CHAPTER

22

Influence of Gut Microbiota on the Brain

Poorna J Visa, K Venkatraman, AV Srinivasan

ABSTRACT

The intercommunication between commensal microbiota and its host is necessary for regulation of various aspects of host physiology. These include immune function, nutrient processing, brain development and function. Gut microbiota influence the stress responses acting through the hypothalamic pituitary adrenal (HPA) axis or the sympathetic adrenal medullary axis resulting in immune function alteration. Alterations in gut microbiota is noted in neuropsychiatric disorders associated with inflammatory state changes such as major depressive disorder, schizophrenia, bipolar disorder as well as in autism and mood disorders. C.jejuni enhances anxiety like behavior by stimulating C- fos protein in selected regions of brain. Central nervous system (CNS) has the capacity to alter gut permeability, motility and secretion by stimulating the HPA axis, autonomic and neuro endocrine pathways which in turn can modulate gut microbial composition. Neuropsychiatric conditions like depression and autism occurs in high degree of concomitance with Gastro intestinal (G.I) disorders. Metabolic products of the microbial community mediates the state of health and illness. A positive the correlation between clostridia species and specific autistic characteristics has been established. Germ free mice are linked with reduced social skills, and this is associated with altered levels of von economo neurons (VEN) neurons. Dysbiosis is the recent concept postulated as a trigger for abnormal protein deposition in neurodegenerative disease like Parkinson disease and as a immunological trigger in RRMS. H.pylori infection increases antral relaxation, acting through the cholinergic nervous system or non-adrenergic – noncholinergic nervous system. Alteration in the process of colonization, in neonates could predispose to disease in later life. Normal microbiota plays a vital role in the development of the brain systematized by specific time constraints. Gut microbiome takes part in the myelination process involving the region of prefrontal cortex. Therefore alteration of gut microbiome is linked to aberrant myelination and neuropsychiatric symptoms.

INTRODUCTION

In today's stressful life with the increased incidence of stress related neuropsychiatric illness, advancement in the insight into gut microbiota, would prove indispensable. Our gut is composed of more bacterial organisms than the number of eukaryotic cells. Colonization with microbes could occur earlier than expected, where bacteria are identified in the placenta, umblical cord and amniotic fluid[4]. Gut microbiota plays a vital role in the post natal maturation of the immune system, digestion and absorption of macromolecules, protection of gut from pathogens and behavioral development.[1,2] There is a relationship between a person's emotional state and gastric acid secretion. This association is regulated by immune, metabolic, neural and endocrine pathway.[1-3] These concepts reflect the interplay between gut microbiota and brain.

Experiments to establish relationship between gut function and mood started as early as 18th century, when an army surgeon studied the gastric fluid secretions from a retained stomach orifice of an 18 year old, as part of his experiments, who he had operated on previously for an accidental gunshot wound, perforating the stomach among other injuries[5]. There is robust data showing co-existence of stress related CNS disease like depression with peripheral diseases like irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD)[6]. Maternal separation (MS) brings about stress associated G.I and mood disorders[6]. Disrupted normal microbiota plays a key role in the development of mood disorders and autism[7]. Bifidobacterium species was particularly susceptible to emotional stress[6]. There is no exact mechanism known by which gut microbiota influences the brain or whether the changes in the gut flora are the cause or effect of a disesase[8]. There are however links such as hormones, immune molecules and specialized metabolites found to play a role in the influence of gut microbiome on psychiatric neurological conditions like depression, anxiety, autism, schizophrenia and other neurodegenerative disorders[6]. The gut microbiota plays an important role in the maturation of the immune system. It triggers the innate immunity causing the maturation of gut associated lymphoid tissue, and acquired immunity by stimulating the local and systemic immune responses[6]. Persistent stimulation of the immune system by gut microbiota leads to low grade physiological inflammation, for defence against pathogens[6].

Gut Microbiota and Mood disorders

Role in anxiety: Serotonin is not only produced in the brain but also released largely by the enterochromaffin cells in the gut[2], which could be the basis for the concept, that the antidepressant drugs work better along with dietary modifications. Plasma levels of serotonin were 2.8 fold higher in conventional animals than germ free animals[2]. Increased plasma serotonin levels are an indirect effect of host microbe interaction[2]. Karolinska team found out

that in the striatum of germ free mice there was a higher production of important neurochemicals like serotonin[8] probably contributing to the lesser anxious behavior in germ free mice than mice colonized with indigenous microbes[8]. Gut microbiota by modulating neuronal proteins like synaptophysin and PSD – 95 influences anxiety like behavior in adult life. Further administered glucocorticoids reduce synaptophysin expression in the fetal brain, suggesting that stress hormones influenced by the gut microbiota play a role in this[9].

C.jejuni increases anxiety like behaviour[13]. C.jejuni infection induces c–fos protein expression in the basolateral nucleus of the amygdyla (BLA), paraventricular nucleus of the hypothalamus (PVN), central nucleus of the amygdyla (CEA), and bed nucleus of the stria terminalis (BST) [13]. These regions are involved in processing autonomic, neuroendocrine and behavioural responses to internal challenges like infection, processive and exteroceptive challenges and are responsible for shift in exploratory strategy with preference for safety over foraging.[13] Bacterial endotoxin could alleviate anxiety acting through cytokines[14]. Mice inoculated with C.jejuni, prior to the activation of cytokines, showed C- fos induction at the nucleus tractus solitarius region of the brain, but no c-fos induction in the enteric ganglion cells, indicating role of vagal sensory neurons, activated by immune cells, paneth cells, or by neuroactive bacterial products, in the early period following infection[14].

Role in depression: Depression is associated with G.I disorders like stress, Crohn's disease and ulcerative colitis[6, 7]. It is speculated that this is caused due to an imbalance between the sympathetic and parasympathetic discharge from the central nervous system, which would also contribute to behavioral impairment[10]. There is a hypothesis linking T cell deficiency to behavioral impairment[10]. However whether these cells act from the periphery or cross the blood brain barrier is unknown. Lactose malabsorption albeit does not produce an effect by itself, but along with fructose malabsorption intervenes with L- tryptophan metabolism and therefore low serum tryptophan levels were especially found in the serum of fructose malabsorbers[11,12]. Reduced L-tryptophan predisposes to depression[11]. Therefore depression is influenced by changes in metabolic activity and composition of gut microbes[2, 6,11,12]. It is postulated that toxic bacterial by products reaching the colon causes a disruption of neurotransmitter metabolism and contribute to depression[12]. Collado et al using the heuristic maternal separation model showed that, maternal separation caused behavioral impairment relating to depression which was accompanied by biochemical changes like reduced noradrenaline in the brain, increased cortisol releasing factor mRNA levels in the amygdaloid cortex, and elevated release of cytokine IL – 6 after immune stimulation in MS rats[4].

Probiotic Bifidobacterium has a therapeutic effect on stress related psychiatric diseases affecting the CNS function like depression and anxiety[6]. Probiotics exert their antidepressive therapeutic influences in rodents by bringing about various changes in neurotransmitters, growth factors and neuromodulators[15]. Probiotic Bifidobacterium therapy produced normalization of immune response, restoration of noradrenaline levels and reversal of behavioral impairments[6]. Studies have shown possible therapeutic approaches which are as follows: Keshavarzi, Zakieh, et al showed that plant aloe vera decreased gastric acid output[10]. This effect could be due to the composition of the plant contributing to increased mucous blood flow and elevated intestinal mucin and bicarbonate secretion.[10]

Gut Microbiota and Stress Response via HPA axis

Neuroendocrine pathways stimulating stress response are the HPA axis acting through glucocorticoid secretion and the sympathetic branch of the autonomic nervous system acting through the release of epinephrine and norepinephrine[3]. The products of the endocrine system and products of the nervous system bind to receptors on the various immune cells, like monocytes, macrophages, lymphocytes and granulocytes. This causes a change in CD4 T helper cells profile from Th1 cells to Th2 cells resulting in dysregulation of cell mediated immune system (Th1 cells) and activation of antibody production (Th2 cells)[13].

Stress exposure alters gut activity by causing stomach and small intestinal hypo motility which causes bacterial overgrowth, along with a decrease of lactobacilli and Bifidobacterium level in the gut, also referred to as dysbiosis.[12] Stress increases mucin secretion which acts as a substrate for bacterial overgrowth. Stress also stimulates colonic inflammation and increases innate immunity. Probably the bacterial cell wall components[3,17] stimulate the immune cells of the gut to produce cytokines which by acting on regions of the central nervous system involved in the HPA axis can modify the neuroendocrine activity and behavioral responses[3]. Administration of exogenous glucocorticoid betamethasone, caused loss of important proteins involved in brain morphogenesis and function, such as microtubule associated protein and synaptophysin[9,18.] . This substantiates the hypothesis of gut brain communication through the HPA axis in stress. Stress is also associated with immune system impairment as the immune system is integrated in the bidirectional network with the HPA axis. Further factors that support the concept linking stress, immune activation, and gut microflora changes in association with gut brain axis can be emphasized by studying the effect of probiotic use. Exposure to early post natal stress results in impairment of the HPA axis causing hyper responsiveness to ensuing stress stimuli, resulting in long term excessive stimulation of glucocorticoids[19]. Persistent increased stimulation of HPA axis results in increased neonatal corticosterone levels[19]. This state if continued to exist in female adults will in their later life manifest as adult elevated Adrenocorticotropic hormone (ACTH) levels. Maternal probiotic inoculation protects against immune dysfunction and disruption of adult gut microflora following exposure to neonatal maternal separation

or adult restraint stress[19]. This it achieves by increasing plasma IgA levels. IgA takes part in the first and second line defence against the pathogens breaching the mucosal surface[19].

Maternal separation affects the normal balance of gut microbiota. This occurs due to increase in aerobic bacteria like enterococci, anaerobic bacteria like clostridia and gram negative bacteria like E.coli while levels of beneficial bacteria like lactobacilli and bifidobacterium is maintained unchanged[19]. Study shows that although neonatally stressed adults did not show any change in their gut microflora, when these animals were exposed to an acute stressor in adulthood it resulted in decreased fecal counts of anaerobic bacteria and clostridia. This shows that exposure to stress in early life, sensitizes certain gut microbiota to stress exposure to later life[19].

Gut Microbiota and Autism

Autism begins in early childhood and is associated with deficits in social, communication and imaginative development. Changes in the metabolic products and composition of the gut microbiome have been connected to the pathophysiology of autism spectrum disorder (ASD) [7,20,21]. There is a frequent association of G.I problems in children with autism as compared to the general population[7,20]. GI problems occur in parallel with behavioral symptoms more in autistic patients than healthy individuals[22]. Significant colonization with distinct Clostridial species in gastrointestinal flora and evidence of Clostridial species in the fecal sample[21] were found in autistic children in comparison to healthy children[21,22]. It is postulated that serum concentration of lipopolysaccharide is considerably higher in autistic patients in comparison with healthy individuals,[7] this was inversely related to socialization scores in a independent manner[7]. Further studies prove that interventions with antibiotics and probiotics improve symptoms in ASD subjects[19,20]. These findings support the concept of modulation of gut barrier integrity and the role of microbiota in the origin of ASD[7]. Bacterial mediated production of indole containing metabolites like indole -3 – propionic acid is based on the presence of the particular bacterium, Clostridium sporogens of gut microflora. The accumulated propionic acid induces mitochondrial dysfunction that leads to energy failure affecting the brain and gut of the autistic children.

Treatment with Vancomycin caused substantial improvements in ASD but was transient as following two weeks after treatment cessation, there was regression probably due to the continued existence of spores of clostridia[20]. This short term supression of autism related symptoms was probably due to the effect of the antibiotic in eliminating the neurotoxin produced by the bacteria[20]. This indicates colonization or action of neurotoxin produced by clostridial species to play a role in ASD[20]. Symptoms of ASD is strongly correlated to neurocognitive impairment, in preterm children[3,20,21]. In a study conducted by L.Desbonnet et al, germ free (GF) mice showed altered sociability including social avoidance, reduced preference for social novelty and repetitive self-grooming behavior. This study shows the important role played by microbiota in the presentation of distinct normal social behavior which is impaired in neurodevelopmental disorders like schizophrenia and autism. There was reduced activity in the right anterior insular region in social tasks as opposed to non social tasks of the brain in autistic subjects when compared to the controls[8,22,23]. This suggests that VEN might play a role in autism[8,23]. Recent studies also highlight the role Hyperbaric Oxygen Therapy (HBOT) to reduce the mitochondrial dysfunction and have a good outcome in the behavioral improvement and gut functions of the ASD children [29].

Association Between Gut Microbiota, Myelination and Neuropsychiatric Disorders

Study has shown the association of microbiome with cortical myelination. Appropriate presence of functional microbiota is essential for the correct cortical myelination at well suited times of neurodevelopment[24]. This study revealed hypertrophic neuronal signaling in GF mice leading to hypermyelination in the prefrontal cortex (PFC). These changes in myelination could be reversed on colonization with conventional microbiota following weaning[24]. Therefore microbiota could serve as therapeutic targets in psychiatric disorders which are linked to dysmyelination process and have been advocated for remyelination in myelination diseases[24].

Gut microbiota and Parkinsonism:

The severity of motor impairment is related to gastroparesis which occurs frequently in Parkinsonism disease (PD). Reduced abundance of Prevotellaceae bacteria and increased abundance of Enterobacteria were found to be prevalent in the postural instability with gait difficulty phenotype of Parkinson disease than the tremor dominant type[30]. Prevotellaceae bacteria is commensal are involved in mucin synthesis in the gut mucosal layer and production of neuroactive short-chain fatty acids (SCFA) through fiber fermentation. Reduced abundance of Prevotellaceae results in decreased mucin synthesis and increased intestinal permeability leading to the greater local and systemic exposure to bacterial antigens and endotoxins, which in turn would trigger or maintain excessive alpha-synuclein expression in the colon or even promote its miscoding. These misfolded alpha-synuclein may spread along the vagal nerve to the brain stem in a caudo-rostal pathway and reach the dorsomotor vagus nuclei, from there spread to the pedunculopontine nuclei and rostrally to the substantia nigra and basal forebrain explaining the gastrointestinal symptoms like gastroparesis, bloating and constipation prior to the motor symptoms in Parkinson disease. PD through its gastroparesis and impaired GI motility may predispose to SIBO (small intestinal bacterial overgrowth). SIBO independently predisposes to worse motor function. It is possible that SIBO contributes to motor dysfunction by disrupting small intestinal integrity leading to immune stimulation and/or alteration in L-dopa absorption[30]. Also SIBO may cause changes in the gut permeability which

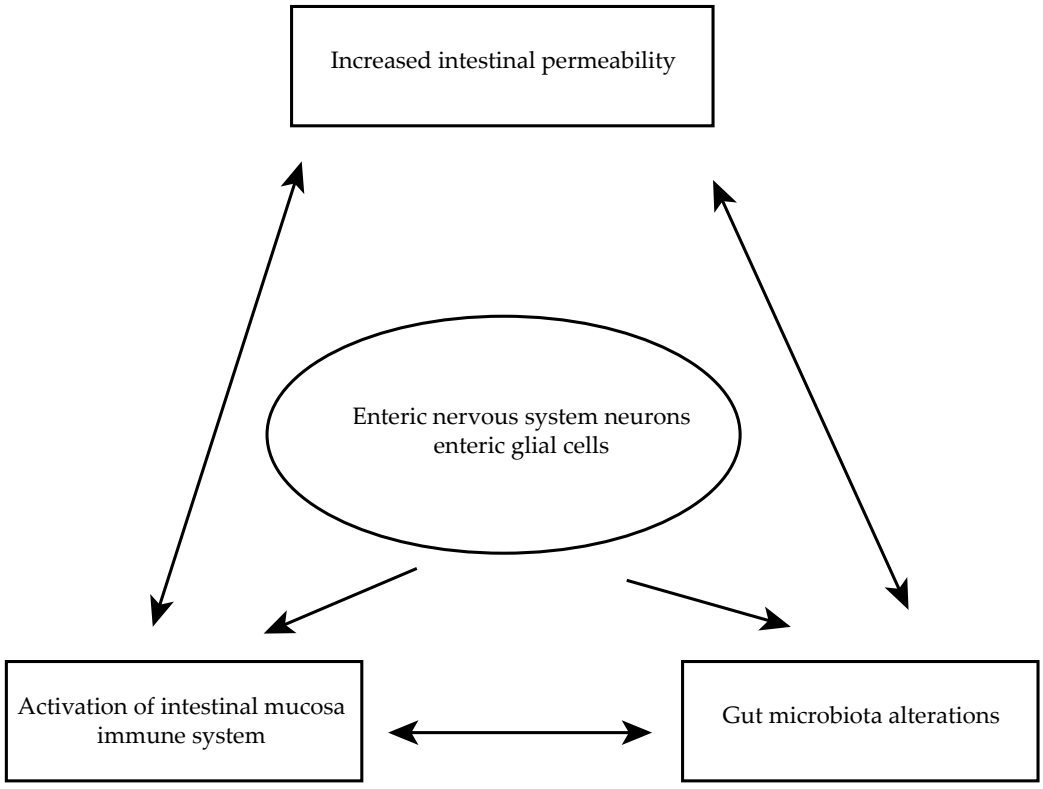

Fig. 1: The link establishing the gut dysbiosis, intestinal permeability and neurological dysfunction in Parkinsons disease

promotes translocation of bacteria and endotoxins across the intestinal epithelium, inducing the pro-inflammatory response along with accumulation of misfolded alpha-synuclein enteric neurons. Lipopolysacharide derived from gram negative enterobacteria may cause delayed and progressive nigral pathology. Neural dysfunction correlated more with inflammatory response than the extent of bacterial colonisation[14]. Thus a link between gut dysbiosis, intestinal permeability and neurological dysfunction has been well documented in PD (Figure 1).

H. pylori is correlated with detrimental motor function of Parkinson disease [25]. H pylori infection, caused increased muscle contractility which may be due to increased polymorphonuclear cells (PMN) infiltration[14]. Also antral relaxation was higher which was maybe due to incresed neuronal inhibition or decreased neuronal excitation. H pylori infection caused reduced acetyl choline (ACh) release[14]. This lower levels of ACh release was due to either disrupted synthesis or storage of ACh.[14] Hence abnormal cholinergic nerve function could be the reason for the higher degree of antral muscle relaxation[14]. H pylori by increasing levels of vasoactive intestinal polypeptide (VIP), and causing changes in nitric oxide (NO) containing neurons, contribute to increased antral relaxation[14].

Influence of Maternal Microbiota on the Fetus and its effect on the Brain Development

Neonates are first brought in contact with microbes from maternal microbiota[4]. There are three specific types of microbiota called the enterotypes, manifested at a early stage in the neonates and therefore have a long term effect on health and immunity of the neonate[4].

Breast milk is an efficient and sustained source of streptococci, staphylococci, lactic acid bacilli, and bifidobacterium[4]. This growth of beneficial bacteria is also contributed by oligosaccharides[4]. Exposure to microbes before birth, during pregnancy and lactation has an impact on the metabolic and immunologic profiles of the pregnant uterus. Commensal microbiota not only plays a role in the development of the immune system but is also necessary for brain development[4]. However this regulation has definitive time restraint. Gut microbiota can influence the development of the brain and modulates behavior like decreasing physical activity and increasing anxiety[4]. Exposure to IL – 6, in utero could alter brain development resulting in permanent behavioral damage[4]. Therefore it is important to understand the time period when changes to the immune function could make the brain susceptible for injury and the period most appropriate for restoration of the brain function[4].

Gut microbiota and hologenomic theory of evolution

Hologenome and holobiont occur in concord to act as a selection unit in evolution. The diverse microbial population by holding a symbiotic relationship with the host causes changes in the hologenome which makes the holobiont more fit and adaptive to the changing enviornment[28]. The capacity for these variations in the hologenomes should be thought of, considering the fact that there are 150 times more bacterial genes than human genes in the gut[28].

The host and symbiont become totally dependent on

each other especially at times of absolute mutualism. Among other benefits most importantly this symbiotic relationship favour colonisation of commensal microbiota instead of pathogenic microbes thereby protecting against infections[28].

Acquisition of new symbionts from the enviornment leads to incorporation of novel genes into the hologenome[28]. This concept opens doors to synthesising genetically modified probiotics. This would aim at achieving favourable hologenomic modifications by changing symbionts genome. This in turn would help the host to survive in adverse conditions.

Gut microbiota in the pathogenesis of Multiple Sclerosis

Multiple sclerosis, an autoimmune disorder characterised by immune mediated destruction of the oligodendrocytres of the central nervous system neurons has been characterised by demyelination and axonal damage. Several hypothetic models have been put forth to explain the autoimmune trigger for this disorder and the latest and convincing model is the altered gut microbiota. Several organisms including Parabacteroides, Prevotella (Bacteroidetes), Adlercreutzia which are involved in the metabolism of phytoestrogens, plant-derived xenoestrogen and Collinsella (Actinobacteria), and Erysipelotrichaceae (Firmicutes), involved in the bile acid metabolism were found to be decreased in abundance in the relapsing remitting multiple sclerosis (RRMS) patients compared to the healthy controls. Metabolites derived from the metabolism of phytoestrogens (lignan and isoflavone) and bile acids play an important role in maintaining homeostasis at mucosal surfaces through the induction of anti-inflammatory responses. Thus a perturbation of the gut microbiota results in the altered homeostasis and thus leads on to the activation of immune responses that might trigger a autoimmune trigger in RRMS[31].

Role of Nervous system in Gut infection

The GI tract acts as a key interface between the body and the pathogen, the lining epithelium, holds the ability to recognise pathogens, followed by stimulating the host defence mechanism and thereby eliminating the pathogen. The epithelium incorporates macrophages, mast cells and dendritic cells which are innervated by nerve fibres belonging to the enteric ganglia, vagus and spinal visceral sensory fibres. It is postulated that the signals generated by these nerve fibres are responded by the immune cells which are located in close proximity to the nerve fibres[14]. Also peripheral neurons could take part in this by being activated through bacterial products or through bacterially derived neuroactive substance like the GABA or histamine[14]. Early vigorous host defence is through neural response which is probably followed by immune mediated brain signaling via the cytokines[14]. Bacterial stimulation of vagal sensory neurons indicates the possibility of peripheral nerves to direct local immune or inflammatory condition to play a role in signaling the immune activity to brain pathway which in turn could contribute to important information like the location of infection corresponding to host defence[14].

CONCLUSION

Gut microbiota has a tremendous influence on the brain. This review focuses on the influence of gut microbiota on diseases concerned with the central nervous system such as depression, autism, Parkinsonism, demyelination diseases and other psychiatric diseases associated with sociocognitive deficits. Various pathways involved in the gut brain axis including some details of the immune and inflammatory system pertaining to the aforementioned conditions is mentioned. This review paves way to the idea that administration of aloe vera, or probiotics, or implementation of novel hologenomics and likewise alternative strategies alongside antibiotics could maintain the balance in gut microbiota. This in turn throws light on the speculation of extending the therapeutic benefits of antibiotics from short term to long term treatment of autism spectrum disorder. Therefore this review advocates the need for further research in this area with the perception; that the harmony in the gut microbiota would enable to improve the treatment of neuropsychiatric diseases from bench to the bedside.

ABBREVIATIONS

1. CNS – Central nervous system
2. G.I – Gastrointestinal
3. VEN – Von economo neurons
4. IBS – Irritable bowel syndrome
5. IBD – Inflammatory bowel disease
6. BLA - Basolateral nucleus of the amygdyla
7. PVN - Paraventricular nucleus of the hypothalamus
8. CEA - Central nucleus of the amygdyla
9. BST - Bed nucleus of the stria terminalis
10. GABA – Gamma amino butyric acid
11. IL – 6 - Interleukin 6
12. Th1 and Th2 – T helper cell 1 and T helper cell 2
13. HPA axis – Hypothalamic pituitary adrenal axis.
14. ACTH – Adrenocorticotropic hormone.
15. Ig A - Ig A antibody
16. GF - Germ free
17. ASD – Autism spectrum disorder
18. PFC - Prefrontal cortex
19. Ach - Acetyl choline
20. VIP - Vasoactive intestinal polypeptide
21. NO - Nitric oxide
22. CGRP - Calcitonin gene related peptide
23. SP - Substance P

24. ACC - Anterior cingulate cortex
25. SIBO- small intestinal bacterial overgrowth
26. PD- Parkinson disease
27. RRMS- Relapsing Remitting Multiple Sclerosis

REFERENCES

1. Ghosh, Amit, et al. "Microbes-Gut-Brain Axis: A Possible Future Therapeutics Target for Gastrointestinal and Behavioral Disorder." *International Journal of Health Sciences and Research* 2015; 5.1:321-29.
2. Wikoff, William R., et al. "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites." *Proceedings of the National Academy of Sciences* 2009; 106.10:3698-3703.
3. Galley, Jeffrey D., and Michael T. Bailey. "Impact of stressor exposure on the interplay between commensal microbiota and host inflammation." *Gut Microbes* 2014; 5.3:390-396.
4. Collado, Maria Carmen, et al. "Microbial ecology and host-microbiota interactions during early life stages." *Gut Microbes* 2012; 3.4:352-365.
5. Beaumont W. Experiments and Observations on the Gastric Juice and the Physiology of Digestion. Plattsburg: F.P. Allen; (1833).
6. Desbonnet, L., et al. "Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression." *Neuroscience* 2010; 170.4:1179-1188.
7. Mangiola, Francesca, et al. "Gut microbiota in autism and mood disorders." *World Journal of Gastroenterology* 2016; 22.1:361.
8. Smith, Peter Andrey. "The tantalizing links between gut microbes and the brain." *Nature* 2015; 526:312-314.
9. Heijtz, Rochellys Diaz, et al. "Normal gut microbiota modulates brain development and behavior." *Proceedings of the National Academy of Sciences* 2011; 108.7:3047-3052.
10. Keshavarzi, Zakieh, et al. "The effects of aqueous extract of Aloe vera leaves on the gastric acid secretion and brain and intestinal water content following acetic acid-induced gastric ulcer in male rats." *Avicenna Journal of Phytomedicine* 2014; 4.2:137.
11. Ledochowski, M., et al. "Carbohydrate malabsorption syndromes and early signs of mental depression in females." *Digestive Diseases and Sciences* 2000; 45.7:1255-1259.
12. Ledochowski, M., B. Sperner-Unterweger, and D. Fuchs. "Lactose malabsorption is associated with early signs of mental depression in females (a preliminary report)." *Digestive Diseases and Sciences* 1998; 43.11:2513-2517.
13. Goehler, Lisa E., et al. "Campylobacter jejuni infection increases anxiety-like behavior in the holeboard: possible anatomical substrates for viscerosensory modulation of exploratory behavior." *Brain, Behavior, and Immunity* 2008; 22.3:354-366
14. Berčík, Přemysl, et al. "Immune-mediated neural dysfunction in a murine model of chronic Helicobacter pylori infection." *Gastroenterology* 2002; 123.4:1205-1215.
15. Jagmag, Shail Adrian, et al. "Exploring the Relationship between Gut Microbiome and Depression." *Trends Gastroenterol* 2016; 1.001.
16. Padgett, David A., and Ronald Glaser. "How stress influences the immune response." *Trends in Immunology* 2003; 24.8:444-448.
17. Sudo, Nobuyuki, et al. "Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice." *The Journal of Physiology* 2004; 558.1:263-275.
18. Antonow-Schlorke, Iwa, et al. "Glucocorticoid exposure at the dose used clinically alters cytoskeletal proteins and presynaptic terminals in the fetal baboon brain." *The Journal of Physiology* 2003; 547.1:117-123.
19. Barouei, Javad, Mahta Moussavi, and Deborah M. Hodgson. "Effect of maternal probiotic intervention on HPA axis, immunity and gut microbiota in a rat model of irritable bowel syndrome." *PLoS One* 2012; 7.10:e46051.
20. Parracho, Helena MRT, et al. "Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children." *Journal of Medical Microbiology* 2005; 54.10:987-991
21. Mayer, Emeran A., et al. "Gut microbes and the brain: paradigm shift in neuroscience." *The Journal of Neuroscience* 2014; 34.46:15490-15496.
22. Desbonnet, L et al. "Microbiota Is Essential for Social Development in the Mouse." *Molecular Psychiatry* 2014; 19.2:146–148.
23. Allman, John M., et al. "The von Economo neurons in frontoinsular and anterior cingulate cortex in great apes and humans." *Brain Structure and Function* 2010; 214.5-6:495-517.
24. Hoban, A. E., et al. "Regulation of prefrontal cortex myelination by the microbiota." *Translational Psychiatry* 2016; 6.4:e774.
25. Parmar, Arpit. "Gut–brain axis, psychobiotics, and mental health." *Asian Journal of Psychiatry* 2016; 22:84-85.
26. Critchley, Hugo D., Christopher J. Mathias, and Raymond J. Dolan. "Neural activity in the human brain relating to uncertainty and arousal during anticipation." *Neuron* 2001; 29.2:537-545.
27. Mayer, Emeran A. "Gut Feelings: The Emerging Biology of Gut–brain Communication." Nature reviews. *Neuroscience* 2011; 12.8:10.1038/nrn3071.
28. Singh, Yadvir, et al. "Emerging importance of holobionts in evolution and in probiotics." *Gut Pathogens* 2013; 5.1: 1.
29. Daniel.A.Rossignol. Interrelationships among the Gut, Mitochondrial Function, and Neurologica Sequelae. Autism science digest. The journal of autismone : issue 04 :57-65
30. Agata Mulak, Bruno Bonaz.Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol* 2015; 21:10609-10620
31. Jun Chen, Nicholas Chia. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. www.nature.com Scientific Reports | 6:28484 | DOI: 10.1038/srep28484.