The Gut-Brain-Microbiota Axis: A New Frontier in Human Health

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INTRODUCTION

Gut-Brain-Microbiota Axis

The gut-brain axis refers to the bidirectional interrelationship between the central and enteric nervous systems in humans and other animals. Only very recently has this relationship gained traction in the research community. Both the brain and the gut can mutually influence the functioning of one another. This gut-brain connection is mediated by the 100 trillion microorganisms of between 500-1000 species that inhabit the human intestine (Marques et al., 2014). The human hosts the microbiota in a commensal relationship, and the human intestine is the habitat for the microorganisms (Collins and Bercik, 2009). These microorganisms themselves play important roles in signalling and communication along the axis. Collins and Bercik therefore include the microbiota in the gut-brain axis, renaming it the gut-brain-microbiota axis (2009).

This paper will examine gut-brain-microbiota research into four human pathologies: irritable bowel syndrome, depression, generalized anxiety, and autism. I will begin by establishing a context: I will define the functions of the microbiota, the connections among members of the axis, and research methods and seminal findings in the field of the gut-brain-microbiota axis. Then I will review research into irritable bowel syndrome, depression, generalized anxiety and autism. After my review, I will discuss future directions, research priorities, and treatment options arising from this revolutionary field of research.

The intestinal microbiota participate in several physiological processes, including immune functioning, signalling, metabolism, nutrient generation, and neurotransmitter

generation. Microbiota participate in metabolic and biosynthetic pathways, producing energy molecules, nutrients and other substances for the host (Mayer et al., 2015). Importantly, some microbiota ferment indigestible fibre into short-chain fatty acids which can be metabolized and used for energy (Cani et al., 2013). Imbalances among the fermenters have been implicated in pathology (Jeffery et al., 2012; Adams et al., 2011). Microbiota also produce neurotransmitters. Several genera and species of commensal microbes have been characterized in terms of the neurochemicals they produce. *Bifidobacteria* and *Lactobacilius* synthesize GABA from monosodium glutamate; *Candida*, *Streptococcus*, and *Escechera* produce serotonin; *E. coli*, *Bacilius*, and *Saccharomyces* produce norepinephrine; and *Serratia* and *Bacilius* produce dopamine (Evrensel and Ceylan, 2015). Normal GI tract colonization by microbiota is required for normal concentrations of catecholamines in the intestinal lumen (Asano et al., 2012), so imbalances in microbes may lead to imbalances in neurochemicals, potentially leading to pathology. Metabolites and signalling molecules produced by the microbiota can communicate with the host via receptors in the intestinal epithelium (Mayer et al., 2015). And relatedly, the host can modulate the activity of the microbiota by way of various signalling molecules, including "catecholamines, serotonin, dynorphin, GABA, and cytokines" (Mayer et al., 2015). Additionally, and importantly, the microbiota are required for normal immune system development (Foster and Neufeld, 2013).

While relatively little is known about the precise nature of the connections between the gut, brain, and microbiota, at least one mechanism has been identified. The vagus nerve is essential for gut-brain communication. Bravo et al. found a reduction in anxious behaviour upon treatment with Lactobacillius rhamnosus (a probiotic strain). This effect was completely blocked

in vagotomized mice, indicating that the vagus nerve is a major pathway connecting the microbiome and behaviour (2011). It is now accepted that physiological changes in the intestine are transmitted to the brain through the vagus nerve, and vice versa (Evrensel and Ceylan, 2015).

Methods and Models

Murine models are widely used to study the gut-brain axis. There are several methods commonly used in rodent gut-brain research: the specific-pathogen-free (SPF) vs. germ-free (GF) paradigm, cecal/fecal transplant, and administration of antibiotics or probiotics. The specific pathogen-free (SPF) vs. germ-free GF model compares normally-reared rodents with colonized GI tracts free of pathogens (specific-pathogen-free) to rodents born surgically and housed in completely microbe-free conditions such that they never develop an intestinal microbiota (germ-free). This method has been used to reveal the importance of having a microbiota, but is limited in its predictive power because it compares only two groups. Cecal and fecal transplant involve placing either the contents of the cecum or a solution of fecal matter from one animal into another (Bercik et al., 2011). It has been used to transfer behavioural (Bercik et al., 2011) and physical (Moran, 2014) phenotype between rodents, and to treat *C. difficile* infection in humans (Brandt et al., 2012). Antibiotics can also be used to disturb the microbiota (Bercik et al., 2011). It is important to examine the effects of antibiotic use on the axis, such as decreased microbiome diversity (Marques et al., 2014), since antibiotics are so commonly administered in human health contexts. Probiotics can be used to support the microbiota. The title "probiotic" refers to live organisms that, in sufficient quantities, bestow health benefits on the host (Collins and Bercik, 2009). Researchers have used probiotics in murine and human research.

Recent advances in molecular genetics, such as 16s rRNA pyrosequencing, have made identification of intestinal signatures possible. 16s rRNA sequencing characterizes and identifies prokaryotes by sequencing the 16s region of their rRNA (Janda and Abbott, 2007). The 16s region is a good means of identifying species because it is evolutionarily conserved, and mutations in this region are thought to effectively indicate measures of evolutionary distance (Janda and Abbott, 2007). The ability to characterize the microbiome with relative methodological ease has enabled research into intestinal signatures of pathology, and has enabled important research in the field of the gut-brain connection.

Human Health

Gut-brain research shows great promise for human health. Dysfunction of the gut-brain axis is implicated in a wide range of puzzling human pathologies including functional gastrointestinal disorders, psychiatric conditions, autoimmune disorders, neurodevelopmental disorders, and obesity. The following section will review current gut-brain research into irritable bowel syndrome, depression, generalized anxiety, and autism. Afterward, I will make recommendations for research priorities and discuss future directions.

FUNCTIONAL GASTROINTESTINAL DISORDERS

Irritable Bowel Syndrome

Perhaps the most obvious link between human health and the gut-brain axis is in functional gastrointestinal disorders (FGIDs). Irritable bowel syndrome (IBS) and inflammatory bowel disorder (IBD) are two common FGIDs. Here I will focus on irritable bowel syndrome as

it has been studied more. IBS is an idiopathic functional gastrointestinal disorder with symptoms of abdominal pain and changes in bowel habit. It affects a sizeable 10-15% of the general population (Grenham et al., 2011). Some evidence has accrued in support of a role of the gut-brain-microbiota axis in the complex etiology and treatment of IBS. I will first discuss psychiatric evidence and then microbiome-related evidence. I will then review attempts to characterize the microbiome of IBS patients and assess the state of research into this condition.

Both IBS and IBD are known to have extensive psychiatric comorbidity (Whitehead et al., 2002; Walker et al., 2008). Fully 90% of IBS patients meet criteria for a psychiatric disorder such as anxiety and depression. Additionally, a significant number of anxiety patients also have IBS (Gros et al., 2009). IBS responds to psychotropic medication: tricyclic antidepressants, SSRIs, SNRIs, and atypical antipsychotics have all been shown to improve symptoms (Dekel, Drossman, and Sperber, 2013). Cognitive behavioural therapy has also been found to improve symptoms of IBS, further hinting at the importance of brain to gut communication (Boyce et al., 2000). Therefore, a large body of psychiatric evidence implies that the gut-brain connection plays a role in IBS.

Grenham et al. describe "postinfectious IBS", or the development of irritable bowel syndrome following bacterial gastroenteritis, i.e. GI tract infection with pathogenic bacteria (2011). Gastroenteritis perturbs the composition of the gut flora and may permanently alter its composition afterward. Marshall et al. (2006) investigated a cohort of individuals infected with gastroenteritis as a result of the Walkerton outbreak in 2000. They found that 36% of this cohort met criteria for IBS 2 years post-infection compared to 10% of controls (2006). Infection is also

used in some murine models of IBS (Moloney et al., 2016). This evidence indicates that IBS is linked to the microbiota.

IBS is not easily treated. Treatments include pharmacological and lifestyle therapies. Patients may be advised to change their diet and exercise programme, and may be prescribed antispasmodics to improve symptoms, as well as psychiatric medication and/or CBT as described above (Nikfar et al., 2008). Meta-analysis of 8 randomized controlled trials revealed that administration of probiotics (live beneficial microorganisms) is an effective treatment for IBS (Nikfar et al., 2008). This further implicated the microbiota in the etiology of IBS. Interestingly, the studies analyzed here used different probiotic strains but found similar effects, hinting that "probiotics" are less about particular strains and more about exerting a beneficial influence on the gut microbiota in general.

There has been some research into intestinal signatures in IBS patients, but no clear theory has emerged yet. It is generally noted that IBS patients have lower than normal microbiota diversity (Jeffery et al., 2012). Interestingly, lower diversity is also found after antibiotic usage, but antibiotics are sometimes recommended for IBS treatment to eliminate pathogens. This hints at different subtypes of IBS. Relatedly, Jeffrey et al. found two distinct clusters of IBS microbiota signatures upon 16S rRNA sequencing and subsequent BLAST search (2012). One group showed normal flora and were essentially indistinguishable from controls, and another group showed a marked increase in the ratio of Firmicutes to Bacteroidetes phyla. The elevated ratio was caused by both elevated Firmicutes and decreased Bacteroidetes (2012). Firmicutes are fermenting bacteria which product short-chain fatty acids from carbohydrates. Since these are

elevated, we would expect this group to have higher levels of fermentation in the gut (2012). In this study, the normal-like group was associated with a higher incidence of comorbid depression, while the high F:B ratio group had a prevalence of depression comparable to that of the general population. The authors suggest that this finding may lead to a theory of two subtypes of IBS: a psychological subtype and a microbiotal subtype (2012).

Because of the role of the microbiota in the etiology of IBS, the microbiota are a potential future therapeutic target: treatments could aim to modulate the microbiome in a beneficial way through probiotics, antibiotics, and dietary interventions as appropriate. Additionally, the connection between psychiatric illness and IBS indicates that therapies that improve IBS may improve the comorbid conditions and vice-versa. Serotonergic dysfunction is another potential point of connection between these conditions. Since 95% of the serotonin in the body is found in the intestines, it may be relevant to the pathology of both gastrointestinal disorders and psychiatric conditions (Moloney et al., 2016). It will be important to consider these in tandem: next I will discuss two common psychiatric conditions.

PSYCHIATRIC DISORDERS

Overview

It is becoming clear that there is a strong connection between psychiatric illness and the gut-brain axis. Dysfunction of the gut-brain axis has been associated with depression, anxiety, and schizophrenia (Luna and Foster, 2015; Nemani et al., 2015). Additionally, it is well-established that psychiatric conditions are highly comorbid with functional gastrointestinal disorders as outlined in the previous section. As well, it is thought that stress plays a role in both

gut-brain-microbiota dysfunction and psychiatric illness. In this section I will consider gut-brain research into two of the most common psychiatric illnesses: depression and generalized anxiety.

Depression

Major depressive disorder (MDD) is a mental illness characterized by feelings of hopelessness, lack of motivation, sadness, and loss of interest in activities (American Psychological Association, 2013). It occurs in 5% of the population at any given time, and its lifetime prevalence is approximately 17% (Blazer et al., 1994). It is usually treated with a combination of cognitive behavioural therapy, other talk therapy, and medication, but these have only modest success rates. Research into the gut-brain connection may prove fruitful in identifying risk factors and treatment targets for depression. I will begin by outlining preclinical research and then report the small amount of clinical human data available.

Stress is known to influence the development of depression (Foster and Neufeld, 2013). Sudo et al. first demonstrated the link between the microbiota and the HPA axis in their now-classic 2004 article. The HPA axis response to stress was elevated significantly in germ-free mice compared to specific-pathogen-free controls, indicating that colonization is important in the normalization or calibration of the stress response. Sudo and colleagues then associated these germ-free mice with either commensal *Bifidobacterium infantis* or enteropathogenic *Escherichia coli* and found that the HPA response normalized for the former and was exaggerated further for the latter. Importantly, fecal matter transplant from SPF mice to GF mice reversed the elevated HPA response when given at an early, but not at a late stage of development (2004). This indicates that the stress response may be plastic for only a certain stage of development, and that

critical periods of heightened environmental sensitivity may therefore be important in stress-related illnesses including depression.

The maternal separation model is a validated murine model of depression. Rats who have been separated from their mothers also have been shown to have altered microbial profiles, including decreased levels of *Lactobacilius* strains (Evrensel and Ceylan, 2015). Desbonnet et al. investigated the use of probiotic therapy in the maternal separation model of depression in mice (2010). They treated maternally separated mice with either *Bifidobacterium infantis* (a probiotic strain) or citalopram (an SSRI). Mice then underwent the forced swim test. While *B. infantis* did not perform quite as well as the antidepressant, it did cause a significant improvement in depressive behaviour. The authors conclude that *B. infantis* could be helpful in the treatment of depression (2010). The connection between maternal separation and depression is interesting because the mother affects the development of the flora so much, colonizing the gut with the bacteria in the birth canal and with breastmilk (Adlerberth, 2009). It is possible that HPA axis hyperactivation and depression could be an adaptive response indicating a lack of maternal bonding, so it would be beneficial to validate these tests using different murine models of depression. As I will discuss later, it is important to characterize causation correctly.

Park et al. used an olfactory bulbectomy murine model of depression to investigate changes in the microbial profile associated with depression (2013). The research group performed either olfactory bulbectomy or sham surgery on mice. They then performed behavioural testing for depression and anxiety on the mice: the tail suspension test, the step down test, and the open field test. They found that olfactory bulbectomized rats showed significantly

more depressed and anxious behaviour than controls. They then analyzed the microbial profile of the mice using 16s rRNA sequencing. They found a similar value of ~60% for Pearson's correlation coefficient within each experimental and control group, but only 49.1% between the groups. This indicated that depressed mice had altered intestinal signatures when compared to controls. Additionally, the researchers replicated these findings in a CRH administration model of depression (2013). Although this is association evidence, it reveals a correlation between depression and the microbiota which is both in need of, and worthy of further investigation.

There have been some studies on the gut-brain connection in depression in humans. Like native bacteria, probiotics produce neurotransmitters such as 5HT, GABA, and ACh and can therefore be used to modulate neurotransmission in a psychologically beneficial way (Dinan, Stanton, and Cryan 2013). They may also modulate the native flora and exert their effect that way. Dinan, Stanton, and Cryan have coined the term "psychobiotic" to refer to live microorganisms producing a mental health benefit (2013). Messaoudi et al. performed a randomized controlled trial in healthy subjects using two preclinically validated probiotics: *Lactobacillus helveticus* and *Bifidobacterium longum*. They tested participants on several measures of stress, anxiety, and depression as well as measuring their urinary free cortisol both before and after 30 days of probiotic or placebo treatment. The probiotic-treated group improved on the Hospital Anxiety and Depression Scale (HADS) test, which measures anxiety and depression, the Hopkins Symptom Checklist (HSCL-90), which measures many psychiatric symptoms, and on measures of urinary free cortisol, which indicates levels of stress (2010). This evidence suggests that the microbiota can influence depression. I will next discuss anxiety disorders.

Anxiety Disorders

Anxiety is a normal and adaptive behaviour seen in many organisms when faced with a potentially dangerous situation. It becomes pathological when the anxious state is expressed when no danger is evident. Generalized Anxiety Disorder is the most common anxiety disorder, affecting 2-5% of the general population, and up to 10% of females over age 35 (Wittchen, 2002). Interventions such as cognitive behavioural therapy, mindfulness based therapy, and pharmaceutical therapy with SSRIs or benzodiazepines are commonly used to treat the illness, and boast a modest success rate. Nonetheless, anxiety remains a difficult mental health problem. Preclinical gut-brain research has revealed potentially illuminating information about the etiology of anxiety disorders.

A study by Neufeld et al. indicated that the microbiota may mediate an anxiety response critical period (2011a). Neufeld et al. investigated the effects of germ-free status on plasticity and behaviour. Germ-free mice were found to show less anxious behaviour in the elevated plus maze compared to specific-pathogen-free mice, even when controlling for general locomotor activity. They also found increased BDNF expression in the dentate gyrus and decreased NMDA receptor 2B expression in germ-free animals (2011a). NMDA-r2B is essential to plasticity in the amygdala, so the authors infer that impaired fear learning could explain their findings (2011). Additionally, BDNF levels are decreased in response to stress, and higher BDNF is associated with lower anxiety, so the finding of increased BDNF in addition to increased anxiety is consistent with established research (2011). Interestingly, these effects were not reversible upon later recolonization (Neufeld et al., 2011b), indicating that the microbiota may mediate an

anxiety response critical period. Although these findings are limited because they use the germ-free paradigm, they indicate that microbiota modulation can result in changes in expression of plasticity-related genes, and that a critical period may be relevant to the development of a normal anxiety response. This research also relates to the research by Sudo et al. (2004) on the effect of germ-free status on the HPA axis, stress, and depression.

Lyte et al. hypothesize that pathogenic microorganisms may directly influence the development of anxious behaviour (1998). Mice treated with sub-infectious levels of *Campylobacter jejuni* (a strain of pathogenic bacteria) showed increased anxiety-like behaviours despite having no immune response to the pathogen, and no evidence of infection in the blood (Lyte et al., 1998). Goehler et al. propose that the anxious signals in response to subclinical infection are transmitted by viscerosensory nerves from the gut to the brain (2007). They detected c-Fos expression in vagal sensory neurons increasing from 5h-8h post-exposure to the pathogen, indicating their activation. Vagal sensory neurons express a number of potentially relevant receptors through which they could detect pathogenic microbes, including prostaglandin, histamine, cytokine, and tumour necrosis factor receptors. Goehler et al. conclude that vagal sensory neurons are a likely mechanism for the detection of pathogenic bacteria, and may explain how pathogens can induce anxious behaviour (2007). The researchers also investigated patterns of brain activation following *C. jejuni* infection, and found, among other areas, activation of the central nucleus of the amygdala, the paraventricular hypothalamus, and the bed nucleus of the stria terminalis, all of which are associated with integration of fear, stress, and anxiety with endocrine and autonomic processes (2007). Taken together, this line of research points at an important potential risk factor for development of anxiety: acquisition of sub-

infectious levels of pathogens. It is worth investigating whether elimination of the pathogen could eliminate the behavioural response. Researchers should compare the numbers and kinds of pathogenic bacteria in the gut flora of human anxiety disorder patients and controls.

It is often thought that anxiety is an exclusively "top-down" process: this is the premise of CBT, which attempts to change cognitions which supposedly mediate the experience of anxiety. Yet gut-brain research indicates that this is not the whole story: anxious behaviour seems to be influenced by "bottom-up" processes, such as subclinical infection (Lyte et al., 1998). These bottom up processes are generally inflammation- and viscerosensory-driven (Goehler et al., 2007). Inflammation of the GI tract may account for the comorbidity discussed previously between FGIDs and psychiatric illness. It is essential to account for the bidirectional nature of the gut-brain connection when discussing illnesses resulting from its pathology.

NEURODEVELOPMENTAL DISORDERS

Autism Spectrum Disorder

Autism is a neurodevelopmental disorder which can range in severity from slight to extreme. It is therefore known as "Autism Spectrum Disorder" (ASD), because patients fall somewhere within a spectrum. Symptoms include behavioural disturbances, impaired speech, social impairments, and repetitive movements (American Psychological Association, 2013). It appears that the prevalence of ASD has been increasing over the past 20 years. It is therefore thought that autism is a GxE interaction, where the presence of certain genes increases the risk but the expression is dependent on some environmental factor. Just what the relevant environmental factors are is still a matter of contention. Since autism is a neurodevelopmental

disorder it is important to consider the findings in other areas of gut-brain research that suggest microbiota-mediated critical periods for neurological development. A developmental critical period could be important in ASD. If this was the case, it would be especially important to recognize, prevent, and treat autism before the critical period is over.

It is well-known that autism and gastrointestinal disturbance are highly comorbid. So common is this comorbidity that gastrointestinal troubles are considered to be symptoms of autism. Importantly, the severity of gastrointestinal symptoms correlates with the severity of the autism (Adams et al., 2011). These GI symptoms may worsen other symptoms by distressing the patient, or may reflect a common cause. ASD children are also known to have a significantly higher history of antibiotic use compared to normal children (Adams et al., 2011). This hints at a role for the microbiota in the etiology of autism. With the rise of the interest in the gut-brain axis, autism researchers have begun to explore the possibility of a role for the gut-brain connection in the explanation of autism.

Researchers have developed mouse models of autism, generally involving prenatal insult. The VPA (vaplroic acid) model of autism is commonly used. It consists of treatment of pregnant dams with VPA at gestational day 11. Resulting offspring show increased autism-like behaviour on social behavioural tests, such as increased avoidance of unknown conspecifics (de Theije et al., 2014). In their 2014 study, de Theije et al. revealed that VPA exposure in utero caused both the expected autism-like phenotype, and increased intestinal inflammation, as measured by activation of astroglia and microgila (markers of GI neuroinflammation). Additionally, increased inflammation was seen in the hippocampus, but not in the amygdala or prefrontal cortex.

Intestinal and brain inflammation could be an important clue in the role of the gut-brain connection in autism, but it is important to determine the nature of the causality. Is inflammation a symptom of a brain disturbance, a cause of it, or is a common cause influencing both inflammation and ASD behavioural deficits?

Perhaps the most compelling evidence for the gut-brain connection in autism is in human research. In 2005, Parracho et al. revealed that *Clostridium* bacteria are of particular interest in ASD. They compared the gut flora of ASD subjects with that of healthy controls and healthy siblings. ASD participants had higher levels of *Clostridium* clusters I and II compared with controls, and the composition of species within the *Clostridium* genus varied between the two groups, with some species found exclusively in ASD participants and some exclusively in healthy participants (2005). These differences in the diversity within the *Clostridium* genus could be an important clue for the etiology of ASD. Adams et al (2011) found lower levels of short-chain fatty acids in the stools of ASD participants, indicating lower activity of fermenters. Another study compared the gut microbiota of ASD and healthy children, and found lower diversity within the ASD group as well as lower levels of fermenters *Prevotella*, *Coprococcus*, and *Veillonellaceae* (Kang et al., 2013). These changes were not associated with gender, age, or diet: only to ASD status. The authors were also able to distinguish autistic children from non-autistic children along a new measure of the ratio of members of the *Prevotella* genus to members of the *Bacteroides* genus (2013). It is clear that ASD shows a strong correlation with gut dysbiosis.

Administration of the antibiotic vancomycin temporarily improves symptoms of ASD, which reliably recur after cessation of treatment. Finegold argues that this is due to regrowth of toxin-producing *Clostridium* species from spores, which are unaffected by the antibiotic (2008). It is known that *Clostridium tetani* produces a neurotoxin responsible for the illness known as tetanus (Hatheway, 1990). This neurotoxin interferes with neurotransmitter vesicle release by proteolytic cleavage of synaptobrevin (Bolte, 1998). In the spinal cord it inhibits GABA release onto motorneurons, resulting in the excessive motor activity known as tetanus (Hatheway, 1990). Bolte suggests that tetanus neurotoxin could travel from the intestines to the brain via the vagus nerve, inhibiting neurotransmitter release there instead. She argues that this could account for the behavioural symptoms of ASD (1998). Finegold admits that *C. tetani* has not been recovered from the guts of ASD patients but argues that the principle could hold for other neurotoxic species of *Clostridium* (2008). It is established that many species of *Clostridium* produce neurotoxins (Hatheway, 1990), so this is plausible and worthy of investigation.

DISCUSSION

It is striking that the disorders associated with the gut-brain-microbiota connection are also the ones the medical community understands the least: functional gastrointestinal disorders, psychiatric illnesses, and neurodevelopmental disorders; other poorly-understood conditions which I did not discuss such as obesity and autoimmune disease are also associated with the gut-brain-microbiota axis. In my discussion I will first outline commonalities revealed by my research, then outline future directions including research priorities, and finally discuss treatment options.

Commonalities

A shared difficulty in understanding the disorders discussed here is their heterogenous character: the diseases manifest so differently from person to person that it is unclear whether it is the same disease. Many of the studies reviewed here suggest that subtyping a particular illness could address this problem. Parracho et al. found inconsistent results upon administration of probiotics to a group of autistic children, and they suggest attempting to separate them into subgroups (2010). Additionally, Jeffrey et al. suggested that there could be at least two types of IBS, a psychological and a microbiotal subtype (2012). Perhaps the gut-brain connection is the missing information that will contribute to a more accurate taxonomy of diseases. In addition, some risk factors are shared among pathologies: gastroenteritic infection, antibiotic overuse, and psychological stress are all risk factors for multiple illnesses. When the etiologies are better understood, disorders with shared aspects of causation may be grouped together and treated in the same way. For example, IBS and psychiatric disorders are both linked to gastroenteritic infection, and are comorbid, so they benefit from the same treatments, as has already been shown (Dekel, Drossman and Sperber, 2013). Gut-brain information could fundamentally change the taxonomy, treatment, and prevention of disease.

Future Directions

Gut-brain research is in its infancy, and the greater number of my citations are from this decade. While pioneering work is exciting, it is important to note how much work remains to be done. In each of the areas of human health I studied, I found a relative dearth of clinical trials. Mayer et al. argue that gut-brain-microbiota research is still largely in a preclinical state, and warn against premature conclusion-making (2015). Regardless, it is important to begin plotting

out the future of gut-brain research so that it can improve human health as soon as possible. Based on this review, the most important research priorities are as follows: 1) Improving scientific understanding of the nature of causality within the gut-brain-microbiota axis; 2) Determining microbiotal markers of specific disease states and markers of general dysbiosis; 3) Developing effective preventative and treatment measures for human gut-brain-related illness.

Priority 1, improving scientific understanding of the nature of causality within the gut-brain-microbiota axis, is especially important because the gut-brain-microbiota axis involves an incredible number of variables with varying degrees of influence. The axis continues to grow in its complexity as we study it. For example, it has long been known that the gut and the brain can mutually affect one another, but only relatively recently have we become aware that the microbiota can affect the gut and the brain, and that the gut and the brain can affect the microbiota. We are aware of 700-1000 species that colonize the adult human gut. It is therefore highly plausible that interactions among species could produce emergent effects; for example it is known that the ratio of Firmicutes to Bacteroidetes is relevant to disease states (Jeffrey et al., 2012) and this depends on both Firmicutes and Bacteroidetes populations. What is more, the microbiota secrete various signalling substances and possess many receptors that respond to host signalling molecules, further complicating the matter. Finally, the microbiome is an ecosystem dependent on host nutrition, and therefore the diet is yet another highly complex variable affecting the system. Many of the studies I have discussed have been coarse and correlational. As research progresses, it will be important to unravel this causal convolution, at least to the point where it will lead to therapeutic potential.

Priority 2, determining microbiotal markers of specific disease states and markers of general dysbiosis, will enable effective diagnosis and targeted treatment of gut-brain-microbiota related health concerns. This is beginning to be addressed. Markers of pathology have been identified in IBS, anxiety, and autism. However, it remains unclear how specific intestinal signatures can get. It would be ideal if scientists could generate a model which could predict disease states from microbiome sequencing and vice-versa. This would be a powerful diagnostic tool, and is becoming practically possible with the development of machine learning algorithms. It will be theoretically difficult because it will need to involve multivariate statistics for the causal reasons described previously. It will also be important to identify not only markers of disease, but markers of health and wellbeing, such as high diversity among microbiota (Adlerberth, 2009), such that health and disease states can effectively be compared.

Priority 3, developing effective preventative and treatment measures for human gut-brain-related illness, depends upon the first two priorities. Causal clarity will reveal the causes and contributions to illness, and therefore therapeutic potential. Diagnostic measures such as high resolution microbiome sequencing are required for disease prevention. Because gut-brain research has so far largely been about development and the etiology of illness, it is likely that therapies will take a more preventative approach to the illnesses. While treating ill people is important, it is equally as important to prevent people from becoming ill where possible. This is a shortfall in the field of medicine which must be addressed in coming years. A preventative approach involves risk management and preventative testing. Risks for gut-brain illness start mounting right from birth: babies born by Cesearean section develop different flora from those delivered vaginally, because vaginal birth allows entry of microflora in the vagina into the mouth

of the baby (Adlerberth, 2009). It is known that Cesearean children are at a higher risk for health problems such as allergies (Adlerberth, 2009). The first bacteria are of extreme importance because they are the colonizers that exert the most influence on the composition of the flora. Additionally, breast-fed babies develop healthier gut flora and immune systems than those fed formula, because breast milk contains the mother's microflora. Those fed formula are at increased risk of overcolonization by *Clostridia* (Adlerberth, 2009). Further risks include antibiotic use and infection by pathogens. Since so many of these risks occur early in life, it makes sense that the critical period has emerged as so important. Developmental gut-brain risks may be especially important in autism, as mentioned previously. As pyrosequencing becomes cheaper and easier to perform, and as we gain an idea of the features of a robust and functional microbiome, it is plausible that microbiome sequencing could become a routine test, alongside the commonplace blood and urine tests at the doctor's office. When patients display markers of pathology, they could be treated accordingly.

Treatments

Several promising gut-brain axis treatments have emerged in my research. I believe that microbiota modulation by means of probiotics, antibiotics, fecal matter transplant, and diet will become treatment targets for illnesses in which dysbiosis is present. Probiotics have effectively treated depression (Messaoudi et al., 2010) and IBS (Nikfar et al., 2008) in humans, and could potentially form part of a holistic approach to the treatment of both physical and mental illness. Dinan, Stanton and Cryan predict that "psychobiotics" will revolutionize the treatment of mental illness (2013). Additionally, the work by Goehler et al. (2007) and Lyte et al. (1998) has revealed the importance of considering the "bottom-up" contributors to the etiology of mental illness.

Elimination of pathological microorganisms could improve or eliminate the illness. Finally, and perhaps most promisingly, gut-brain disorders may be treated by fecal matter transplant, which boasts impressive cure rates for *C. difficile* infection (Brandt et al., 2012). It is thought that the healthy bacteria from the donor outcompete any bad bacteria and restore balance within the ecosystem, and researchers are hopeful that this principle can apply to other forms of dysbiosis (Vrieze et al., 2013). Fecal transplant has already been shown to improve autoimmune disease (2013), so it is plausible that it could restore health in other gut-brain-related disorders discussed in this article.

I think that the diet in particular will emerge as an important therapeutic target for microbiota-related disorders because it is readily modifiable: it can provide or restrict substrates for microflora metabolism and can therefore be tailored to support certain kinds of flora and crowd out others. While researchers are not yet making dietary recommendations for specific conditions, preliminary research on the role of the diet in the microbiome is promising. Marques et al. describe the microflora-modulating power of prebiotics, complex saccharides found in foods which are fermented by and feed the microflora (2014). Anecdotally, many people get results from attempting various dietary regimes to treat their gut-brain issues. Dr. Natasha Campbell-McBride has developed the "Gut and Psychology Syndrome", or GAPS diet (Campbell-McBride, 2004). She recommends the strict diet for the treatment of autism and psychological illness. She claims that the diet excludes foods that feed pathogens and irritate the gut, and includes highly nutritious and protective "ancestral" foods such as bone broth and grass-fed beef liver, as well as a high-dose probiotic. Unfortunately Dr. McBride's work has not been universally accepted. Much more attention needs to be given to the human diet because it has

great potential for modulating the intestinal ecosystem. In the future, it is possible that gut-brain research will be able to develop prescription diets for the treatment and prevention of IBS, depression, anxiety, autism, and other illnesses.

CONCLUSION

This article has reviewed theoretical, murine, and human gut-brain-microbiota research into IBS, depression, anxiety, and autism. Several prominent themes emerged from the review, including shared risk factors, potential treatments, and an overarching need for specific kinds of future research. I argued that future research should focus on achieving causal and theoretical clarity, determining microbiotal markers of health and disease, and developing effective preventative and treatment measures for gut-brain illnesses. Gut-brain-microbiota research shows great promise for the treatment and prevention of some of the most challenging human illnesses. The author looks optimistically forward to future advances in this field.

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