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The Role of the Gut-Brain Axis in Alzheimer’s Disease: A Narrative Review

by

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An undergraduate honors thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in University Honors and Public Health Studies: Pre-Clinical Health Science, General Science

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Introduction

Alzheimer’s Disease (AD) is a type of dementia that accounts for 60% to 80% of all dementia cases per year, and was the seventh leading cause of death in the United States in 2021. It is estimated that 13.8 million Americans will have AD by 2060, compared to the estimated 6.5 million living with AD today (Alzheimer’s Association, 2022). The clinical characteristics of AD include severe deficits in memory, cognitive, and motor functions, causing detrimental changes to an individual's quality of life. The direct cause of AD is unknown, but research on its progression and risk factors has revealed that the onset of the disease occurs 10-20 years before the start of clinical symptoms (Long & Holtzman, 2019). Diet, exercise, educational history, aging, chronic infection, chronic inflammation, and sleep problems are risk factors (Pluta et al., 2020). It is projected that the total number of people with AD will reach 131.5 million by 2050 globally, compared to the 43.8 million reported in 2016 (Kesika et al., 2021). The goal of this narrative review is to discuss the mechanisms of gut-microbiota brain communication, how these gut to brain communication pathways are related to AD pathogenesis, and the potential for microbiome-based treatments and prevention strategies that may offer better outcomes for patients than current treatment methods.

The progression of Alzheimer’s Disease (AD) from asymptomatic to mild cognitive and functional impairment, to dementia, provides a window of opportunity for intervention to slow or prevent the disease (Belizario, 2018). The exact mechanisms by which gut microorganisms affect neurodegenerative disease pathogenesis is unknown, however, evidence shows a clear relationship between an altered microbial composition and neurodegeneration. This occurs through interactions of the gut microbiota (GM) that leads to intestinal permeability, stimulation of an inflammatory response, and regulation of the blood-brain barrier. Most GM co-exist with humans without harmful effects. However, when equilibrium is disturbed, pathological
conditions can begin to develop. GM are necessary for food digestion, metabolism, and production of molecules such as vitamins, amino acids, short-chain fatty acids (SCFAs), and metabolites (Belizario et al., 2018). For example, GM synthesize microbial enzymes such as disaccharidases and polysaccharidases, and synthesize vitamins B and K as well as essential amino acids such as glutamine and arginine. GM also can metabolize drugs and hormones, stimulate cytokine and interferon production, and can degrade bacterial toxins as well (Antushevich, 2020). Additionally, the brain’s immune cells, microglia, are under constant regulation by the gut microbiome (Dinan & Cryan, 2017). Defects of microglia are observed in germ-free mice (GF), leading to a compromised innate immune response (Vendrik et al., 2020). GF mice lack all microorganisms, such as bacteria, viruses, and eukaryotic microbes, and can thus be used to model gut microbiota behavior in disease models of AD. This is achieved through precise breeding in an isolated environment and constant monitoring for contamination (Kennedy et al., 2018). Given the relationship between the gut microbiome and the brain, as well as unsuccessful interventions to treat and prevent AD, considering the gut-brain axis in AD pathology and treatment can offer more beneficial outcomes. Therapeutic interventions such as probiotic supplementation, and fecal microbial transplantation have shown positive outcomes in cognitive function in animal models of AD.

The Gut-Brain Axis

The gut-brain axis (GBA) refers to the bidirectional communication pathways between the brain and gut microbiome. The human gut microbiome contains viruses, bacteria, fungi, and parasites. The term gut microbiome refers specifically to the microbial genome in the gastrointestinal (GI) tract. Most genes of bacteria in the gut microbiome encode for enzymes and structural proteins that influence the function of cells (Appleton, 2018). The gut microbiome takes up the majority of the human microbial population, with 1000 different bacterial species and $10^{13}$ to $10^{14}$ microorganisms, as well as 100x as many genes as are in the human genome.
Its functions include protecting against pathogen invasion and regulating host metabolism and immune responses (Liu et al., 2021). The two phyla, Firmicutes and Bacteroides account for at least ¾ of the microbiome. While individuals all have a unique microbial profile, healthy individuals do show similarities in relative abundance and distribution of microbiota (Carabotti et al., 2015).

Currently, there is not a precise definition of what a healthy, balanced microbiome entails. Because of this, defining an imbalanced microbiome is also not agreed upon in the literature (Tiffany & Bäumler, 2019). Dysbiosis can be described by an imbalance of commensal bacteria, those that offer protection, and pathogenic bacteria that threaten the host. Dysbiosis can begin at any stage in life, through disturbances such as antibiotic exposure, lack of breastfeeding, mode of birth delivery, and stress/infections during pregnancy (Dinan & Cryan, 2017). Increased age and disturbances in host genetics and the immune system can also lead to dysbiosis (Cani & Knauf, 2016). An individual's diet is especially impactful on the gut microbiome. This involves all aspects, including dietary intake of calories, carbohydrates, fats, proteins, vitamins, and total fiber consumption. Dysbiosis is associated with many conditions such as inflammatory bowel disease, metabolic syndrome, colon cancer, autism, anxiety, and depression (Barrio et al., 2022). In rodent and human studies, antibiotic use, diet, and stress were shown to impact the neurodevelopment and behavior of offspring. Research has suggested that altered neuronal and microbial organization in pre and postnatal stages of development can lead to neurodevelopmental disorders (Park & Kim, 2021). Long-term use of antibiotics has also shown to have detrimental effects on the enteric nervous system (ENS) and the brain in preclinical studies (Cryan et al., 2019). In the clinical setting, gut dysbiosis and central nervous system (CNS) disorders are often seen concurrently, such as with autism, anxiety, depression, and gastrointestinal disorders.
The bidirectional communications system of the gut brain-axis involves the ENS, the immune system, the vagus nerve, and microbial metabolites. Understanding the importance of the 10th cranial nerve, the vagus nerve, can provide a clear and direct picture of how closely linked the gut and brain are, and one of the major ways in which they act as a true bidirectional communication system. The vagus nerve is a part of the parasympathetic nervous system and is responsible for autonomic functions, such as control of mood, heart rate, digestion, and the immune response. The vagus nerve carries information from the digestive system to the brain and vice versa. Because the major functions of the vagus nerve are afferent, sending information to the brain from organs such as the gut, liver, heart, and lungs, it is proposed that this suggests that these inner organs are major sources of sensory information. The neurons of vagal efferent fibers, sending information away from the brain, emerge from the dorsal motor nucleus of the vagus nerve in the medulla and innervate muscular and mucosal layers of the gut. The vagus nerve connects intestinal nerve cells with neurons in the brain. Brainstem nuclei can monitor bowel functions and spread signals to other areas of the brain, because of the presence of vagus nerve nuclei in the brainstem (Pluta et al., 2020). Vagal afferent fibers can sense indirect microbial signals through the diffusion of microbial metabolites, or through cellular and humoral communication. For example, these vagal fibers can sense products such as lipopolysaccharides and SCFAs produced by microbiota (Bonaz et al., 2020). Thus the vagus nerve is a crucial component of the GBA, showing a link between the gut and neuroinflammatory disease.

Microbiota are key contributors to gastrointestinal homeostasis in this system. The ENS is the nervous system of the GI tract and communicates with the CNS through neurotransmitters and the vagus nerve (Belizario et al., 2018). Its functions include coordinating gut functions such as motility and control of fluid (Cryan et al., 2019). The ENS produces hormones and peptides that can cross the blood-brain barrier and act synergistically with the vagus nerve. Notably, the ENS has more neurons than the spine (about 100-500 million
neurons, the largest accumulation in the body), and produces over 30 neurotransmitters. GF mice were found to have lowered excitability of intrinsic primary afferent neurons. When exposed to intestinal microbiota, the effects were reversed and the electrophysiological properties of the ENS neurons were returned to normal (McVey, 2013). GF mice and mice treated with antibiotics have revealed the important role of an intact microbiome in the proper functioning of the ENS, such as altered ENS neurochemistry and structure (Cryan et al., 2019). The density of neuronal populations in the GI tract is important for its proper function. Germ-free mice observed to have decreased neuronal population in the colon were normalized by administration of the bacterial species *Bacteroides thetaiotaomicron* alone. This suggests that this common gut microbe is essential in the regulation of enteric neuronal cell populations (Aktar et al., 2020).
Gut microbiota communicate with the brain via neural, immune, metabolic, and endocrine pathways. An altered microbiota composition can lead to bacterial products such as serotonin (5-HT) or fatty acids crossing the gut barrier and reaching the brain via the bloodstream (1), via mucosal immune cells releasing cytokines (2), via vagus nerve pathways (3), or via the release of gut hormones by enteroendocrine cells (4), such as noradrenaline that has the ability to influence signaling between bacteria, which can affect microbial composition and activity (5) (Collins et al., 2012).
Stimulation of the immune system begins at the intestinal mucosal surface, where the microbial cell wall stimulates the innate immune system to produce cytokines. Along with cytokines, Cryan et al. (2019) highlight that microbiota-host interactions also lead to the release of chemokines, neurotransmitters, neuropeptides, endocrine messengers, and microbial by-products. These can infiltrate the blood and lymphatic systems, influence neural messages carried by the vagal and spinal afferent neurons, and thus can constantly communicate with the brain (Cryan et al., 2019). Structures that are present in the GI tract such as the lamina propria, are part of the mucosal immune system and protect against pathogens. GF mice are important for understanding the relationship between GM and immunity (Fung, 2020). GF mice, and mice treated with antibiotics (ABX), have shown to be significantly more susceptible to infection due to impaired immune function. For example, innate lymphoid cells, cells important for the innate immune system’s response to infection, have been shown to have impaired development. Further, interleukin-22, an important mediator in inflammation and bacterial infection is reduced in GF mice and ABX mice (Fung, 2020).

The GBA and Alzheimer’s Disease

Alzheimer’s Disease (AD)

Mild cognitive impairment (MCI) is a cognitive state between normal cognitive aging and dementia and has been shown to progress to AD at a rate of 10%-15% per year (Yang et al., 2019). In AD, neurons in the brain regions responsible for learning, memory, and language are damaged. This damage can occur up to 20 years before the start of symptoms related to learning, memory, and language (Alzheimer’s Association, 2022).
AD is characterized by amyloid-beta (Aβ) plaques and neurofibrillary tangles composed of the protein tau (Kowalski & Mulak, 2019). The Aβ plaques are found extracellularly and the hyperphosphorylation of the protein tau aggregates into neurofibrillary tangles intracellularly. Tau is a microtubule-associated protein (MAP), important for stabilizing neuronal microtubules, which are vital for functions such as intracellular signal transduction. Additionally, hyperphosphorylated tau and resulting microtubule breakdown from tactless signals leads to ineffective intracellular transport and thus neuronal degradation. Tau aggregation leads to neuroinflammation and the pathological effects of neurodegenerative diseases (Muralidhar et al., 2020). Evidence for misfolded proteins spreading along the GBA is supported by the finding of the amyloid protein alpha-synuclein (a-syn) in the neurons of the gut wall. The dorsal motor nucleus of the vagus nerve is one of the first affected brain regions that contain a-syn deposits. This provides a mechanism by which neuronal cells can accumulate the misfolded proteins from the gut up to brain regions (Kowalski & Mulak, 2019). Regarding amyloid plaques, it is not well understood what causes their formation in conditions such as AD. Aβ is a cleavage product of amyloid precursor protein (APP), a transmembrane protein involved in neuronal development (Kowalski & Mulak, 2019). Aβ is recognized as an antimicrobial peptide (Kowalski & Mulak, 2019) and is not toxic under normal physiological conditions (Kametni & Hasegawa, 2018), but when accumulated as plaques, due to an imbalance between Aβ production and clearance (Hansen et al., 2018), they become recognized as foreign material by the brain (Figure 2). This recognition initiates an inflammatory and immune response by activating the microglia and release of cytokines, which eventually lead to cell death and neurodegeneration (Khan et al., 2020).
Amyloid-beta (Aβ) plaques are not formed when microglia can properly clear soluble Aβ fibrils. Soluble Aβ is formed when the amyloid precursor protein (APP) is cleaved by the enzyme α-secretase. If APP is cleaved by enzymes β-secretase and γ-secretase, insoluble Aβ is formed and cannot be cleared, forming plaques (Megur et al., 2021).

The amyloid hypothesis is the notion that AD is primarily caused by the accumulation of Aβ (Kametani & Hasegawa, 2018). This hypothesis has led most research on AD and clinical trials (Kurkinen, 2021).
However, the amyloid hypothesis has shown to be less than ideal as clinical trials have failed (Kowalski & Mulak, 2019). One of the limitations to the Aβ hypothesis is that Aβ plaques may not be inherently cytotoxic, as mouse models with Aβ deposited in the brain have shown to not result in NFT formation or nerve cell death. Amyloid plaques in elderly patients without dementia have shown to be just as prominent in elderly with a form of dementia (Kametni & Hasegawa, 2018). Recently, research on the role of GBA in AD pathology has continuously increased over the years and has shown the potential to offer more effective treatment for patients as well as potentially offering a more comprehensive diagnostic tool, through the identification of dysbiosis, for example (Liu et al., 2020).

Alzheimer’s Disease & Gut Microbiota

Many researchers highlight the need to rethink AD pathogenesis, as recent clinical trials have been disappointing due to a potential gap in AD understanding (Kesika, et al., 2021), (Kurkinen, 2021). It is well established that there is a relationship between AD and GM, and changes in gut bacteria can negatively impact cognition (Li et al., 2020). In a study by Li et al. (2019) dysbiosis was observed in patients in the MCI stage, and with AD (Li et al., 2019), suggesting that gut microbial dysbiosis can be a preclinical symptom of AD (Qian et al., 2021). In mouse models, a study revealed that gut dysbiosis began at 5 months of age along with impaired cognitive behavior and amyloid deposition (Liu et al., 2020). Notably, the strains of Lactobacillus, Bifidobacterium, and Clostridium reduced Aβ deposition, neurofibrillary tangles, and neuroinflammation, while improving cognitive dysfunction (Liu et al., 2020). The first report of gut-microbiota diversity influencing neuroinflammation and amyloidosis was in 2016, in a murine model of AD (Kowalski & Mulak, 2019). While it is not well understood what causes the amyloid plaque formation, GM are a source of a significant number of amyloids (Kowalski & Mulak, 2019). It has been observed by Li et al. that GM promote amyloid deposition and
reduce the expression of an inhibitor of the signaling pathway ERK1/2, which enhances the transcription of Aβ (Li et al., 2020).

Gut bacteria produce various molecules that have important roles in host physiology. An altered microbial composition can impact how these products function and interact with other pathways. Amyloid fibers produced by bacteria, such as *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Mycobacterium Tuberculosis*, and *Staphylococcus aureus*, can form biofilms, strongly binding to each other and in turn resisting destruction by immune factors (Megur, 2021). Additionally, Bacteria produce neuroendocrine signaling molecules that affect physiology, a possible route of communication between the host and the microbiome. Bacteria from the genus *Escherichia* synthesize norepinephrine, *Bacillus spp* synthesize dopamine and norepinephrine, and *Escherichia spp* and *Lactobacillus spp* produce gamma-aminobutyric acid (GABA) (Doifode et al., 2021). This is significant as GABA is a major inhibitory neurotransmitter in the CNS whose dysfunction can contribute to cognitive impairment (Doifode et al., 2021). GABA, serotonin (5-HT), and dopamine are all major examples of bacterial products that are involved in mood, behavior, and cognitive function as neurotransmitters (Table 1) (Liu et al., 2020). In a study by Zhuang et al., the bacteria *Ruminococcus* species was found to be increased in AD patients. This bacteria degrades mucus, an important element of the protective gut barrier. Contrastingly in this study, the strain *Bacteroides fragilis* was not found, an anti-inflammatory bacteria that strengthens the intestinal barrier and prevents gut leakiness. This gut microbial alteration can lead to gut leakiness due to the mucus degradation and in turn microbial products into the body (Zhuang et al., 2018). When exposing neuroblastoma cells to gram-negative or gram-positive bacteria, significant concentrations of Aβ were produced, providing evidence that bacteria trigger Aβ production (Meier-Stephenson, 2022). Cognitive dysfunction is believed to be due to the deposition of beta-amyloid
plaques outside of the neuron, and neurofibrillary tangles inside the neuron, leading to neuroinflammation among others dysfunction such as vascular degeneration (Kesika, et al., 2021).

Short-chain fatty acids (SCFAs) are important metabolites of gut microbiota involved in the GBA. They are metabolites of anaerobic gut bacteria through fermentation of complex carbohydrates (Nogal et al., 2021) and can affect the brain by influencing emotional levels, improving attention, memory, and motivation, as well as improving sleep (Megur et al., 2021). SCFAs are important when considering the role of the GBA in AD as they are involved in many processes of the CNS, and AD patients have decreased levels of SCFAs (Park & Kim, 2021). SCFAs affect gene expression and cell signaling by controlling acetylation and phosphorylation of host proteins such as histones (Park & Kim, 2021). SCFA metabolites include acetate, propionate, and butyrate (Liu et al., 2020). SCFAs can directly affect immune cells and immune modulators to maintain homeostasis, regulate the differentiation, recruitment, and activation of inflammatory cells, cross the BBB, and may communicate along the microbiota GBA by activating G protein-coupled receptors (Guo, 2022). Gut microbe metabolites such as SCFAs modulate microglial function and maturation. When the gut becomes more permeable, microbial exudates such as amyloids and LPS can enter the circulatory system, leading to an abnormal level of proinflammatory cytokines (Doifode et al., 2021). Increased blood-brain barrier permeability in GF mice was shown to be alleviated by SCFAs, and defective maturation of microglia in GF mice was improved by SCFA treatment (Doifode et al., 2021). GF mice with defective microglial cells were also ameliorated with SCFA treatment in a study by Park & Kim (2021). Gut microbes such as Bifidobacteria and Lactobacilli convert nitrite and nitrate to nitric oxide, and gut Bacilli and Streptomyces can also synthesize nitric oxide. Alteration in gut microbes that can thus result in an increase in nitric oxide can cause axonal degeneration, neuroinflammation, and neurodegeneration (Khan et al., 2020).
Neurotransmitters involved in the GBA

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Function</th>
<th>GM involved in synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-aminobutyric acid (GABA)</td>
<td>Amino acid neurotransmitter in the central nervous system. Involved in behavior, cognition, stress response, depression, and anxiety (Cui et al., 2020). Modulates intestinal motility and inflammation (Chen et al., 2021).</td>
<td><em>Bifidobacterium, Bacteroides fragilis, Parabacteroides, and Eubacterium</em> (Chen et al., 2021).</td>
</tr>
<tr>
<td>Serotonin (5-HT)</td>
<td>Promotes intestinal motility (Chen et al., 2021). Modulate host behavior, mood, sleep, and digestion (Cryan et al., 2019).</td>
<td><em>Staphylococcus and Clostridial species</em> (Chen et al., 2021).</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Plays a role in gastric secretion, mucosal blood flow (Chen et al., 2021), memory and motivation (Cryan et al., 2019).</td>
<td><em>Staphylococcus</em> (Chen et al., 2021).</td>
</tr>
</tbody>
</table>

GABA, serotonin, and dopamine are major neurotransmitters involved in the communication pathways between the gut and brain. Various gut bacteria are involved in their synthesis, and these neurotransmitters have important impacts on host physiological function.

**Neuroinflammation**

The role of neuroinflammation in AD progression is important as it can provide more insight into how GM are involved in neurodegeneration. A thick layer of mucus, a semipermeable layer of enterocytes, and a gut-vascular barrier composed of endothelial cells and enteric glial cells work to prevent the uncontrolled passage of luminal content into the bloodstream and underlying tissue from the gut. A compromised barrier between the CNS and periphery, combined with a compromised gut barrier (“leaky gut”) is an open opportunity...
for gut-derived molecules, toxins, and pathogens to reach the brain and activate the inflammatory response (Agirman et al., 2021). The link between gut microbiota, neuroinflammation, and AD is seen in a study by Wang et al. (2019), where mouse models of AD progression revealed that gut microbial alterations led to the accumulation of isoleucine and phenylalanine, in turn stimulating the proliferation of pro-inflammatory Th1 cells of the immune system, which then can stimulate microglia activation (Wang et al., 2019). Elevated levels of these amino acids during AD progression promote infiltration of Th1 cells into the brain via the circulation and can crosstalk with microglial cells, resulting in neuroinflammation and cognitive impairment. This is particularly significant as the combination of elevated amino acids and bacteria associated with TH1/M1 cells can be used as a diagnostic biomarker for AD patients (Wang et al., 2019).

Microglia serve as phagocytes in the CNS and are important for tissue maintenance, injury response, and protection against pathogens. Hansen et al. (2018) propose that through aging and/or factors not clear in the present, microglia become less effective and in turn cannot adequately clear excess Aβ. Microglia can turn from beneficial phagocytes to destructive, leading to the elimination of synapses and secretion of neurotoxic cytokines leading to injured neurons. Microglia that are not able to properly clear excess Aβ appear to be deficient in a cell surface receptor called TREM2, that when activated initiates microglia proliferation and phagocytosis. TREM2 mutation is associated with AD and increases the risk up to threefold (Hansen et al., 2018). In response to stimuli such as infection, trauma, and depression among others, Aβ is released triggering the innate immunity cascade. During the early stages of AD, there is a low concentration of Aβ which activates microglia to promote Aβ clearance via phagocytosis. Excessive microglial stimulation and increased neuroinflammatory signaling lead to neuronal and glial cell death (Kowalski & Mulak, 2019).
The diversity of GM is essential for the maintenance maturation and proper functioning of microglial cells and thus CNS functioning (Doifode et al., 2020). Patients with amyloidosis and cognitive impairment show higher levels of pro-inflammatory cytokines and a reduction of anti-inflammatory cytokines. In a study examining the pro-inflammatory cytokine profile and gut microbiota of elderly patients, those with cognitive impairment showed higher levels of pro-inflammatory cytokines compared to controls as well as patients with cognitive impairment but no brain amyloidosis. Furthermore, a positive correlation was observed between specific pro-inflammatory cytokines and an abundance of the inflammatory bacteria taxon Escherichia/Shigella (Cattaneo et al., 2017).

**The Blood-Brain Barrier**

The blood-brain barrier (BBB) is a specialized environment in the CNS that acts as a semi-permeable barrier, controlling the passage and exchange of molecules and nutrients between the circulating blood and the brain. The multilayer unit is comprised of specialized brain endothelial cells, linked by tight junctions that control the passage of molecules and nutrients between the circulatory system and the brain (Liu et al., 2020). The aging process can weaken this system, opening up the possibility for entry of detrimental GM into the brain. This can promote neurodegeneration and pathological changes in AD (Liu et al., 2021). The BBB in post-mortem studies of AD patients revealed BBB damage and accumulation of blood-derived products in the brain, and MRI studies of the living human brain showed that BBB breakdown was associated with learning and memory (Kowalski & Mulak, 2019). Liu and colleagues argue that the first step involving microbiota in the pathogenesis of AD is the dysfunction of the BBB. Many products of GM that cross the BBB promote beta-amyloid production, which also promotes amyloid plaques. Thus, GM has been suggested to be a regulator of the BBB, beginning as early as intrauterine life. Therapeutic drugs that are based on the structural and immunological components of AD; B-Amyloid deposition, formation of neurofibrillary tangles, and sustained
glial-mediated inflammation in the brain, have had less than promising results. This is likely due to the multifactorial nature of AD pathogenesis (Liu et al., 2021).

One example of a GM-derived substance that is detrimental to the BBB and CNS is lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria. AD patients present with a higher relative abundance of LPS-producing bacteria such as Burkholderiaceae, Staphylococcaceae, Porphyromonas gingivalis, and Propionibacterium acnes (Xu et al., 2021). Altered GM elevates LPS levels in circulation, and causes systemic inflammation and BBB disruption. A disturbance of the BBB results in the accumulation of LPS. LPS indirectly initiates amyloidogenesis and accumulates when the BBB is altered, resulting in neuroinflammation and memory deficiency (Kar et al., 2022). Additionally, circulating LPS can alarm the immune system as it is a superantigen, promoting systemic inflammation. This results in endothelial apoptosis, membrane abnormalities, mitochondrial damage in brain endothelial cells, and alterations of BBB transport pathways.

Inflammation induced by LPS administration in maternal rats resulted in Aβ accumulation. LPS administration was found to increase APP levels and probiotic administration ameliorated this effect in the rat brain tissues (Kar et al., 2022). Altered GM can also increase cytotoxic bile acids such as deoxycholic acid, which can cross the BBB and deposit into the brain, resulting in apoptosis, increased reactive oxygen species (ROS), and inflammation (Khan et al., 2020). Maintaining the integrity of the BBB is necessary for the proper maintenance of brain homeostasis and the prevention of foreign objects invading brain tissue. Microbiota also produces substances that are important for the BBB such as SCFAs. When GM are altered, this can result in decreased amounts of substances such as SCFA which are critical for BBB health and thus lead to damage. GF mice presented with weakened BBB integrity were normalized with SCFA treatment (Park & Kim, 2021). BBB breakdown occurs in the aging human brain, beginning in the hippocampus, and may contribute to cognitive impairment. This has been shown to occur in AD patients before dementia, neurodegeneration, or brain atrophy.
even occurs (Liu et al., 2021). Modifying gut microbiota has the potential to be a therapeutic approach toward the prevention of BBB breakdown in the preclinical stage of AD.

Treatments Targeting the Gut-Brain Axis in Alzheimer’s Disease

Probiotics

Probiotics have become increasingly popular in recent years for use in clinical trials as well as by the average consumer looking to improve their health (Salminen et al., 2021). However, the regulation of probiotics is not as thorough as it should be (de Simone, 2019). The label “probiotic” is easily misused and is conflicting in the probiotic industry. This is because the results from a study on a specific probiotic can be extended to other probiotics, and may not take into adequate consideration the dose, specificity, combination of strains, methods of creating the specific formula, and duration of intake (Simone, 2019). The current definition of probiotics states that probiotics are living microorganisms when administered in adequate amounts, confer a health benefit to the host (Salminen et al., 2021). Psychobiotics can be defined as probiotics that provide health benefits to the host through interaction with CNS and commensal bacteria when ingested in adequate amounts (Barbosa et al., 2020). The dose of probiotics administered should be precise and appropriate to the target host. The decision about what dose to administer is made based on considerations of strain, route of administration, and other factors. Studies most commonly use a dose of 10^8 to 10^11 colony-forming units (CFU) (Den et al., 2020).

To date, several studies have explored the use of probiotics to treat the symptoms and progression of AD. Den et al (2020) found that probiotic supplementation may enhance cognitive function in AD patients by decreasing levels of inflammation. The mechanisms explaining how psychobiotics can control and/or prevent
neurodegeneration are not well understood, and many studies have conflicting results (Den et al., 2020). A randomized, double-blind, controlled clinical trial conducted on 60 AD patients found that after 12 weeks, AD patients showed significant improvement in cognitive function as well as metabolic status (Akbari et al., 2016). The probiotic supplement administered was a 200ml/day milk composed of *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum* (Akbari et al., 2016).

Because the gut can communicate with the brain via hormones and neurotransmitters, studies that examine how probiotics impact the behavior of hormones and neurotransmitters add insight into how this affects host physiology. A study by Scardaci et al. (2021) aimed to examine microbiota at a molecular level when exposed to the hormone norepinephrine (NE), specifically using the probiotic strain Enterococcus faecium NCIMB10415. This strain belongs to the species *Enterococcus faecium*, known for its role as gut commensal. The strain *Enterococcus* has a controversial history, due to reports of causing fatal infections in immunocompromised hospitalized patients. However, it is also used in commercialized probiotics to improve antibiotic-induced dysbiosis, IBS, and hypercholesterolemia, among others. The authors found that the probiotic strain was able to sense and respond to NE by changing its growth pattern, protein profiles, and physiology, which induced a change that could favor the survival and colonization of host tissues. The authors note that these results are supportive of the notion that probiotics can enhance environmental resistance by activating a stress response after exposure to a hormone, such as NE in this case (Scardaci et al., 2021).

As noted previously, mice models offer a great opportunity to examine the role of the gut in diseases such as AD. They also allow researchers to examine how certain probiotic strains impact the GM and in turn various physiological processes. In a study focusing on *Lactobacilli* and *Bifidobacteria*, probiotic supplementation of 2g of *Lactobacillus acidophilus, Lactobacillus fermentum, Bifidobacterium lactis, and*
Bifidobacterium longum for 8 weeks resulted in improved memory and learning by modifying the microbiota of rats and improving oxidative stress biomarkers (Athari et al., 2018). Similarly, β-amyloid administered rats were found to have improved spatial memory and synaptic plasticity following probiotic supplementation of Lactobacillus acidophilus, Bifidobacterium bifidum, and Bifidobacterium longum in a capsulated form which resulted in improved oxidative stress and pro-inflammatory biomarkers. A proposed mechanism of this is that the probiotics improved neuronal circuits by reducing the effects of the oxidative stressors and pro-inflammatory cytokines that are released by activated microglia. This is important due to the role of microglia outlined previously, along with oxidative stress being strongly associated with AD (Rezaei et al., 2019). Asl et al. administered B-amyloid to mice to evaluate the effects of probiotic bacteria on the behavioral and electrophysiological aspects of the animal's cognitive functions. The mice were given a mixture of the bacteria Lactobacillus acidophilus, Bifidobacterium bifidum, and Bifidobacterium longum. Results showed that the Alzheimeric mice treated with the probiotic mixture had improved spatial memory and synaptic plasticity compared to the Alzheimeric mice without probiotic supplementation (Asl et al., 2019). Wild-type control mice were compared to mice with the human Aβ sequence administered into the APP gene. Probiotics were also used to control gut microbiota, using fecal samples to examine its microbial diversity. Brain pathology was associated with intestinal dysbiosis and increased intestinal inflammation. After probiotic administration, GM was able to be modulated in a way that decreased intestinal inflammation and gut permeability. However, the benefits of probiotic supplementation were limited to the gut and did not concur an effect relating to the brain such as Aβ plaque load, neither in cytokine levels (Kaur et al., 2020).

Fecal Microbiota Transplantation
Fecal microbiota transplantation (FMT) is the most effective GM intervention. In FMT, a solution of fecal matter is administered from a donor into the intestinal tract of a recipient. FMT has been shown to have beneficial effects in autism spectrum disorder in clinical trials (Vendrik et al., 2020). Multiple sclerosis, Parkinson’s disease, and AD have shown beneficial results in animal models (Vendrik et al., 2020). FMT has been successful in the treatment of gastrointestinal diseases such as *Clostridioides difficile*, which after FMT, results in the elimination of symptoms through normalization of the altered microbial composition (Antushevich, 2020). Kim et al. (2021) showed that after FMT was performed on normal mice using feces from AD mice, neurogenesis was decreased, resulting in memory impairment, and increased pro-inflammatory cytokines were also observed. However, neuronal cell death, a feature of neurodegeneration, was not observed (Kim et al., 2021). In AD, more pro-inflammatory gut bacteria and pro-inflammatory cytokines are found in the feces of patients with cognitive impairment and brain amyloidosis compared with patients with cognitive impairment but no amyloidosis. Notably, FMT studies in humans with AD have not been published. However, animal models provide insight into how FMT can affect neurological disorders and their symptoms such as in AD (Vendrik et al., 2020). Before FMT, it is ensured that the donor does not have any health conditions such as autoimmune diseases, diabetes, metabolic syndromes, inflammation, be obese, and is not partaking in behavior that impacts the gut microflora such as drinking alcohol or being on drugs such as immunosuppressants, antibiotics, aspirin, or even probiotics. Interestingly, recent studies have shown that males and females utilize the same microbiota differently, and thus this should be taken into account during FMT (Antushevich, 2020). In a study analyzing the fecal samples from 60 AD patients, the feces of AD patients was significantly different from healthy controls, specifically by a reduction of *Odoribacter, Anaerobacterium*, and *Papillibacter* and an increase in *Bifidobacterium, Sphingomonas, Lactobacillus*, and *Blautia* in AD patients (Zhou et al., 2021). In a mouse model of AD, FMT showed to improve cognitive deficits and reduced Aβ in the brain. Decreased phosphorylation of tau protein, decreased neuroinflammation, and increased synaptic plasticity were also
observed. Additionally, gut microbial alterations were reversed after FMT. *Proteobacteria* increases during aging and is related to inflammation. In the mouse model of AD, *Proteobacteria* was at a higher abundance. This was reversed following FMT (Sun et al., 2019). Mice with AD characteristics such as amyloid plaques, neurofibrillary tangles as well as memory deficits, showed a distinct composition of GM, an impaired epithelial barrier, and chronic systemic and intestinal inflammation compared to healthy controls. After FMT in these mice, Aβ plaque formation, neurofibrillary tangle, and cognitive impairment were improved (Kim et al., 2020). Similarly, Zhan and colleagues found that improving GM composition in a mouse model via FMT was effective as a therapeutic strategy for age-related cognitive dysfunction (Zhan et al., 2018). More studies on humans are needed to make accurate conclusions and to understand the extent to which FMT can improve symptoms of neurological conditions. It should also be considered that studies have shown that the way microbial composition changes in AD patients and in AD mouse models are different (Kim et al., 2021).

**Conclusion**

While research shows a clear relationship between the GBA and neurodegeneration, particularly in AD, the exact mechanism remains unknown (Kaur et al., 2021). Dysbiosis can occur at any stage in life and has a significant impact on host health. Evidence suggests that altered neuronal and microbial organization in pre and postnatal stages of development can lead to neurodevelopmental disorders. Factors that influence gut microbial composition such as antibiotic or probiotic use, diet, and/or stress during pregnancy impact the microbiome, neurodevelopment, and behavior of offspring in rodent and human studies (Silva et al., 2020). GM can trigger neuroinflammation through interactions with the ENS, vagus nerve, and microbial metabolites that cross the BBB. It is also plausible that dysbiosis can be stimulated by neurodegeneration and neuroinflammation, causing worsened symptoms in AD patients. It is unclear whether microbiota affects primarily AD progression or the
inflammatory aspects of AD. Additionally, whether dysbiosis causes AD pathology or is a co-phenomena that worsens symptoms remains to be understood. Due to the complexity of the gut microbiome and the number of factors that can influence its composition, diet, being a major confounding factor in research of the gut microbiome, should be accounted for in research studies as an individual's dietary habits can change constantly, quickly influencing microbial composition (Quigly, 2017). Thus, clinical trials on humans are needed to assess whether patterns seen in animal models translate accurately. With more studies using animal models being conducted supporting the hypothesis that an altered gut microbiome impacts neurodegeneration, and the attempt to re-balance its altered state can positively impact symptoms, therapies aiming to restore a healthy gut microbiome may be of significant advantage for AD patients.

Abbreviations

Aβ Amyloid-beta
ABX Antibiotics
AD Alzheimer’s Disease
APP amyloid precursor protein
BBB Blood-brain barrier
CFU Colony-forming unit
CNS Central nervous system
ENS Enteric Nervous System
FMT Fecal microbiota transplantation
GABA Gamma-aminobutyric acid
GBA Gut-brain axis
GF Germ-free
GI Gastrointestinal
GM Gut microbiota
LPS Lipopolysaccharide
MCI Mild cognitive impairment
NE Norepinephrine
SCFAs short-chain fatty acids
TREM2 Triggering receptor expressed on myeloid cells 2
https://doi.org/10.1002/alz.12638


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