THE GUT MICROFLORA-BRAIN CONNECTION AND NEUROLOGIC DISORDERS – A REVIEW OF THE EVIDENCE – PART I

INTRODUCTION

There is certainly little question that, over the last 10-20 years, one of the most popular topics of interest among nutritional and functional medicine practitioners has been the role of gut microflora and human health. Furthermore, there is also little question that the vast majority of conversations, seminars and published papers on the subject have addressed gut microflora purely in terms of its role in GI health, particularly in reference to the ever increasing number of patients who present with issues such as irritable bowel syndrome (IBS), small intestinal bacterial overgrowth (SIBO), and inflammatory bowel disease. For me, this is ironic because most of us are aware, at least on a conceptual level, that the microflora residing in the gut are well documented to have a powerful influence on the health and well-being of many organ systems outside the gut. Why is it that we do not talk more about the role of gut microflora in influencing biochemistry and physiology not involving the GI tract? I would surmise that one reason is that most of the information we receive on the gut microflora-systemic health relationship is either highly technical, highly academic or both, with little or no emphasis on practical, clinical applications. Evidence of this was readily apparent to me as I began looking into published papers on the gut microflora-brain relationship. It seems I was spending hours reading paper after paper reporting an endless stream of animal studies on this connection that, to me, have little or no practical value in terms of addressing the needs of patients suffering from behavioral or neurodegenerative disorders.

Fortunately, after literally dozens of hours of reading papers, I was able to find a few that were applicable to the clinician who is looking for additional answers to addressing the needs of patients suffering from behavioral and neurodegenerative disorders. Does what I am about to present replace all that we have traditionally done in terms of optimizing neurotransmitter activity and brain health plus all that I suggested in my previous newsletter series on brain health, inflammation, and insulin resistance? Certainly not. For, there is no question that this approach has provided assistance for many patients. However, what I would like to suggest is that, when your efforts to gain satisfactory results with patients suffering from behavioral and neurodegenerative ailments have not yielded the outcome you and your patients have expected, take a look at the gut, with specific emphasis on gut microflora.

What I am going to present in this series is not only evidence that this approach to brain health has validity, but at least some basic ideas about the practicalities of optimizing the gut microflora-brain connection to yield the best possible results in the patients for whom nothing else has worked as expected.

BASIC PRECEPTS OF THE GUT MICROFLORA-BRAIN CONNECTION

In part I of this series I would like to present highlights of papers that give a basic overview of the gut microflora-brain connection and why it is important clinically. The first paper is “Gut microbiota-brain axis” by Wang and Wang (1). The first quote from this paper that I would like to feature points out the importance of the gut microflora-brain connection:

“Although the exact mechanism of gut microbiota-brain axis has not yet been fully understood and clarified, the evidence from animals and human studies has showed that gut microbiota can play an important role in brain behavior and cognitive development by producing hormones, immune factors, and metabolites, which also indicated that altering the gut microbiota may improve or even cure brain diseases.”
The gut microbiota and the brain interact with each other, and the gut microbiota can be regarded as an independent variable in gut microbiota-brain axis, its effect on the brain regarded as a dependent variable.”

The next section of the paper provides some historical context as to events that led to an awareness that there was a connection between gut microflora and brain function:

“…a public health emergency aroused people’s attention to the possible relationship between the gut microbiota and the brain. In the year 2000, the flood occurred in the town of Walterton, Canada, making the drinking water polluted by Escherichia coli and Campylobacter jejuni. Among the 4561 infected participants, 2451 of them completed a reassessment 8 years later, and 1166 of them were diagnosed with irritable bowel syndrome (IBS). Among these IBS patients, anxiety and depression were found to be independent risk factors for continuous IBS.”

The next quote highlights notable animal research that followed which provided further support for the idea that there, indeed, was a connection between gut microflora and brain health:

“In the year 2011, a study by Diaz Heijtz et al. showed that compared with conventional mice who were growing in specific-pathogen-free (SPF) environment, germ-free (GF) mice under the experimental conditions had less anxiety-like behaviors and increased 5-HT synthesis in the thalamus. When moving the adult GF mice to SPF environment, its reduced anxiety-like behavior did not increase, but its offspring’s anxiety returned to the normal state, which indicated that there was a critical time window for the influence of gut microbiota on behavior development.”

How do gut microflora affect the brain? Wang and Wang (1) mention four ways:

“Generally speaking, gut microbiota exerts effects on the brain not only through the nervous system (gut-brain’s neuroanatomical pathway) but also through the endocrine system, immune system, and metabolic system. A bidirectional communication between the gut and the brain is referred to as the gut-brain axis. Interaction of the gut microbiota and gut-brain axis is referred to as the gut microbiota-gut-brain axis (hereinafter referred to as the gut microbiota-brain axis.”

Next the authors discuss each of the four ways gut microflora can interact with the brain in detail.

Neuroanatomical pathways

“The gut can interact with the brain through two neuroanatomical pathways. The first one is mutual information exchange directly between gut and brain by the autonomic nervous system (ANS) and vagus nerve (VN) in the spinal cord; another one is a bidirectional communication between gut and brain through the bi-communication between enteric nervous system (ENS) in the gut and ANS and VN within the spinal cord.”

The authors then point out the following concerning these neuroanatomical pathways:

“Direct neural communication between gut microbiota and the brain is mainly realized through VN, i.e., bacteria stimulates afferent neurons of ENS, and the vagal signal from the gut can stimulate the anti-inflammatory response, preventing against pyosepticemia caused by microorganisms.”

Neuroendocrine–hypothalamic–pituitary–adrenal axis

Animal research has demonstrated that gut microflora are essential for proper development of the HPA axis early in life. Wang and Wang (1) state:

“…feces contained gut microbiota were vital for the postnatal development of appropriate stress reaction, and the timing that microbiota appeared in early life was a very narrow window, which was extremely important for normal development of HPA axis.”

Gut immune system

“Development of the gut immune system depends on gut microbiota. Germ-free mice had no immune activity, but they
could generate immune function when given certain microbiota.”

**Direct metabolic impact through neurotransmitters and neural regulators synthesized by intestinal bacteria**

Wang and Wang (1) state the following concerning a direct metabolic impact of gut microflora:

“Gut bacteria can synthesize gamma amino acid, butyric acid, serotonin (5-HT), dopamine, and short-chain fatty acids (SCFAs), and these substances can exchange between cells and microorganism, especially intestinal cells in the gut and can produce many serotonin neurotransmitters that have an effect on the brain. Bacterial enzymes can also produce neurotoxin products such as D-lactic acid and ammonia. Hence, a lot of necessary neurotransmitters in the body are generated by the gut microbiota, exerting influence on the human body including the brain, among which many neurotransmitters in the human gut microbiota are also critical molecules.”

**Intestinal mucosal barrier and blood-brain barrier (barrier system)**

Wang and Wang (1) also mention one other way gut microflora can affect the brain – through their impact on two barriers – the intestinal mucosal barrier and the blood-brain barrier. This can happen under stressful circumstances that lead to increased intestinal permeability. This increased permeability, in turn, can lead to endotoxins produced by gut microflora to enter the general circulation, leading to production of inflammatory cytokines. These inflammatory cytokines can consequently have an impact on the blood-brain barrier:

“Peripherally produced inflammatory factors could increase the permeability of the blood-brain barrier, thus making it possible for peripherally produced inflammatory factors to directly influence the brain.”

For an overview of the ways gut microflora can affect the brain, please see figure 1 on page 8 of this newsletter. The figure comes from the Wang and Wang (1) paper.

The next paper I am going to discuss provides more detail on the ways gut microflora directly impact neurotransmitter status. In “Microbes and the mind: emerging hallmarks of the gut microbiota-brain axis” by Bauer et al (2) the first direct neuromodulating component produced by gut microflora discussed by the authors is short chain fatty acids.

**Short-chain fatty acids**

“Within the GI tract, microbes metabolize dietary fibre into short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate. An important energy source for the host, these bacterial metabolites also have biological activity.”

Concerning the impact of SCFAs on the nervous system the authors state:

“SCFAs...can interact with neurons in the CNS and enteric nervous system (ENS), regulating heart rate, oxygen consumption and GI motility. SCFAs have been shown to initiate beneficial glucose metabolism through a gut-brain neural circuit, a possible mechanism for the benefits of high-fibre diets.”

**Brain-derived neurotrophic factor**

Another neuromodulating factor affected by gut microflora is brain-derived neurotrophic factor (BDNF). Concerning BDNF, Bauer et al (2) point out:

“BDNF, the most abundant neurotrophin in the human cortex, enhances neuroimmune responses and coordinates synaptic formation, plasticity and function. In addition, this secretory protein regulates neuronal differentiation, proliferation and survival and has a critical role modulating memory and learning formation.”

In relation to its connection with gut microflora, the following is stated:

“In addition to genetic and epigenetic control of BDNF, prebiotics and diet regulate BDNF levels, which suggests a potential microbial-directed mechanism.”

Furthermore, in studies on non-germ free animals, the following was noted:

“In non-germ free studies, alterations of the gut microbiota via infection and antibiotics also influence BDNF expression.”
Gamma-aminobutyric acid (GABA)

Since GABA is readily available as a supplement, the role of GABA as an inhibitory neurotransmitter is fairly well known among nutritional practitioners. What may be less well known, though, is its relationship with gut microflora. Bauer et al (2) begin their discussion on GABA by pointing out some of the basics concerning GABA and its role in neurologic function:

“γ-Aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the CNS. Like BDNF, GABA deficiencies have been linked with anxiety, depression and Alzheimer’s disease. Within the brain, GABA biosynthesis occurs via the glutamine-glutamate-GABA cycle.”

What is the relationship between GABA and the gut? The authors continue:

“However, GABA is also found throughout the enteric nervous system and GI tract. In a recent ex vivo study, Barrett et al. demonstrated that Lactobacillus and Bifidobacterium strains isolated from the human gut microbiota synthesized GABA when grown in glutamate-containing media. Germ free mice also exhibit decreased levels of GABA within the colon, suggesting in vivo production of GABA by the gut microbiota. While intestinally synthesized GABA has not been shown to enter the brain, ‘gut GABA’ may modulate CNS via ENS or vagal nerve activation.’”

In terms of the impact of probiotic supplementation on GABA, this relationship has been examined in animal studies. Bauer et al (2) point out:

“In 2011, researchers examined the role of the gut microbiota on GABA expression within the brain. Mice treated with Lactobacillus rhamnosus, a probiotic with anti-inflammatory properties, exhibited altered GABA receptor expression in the brain accompanied by reduced anxiety- and depressive-like behaviors.”

Before continuing, I would like to go back to the last sentence in the second quote above on GABA that discusses the impact of GABA synthesized in the gut. In stating that, since it cannot cross the blood–brain barrier, it affects the brain through its actions on the enteric nervous system and the vagus nerve, it, by extension, addresses a controversy nutritional practitioners have been debating for years about the impact of supplemental GABA.

On one hand, many nutritional practitioners have and continue to maintain that supplemental GABA has a fairly predictable positive impact in patients experiencing behavioral symptomatology that goes way above and beyond what would be expected from a simple placebo effect. On the other hand, many researchers and conventional medical practitioners insist that, since GABA cannot cross the blood–brain barrier, any change in behavior seen in patients after supplemental GABA administration, no matter how profound, must be due to placebo effect. Since, as noted above, GABA located in the gut can affect brain function via its impact on the enteric nervous system and the vagus nerve, the position of those who maintain that supplemental GABA has a positive impact beyond placebo effect appears to be supported.

Serotonin

Next to GABA, since so many natural substances and pharmaceuticals impact behavior through serotonin modulation, serotonin is fairly well known among virtually all health care practitioners. As with GABA, what may be less well known is the relationship between serotonin and gut microflora. Bauer et al (2) began their discussion on serotonin by describing its basic functions:

“Serotonin (5-hydroxytryptamine, 5-HT) regulates diverse biological processes, including respiration, GI secretion and peristalsis, cardiovascular responses, and behavioural and neurological functions. Alterations in serotonin neurotransmission and expression are linked with multiple psychiatric disorders, notably depression.”

While I’m sure many of you are aware of this, what may be unknown to others is how much serotonin is produced in the gut:

“The vast majority of serotonin, an estimated 95%, is found within the GI tract. Here, 90% of serotonin is localized within the epithelial enterochromaffin cells (ECs) and the remaining 10% remains within the ENS.”

Furthermore, production of the serotonin in the GI tract does involve gut microflora:

“...while serotonin biosynthesis in the brain remains largely independent of the
GI tract, serotonin biosynthesis within the GI tract involves specific microbiota-gut interactions.”

This interaction occurs via the following pathway:

“Spore-forming bacteria within the gut microbiota release metabolites, including SCFAs, signaling EC production of serotonin. These microbially stimulated ECs increase expression of tryptophan hydroxylase 1 (Tph 1), the enzyme catalyzing the rate-limiting step of serotonin biosynthesis from tryptophan.”

Of course, no discussion on serotonin and tryptophan in relation to neurologic function would be complete without mentioning the kynurenine pathway, which I discussed in detail in the previous newsletter series. Even though I discussed the kynurenine pathway over several newsletters, still more needs to be stated in terms of its relationship with gut microflora. Bauer et al (2) state:

“The vast majority of GI tryptophan is not utilized for serotonin production, but rather metabolized via the kynurenine pathway. Alteration of kynurenine:tryptophan ratios has been linked with depression and anxiety. The gut microbiota may modulate this ratio directly, via production of reactive oxygen species which inhibit kynurenine metabolism, or indirectly, via regulation of pro-inflammatory cytokines involved in kynurenine synthesis. Treatment with *B. infantis*, an anti-inflammatory probiotic, reduced the kynurenine-tryptophan ratio in rats, although no shifts in stress behaviours were detected.”

The next quote provides more detail, based on animal studies, on the intricacies of the tryptophan/serotonin/gut microflora relationship:

“Marked alterations in tryptophan and serotonin expression in germ free models support gut microbial regulation of tryptophan metabolism and serotonin signaling. Germ free mice have increased levels of plasma tryptophan. Colonization of germ free mice normalized anxiety-like behaviours and plasma tryptophan levels, but failed to normalize serotonin levels in the hippocampus, suggesting a critical window of microbiota-dependent serotonin regulation in the brain. In addition, germ free mice display decreased levels of GI serotonin compared with microbe-exposed counterparts...”

As you can see, while the mechanisms have not been precisely defined, it appears that gut microflora play a major role in the conversion of tryptophan to serotonin in both the brain and the GI tract.

In concluding their paper, Bauer et al (2) provide two “big picture” thoughts about the connection between gut microflora and the nervous system:

“A growing number of microbiome researchers recognize the gut microbiota as a vital component of the human organism. Thus, the gut microbiota-brain axis does not reflect control of the gut microbiota by the host or vice versa, but rather synergistic *extensions* of the host nervous system. Within this framework, the boundary separating microorganism from host blurs, highlighting our indistinguishability – a radical blurring of self and (microbial) non-self.”

The second “big picture” thought revolves around what was discussed previously – the role of gut microflora in neuromodulation. However, as an extension of this thought, the reverse is true where optimal neuromodulation is crucial for the maintenance of optimal gut microflora integrity. Also, please note in the following quote the important role of gut microflora in optimal microglia function which, as I noted in the previous newsletter series, is crucial for maintaining desirable levels of inflammatory mediators in the brain.

“Gut microbes produce or induce production of neuromodulators, maintaining brain chemistry and cognition. In turn, the host nervous system modulates GI motility and barrier homeostasis, sustaining the microbial community. Through these bidirectional signals, the gut microbiota-brain axis maintains metagorganismal homeostasis of the nervous system. Both the absence or alterations of the gut microbiota impair neural homeostasis, resulting in behavioural and neurochemical shifts. To cite one example, researchers recently demonstrated that the gut microbiota maintains homeostasis of microglia, the innate immune cells of the CNS. Germ free mice display microglial immaturity, a phenotype...”
also observed following antibiotic-induced eradication of the microbiota. In addition, germ free mice exhibit reduced microglial immune responses. Exposure to either a complex microbiota or microbial metabolites largely restored microglial morphology and function, partially rescuing neural homeostasis.”

More information on the relationship between gut microflora and the vagus nerve

The paper by Wang and Wang (1) discussed above provided a brief description of the connection between gut microflora and the vagus nerve. In “Microbiota-gut-brain axis and the central nervous system” by Zhu et al (3) more detail on vagus nerve function and its interactions with gut microflora is provided:

“The vagus nerve of the body can control function of multiple organs, such as heart rate and gut motility; the vagus nerve can also transmit peripheral immune signals to the CNS. The vagus signal from the gut can trigger an anti-inflammatory response against the sepsis induced by microorganisms. Gut microorganisms can affect brain functions through the vagus nerve; after a vagotomy, the microorganisms will not be able to regulate behaviors. After vagotomy in mice, no behavioral change was found even for mice that were treated with probiotics. Similarly, the bifidobacteria treatment, which had been previously reported to be effective, did not improve the behavior of vagotomized mice.”

More information on the relationship between gut microflora and the HPA axis

Wang and Wang (1) also provided some brief information on the relationship between gut microflora and the HPA axis. Zhu et al (3) provide additional data on this important aspect of neurologic function:

“In 2004, Sudo et al. were the first to report that symbiotic gut microorganisms were associated with the hypothalamic-pituitary-adrenal (HPA) axis. In response to stress, the levels of corticosterone hormone and adrenal hormones were higher in sterile mice than in mice harboring normal microorganisms. Gut colonization by Bifidobacterium can attenuate the increased HPA response; however, this inhibition can only be initiated in the early stages of life, indicating that the most primitive exposure to microorganisms is necessary for the inhibition of neural regulation by the HPA axis.”

As you can probably guess, this profound connection between gut microflora and optimal function of the HPA axis when stressful situations occur during early life can have massive clinical implications. In what way? As we know, many if not most of our most challenging chronic illness patients often report two key findings in their patient histories:

- Frequent use of antibiotics before five years of age.
- Exposure to highly stressful situations before five years of age.

As the quote above suggests, patients reporting these two crucial aspects of their early lives are much more likely to have demonstrated excessively high levels of cortisol and other stress hormones early in life. As we all know, this early production of excessive levels of stress hormones has been reported in many studies to lead to disturbances in health than can have lifelong implications.

**Autism spectrum disorder (ASD) and gut microflora**

I would guess that, for many of you, the first diagnosable illness that comes to mind when the relationship between gut microflora and neurologic function is considered is ASD. Zhu et al (3) provide an interesting and compelling discussion on ASD and its connection with gut microflora/brain interactions.

The authors begin their discussion on this topic with the following:

“Autism spectrum disorder (ASD) is a type of extensive brain function development disorder that begins during the early stages of growth (<36 months), and it is manifested as different levels of interpersonal barriers, language development disorders, and stereotyped behavior. Environmental factors (especially gut dysbacteriosis) are involved in the development of ASD, and as many as 70% of autistic patients exhibit gastrointestinal tract-related symptoms;
therefore, it is thought that ASD is associated with impairment of the gut-brain axis. The majority of autistic patients have diarrhea, abdominal pain, constipation, gastroesophageal reflux, and other gastrointestinal symptoms. Mazurek et al. studied 2,973 ASD patients and found that 24% of the patients had at least one type of gastrointestinal disease, and Adams et al. found that ASD child patients showed significantly greater occurrence of diarrhea, abdominal distension, and constipation than normal children, and the gastrointestinal symptoms were also closely associated with the severity of ASD. Moreover, ASD patients have a significantly higher content of the genera Faecalibacterium and Clostridium in the gut than normal children. Desbonnet et al. placed sterile mice and normal mice in two separate spaces, and the sterile mice would develop autistic behavior, whereas recolonization of symbiotic flora was able to restore the partially defective social behavior in the mice. Impaired immune function is a common feature of ASD patients, and the increase in the genera Faecalibacterium may be the main reason for immune dysfunction in ASD patients.”

The next quote I would like to feature discusses the relationship between the short-chain fatty acids (SCFAs) mentioned above and autism:

“Adams found that the levels of SCFAs, which are very important for the development of neurological functions, in the stool samples of autistic children were lower than those of normal children…”

Next Zhu et al (3) discuss, as evidence to support the hypothesis that there is a relationship between gut microflora and autism, bacterial metabolites such as those that can be measured via organic acids testing.

“The bacterial metabolites, 4-ethylphenylsulfate and 3-(3-hydroxy phenyl)-3-hydroxypropionic acid, can cause mice to develop ASD-like symptoms.”

Furthermore, ASD patients often demonstrate higher levels of bacterial metabolites known as lipopolysaccharides (LPS) in blood:

“...the serum lipopolysaccharide (LPS, cell wall components of gram-negative bacteria) levels are significantly higher in ASD patients than in normal subjects, and LPS has a clear association with social disorders. Most children with ASD have a history of infection before age 2, and the frequency of their use of antibiotics is also significantly higher than that of normally developed children. Antibiotics destroy the physiological balance of the inherent gut flora, and the newly colonized microorganisms produce neurotoxins, thereby inducing chronic diarrhea and ASD. For example, an abnormal increase in Clostridia and Bacteroidetes in the gut can promote gastrointestinal symptoms and ASD behaviors. Therefore, both the imbalance of gut flora and the entry of excessive amounts of bacterial metabolites into the brain through the circulation are associated with the onset of ASD.”

In part II of this series I will focus on the interaction between gut microflora and brain function as it relates to other neurologic ailments, with a focus on neurodegenerative diseases.

REFERENCES

FIGURE 1