# THE GUT-MICROBIOTA-BRAIN AXIS AND NEUROPSYCHIATRIC HEALTH

By

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THE GUT-MICROBIOTA-BRAIN AXIS AND

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Abstract:

In this literature review, the evidence and theoretical explanation of the Gut-Microbiota-Brain axis and its role in neuropsychiatric disorders is presented. There is now a paradigm shift in neuroscience toward an increasingly supported understanding of neurobiology in terms of not only the CNS, but also the so-called "second brain" residing in the enteric nervous system of the gut and its resident microbiota. Accordingly, more and more research is looking into the possible role of this second brain in the psychopathology of psychiatric disorders, such as major depressive disorder (MDD) and neurodevelopmental disorders, including attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). This review outlines a burgeoning explanatory model, which, if further supported by more rigorous studies, has the potential for the development of novel psychiatric treatments via modulation of gut-microbiota dysbiosis with pro/pre-biotics, fecal microbiota transplantation (FMT), etc., as well as a theoretically promising avenue to disease prevention via intervention into the gut-microbiota during the neurodevelopmental windows in which we think ADHD and ASD are developed.

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# CHAPTER I

## The Neurobiology of The Gut-Microbiota-Brain Axis

It has been noted that the number of microorganisms in the human body outnumber the number of human cells tenfold, with these microbes jointly containing over 100 times more genes than our own genome (Gill et al. 2006; Handley, Dube, and Miller 2006). A great number of these microbes are located on the lining of the gut, where they comprise a complex ecosystem which has been called the gut microbiota. Research has found that there is a complex interaction between the host and its resident gut microbiota, with effects ranging from energy metabolism, immunological competence and chronic inflammation, to brain functioning and psychological behavior (Robles & Guarner 2013). Indeed, this microbiota has been referred to by some as another anatomical organ because of its extensive roles in the bi-directional axis with the brain, of which will be the topic of this literature review (Clarke et al. 2014).

### *Structure of the Gut Microbiota*

As used here, the term "microbiota" refers to all of the microbial organisms resident in the gut, including archaea, eukaryotes, and viruses in addition to bacteria. Of course, chief among them are the bacteria, which according to a metagenomics analysis

of the gut microbiota in 124 human subjects found 99% of the genes were bacterial with the total number of species found amongst the 124 subjects being between 1,000-1,150 (Qin et al. 2010; Echkburg 2005). The factors impacting the composition of an individual's gut microbiota include genetic predisposition, nutrition, stress, environmental factors, history of infection, use of antibiotics, age, and level of physical activity (Thursby and Juge 2017) .

The majority of the gut microbiota's colonization begins at birth, as the inside of the uterus is relatively aseptic, the fetus is mostly sterile until inoculation either via the mother's birth canal during vaginal delivery, or if cesarean section is to be performed, then through contact with the external environment of the hospital and the mother's skin. This disparity in mode of colonization has been shown to cause disparate microbiota compositions, such as greater levels of *Lactobacillus, Prevotella,* and *Bifidobacterium* following vaginal delivery, and *Staphylococcus* and *Cornyebacterium* as the predominant genera colonized by newborns following cesarean section (Dominguez-Bello et al. 2010).

*Evidence of Top-Down Communication:*

*Hypothalamic-Pituitary-Adrenal Axis (HPA Axis)*

Psychological stress is a known regulator of physiologic systems throughout the body via activation of the hypothalamic–pituitary–adrenal axis (HPA axis), including alteration of the gut microbiota composition. This stress-mediated effect on the gut microbiome has been demonstrated in various special models. Following provocation of separation anxiety in rhesus monkeys by separating them from their mothers at an early age, their fecal *Lactobacillus* concentrations declined (Bailey and Coe 1999). A mere two hours of

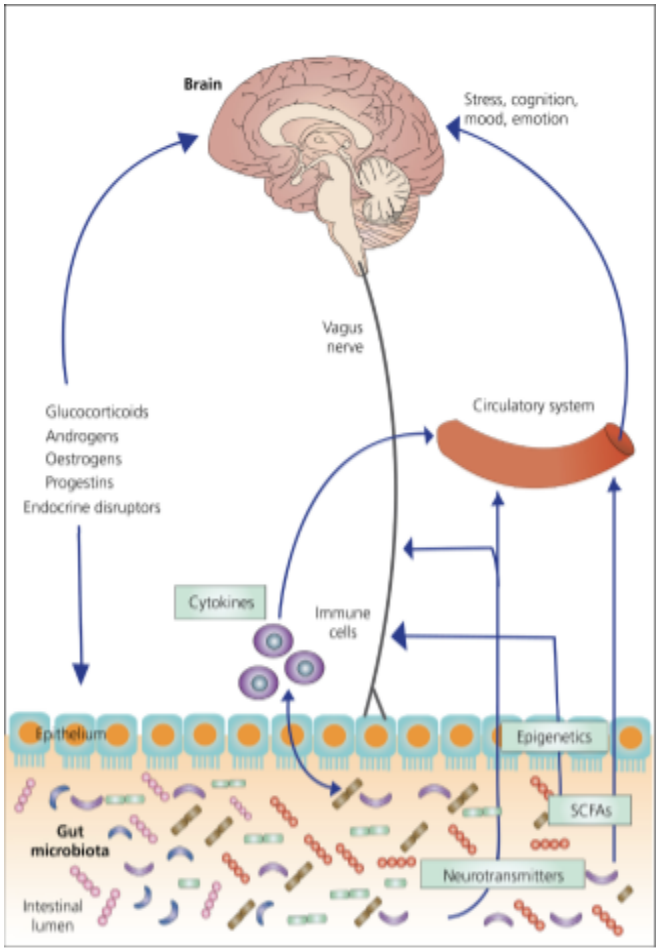


Figure 1: The Gut-Microbiota-Brain Axis illustrated with a few of its many mechanisms of bilateral communication (Tetel et al., 2018)

social disruption in a mouse model similarly affected the gut microbiota by a decrease in *Lactobacillus* population (Galley et al. 2014). In a human model, the *Lactobacillus* fecal concentrations were tracked in healthy students with the result of lower levels of this commensal bacterium during a period of extreme academic stress relative to lower stress periods of the semester (Knowles, Nelson, and Palombo 2008). This effect on the microbial communities in the gut is believed to be partially mediated by cortisol secretion affecting gut integrity, mucus secretion, and motility (Rubio and Huang 1992). The increase in intestinal permeability, leaky gut, causes acute and chronic local immune activation as illustrated in figure 1, cascading local and systemic inflammatory and other immunological responses.

*Evidence of Bottom-Up Communication:*

*Immuno-modulation*

Immunogenic bacteria in the gut can readily cause chronic low-grade inflammation, causing release of pro-inflammatory cytokines and substances that may gain access to the central nervous system. One pro-inflammatory substance produced in the gut, LPS, may interface with the CNS via recognition by its TLR-4 receptor, which is expressed across the brain in microglia, macrophages, and monocytes. This LPS-brain

interaction alone has been implicated in the psychopathology of depression comorbid with irritable bowel syndrome (Daulatzai 2014).

*Vagal Transmission*

The vagus nerve serves as the primary form of afferent neuronal communication between the gut and the brain. Studies conducted following vagotomy, severance of the vagal nerve, show hindered cytokine induction up the vagus nerve following LPS activation of IL-1β, which would normally induce inflammatory-related illness (Bluthé et al. 1994). Evidence for how important the vagus nerve is in gut-microbiota-brain communication was elucidated in a murine model study looking at two groups of rodents: both administered the probiotic, *Lactobacillus rhamnosus*, and only one group vagotomized. The results of this study, with significant anxiety, depressive behavior, and corticosterone level reductions in only the non-vagotomized group, showing the vagus nerve serves as a pivotal mechanism for gut-microbiota-brain interactions (Bercik et al. 2011).

*Microbial Metabolites*

Of the many metabolites produced by the gut microbiota, short-chain fatty acids (SCFAs) are especially relevant to the gut's interaction with the central nervous system. SCFAs produced by microbes in the gut, such as propionic acid and butyric acid, are

known to upregulate gene expression of an enzyme, tyrosine hydroxylase, which inhibits the synthesis of the neurotransmitters noradrenaline and dopamine, as well as upregulate dopamine-β-hydroxylase, an enzyme which converts dopamine to noradrenaline (Nankova et al. 2014). The medical implications of this known role of SCFAs is especially apparent for the many psychiatric disorders thought to be mediated by dopamine and noradrenaline levels, such as attention-deficit/hyperactivity disorder and depression. Furthermore, a study on germ-free mice showed long term treatment of propionic acid decreased *in vivo* concentrations of serotonin, GABA, and dopamine (El-Ansary, Ben Bacha, and Kotb 2012).

*Production of Neural Substrates*

In addition to microbial metabolites that indirectly act on the central nervous system, microbes can also produce a diverse array of substances which affect the brain directly, such as neurotransmitters themselves, as well as their precursors. In addition to the role of the gut in synthesis of the majority of the body's serotonin, the gut also serves as the source of tryptophan, which crosses the blood-brain barrier into the CNS where it is used for further serotonin synthesis (Mawe and Hoffman 2013). It was found that germ-free mice have elevated serum levels of tryptophan and reduced serotonin blood concentrations relative to wild-type, normally colonized mice, which suggests microbiota dysbiosis might result in reduced serotonin synthesis via decreased expression of

tryptophan hydroxylase in the gut compared to mice with a healthy gut microbiota (Yano et al. 2015).

Moreover, gut bacteria locally produce other neurotransmitters, such as catecholamine, histamine, and GABA which can be transmitted across the intestinal lining through enterochromaffin cells to enter into enteric nerve receptors. A major inhibitory neurotransmitter, γ-aminobutyric acid (GABA), which in its dysfunction is connected to the neuropathogenesis of anxiety, depression, autism, and schizophrenia, is produced by the gut bacteria, *Lactobacillus brevis* and *Bifidobacterium dentium* (Barrett et al. 2012). Additionally, it has been suggested that GABA produced in the gut affects the brain by crossing the blood-brain-barrier (BBB) and directly enters the CNS (Takanaga et al. 2001). However, the gut microbiota is also believed to affect CNS GABA levels indirectly through vagal signaling, considering a study showing *Lactobacillus rhamnosus* reducing anxiety and depression-like behaviors and increase GABA concentrations in mice hippocampi, but only when the vagus nerve is left intact (Bravo et al. 2011; Janik et al. 2016). In regard to the microbiota's role in dopamine and noradrenaline concentrations, it is suggested that gut bacteria produce catecholamine from studies showing germ-free mice with strikingly lowers levels of these neurotransmitters in the cecum than are present in commensal-colonized mice (Asano et al. 2012).

*Great Transformation of Disease Types: The Role of the Gut Microbiota*

The species *Homo sapiens* is, in its essence, a superorganism. This much is clear from the extensive symbiotic relationship we hold with our resident gut microbiota. It is becoming increasingly evident from the many scientific fields researching the multi-faceted gut microbiota that a comprehensive understanding of human health requires studying our microbial symbiotes as well. The scientific understanding of human health as the product of a complex interplay between host and microbiota represents an increasingly-supported paradigm in human physiology. As will be elucidated in this paper, this paradigm shift in our understanding shows clear potential towards novel pathophysiological models of psychiatric disorders, as well as a capacity toward a next generation of novel psychotherapeutics based in a completely unique mechanism of action–something needed in the SSRI-dominated field of psychiatry.

In addition to these potential implications from the field of gut microbiota research, epidemiological researchers have suggested evidence of microbiomical differences between populations shows a relationship of our microbiota's vast evolution in the transition from pre-industrial, infectious disease-predominate causes of human morbidity to today's, post-industrial, state of human health increasingly marred by inflammation-driven diseases, such as depression, obesity, and autoimmune diseases (Liang et al., 2018).

With the Industrial Revolution came extensive change in humanity's cultures, diets, behaviors, and it was during this time the field of medicine innovated to the extent that the once seemingly-unconquerable existence of microorganisms was finally susceptible to man-made vaccines and antibiotics. This period in history marks a distinct shift in the types of disease burdening humans. Namely, this shift jumps from the prevailing affliction of the past–infectious disease– toward the present state of human health increasingly affected by a different array of diseases, including autoimmunity, such as in asthma and allergies; metabolic disease, such as diabetes and fatty liver disease; psychiatric disorders, such as anxiety, developmental, and mood disorders; cardiovascular disease, such as coronary artery disease (Liang et al., 2018).

The "old friends" hypothesis was first proposed by the researcher, Rook, in 2008 on the basis of another idea termed the hygiene hypothesis, which suggests early childhood exposure to particular microbes primes the immune system in such a way that prevents the later development of allergic disease. Rook's 2008 tangential concept of "old friends" hypothesized in pre-modern times we lived in a healthy, symbiotic relationship with certain commensal bacterial and parasitic organisms (Rook et al., 2008). His conclusion, of course, is that in modern day we do not have an adequate exposure to these microorganisms, and as a result, our immune system does not properly develop, conceivably, leading to many health problems later in life due to chronic inflammation,

such as mood disorders and autoimmune diseases.

The relationship between our gut microbiota and this transformation of disease, then, appears to reside in our coevolution, or more specifically, in the adaptation of our gut microbiota to our new lifestyles in the absence of a complementary change in our own human genes. This lopsided evolution of one partner in our vital symbiotic relationship has led to a modern, western variant of the gut microbiota–one in which we, their hosts, were not evolutionarily adapted to benefit, as well as we might otherwise with a healthy microbiota established through millennia of natural selection. It is in our nature now to transform our environment, both outside and inside our bodies, at a rate much faster than what our own genes can be driven to adaptation through natural selection, and as a result, we will continue to see human health increasingly at odds with a microbial environment in which our bodies were not adapted to live. In the next chapter of this thesis, I will delve into the state of research describing the neuropsychiatric implications from our rapidly changing, modern, gut microbiota.

# CHAPTER II

# The Neuropsychiatry of the Gut Microbiota

## *Attention-Deficit/Hyperactivity Disorder (ADHD) and the Gut Microbiota*

The currently prevailing psychopathological theory of Attention-Deficit/Hyperactivity Disorder (ADHD) is neuropsychiatric in nature, conceptualizing the disorder as polygenic variations in the functioning of the monoamine neurotransmitter systems and alterations in neural network structures. This neuropsychiatric paradigm is slowly being joined by a neuro-entero-immunological perspective fed by the quickly expanding field of evidence for the bidirectional interplay of the gut-microbiota-brain, which is presently transitioning from preclinical research into human studies. A significant deviation from the previous tradition of thought, this theory emphasizes the role of environmental factors on the development and function of the BGM axis leading to phenotypic implications. The strength of a microbiota-derived etiologic model here is especially seen in its possible explanation to the long puzzling question of the wide variance in worldwide ADHD prevalence, as diet, gut microbiota composition, and other environmental factors vary so much in the world's great heterogeneity of cultures and climates. Additionally, this model, if shown to be correct,

opens up significant avenues for not only effective treatment of the disorder, but also possible prevention.

*A Novel Hypothesis for ADHD Etiology: Autonomic Dysregulation Model*

This explanatory model of ADHD suggests the autonomic dysregulation seen in ADHD is caused by an imbalanced, less rich and less diverse gut microbiota early in life, as well as perpetuated throughout adulthood (Sandgren and Brummer 2018). This theory for the fundamental causes of ADHD relays how early life environmental differences lead to variations in microbiota composition responsible for the alterations in metabolic and immunologic functioning that causes the various symptoms of ADHD. During the early life periods of neurodevelopment, autonomic dysregulations lead to altered immunologic functioning, as well as altered metabolism and formation of pathophysiologically relevant monoamines such as dopamine, serotonin, and the neurotransmitter-derivative, tryptophan. Figure 2 illustrates many of the wide-ranging factors thought to play roles in the development of early childhood gut microbiota, as well as indicating that a genetic predisposition toward the disorder combined with the various causes that lead to gut dysbiosis may result in altered neurodevelopment and ADHD.

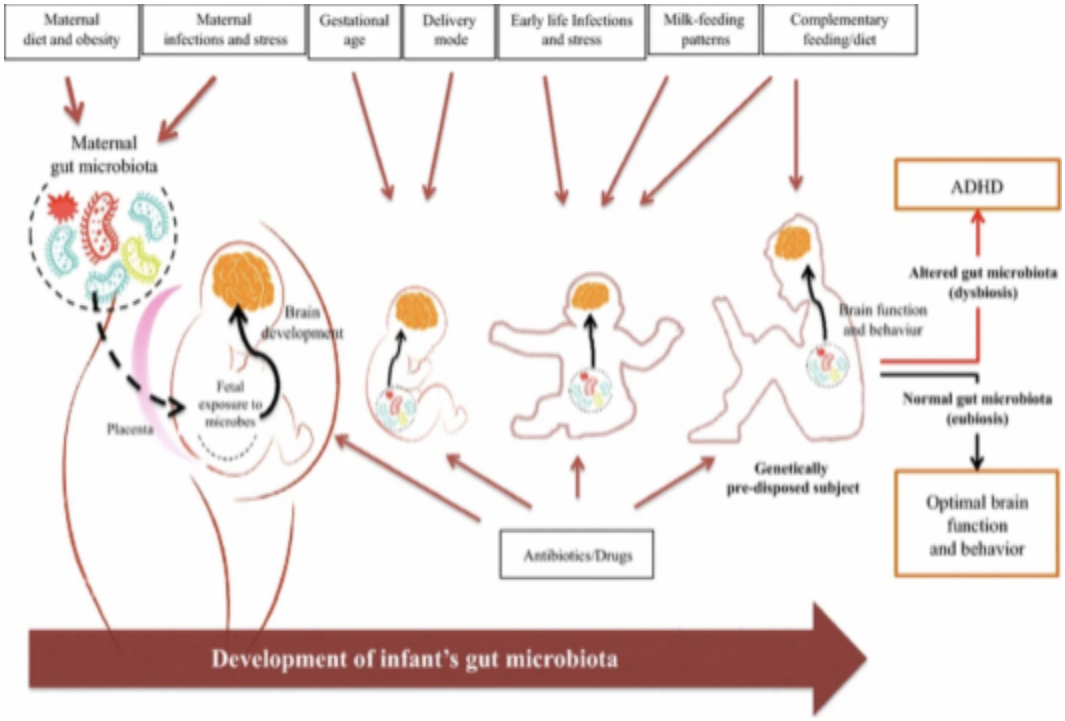


Figure 2: Development of Infantile Gut Microbiota. A representation of the various environmental factors that influence the development of the gut microbiota in early childhood. The combination of genetic predisposition and factors including delivery mode, milk-feeding patterns, antibiotic use, etc., which can cause dysbiosis is shown to cause development of ADHD (Cenit et al., 2017).

*Mechanisms of Autonomic Dysregulation:*

*Altered peripheral metabolism and formation as mediator of CNS GABA concentrations*

Central to this neuro-entero-immunological theory of psychopathology in ADHD is the idea of a suboptimal (lowered/imbalanced) microbial inoculation in early life resulting in a dysbiotic gut microbiota development, altering not only early life

neurodevelopment, but also the continued metabolic, and other, activity of gut microbiota throughout life. One possible mechanism of pathology in ADHD is reduced levels of the inhibitory neurotransmitter GABA, and this is gastroenterologically relevant as GABA is a common metabolic product of microbial fermentation (Dhakal, Bajpai, and Baek 2012).

Magnetic resonance spectroscopy (MRS) research has indicated reduced CNS levels of GABA in ADHD children (Edden et al. 2012). It has been shown that the GABAergic system is responsive to both short- and long-term stress sensitivity in rodents, possibly mediated by the neuroactive steroid hormones, such as allopregnanolone (Skilbeck, Johnston, and Hinton 2010). Additionally, a study on healthy rodents indicates emotional behavior and expression of GABA receptors in the brain can be modulated by the introduction of a probiotic, *Lactobacillus*, and mediated by the vagus nerve (Bravo et al. 2011). In accordance with these findings, it is possible an altered GABA metabolism or formation in the CNS may be caused by alterations in the periphery.

*Altered metabolism and formation in the periphery as mediator of CNS monoamines (dopamine, serotonin, norepinephrine) concentrations*

In line with predominating theories of ADHD pathology is the theory of decreased dopamine (DA) levels in the CNS (Sandgren and Brummer 2018). Various

studies have shown that this insufficiency in DA concentrations may have its roots in the periphery (Cortese 2012). A study comparing germ-free (GF) mice to control, wild-type gut microbiota possessing mice gives support to the hypothesis of peripheral mediation of DA, where GF mice demonstrated elevated turnover of monoamines such as dopamine, norepinephrine, and serotonin in the striatum, as well as altered expression of anxiety and plasticity-related genes in multiple brain regions (Diaz Heijtz et al. 2011).

In addition to the pathophysiological role of altered CNS dopamine levels in ADHD, serotonin has been implicated in impulsivity and hyperactivity symptoms seen in the disorder (Banerjee and Nandagopal 2015). Serotonin is widely established to be a central component of bidirectional communication of the gut-microbiota-brain axis (O'Mahony et al. 2015). In addition to the role of the gut in synthesis of the majority of the body's serotonin, the gut also serves as the source of tryptophan, which crosses the blood-brain barrier into the CNS where it is used for further serotonin synthesis (Mawe and Hoffman 2013). Important implications of these findings are suggested in the role of CNS serotonin modulation of learning and memory function via effects on dopaminergic, cholinergic, and GABAergic neurotransmission (Seyedabadi et al. 2014). This is relevant to ADHD pathology and gastrointestinal immunologic functioning, as research has suggested serotonin signaling properties become altered following intestinal inflammation, resulting in decreased functioning and expression of serotonin-selective reuptake transporter (SERT) (Mawe and Hoffman 2013). In vitro evidence has indicated

decreased tryptophan transport in boys in combined type ADHD, possibly implying decreased serotonin levels in the brain (Johansson et al. 2011).

*Altered Immunological Functioning*

It has been suggested that chronic pro-inflammatory immune dysregulation, possibly resulting from genetic predisposition, mediates the pathogenesis of ADHD (Verlaet et al. 2014). Evidence for this hypothesis is found in, among others, studies showing a significant prevalence of allergic diseases (asthma, urticarial, atopic dermatitis, allergic rhinitis) in individuals diagnosed with Attention-Deficit/Hyperactivity Disorder (Chen et al. 2017). Higher intake of the probiotic fermented food, kimchi, omega-3 fatty acids, polyunsaturated fatty acids (PUFAs), and lower intake of fast food, were associated with lower odds of having ADHD (Woo et al. 2014). Furthermore, immunologic dysregulation has been implicated in not only in the etiology of ADHD, but also in its on-going pathophysiology. As with Autism Spectrum Disorders, it has been suggested that sensitivities to certain foods, and their subsequent reactions, may act as antigens to induce more severe ADHD symptoms (Pelsser et al. 2011). Interestingly, in one study, this food sensitivity-related allergic reaction was shown to be non-allergic or cell-mediated (non-IgE, non-IgG mediated) (Pelsser et al. 2011).

*Implications of Metabolic and Immunological Dysfunction Findings*

These results seem to suggest potential avenues of therapeutic intervention acting on the very immunological dysfunction and neurotransmitter metabolism, which seems to be implicated in the etiology of the disorder. Even more exciting for the state of neuropsychiatry than additional therapeutic methodologies, is the prospect of preventative action via early life gut-microbiota inoculation of anti-inflammatory and monoamine-promoting probiotics and prebiotics. For a neurodevelopmental disorder with a growing prevalence in western societies, this novel explanatory model addresses an increasing societal burden, possibly marking the shift toward a future of child psychiatric medicine empowered for the first time with this sort of psycho-vaccination.

## *Autism Spectrum Disorder (ASD) and the Gut Microbiota*

The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders defines Autism spectrum disorder (ASD) as a developmental disorder that affects social communication and behavior development without a clear etiology. Proposed etiologic explanations have spanned many paradigms, including epigenetic effects, congenital causes, immune dysregulation, exposure to environmental toxins, and, more recently, researchers have proposed a link between microbiota dysfunction and the development of ASD (Alibek et al., 2019). Due to findings on the increased incidence of gastroenterological disorders in individuals with ASD, researchers began looking toward the gut, and its resident microbiota, for possible clues to the etiology of the disorder (Holingue et al. 2018).

### *Gut Microbiota Composition and ASD*

There are a plethora of animal studies documenting alterations to the gut's microbial composition associated with neurobehavioral changes, particularly with anxiety and depression related effects, but recently there has been an emergence of these studies looking into autism-related effects (Warner 2019). Gut microbiota composition at year

one of age was found to predict cognitive performance at two years of age, with an especially high predictive power toward communicative behaviors (Carlson et al. 2018). A 2020 systematic review on the topic, found a trend of increased ratio of Firmicutes/Bacteroidetes and decreased *Bacteroides* in ASD individuals relative to neurotypical controls; and, as corroborated by every study reviewed, the researchers found a significantly decreased absolute abundance of *Bifidobacterium* genus in children with ASD compared to non-sibling controls (Ho et al. 2020). However, this same systematic review also found the majority of its reviewed studies had contrasting results with regards to most trends in microbial composition in ASD. With this finding, the study's authors conclude the gut microbiota by itself is not a sufficiently predictive biomarker for ASD, but instead suggest the integration of multiple omics data (i.e., metabolomics, transcriptomics, proteomics, microRNAs, and exosomes) to capture a more robust image of the gut microbiota's composition in ASD.

*Epi-/genetics in ASD*

The conventionally popular hypothesis of a strong hereditary cause, as supported by early studies of monozygotic and dizygotic twins with concordance rates up to 90% (Bailey et al. 1995), may have been overestimations of hereditary effect, as the researchers argue in a more recent study (Hallmayer et al. 2011) that found heritability of

ASD in 193 twin pairs was approximately 50%.

*Gestational Health in ASD*

While researchers have long believed the amniotic fluid and placenta to be free of microbial life (Jiménez et al. 2008), recently the *in utero* environment has been demonstrated to be *not* sterile (Digiulio et al, 2008). Furthermore, the fetus itself is now known to host a gut microbiota, as researchers have found the meconium, or the first neonatal feces, to contain a range of bacteria, predominant of which were *Enterococcus* and *Staphylococcus* (Jiménez et al. 2008).

In addition to the research looking into the newly appreciated gestational microbiota, researchers have investigated the effects of maternal health on the health of gestational microbiota and the resulting health of neonates. In one study, obesity and diabetes during pregnancy was found to produce characteristic changes in the gut microbiota of the neonate, with these alterations being associated with ASD (Connolly et al. 2016). Moreover, this study also found a high-fat diet alone to be enough to alter the neonate's microbiota in the characteristic manner associated with ASD.

*Cesarean Section and ASD*

Just as researchers began to look into the rise in cesarean section (c-section) rates as a potential risk factor in prevalence of ADHD, studies have now been performed which implicate this mode of delivery in the etiology of ASD as well (Curran et al. 2015). Compared with vaginal delivery, one study demonstrated an odds ratio of 1.26 for development of ASD following c-section (Yip et al. 2017).

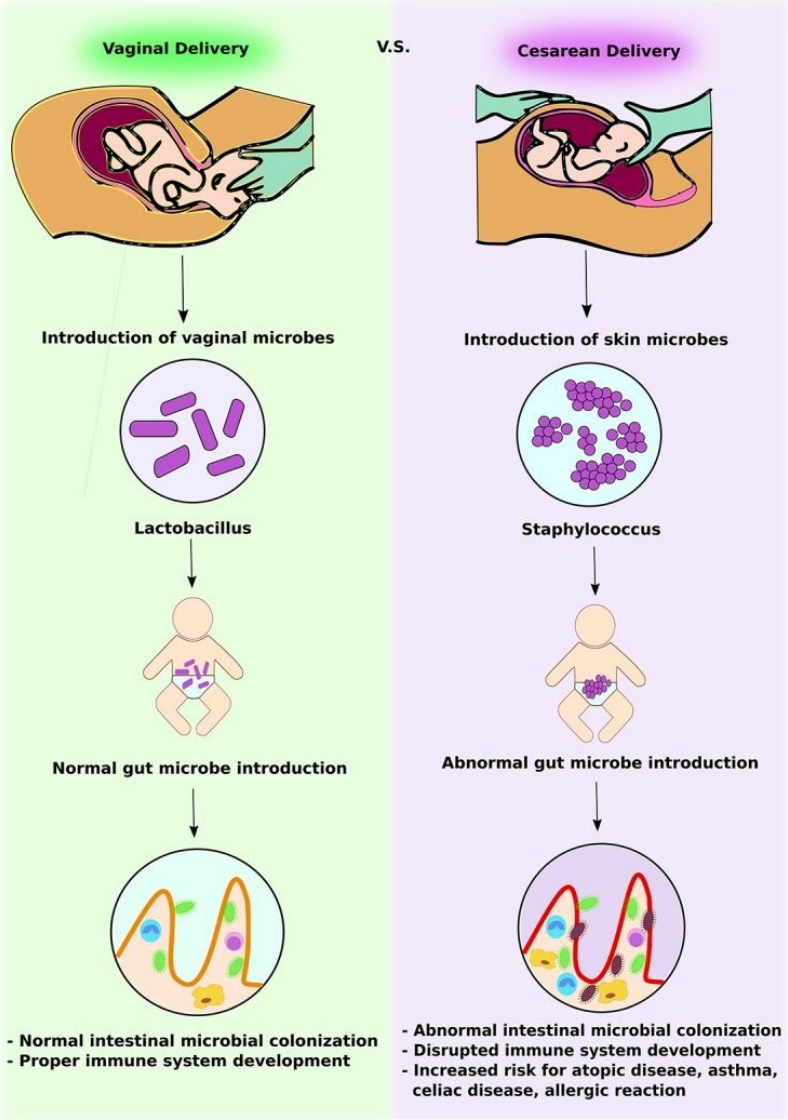


Figure 3: Microbial Colonization Variation by Mode of Delivery. In vaginal delivery, *Lactobacillus* predominates as the major genus of bacteria to first colonize the infant. In cesarean section, however, the skin bacteria *Staphylococcus* predominates. The introduction of skin microbes may then lead to dysbiosis of the gut, resulting in increased risk for immunological disorders such as atopic disease, asthma, celiac disease, and allergic reaction (Eshraghi et al., 2018).

*Inflammation in ASD*

An increasing amount of research is investigating inflammation as a key player in ASD etiology and ongoing pathophysiology. A systematic review analyzed the research trends of 437 publications on ASD from 1971 to 2010, finding 95% (416) implicated an association between ASD and inflammation (Rossignol and Frye 2012). Accordingly, the gut microbiota has also been implicated in systemic inflammation, via activation of the vagus nerve, as well as production of immuno-modulatory short chain fatty acids (SCFAs) (Richard E. Frye et al. 2017). SCFAs, such as propionic acid and butyric acid, have shown to modulate the immune system in several manners, including the regulation of T-cell and cytokine activation, increasing expression of immune-activating genes, as well as the homeostasis of microglial cells during neurodevelopmentally critical periods (Richard E. Frye et al. 2017; R. E. Frye et al. 2016; Erny et al. 2015).

## Microbiota-based Interventions for ASD

At this time, there exist no definitively effective interventions for ASD, but there have been many small studies with preliminary data that suggest efficacy for probiotics and prebiotics on improving ASD symptoms.

Table 1.) Examples of Typical Quality/Size of Pro/Pre-biotic Studies

| **Model** | **Treatment** | **Effect** | **Limitations** | **Reference** |
|---|---|---|---|---|
| 22 children diagnosed with ASD | *Lactobacillus acidophilus* | Significant improvement in ability of concentration and carrying out orders | No control group | (Kaluzna-Czaplinska and Blaszczyk, 2012) |
| 11 children diagnosed with ASD | Vancomycin and *Lactobacillus acidophilus*, *L. bullgaricus*, and *Bifidobacterium bifidum* | Improvement of short term behavioral scores (from evaluation by treatment status blinded clinical psychologist) | Results largely waned at 2-8 month follow-up | (Sandler et al., 2000) |
| 41 patients diagnosed with ASD | Omega-3 fatty acids (EPA + DHA) | Improvement of ASD core symptoms, social problems and attentional issues | Open-label study | (Ooi et al., 2015) |
| 387 children diagnosed with ASD | gluten-free and/or casein-free diet | Improvement of ASD behaviors, physiologic symptoms, and sociality | A retrospective analysis of parental reports | (Pennesi and Klein, 2012) |

## *Major Depressive Disorder (MDD) and the Gut Microbiota*

Major Depression is characterized by a low mood and/or anhedonia, the inability to experience pleasure, for more than two consecutive weeks, and is a leading cause of morbidity, disability, and mortality (aan het Rot, Mathew, and Charney 2009). Theories of the physiological cause of the disorder range widely, from the most popular theory among clinicians– that of altered neural functioning, including imbalances in neurotransmitter levels (aan het Rot, Mathew, and Charney 2009)– to explanations of altered functioning in the HPA axis, the immune system and, finally, the gut-microbiota-brain axis (Chaudhury, Liu, and Han 2015; Singhal and Baune 2017). These four predominating theories on the etiology, or the manner of causation, of depression form the basis of the following chapter.

### *The History of Pathophysiological Theory of Major Depressive Disorder: Theories of Altered Neural Functioning*

Of the three main hypotheses within the umbrella of neural malfunctioning theories of depression, the most popularly known ***monoamine hypothesis***, which suggests imbalances in key mood-related neurotransmitters play a role in the development of the disorder (aan het Rot, Mathew, and Charney 2009). Chief among

these neurotransmitters believed to help regulate mood are serotonin and norepinephrine. It has been shown that in people with a personal and family history of depression, pharmacologically-induced low levels of serotonin, via administration of a tryptophan depleted diet of amino acids, does indeed induce episodes of depression. However, this theory, in spite of its wide clinical acceptance, is undermined by methodologically-comparable studies on patients without personal or familial histories of depression, whereby the depletion of their serotonin levels does not cause a resultant drop in mood (aan het Rot, Mathew, and Charney 2009).

The next etiological hypothesis of depression within the explanatory umbrella of neural models is the ***neuroplasticity hypothesis***, which suggests symptoms of depression arise from an impaired neuroplasticity induced by CNS-based risk factors such as neurotransmitter imbalance and decreased brain derived neurotrophic factor (BDNF) levels (B. Liu et al. 2017; Williams 2016). The BDNF protein is a member of the neurotrophin family of growth factors, which is essential for the growth and survival of neurons in the brain. The rationale for this hypothesis can be partly seen in the delayed onset of symptom reduction from standard antidepressant (AD) treatment, whereby increased synaptic serotonin, or norepinephrine and dopamine, concentration does not itself account for the antidepressant efficacy of the treatments (B. Liu et al. 2017). On a clinical level, this phenomenon can be understood by the oftentimes 4-6 week delay in

clinical onset of symptom reductions. This multi-week delay in treatment occurs in spite of increased synaptic concentrations of monoamines starting from the very first administration. It then follows that there must be some other neurological effect from these monoamine-increasing antidepressants which can mediate the amelioratory results, especially an effect that is time-delayed as to account for the 4-6 week delay of symptom reduction onset. This is where the neuroplasticity hypothesis comes in. In this explanatory framework, standard antidepressant therapies such as selective serotonin reuptake inhibitors (SSRIs) exert an unintentional yet clinically-mediating effect on the brain's neuroplasticity that account for the ultimately effective, yet delayed, characteristic of the treatment.

The neural circuit hypothesis suggests depression is the result of miscommunications across specific neural circuits of the brain. This multi-circuit miscommunication is caused by irregular interaction of neural structures thought to be fundamental to mood states, such as dopaminergic (dopamine-releasing) neurons in the ventral tegmental area (VTA), a major neural structure of dopamine production, and 5-HT (serotonin-releasing) neurons in the dorsal raphe nucleus, the serotonin counterpart of the VTA. Studies on deep brain stimulation, the electromagnetic manipulation of the electric pulses in neuron firing, have shown that their therapeutic, antidepressant effects

are the result of normalization of the communication abnormalities in these neural circuits (Chaudhury, Liu, and Han 2015; Williams 2016).

*Theories of Altered HPA Axis Functioning*

One hypothesis in the biological development of depression implicates an exaggerated case of the normal physiological response to stress across the HPA axis– the intersection of the central nervous system and the endocrine system. This extreme sensitivity in the HPA axis results in increased release of corticotropin-releasing factor (CRF) and vasopressin (AVP) by the hypothalamus. As a result of CRF and AVP release, the anterior pituitary gland secretes adrenocorticotropic hormone (ACTH), which stimulates the release of adrenocortical hormones, including glucocorticoid (GC), which causes a negative feedback circuit, as GC inhibits the secretion of CRF and AVP by the hypothalamus (Barden 2004; Kunugi et al. 2010). The theory of depression by altered HPA functioning suggests excessive circulation of GC, which causes desensitization of the glucocorticoid receptor (GR), resulting in a dysfunction of the healthy negative feedback circuit to keep the HPA stress response in check. Indeed, this dysfunctional negative feedback circuit is seen in over half of depressed patients, leading to a chronic increase in GC and ACTH, and even hyperpotassemia, elevated levels of potassium ($K^+$) in some patients (Barden 2004; Rook and Lowry 2008). Additionally, it has been found

that HPA axis dysfunction reduces BDNF expression (Kunugi et al. 2010) decreases Glu receptor expression, disturbs neuroplasticity and neural circuits (Chaudhury, Liu, and Han 2015; Singhal and Baune 2017), and even inhibits 5-HT synthesis (Maes et al. 2011). These various disturbances in normal physical functions result in the psychological effect of depression because of their central role in the regulation of mood states and stress responses.

*Theories of Altered Immunological Functioning*

The cytokine hypothesis–as shown in figure 3– theorizes depression is the result of an excessive inflammatory response, resulting from increases in pro-inflammatory cytokines such as interleukin-6 (IL-6) and TNF-α and decreases in anti-inflammatory cytokines such as IL-10 and TGF- β. Cytokines are chemicals secreted by cells of the immune system, which have certain effects on other cells, such as initiating inflammatory responses. The overall inflammatory response leads to inhibited negative feedback of the HPA axis, causing the bundle of physiological reactions described above, including reduction of serotonin synthesis, disturbed glutamatergic systems, increased blood-brain barrier permeability, and, therein hypothesized, depression (Maes et al. 2011; Schiepers, Wichers, and Maes 2005; O'Brien, Scott, and Dinan 2004; Wichers and Maes 2002; Leonard 2018; Haroon and Miller 2017).

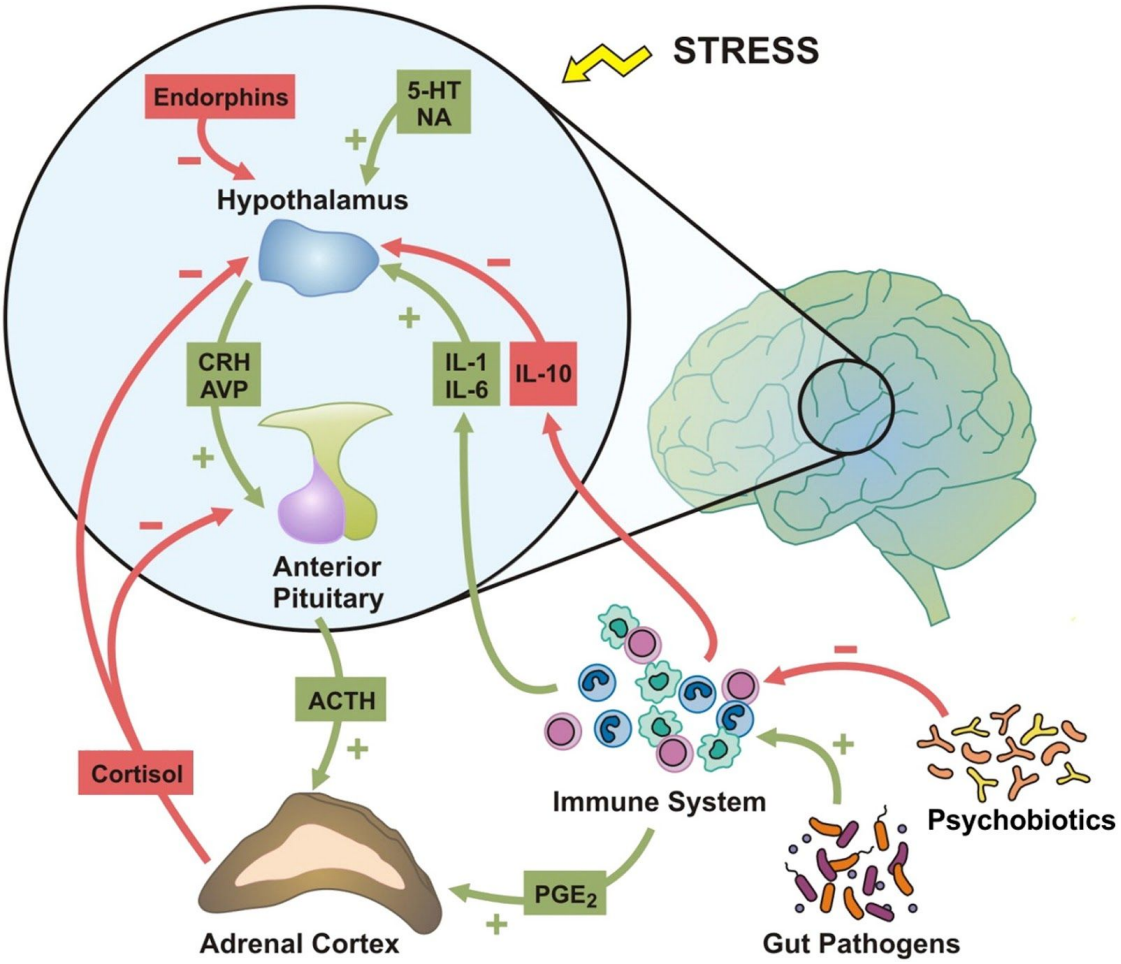


Figure 3: Stress and the Gut Microbiota. Stress is a ubiquitous phenomenon in the etiology of major depressive disorder. Psychological stress acts in a top-down manner to activate the HPA axis by directly inducing release of corticotropin-releasing hormone (CRH) and vasopressin (AVP) along with more indirect pathways starting from the gut. Gut pathogens, for example, can cause altered gut barrier functioning, or "leakiness", which results in transmission of pro-inflammatory molecules, such as lipopolysaccharide, into the bloodstream. This immune activation can act on the hypothalamus with increased interleukin-1 (IL-1) and interleukin-6 (IL-6) levels, as well as directly stimulation of the adrenal cortex by prostaglandin $E_2$ ($PGE_2$).
Psychobiotics can act against this proinflammatory state by elevation of anti-inflammatory cytokines interleukin-10 (IL-10) and suppression of pro-inflammatory cytokines and HPA axis activity. (Dinan et al., 2013)

*Altered Gut-Microbiota-Brain Functioning: A More Fundamental Explanation*

As can be seen in a broad view of the above etiological theories of major depressive disorder, this psychopathology is not merely a mental disorder confined to the brain, but it is a systemically-defined biological disease characterized by physiological systems incorporating immunological, hormonal, and epigenetic causes– among many others. Intuitively, scientists conceive of disorders with seemingly far-flung etiological foundations as containing a single yet-to-be-determined foundation and elegant etiological explanation. Applying this intuition to the etiologically multi-system major depressive disorder, the scientific inclination to choose a theory which elegantly connects all the mechanistic dots has brought many researchers to view the gut-microbiota-brain axis as the intersection and major driving force behind these seemingly disjointed events, which culminate in the presentation of depression. Indeed, all of the above described pathological mechanisms of depression interplay with each other, and the biological theater of this interaction seems to be predominantly located within the various pathways of the gut-microbiota-brain axis, begging a proper psychopathological model of depression be located on this axis as well.

*The Evidence for the Microbiota-Gut-Brain Axis Model of Depression: Differences in*

*Phenotypic Composition of Microbiota in Depressed versus Health Subjects*

There has been a marked proliferation of studies investigating the microbial differences in the guts of depressed subjects–both in rodent models and human patients. In some of the research on human subjects, it was found that depression was associated with reductions in both microbiota diversity and richness (Jiang et al. 2015; Kelly et al. 2016). While phenotypic differences in microbiota composition between control and depressed models are still in debate (Lin et al. 2017) and cannot be precisely drawn, a few broad (phylum and genus level) distinctions have been identified. In phylum level differences, depressed subjects have shown to contain increased richness of Proteobacteria and Bacteroidetes and decreased richness of Firmicutes; on the order of family, depression is associated with an increased abundance of *Prevotellaceae*; on the genus level, there is an increased abundance of *Prevotella* and decreased abundance of *Faecalibacterium, Ruminococcus*, and notably, *Lactobacillus* and *Bifidobacterium* (Jiang et al. 2015; Y. Liu et al. 2016)**.**

*Depressive Symptoms Can Be Transmitted Following Fecal Microbiota Transplantation*

Additional support for the tenability of this model as a pathophysiological paradigm of depression's biological underpinnings, as well as the groundings for effective clinical treatment, may come most directly from research on the transfer of

psychological states following transplantation of fecal microbiota from diseased subjects. It has been found that transplanting fecal microbiota from depressed human patients to germ-free mice results in the presentation of increased depressive symptoms in the recipient mice (Zheng et al. 2016). A similar study illuminated the specific depressive symptomatic changes in antibiotic cocktail-treated and microbiota-depleted rodents who received transplants of fecal microbiota from depressed human patients. This study's results found transfer of depressive symptoms similar to their human microbiota providers, such as increased anxiety, anhedonia and tryptophan metabolism disturbances (Kelly et al. 2016). The transfer of psychological states and physiological symptoms of depression through transference of microbiota is further and strong evidence of the regulation of mental states by the gut microbiota. Additionally, this transference phenomenon may exist in the acquisition of microbiota from parents via longitudinal and horizontal gene transfer, further explaining the hereditary nature of psychiatric illness (Yatsunenko et al. 2012).

*Dysbiosis of the Gut Microbiota and Susceptibility to Depression*

Perturbations of the gut microbiota composition are becoming all the more common in 21st century western societies. Proliferating antibiotic use, increases in levels of chronic stress, and increasingly processed, poor diets, all serve to perturb human gut microbiota, increasing incidences of depression and other mental illness (Frei et al. 2012;

Sonnenburg et al. 2016; Sandhu et al. 2017). Despite the huge advances in human health from the threat of infection, antibiotics have indeed served to damage beneficial microbes, thereby perturbing the gut microbiota and the connected biological systems along the gut-microbiota-brain axis, resulting in increases in incidence of mental diseases, as well as somatic illness (Hu et al. 2015; Fröhlich et al. 2016; Wang et al. 2015). It has been shown that infants treated with antibiotics within the first year of life are more likely to suffer from behavioral problems and depression (Slykerman et al. 2017). Furthermore, the link between chronic stress and depression has been shown to not only be mediated by the brain's stress response system, but also by dysfunctions in the gut-microbiota-brain axis (Holdeman, Good, and Moore 1976). It was shown that chronic restraint stress disturbs the gut microbiota, leading to bottom-up effects such as decreased hippocampus 5-HT content, decreased mRNA expression of BDNF, increased stress hormones plasma levels, declines in circulatory IL-10 levels, resulting in depression (Liang et al. 2015). Not surprising to dietotherapy theory, diet has been implicated in the disruption of healthy gut-microbiota in the context of depression with poor diet and its subsequent microbiota perturbation leading to an increased incidence in depression (Yatsunenko et al. 2012; Frei et al. 2012). The Western diet, which includes highly refined foods, excessive levels of saturated fats, sugar, and food additives, has been shown to perturb the healthy gut microbiota and increase depression susceptibility

(Slyepchenko et al. 2017).

*Traditional Antidepressants and Their Mechanistic Connection to the GMB Axis*

There has been a growing research interest into probing the wide range of antidepressant therapies for antimicrobial effects on the gut microbiota, leading to the emerging hypothesis of antidepressant efficacy being at least partly mediated by the gut-microbiota-brain axis (Macedo et al. 2017; Yuan et al. 2015). This growing research interest is understandable considering the first antidepressant drug, isoniazid, was originally an antimicrobial agent used in *Mycobacterium tuberculosis* infections (Macedo et al. 2017). Indeed, looking across the many later developed antidepressants, antibiotic properties abound: first-generation tricyclic antidepressants (TCAs) inhibit many bacteria, including *E. coli*, *Yersinia*, and *Plasmodium*; selective serotonin reuptake inhibitors (SSRIs), the currently most popularly prescribed antidepressant inhibits Gram-positive bacteria; the newly developed antidepressant ketamine inhibits *Staphylococcus*, *Enterococcus*, and *Candida albicans*. Even more, it has been shown that the common antibiotics, such as minocycline and ceftriaxone sodium, possess antidepressant effects (Macedo et al. 2017). Together, the commonality in effects these two classes of medications share might imply a, at least partly, shared mechanism of

action.

*Modulation of the Gut Microbiota as a Treatment of Depression*

In addition to research showing compelling evidence for dysbiosis of gut microbiota leading to the development of disease, there is also increasing evidence for modulation and restoration of the microbiota bringing improvement to these diseases. There are four primary methods that have been shown to effectively recover healthy microbiota composition, which are prebiotics, probiotics, healthy diet (defined below), and fecal microbiota transplantation (FMT) (Sandhu et al. 2017; X. Liu, Cao, and Zhang 2015).

Probiotics and prebiotics, which are the live microorganisms which confer health benefits to a host and the substrates needed by these health-conferring, commensal microorganisms, respectively (Gibson et al. 2017). Due to the effects of these probiotics along the entirety of the gut-microbiota-brain axis and their ability to affect psychological well being, they are sometimes termed psychobiotics (Dinan, Stanton, and Cryan 2013). This designation of "psychobiotic" is earned by both clinical and animal studies showing

probiotics capable of alleviating depression at levels comparable to traditional antidepressant therapies (Akkasheh et al. 2016). Moreover, the antidepressant effect seen in administration of probiotics has been shown in animal models to be mediated specifically the gut-microbiota-brain axis (Bharwani et al. 2017; Liang et al. 2015). These probiotics shown to possess psychological benefit in these studies include *Bifidobacterium bifidum* , *Lactobacillus casei*, and *Lactobacillus helveticus* (Akkasheh et al. 2016; Liang et al. 2015). On top of direct modulation of the gut microbiota via supplementation of the health-conferring bacteria which may be in insufficient richness, modulation of the gut-microbiota-brain axis via supplementation of the raw substrates used generally across commensal bacteria, thus increasing richness from a microbial-nutritional level (X. Liu, Cao, and Zhang 2015). The most popular of these prebiotics being fibers, fructose-oligosaccharide and galacto-oligosaccharide, and omega-3 fatty acids (Gibson et al. 2017).

Similar to modulation of specific key microbial substrates, as in prebiotic supplementation, changes in diet can confer comparable effects on gut microbiota functionality; specifically, the changes to gut microbiota which result in increased MGB axis functioning are thought to be increases in diversity, stability and richness of the commensal microbiota (Gibson et al. 2017). Diets can achieve these prebiotic-like substrate-mediated effects by containing plentiful unsaturated fats, plentiful fiber, and fermented foods–such as in the Mediterranean diet.

The fourth main method for modulating the gut-microbiota is fecal microbiota transplantation from a healthy donor to the gut of a diseased gut, seeking to restore a healthy microbiota composition. This procedure has been shown to be effective in the treatment of *Clostridium difficile* infection, ulcerative colitis, and inflammatory bowel disease (Evrensel and Ceylan 2016).

Taken together, the current research on pro/pre-biotics, healthy diet, and FMT suggest these procedures, via their amelioratory effects on the functioning of the gut-microbiota-brain axis, may be promising future treatments for major depressive disorder (Dinan, Stanton, and Cryan 2013).

# CHAPTER III

# Conclusions

## *Limitations and Future Research Directions*

It is clear, however, by the overwhelming abundance of non-human studies in this field of research, that additional studies are required with human subjects so we can further illuminate the mechanisms and roles our human gut microbiota serve in the full range of neuropsychiatric, metabolic, and other diseases in which the animal model studies suggest might be implicated. Another limitation of this field of research is in the scarcity of replicated findings. However, it has been noted that the field's lack of replicated findings may not suggest false results, but instead speaks to methodological differences in sample collection and data analysis. There are many different techniques used to collect samples for microbiota analysis, ranging from a stool sample up to the much more invasive submucosal biopsy. The vast majority of published studies on the diversity of the gut microbiota utilize stool samples for microbial analysis in place of submucosal biopsies (Fraher et al., 2012). This over reliance on stool sampling may be a major limitation on the field's findings, as it has been widely shown that the mucosal microbiota differs strikingly from that within the stool. Further, this variation in

microbiota profiles between the two sampling methods is relevant to the state of the research, as we know stool samples tend to possess an abundance of luminal species while lacking entire mucosal populations, which facilitate the chief interactions with the immune system (Shi et al., 2017).

### *A Paradigm Shift in Neuropsychiatric Theory*

The results presented here show a growing interest in the scientific community to see neuropsychiatric health in more holistic, multi-system terms, increasingly appreciating the "second brain" of the enteric nervous system and its essential resident gut microbiota. These results seem to suggest potential avenues of therapeutic intervention acting on the immunological dysfunction, microbial metabolite production, and neurotransmitter metabolism which are all crucially linked with the physiology of the central nervous system. Beyond research investigating the gut microbiota for possible treatment modalities, there is much potential for prophylactic measures and novel methods of risk screening of different psychiatric disorders based on microbiomic profile.

### *Public Health Implications*

By virtue of the many factors affecting gut microbiota composition, variation

among populations and individuals is inevitable. These differences can be seen in dramatic microbial differences in a study investigating gut microbiota of children living in Burkina Faso, who had a *Firmicutes*/*Bacteroidetes* ratio of 0.47, while children in Italy had a F/B ratio of 2.8 (Filippo et al. 2017). Additionally, even more than the variation you would expect between the stark living differences between children in Italy versus Sub-Saharan Africa, there is good data to suggest there are differences among ethnic groups living in the United States as well. In a 2018 study comparing the gut microbiota across 1,673 healthy individuals in the United States, researchers found significant differences in taxonomic composition between 4 ethnicities (Brooks et al. 2018). These researchers found there are at least 12 microbial taxa which reproducibly vary in abundance between ethnicities.

It is clear from studies such as these, not only is there great taxonomic variation among individuals and groups, but these differences correlate tightly with social determinants of health, which disproportionately affect members of minority and lower socioeconomic status (SES) groups. As such, the gut microbiota may have potential to mediate public health disparities, giving further exigency to an already important scientific initiative. For this reason and the others described above, it is imperative for the fields of microbiomics and neuroscience to continue investigating the roles played by the gut microbiota in human health.

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