Vagal nerve activation**

The vagus innervates a large proportion of the digestive tract and is known to be responsive to a number of endogenous chemicals in the digestive tract. Vagal afferent fibres project into the nucleus tractus solitarius, brain stem and forebrain structures, including the hypothalamus (Berthoud et al., 2011), which may allow regulation of stress responses of the HPA axis. Hormones and neurotransmitters known to activate vagal afferents include cholecystokinin, leptin (Peters et al., 2006), peptide YY$_{3-36}$ (Koda et al., 2005), glucagon-like peptide-1 (Abbott et al., 2005), ghrelin (Date et al., 2002), adrenalin (Miyashita and Williams, 2006), glutamate (Uneyama et al., 2006) and serotonin (Hillsley and Grundy, 1998). Serotonin is of particular interest because it has regulatory roles for gut motility and secretion, but is also an important neurotransmitter in affective disorders such as depression (O’Mahony et al., 2015). In depression, both human and animal subjects have been found to have altered composition of the gut microbiota in comparison with control subjects (Dinan and Cryan, 2013; Park et al., 2013; O’Mahony et al., 2015). These alterations in the composition of the gut microbiota may result in altered vagal activation, which may contribute to the symptoms seen in depression.

**Interaction between microbiota and vagal nerve function**

The importance of the microbiota secretions activating vagal afferents that then signal to the central nervous system and brain regions, such as the hypothalamus, has been shown in animal models, particularly rodent models of anxiety. Some mouse models of anxiety are induced by oral agent administration (Painsipp et al., 2011; Hassan et al., 2014), or there may be a genetic predisposition in some rodent strains (Carola et al., 2004), allowing the use of behavioural testing to assess the efficacy of subsequent treatments. The application of specific bacteria into the digestive tract can abrogate these anxiety-like behaviours, with measurable changes in enteric neuron excitability (Bercik et al., 2011) and GABA receptor expression in the brain (Bravo et al., 2011). These normalising effects are
lost following vagotomy (Bercik et al., 2011; Bravo et al., 2011). Thus, it has been hypothesised that vagal activation by the gut microbiome is necessary for regulation of normal mental health (Dinan and Cryan, 2013). It has also been suggested that pathologic microbes may modulate the same mechanism to potentiate depressive and sickness behaviours (Maes et al., 2012). Therefore, it is reasonable to hypothesise that pathogenic microbes may modulate activation of the vagal afferents, causing subsequent pathologic changes in the central nervous system, which may then propagate systemic changes such as disease symptoms (Figure 2).

Gut microbiota and humoral communication

The use of germ-free mice has indicated the probable use of humoral agents from the gut microbiota to communicate with the host (Figure 2). For example, germ-free mouse models lack a normal anxiety response if normal gut microbiota do not colonise the gastrointestinal tract early in life (Neufeld et al., 2011; Clarke et al., 2013). In this model, there is a higher hippocampal concentration of serotonin, and in males, there is a higher plasma concentration of its precursor, tryptophan (Clarke et al., 2013). Serotonin and other tryptophan metabolites are produced by a range of microbes in the digestive tract and enter the circulation (O’Mahony et al., 2015; Morris et al., 2017). Thus, it is hypothesised that these products produced by the gut microbiota act as humoral modulators of the central nervous system (Clarke et al., 2013). This is consistent with a previous report showing that serotonin activation of the dorsal raphe nucleus promotes secretion of CRH (Marcinkiewcz et al., 2016), and the higher circulating concentrations of corticosterone (the rodent equivalent of cortisol) in the germ-free mice (Neufeld et al., 2011; Clarke et al., 2013). However, the reduced anxiety response is inconsistent, and warrants exploration of serotonin receptor expression in the brains of germ-free mice, which may provide some explanation for the apparent selective sensitivity to serotonin. While the use of these animal models has some limitations when findings are applied to human physiology, they do appear to be providing important mechanistic information about how the gut microbiota affect the brain by clearly showing that products of the gut microbiota affect the central nervous system and produce measurable behaviours such as reducing anxiety-like responses (Clarke et al., 2013). This is significant as it shows that the gut microbiome is capable of inducing changes in the central nervous system and consequent behavioural responses to environmental stimuli. Therefore, by modulating the availability of neurotransmitters (or their precursors) and receptors in the brain, the gut microbiome has the potential to regulate mental health status.

As well as producing neurotransmitters, the gut microbiota also produce hormone analogues and other biologically active products. Among these biologically active products are tyrosine derivatives, such as dopamine and adrenaline (Asano et al., 2012), as well as various short-chain fatty acids (Nankova et al., 2014). While dopamine and adrenaline are capable of acting locally to affect gut functions such as motility and secretion (Asano et al., 2012), the short-chain fatty acids are capable of entering the systemic circulation if absorbed via the large intestine (Nankova et al., 2014). Once in circulation, they are distributed throughout the body and are preferentially taken up by various tissues affecting their function and the individual’s health (Koves et al., 2008). For example, altered fatty acid concentrations may contribute to insulin resistance, resulting in a chronic condition, type 2 diabetes mellitus (Koves et al., 2008). This may be one pathway by which the gut microbiota may contribute to an individual’s well-being.

Of note, short-chain fatty acids that remain in circulation, avoiding uptake and metabolism by the peripheral tissues, are capable of entering the central nervous system. Short-chain fatty acids, including propionic acid, are transported across the blood-brain barrier, directly entering the brain via a saturable transport mechanism (Conn et al., 1983). These products have been shown to modulate the behaviour in animals in ways that mimic anti-depressive and anxiolytic effects following peripheral and central (intracerebroventricular) administration (Nankova et al., 2014). Importantly, altered concentrations of these short-chain fatty acids in the brain change the expression of neuromodulatory genes, such as CREB, which have been implicated in the development of autism spectrum disorders (Nankova et al., 2014). While it has not been conclusively shown that gut-derived short chain fatty acids reach the brain, this appears to be a promising area for future research.

Synthesis of findings and directions for future research

Each of the two hypotheses posited in the Introduction to this paper has been shown to have substantial evidence to support at least the pathways that may be activated to achieve the outcomes (i.e. changes in gut microbiota,
or changes in mood state). Thus, it is most unlikely that, at this stage, either one of these two pathways can be accepted as validated over the other. This leaves both hypotheses as worthy of consideration. While this might appear to support the third position (i.e. that the gut microbiota and depression occur co-temporaneously), it is unlikely due to the evidence presented above that each can precede the other. With each of those pathways receiving some research support, coincidental occurrence of the end points of each of those pathways does not currently have a strong basis.

Alternatively, it may be that each pathway operates, but under different circumstances. That is, some particular events/stressors may occur that trigger mood changes, and those mood changes subsequently change gut microbiota. The reverse pathway might occur under different conditions, so that digestive or infection-based challenges instigate gut microbiota changes which later contribute to depressive behaviour. The role of stress _per se_ in depression needs to be controlled for in studies of these pathways to avoid a confound of ‘causal’ factors. However, at present, the nature of each of those particular sets of environmental stressors/events in humans is unknown, and represents a potential focus for future research into naturalistically occurring depression among human populations. Longitudinal data could assist in further deciphering these issues and (potentially) providing more detailed stress/events – depression/gut microbiota equations.

It is also relevant to question the value of continuing to undertake this research by way of a unitary definition of depression. That is, when the nine major diagnostic criteria for MDD as set out in the Diagnostic and Statistical Manual of Mental Disorders (5th revision) (DSM-5) are added to the extra features described on pp. 162–165 of the DSM-5, it has been noted by Ø stergaard et al. (2011) that the possible number of combinations of those criteria that fulfil a diagnosis of MDD is nearly 1500. Because (as mentioned above) the current major treatments for MDD are only effective about 74% of the time (Rush et al., 2006), the need to consider depression from a multi-faceted model of symptom clusters presents a potential research agenda. That approach has been urged upon researchers and clinicians alike for several years (Insel, 2013), and there have been some interesting reports of the nature of different ‘subtypes’ of depression (Parker et al., 2002; Parker, 2005), focussing upon ‘atypical’ depression, ‘melancholic’ depression and four subtypes based upon the clinical content of the particular symptoms present (Sharpely and Bitsika, 2014). Other models of depression subtypes might include those endophenotypes based upon genetic factors, HPA-axis responsivity, immunological factors and treatment outcomes. Matching of such depression subtypes to specific stressors/events and tracing the prevalent gut microbiota-depression/depression-gut microbiota pathway from those stressors/events by way of depression subtypes represents a potentially fruitful avenue for research.

Evolving from the points made in the preceding paragraph, although MDD is the most common form of depression used in clinical and research settings (Hasin et al., 2005), it was established two decades ago that patients with fewer than the five symptoms required for MDD suffer from a disease-related burden that is undifferentiated from patients with the full MDD diagnosis (Judd et al., 1996, 1997). Subsyndromal depression (SSD) (Judd et al., 1994) requires any two MDD criteria, whereas MDD requires at least one of the two key symptoms of depressed mood or anhedonia to be present, plus sufficient other symptoms to a total of at least five. Patients with SSD with just two of the MDD diagnostic criteria were found to have no large consistent differences in impairment compared with patients who fulfilled the complete criteria for MDD across eight domains of functioning (Judd et al., 1996); both depressive groups suffering significantly more than participants with no symptoms of MDD (Judd et al., 1998). The incidence and effects of SSD are particularly relevant in older persons who have SSD because they also have a 5.5-fold chance of developing MDD within 1 year compared to people who have none of the symptoms of MDD at all (Lyness et al., 2006), and show significantly greater levels of psychological disability, hopelessness and death ideation (Chopra et al., 2005). Other data suggest that elderly patients with SSD ‘are as ill as those with minor or major depression (in terms of) medical burden’ (Lyness et al., 2007) but it is prevalent, underdiagnosed and undertreated (Goldney et al., 2004; Vanitallie, 2005). It may be that some symptoms of MDD (enough to qualify the individuals for a diagnosis of SSD but not MDD) may be associated with one of the pathways and other symptoms are associated with the alternative pathway. That model could produce the combined result of both pathways being present in some (highly symptomatic) patients, potentially clarifying the relative roles of the two hypotheses described above.

It may also be of value to investigate the interaction between different treatment regimes and the gut microbiota-depression/depression-gut microbiota pathways. That is, does medication or psychotherapy (or any other therapy such as exercise or transcranial stimulation, etc.) work more effectively when gut microbiota changes precede depression or when depression precedes gut
microbiota changes? It may be that, like most patients with MDD, a series or combination of treatments is most effective, but the question of which order is most efficacious for which of these two pathways remains unanswered.

In conclusion, the gut microbiota affect several aspects of human functioning, and it is not unexpected that mood and mood-related behaviour should be included among those aspects. If, as has been argued for some time, depression is characterised by an adaptive behavioural withdrawal from a noxious and uncontrollable stimulus (Ferster, 1973; Kanter et al., 2008), and that depressive behaviour can bring some advantage to the depressed person (Gilbert, 2005), then the involvement of such a fundamental component of the individual’s physiology as gut microbiota in this process is not to be unexpected. Notwithstanding that logical argument, the tracing of the pathways by which the gut microbiota are involved in mood-related behaviours remains a major challenge for researchers, and holds substantial potential clinical outcomes for persons who experience MDD or related depressive disorders.

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References


