The Potential Effects of Radiation on the Gut-Brain Axis

Carli B. Jones[1]*, Catherine M. Davis[2], and Karen S. Sfanos[1,3,4]

[1]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[2]Division of Behavioral Biology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[3]Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA

[4]Department of Urology, James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA

RUNNING TITLE: RADIATION AND THE GUT-BRAIN AXIS

---

*Corresponding author: C. B. Jones, Department of Pathology, Johns Hopkins University School of Medicine, East Baltimore Campus, 1550 Orleans Street, Room 138, Baltimore, MD 21287. email: cjone228@jhmi.edu.

Jones, C.B., Davis, C.M., and Sfanos, K.S. The Potential Effects of Radiation on the Gut-Brain Axis. *Radiat. Res.*

Abstract

Humans may be exposed to different types of radiation in their lifetimes, typically in the form of low linear energy transfer (LET) radiation that is, for example, used as a treatment for cancer. In addition, astronauts may be exposed to high LET radiation in outer space. Here, we propose that alterations to the gastrointestinal (GI) microbiota may occur upon exposure to low or high LET radiation, and that these alterations may perturb important relationships that exist between the GI microbiota and human health. For example, the GI microbiota can communicate with the brain via various pathways and molecules, such as the enteric nervous system, the vagus nerve, microbial metabolites, and the immune system. This relationship has been termed the "gut-brain axis". Alterations to the composition of the GI microbiome can lead to alterations in its functional metabolic output and means of communication, therefore potentially causing downstream cognitive effects. Consequently, studying how radiation can affect this important network of communication could lead to new and critical interventions, as well as prevention strategies. Herein, we review the evidence supporting a relationship between radiation exposure and disruption of the gut-brain axis as well as summarize strategies that may be used to counter the effects of radiation exposure on the GI microbiome.

Introduction

In recent years, the field of microbiome research has exploded, with studies expanding into almost every discipline that one can imagine. There are countless aspects of human health that are connected to the microbiome – the microbial ecosystems that exist at numerous sites throughout the human body including the gastrointestinal (GI) tract, skin, oral cavity, and genitourinary tract (*1*). These communities can include bacteria, archaea, eukaryotes, and viruses, with bacteria being the predominantly characterized component thus far (*2*). It is now more than evident that the constituents of the microbiome are fundamental to the health of the host, and this relationship has been best studied in relation to microorganisms that reside in the GI tract. The GI microbiome in each individual has important connections to other body systems including the digestive system, immune system, endocrine system, and, notably, the nervous system (*3*). The interplay between the GI tract and the central nervous system (CNS) - termed the gut-brain axis - is currently being explored in relation to a multitude of factors and disorders including cognition and mental health (*4*), mood disorders (*5*), schizophrenia (*6*), metabolic syndrome (*7*), and irritable bowel syndrome (*8*).

Various elements alter the composition and subsequent functional status of the GI microbiome, including antibiotics, pre/probiotic supplements, diet, and the environment. One additional factor that has the ability to alter microbial populations that has been underexplored in relation to the human microbiome is radiation (*9*). Radiation is naturally present in the environment, encountered in daily life; as well as in more extreme situations such as cancer treatment and space travel. Everyday sources of radiation include terrestrial radiation from rocks and building materials, trace amounts in food, cosmic radiation, background radiation from medical diagnostics and therapeutics, and consumer products such as smoke detectors and

ceramic dishes (*10*). It is therefore plausible that the radiation encountered in both daily life and in situations such as cancer treatment or space travel could have a significant influence on the composition and function of human-associated microbiota, including deleterious effects on the gut-brain axis.

This review covers the current evidence supporting the mechanistic relationship between the GI microbiome and the nervous system in both health and disease. Further evidence is also covered on how exposure to low or high LET radiation influences the GI microbiota, leading to potentially detrimental effects on the gut-brain axis.

1. Gut-Brain Communication Pathways

The GI tract and its microbial communities have several methods of communicating with the brain including via the enteric nervous system (ENS), production of microbial metabolites that influence the nervous system, microbe-derived neurotransmitters, and neuroimmune pathways. In this section, we will discuss these methods of gut-brain communication and how they function.

1.1 *The Enteric Nervous System and Branches of the Autonomic Nervous System*

One potential method of communication between the microbiota in the GI tract and the brain is via use of the vagus nerve. The vagus nerve is the tenth cranial nerve, and it allows bi-directional communication between the GI tract and the brain (*11*). The common hepatic branch of the abdominal portion of the vagus nerve is the most discussed in terms of gut-brain communication. This branch innervates the pyloric sphincter, the antrum of the stomach, the pancreas, and the proximal duodenum of the small intestine (*12*). In the brain, this nerve sends information to the nucleus tractus solitarii, which sends information to regions such as the

amygdala, thalamus, and locus coeruleus, all of which are involved in behavioral and emotional function (*13*). Vagal afferent nerves detect nutrients and nutrient-related conditions, such as acidity, cholecystokinin, and mechanical stimuli (*12*). Therefore, it is thought that the GI microbiota can communicate with the brain through peptides and metabolites they produce that signal through this nerve (*14*).

Previous studies have shown that the effects of the GI microbiome on factors such as anxiety, learned fear, and behavior are often not observed in the absence of the vagus nerve, indicating that it plays a critical role in communication with the brain (*15-17*). Bravo *et al*. found that stress-induced corticosterone and anxiety- and depression-related behavior was reduced in adult Balb/c mice after chronic oral administration of *Lactobacillus rhamnosus* JB1 strain, and that this effect was mediated by *L. rhamnosus*-dependent alterations to gamma-aminobutyric acid (GABA)B1b mRNA expression in different regions of the brain (*14*). Likewise, Bercik *et al*. found the same anti-anxiolytic effect when administering *Bifidobacterium longum* to male AKR mice who had dextran sodium sulfate (DSS) induced colitis, which leads to chronic anxiety-like behavior (*17*). Furthermore, *B. longum* metabolites were found to decrease excitability of enteric neurons. In both studies, vagotomy reversed the attenuating effects of the probiotic bacterial administration (*14, 17*). In another study by Tanida *et al*., injection of *Lactobacillus johnsonii* into the duodenum of male Wistar rats decreased sympathetic nerve activity as well as blood pressure, while simultaneously increasing gastric vagal nerve activity (*18*). Taken together, these studies provide a basis of evidence that the GI microbiota activate vagal pathways at the level of the enteric nervous system that signal to the CNS.

1.2 *Microbial Metabolites*

Lipids

Different types of lipid products can exert a range of effects on the host. For example, short chain fatty acids (SCFAs) are one of the metabolic products of the GI microbiota that have the ability to influence cellular processes in the host. SCFAs are produced via fermentation of carbohydrates that are not digestible by the host (*19*). The main SCFAs formed are acetate, propionate, and butyrate (*20*). These compounds are involved in epigenetic regulation of the genome, inducing inhibition or activation of histone modifying enzymes, therefore affecting gene expression. For example, SCFAs can modulate chromatin structure within the nuclei of lymphocytes to favor gene products that result in the proliferation of anti-inflammatory Foxp3+ regulatory T cells (Treg) (*19*). Furthermore, SCFAs can inhibit histone deacetylase (HDAC) near the Foxp3 promoter while simultaneously promoting acetylation of histone 3 at the Foxp3 promoter region, consequently inducing Treg development (*19*).

Polyunsaturated fats can also influence the host. These lipids, such as omega-6 and omega-3 fats, can be produced by certain bacteria in the GI tract (*20*). Omega-3 fats have been found to have anti-inflammatory effects when they are in a higher concentration than omega-6 fats (*19*). Additionally, saturated long-chain fatty acids can have antimicrobial properties in high concentrations, mostly targeting gram positive bacteria (*20*). Therefore, the lipid metabolites produced by the GI microbiota can have regulatory effects on other GI microbiota.

The lipid metabolites produced by the GI microbiota have also been shown to have effects on the brain. For instance, in one mouse study, SCFA supplementation alleviated selective and enduring alterations induced by repeated psychosocial stress such as anhedonia and heightened stress-responsiveness, as well as stress-induced increases in intestinal permeability (*20*). SCFA administration did not improve chronic stress-induced alterations in body weight gain and memory, but it decreased anxiety-like behavior in this study (*20*).

Another microbial metabolite pathway that has gained recognition in recent years is the endocannabinoid system. Ligands of the endocannabinoid system are members of the N-acylethanolamine (NAE) family of bioactive lipids. Two of the most well characterized endocannabinoids are N-arachidonylethanolamine (AEA, or anandamide) and 2-arachidonoylglycerol (2-AG) (*21*). These compounds, as well as many other endocannabinoids and endocannabinoid analogs, bind to endocannabinoid receptors such as cannabinoid receptor type 1 (CB_1), which is highly abundant in the CNS, and cannabinoid receptor type 2 (CB_2) that is found primarily in the GI tract (*22*). The interaction of endocannabinoids with these receptors has been found to have a wide range of effects in regulating fat intake and storage, satiating appetite, pain, inflammation, metabolism, liver function, and neurological effects (*21*). It has been posited that the GI microbiota regulate the levels of endocannabinoids in the GI tract and adipose tissue, and that specific alterations in the composition of microbiota can lead to a wide range of downstream effects (*21*). For example, mice on a high fat diet (HFD) treated with *Akkermansia muciniphila* exhibited increased intestinal levels of endocannabinoids that correlated with an improved metabolic profile and reversal of high-fat diet-induced metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance (*23*). Another similar study showed that HFD-fed mice that received *Akkermansia muciniphila* displayed reduced fat mass and improved glucose tolerance via distinct mechanisms of action on the endocannabinoid system (*24*). Interestingly, it has also been found that chronic cannabis users have a decreased prevalence of obesity (*25*). In all, although the mechanisms of communication are not fully elucidated, it is clear that the GI microbiota can produce and/or regulate important lipid metabolites, causing important physiological changes in the host.

Cysteine Metabolism

Another important molecule involved in microbial metabolism is the amino acid cysteine. Some species of bacteria, such as those belonging to the family *Desulfovibrionaceae,* desulfinate cysteine for use as a carbon source for downstream purposes (*19*). Cysteine can also be converted into glutathione, which can help to protect the host from reactive oxygen species (ROS). In patients with multiple sclerosis, dysbiosis (a pathogenic shift in the composition of the microbiota) can occur in the GI tract, with an increase in bacterial species that sequester cysteine that can lead to a buildup of cysteine and a dearth of glutathione production (*19*). Pathological symptoms may occur as a direct result of the lack of glutathione, therefore leading to decreased protection from the inflammatory response occurring in the CNS. With an excess of ROS, the blood brain barrier (BBB) can experience degradation, causing immune cells to enter the CNS and phagocytose myelin. Hence, it is clear that the metabolism of cysteine by the GI microbiota can have profound consequences on the brain.

Tryptophan Metabolism

Tryptophan, another amino acid, is also involved in a series of metabolic pathways that have important implications for the CNS. Tryptophan is a precursor molecule for serotonin (5-hydroxytryptamine, or 5-HT), as well as kynurenine. Changes in the availability of these two downstream molecules have been found to have effects on both the ENS as well as the CNS (*26*). Ninety five percent of the body's 5-HT is located in the GI tract and is primarily produced by enterochromaffin cells (EC), and many microbial species in the GI tract metabolize 5-HT (*27*). The 5-HT in the GI tract is involved in various reflexive functions, such as motility, secretion, absorption, transit, and colonic tone. 5-HT is also involved in mediating nausea and can modulate levels of food intake (*26*). The other five percent of serotonin is produced in the CNS

via serotonergic neurons that originate in the raphe nuclei in the brain and acts as an important neurotransmitter (*11*). While there is not yet a known pathway of communication between 5-HT from the GI tract and the brain, it is clear that changes in GI levels of 5-HT can influence the brain. A study by Yano *et al*. found that germ free (GF) mice, which lack all microbiota, had less 5-HT in the colon and serum compared to specific pathogen free (SPF) controls, which have a microbiome but lack particular pathogens. The presence of the microbiota elevated tryptophan hydroxylase 1 (Tph1) expression in colonic ECs, which promotes 5-HT synthesis (*28*). Specific metabolites are secreted by a subset of bacteria in the colon, including α-tocopherol, butyrate, cholate, deoxycholate, p-aminobenzoate (PABA), propionate, and tyramine, and it is these metabolites that provide the signal to upregulate Tph1 expression and consequently 5-HT production (*28*). The authors proposed that indigenous spore-forming bacterial (SFB) species were the producers of these metabolites (*28*). Filtered colonic contents from GF mice that were colonized with SFB induced 5-HT production in EC culture, as well as increase Tph1 expression (*28*), which indicates that the lack of SFB in GF mice is a possible mechanism of altered 5-HT synthesis.

The tryptophan that is not metabolized into 5-HT goes through the kynurenine pathway in the liver and is converted to kynurenine via tryptophan-2,3-dioxygenase (TDO) or indoleamine-2,3-dioxygenase (IDO) (*11*). While IDO is expressed in all tissues and can be induced by interferon gamma (IFNγ), TDO is only expressed in the liver and can be induced by glucocorticoids (*26*). Both IDO and TDO have connections to the GI microbiome. TDO activity may be partially dependent on microbial-neuroendocrine interactions, with significant implications for behavior and CNS function (*26*). On the other hand, studies have shown that species of *Lactobacillus* in the GI tract may attenuate conversion of tryptophan to kynurenine via

inhibition of IDO (*29*). This is important because increased levels of kynurenine have been implicated in autism, schizophrenia, depression, and neurodegenerative diseases (*30, 31*). Therefore, the control of kynurenine levels by species of *Lactobacillus* may be critical to neurologic function (*11*). Kynurenic acid is a neuroactive metabolite that can act on the N-methyl-D-aspartate (NMDA) and alpha-7 nicotinic acetylcholine receptors in the CNS and ENS, thus linking it to a role in immunoregulation and anti-inflammatory effects in both the brain and the GI tract, in addition to the regulatory effects that these receptors have on cognition (*32-34*).

Microbe-Derived Neurotransmitters and Receptor Alterations

Another mechanism whereby the GI microbiota may communicate with the brain is via microbe-derived neurotransmitters (*35*). For example, ingestion of *Lactobacillus rhamnosus* was shown to modulate the level of the receptor subunits for GABA, the main inhibitory neurotransmitter in the CNS, in different regions of the brain in mice (*14*). GI microbiota have the ability to regulate GABAergic neurotransmission via the vagus nerve. As previously mentioned, Bravo *et al*. found that chronic treatment with *L. rhamnosus* increased expression of GABA receptor mRNA transcripts in cortical regions of the brain, while decreasing expression in the hippocampus, amygdala, and locus coeruleus, whereas these alterations did not occur in vagotomized mice (*14*). Other neurotransmitters, such catecholamines, can be produced by not only the CNS but also the GI microbiota. Dopamine, which is a catecholamine, is produced by lactic-acid producing bacteria in culture, although peripherally synthesized dopamine is not able to cross the BBB (*36*). Ingestion of a probiotic strain of *Lactobacillus plantarum* increased levels of neurotransmitters such as 5-HT and dopamine in the striatum of the brain (*37*). The increase in striatum neurotransmitters was associated with alleviation of anxiety-like behaviors in a mouse model (*37*).

On the contrary, the absence of microbiota also leads to changes in dopamine and its receptors in the brain (*5, 38*). Germ-free mice exhibit increased *Drd1a* (dopamine D1 receptor) expression in the brain, as well as increased turnover of not only dopamine, but also norepinephrine and 5-HT (*38*). Therefore, it is thought that the GI microbiota are involved in regulating monoamine transmission in the brain.

Neuroimmune Pathways

Microbes in the gastrointestinal tract can also communicate with the brain via neuroimmune pathways. First, some GI bacteria can convert the dietary component L-histidine to histamine, which can inhibit the production of TNF-alpha (*39*). Histamine plays a role in immunomodulation but is also a known neurotransmitter. It has been proposed that production of histamine by members of the GI microbiota may influence signaling in the ENS that includes pain perception and GI tract motility (*39*). Next, gram negative bacteria can stimulate the production of pro-inflammatory cytokines, such as Interleukin (IL)-6 and IL-1beta. Lipopolysaccharide (LPS) on the surface of gram-negative bacteria can bind to TLR-4 on the surface of GI endothelial cells, causing them to express these pro-inflammatory cytokines, which can later communicate with the brain by activating the afferent terminals of peripheral neurons and eventually sending signals to the CNS (*40*). Finally, inflammatory responses in the GI tract have also been found to signal to the brain through the vagus nerve. For example, mice with *Campylobacter jejuni* infections exhibit increased neuronal activation in vagal sensory ganglia and in the nucleus of the solitary tract (*41*). When VSL#3, a probiotic including strains of *Lactobacillus*, *Bifidobacterium*, and *Staphylococcus*, was administered in a mouse model of liver inflammation, sickness behavior that included social withdraw and immobility, as well as systemic inflammation were attenuated (*42*).

1.3 *Effects of GI Microbial Metabolites on the Brain*

The aforementioned microbial metabolites connect the GI microbiome to the ENS and CNS. In addition to signaling that occurs between the GI microbiota and the nervous system, there are effects that the microbiome has on different cell types in the brain, including effects on myelination and neuronal proliferation.

Myelination

Several recent studies have implicated the GI microbiota in the regulation of genes that control myelination in the brain. Cresol, a phenol that is produced by some GI microbes, crosses the BBB. Increased cresol levels were detected in mice with GI microbiota enriched for Clostridiales, *Lachnospiraceae*, and *Ruminococcaceae* (*43*). These mice also exhibited social avoidance behaviors and had downregulation of genes involved in myelin expression and differentiation. Of interest, transfer of the intestinal microbiota from affected mice to microbiota-depleted recipient mice of a different genetic background was sufficient to induce the social avoidance behavior. Since the composition of the GI microbiota was sufficient to induce the depressive-like behaviors in genetically distinct mouse strains, it was concluded that in this case the microbiota overrode genetics (*43*).

The prefrontal cortex (PFC) is a critical region of the brain that is implicated in a wide range of neuropsychiatric disorders including depression, autism, and schizophrenia (*44*). Within the PFC lies the central neuronal circuitry that controls emotional regulation, memory storage, behavioral flexibility, and attention. In a study by Hoban *et al*., a marked upregulation of genes involved in myelination as well as genes involved in transcriptional regulation was found in the PFC of germ-free mice (*44*). Therefore, the absence of microbiota led to hypermyelinated axons

in the PFC. Thus, the GI microbiome may have a strong connection to the myelination state of the PFC and could have an important role in many of its associated neuropsychiatric disorders.

Neuronal Formation, Maturation, and Proliferation

Though there is little work in the field thus far, there is accumulating evidence that the GI microbiome can influence neuronal proliferation, formation, and maturation as well (*45*). For example, germ-free mice have increased adult hippocampal neurogenesis, mostly in the dorsal hippocampus, a region of the brain primarily involved in cognitive functions (e.g., spatial memory) (*46*). Therefore, the GI microbiota may regulate the amount of neuronal proliferation occurring in the brain, which could impact cognition. There appears to be a critical period of time early in life during which the composition of the microbiome influences future adult hippocampal neurogenesis. GF mice tend to have a decrease in occludins and claudins in the BBB, and consequently a loss of integrity of the tight junctions (*45*). Therefore, a dysbiotic state in the gut can potentially influence neurogenesis, microglial activation, as well as neuronal myelination, survival, growth, and differentiation signaling (*45*).

Neuropsychiatric Disorders

Neuropsychiatric disorders have been increasingly linked to changes in the GI microbiome as well as inflammation, which are intimately connected. Manic patients who received antibiotics in the past exhibited increased symptom severity, which points to an intriguing connection between the composition of the GI flora and normal CNS function (*47*). Another example is that GABA can be produced by *Lactobacillus brevis* and *Bifidobacterium dentium* in the GI tract, and GABA function is associated with depression, anxiety, autism, and schizophrenia (*48, 49*). GABA produced in the intestines can cross the BBB. Therefore, this is an area worthy of further investigation.

The etiology of schizophrenia is not well understood, and to date no true molecular markers of the disease have been identified (*47*). The condition is often associated with GI comorbidities, including irritable bowel syndrome, inflammatory bowel disease, and celiac disease (*2*). In 1953, Bender noted an association between celiac disease and schizophrenia (*50, 51*). This was the first link between autoimmune disease in the GI tract and schizophrenia. Genetic polymorphisms in genes related to serotonin have been reported in schizophrenia patients (*52, 53*). For example, polymorphisms in the gene HTR1B, which encodes the 5-HT_{1B} protein, a G-protein coupled receptor whose ligand is serotonin, were found to be correlated with incidence of schizophrenia in the Han-Chinese population (*53*). It is possible, therefore, that alterations in serotonin metabolism due to changes in the GI microbiota (such as indigenous SFB species, see section 1.2) could influence mental illnesses such as schizophrenia.

Anxiety and depression have also been linked to the GI microbiome. Neufeld *et al*. reported reduced anxiety in GF mice in stressful situations compared to control mice (*54*). Bravo *et al*. demonstrated reduced anxiety in rats following probiotic treatment, with the probiotic effect being ameliorated in vagotomized subjects (*14*). Additionally, *Campylobacter jejuni* infection in mice resulted in induction of c-Fos in anxiety-related regions of the brain (*41, 55*). Depression has been associated with deficiencies in tryptophan metabolism (*56-58*), which, as previously described, is closely related to the GI microbiome. There are many species in the GI microbiome that contribute to tryptophan catabolism including *Escherichia coli*, *Bacteroides* spp., *Clostridium* spp., *Lactobacillus* spp., *Ruminococcus* spp., and *Peptostreptococcus* spp. (*59*). In one study, fecal transplants from depressed human patients to rats lead to alterations in tryptophan metabolism and anxiety-like behavior in the recipients, which was evidence of a causal role of the GI microbiome in depression (*60*).

Finally, autism spectrum disorder (ASD) is speculated to have a connection to the GI microbiome. Dysbiosis is often associated with late onset ASD, and GI symptoms are common in patients (*61-65*). Various studies have found increased levels of *Clostridium* species, decreased levels of Bacteriodetes, as well as altered compositions of *Bifidobacterium*, *Lactobacillus*, *Sutterella*, *Prevotella*, *Ruminococcus*, and *Alcaligenaceae* in the feces of patients with the disorder (reviewed in (*2*)).

Importantly, there is bidirectional communication and influence between the brain and the GI tract. While changes in the GI microbiota can affect the brain, changes in the brain can also affect the GI microbiome. For example, stress alters patterns of mucous secretion in the GI tract, which can consequently impact the GI microbiome (*2*). Galley *et al*. also showed that merely two hours of social disruption in mice led to a decrease in the GI levels of *Lactobacillus* (*66*).

2. Radiation Effects on the Gut-Brain Axis

There are several ways to categorize types of radiation. Non-ionizing radiation is incapable of removing electrons from atoms, and this includes radio waves, visible light, and microwaves. Ionizing radiation has sufficient energy to remove electrons from other atoms. Within the category of ionizing radiation, there are sub-categories of high and low energy transfer radiation. Linear energy transfer (LET) refers to the amount of energy lost per unit of path length travelled by the radioactive particle (*67*). Therefore, high LET radiation deposits more energy in tissue and thus has the potential to cause more significant tissue damage than low LET radiation. As the radiation penetrates cells and emits energy, it can cause DNA damage. An example of low LET radiation would be gamma rays, which are often used in treatment for

cancer. These rays are very penetrating and can travel several hundred feet in air (*68*). Conversely, examples of high LET radiation include high energy and charge (HZE) particles, neutron particles, and alpha particles. HZE and neutron particles can both penetrate deeply into the human body, travel long distances, and require heavy shielding for protection. These types of high LET radiation can be encountered in space outside of the Earth's magnetosphere. In this new age of technology, travel, medical treatment, and more, factors such as radiation must be considered when investigating the interaction of the GI microbiome and the brain. Radiation can also be encountered in the environment, and persistent exposure to environmental radiation, such as in the Chernobyl Exclusion Zone, has also been found to alter health metrics, such as the composition of the GI microbiome (*69*). Likewise, examining the gut-brain axis in the context of radiological injuries could be extremely useful when trying to determine which individuals have been exposed to damaging amounts of radiation and which have not (*70*). Analyzing the microbial composition of fecal samples from individuals exposed to radiological and perhaps even nuclear disasters could serve as a minimally invasive and informative biodosimetry tool in the future (*70*).

2.1 ***GI Dysbiosis Caused by Low LET Radiation***

Low LET radiation, such as radiation that is used for radiotherapy for cancer, has been shown to cause dysbiosis in the GI microbiome (Table 1). For example, Ritchie *et al*. exposed male Sprague-Dawley rats to fractionated low LET gamma radiation and measured changes in the GI microbiome. They found increases in bacteria belonging to the bacterial family Lactobacillales and decreased Clostridiales and Firmicutes in animals treated with low LET gamma radiation (*71*). The species richness and diversity between the irradiated and non-irradiated groups was not significantly different. Although the species richness, measured by the

Chao Index, was not significantly different, it was notably higher in rats that received radiation compared to those who did not.

Other studies have found correlations between the changes in the GI microbiome that occur following low LET radiation exposure and the amount of inflammation in the GI tract. Gerassy-Vainberg *et al*. used localized internal rectal radiation to investigate the effects caused by the exposure on pro-inflammatory dysbiosis. They posited that some of the radiation-induced changes in GI microbiota can exert direct pro-inflammatory effects on the GI epithelial cells, increasing their expression of pro-inflammatory cytokines such as TNF-alpha and IL-1beta compared to those with unexposed microbiota (*72*). Based upon these findings, the proposed sequence of events by several groups is that radiotherapy can induce dysbiosis in the GI tract, which consequently triggers the release of pro-inflammatory cytokines such as IL-1beta from the gastrointestinal mucosa, and these cytokines eventually aggravate mucosal damage (*72*). However, it is important to note that the study does not rule out any direct effects of radiation to the GI epithelial cells. Another study by Yamanouchi *et al*. exposed mice to 2 and 4 Gy of X-ray radiation and studied short-term changes to *Lactobacillus* and *Bifidobacterium*. They found an immediate decrease in *Lactobacillus* in both groups, and a large increase in *Bifidobacterium* in the 4 Gy group (*73*). This led the authors to postulate that *Bifidobacterium* may play a potential role in the repair and regeneration of the GI epithelial tissue.

Another interesting observation in studies modeling radiotherapy for cancer is that the compositional changes in the microbiota occur over an extended period of time. In a mouse study by Gerassy-Vainberg *et al*., at two weeks post-irradiation the microbiome composition was still similar to baseline. However, the predominant change in bacterial composition occurred six weeks post-irradiation (*72*). Consistent with this study, in patients with gynecological cancers

receiving pelvic radiotherapy, it was also found that the maximal change in microbial composition was 1-3 months post-irradiation (*74*). Therefore, it is important to take into account that microbial changes may happen gradually, and this should be a consideration when tracking changes in the GI microbiome post-radiation exposure.

2.2 *Cognitive Effects of Radiotherapy for Cancer*

In addition to changes in the microbiome, there is an extensive body of evidence illustrating that radiotherapy can cause cognitive impairments in patients. At first, most research in this field focused on the effects of chemotherapy, calling it a "cancer/chemotherapy related cognitive impairment" (*75*). Patients who receive chemotherapy have often been noted to have anxiety, depression, cognitive impairments, and sleep disturbances. More specifically, cognitive impairments in chemotherapy patients include changes in executive functions (cognitive processes that are necessary for managing oneself and one's resources in order to achieve a goal), learning and memory, visuospatial skills, attention, language, and concentration (*76*). In some cases, these symptoms have been found to last well beyond five years post-treatment (*77*). However, most of the patients receiving chemotherapy and experiencing these symptoms were also receiving radiation therapy.

Importantly, while cognitive impairments have been noted in cases of head-only radiation, impairments have also been found when the radiation was administered to other parts of the body, therefore implying some other factor, such as the GI microbiome, may mediate the damage. For example, Feiock *et al*. studied the cognitive effects caused by radiation as opposed to solely focusing on chemotherapy. Balb/c mice either received a 16 Gy fraction of ionizing radiation to the right hind limb, or received three doses of methotrexate, a chemotherapeutic agent, once per week for three weeks (*75*). They found that non-brain directed radiation was

equally as effective as chemotherapy at causing significant bystander injury in the brain, including multifocal hypometabolism and persistent neuroinflammation. Shibiyama *et al*. also found cognitive impairments in radiotherapy patients even when the radiation was directed at non-brain areas (*78*). In breast cancer patients who received radiotherapy, there was significantly impaired verbal memory and delayed recall, with deficiencies lasting for several months after treatment. The radiotherapy group additionally exhibited elevated systemic levels of the pro-inflammatory cytokine IL-6 (*78*). A prostate cancer study found that cancer-related fatigue during radiotherapy was often associated with cognitive impairment, and the authors posited that there may be specific cognitive domains associated with fatigue and cognitive impairment during external beam radiation therapy (EBRT) (*79*). Finally, Cui *et al*. found that abdominal radiation for pelvic malignancies resulted in increased expression of a specific microRNA that targeted brain-derived neurotrophic factor (*Bdnf*) mRNA, consequently resulting in cognitive dysfunction (*80*). Therefore, low LET radiation as a causative agent in cognitive dysfunction warrants further investigation. While the exact mechanism remains unclear, we propose that these cognitive changes could be linked to radiation-induced changes in the GI microbiome (Figure 1). As previously discussed, dysbiotic changes are observed in the GI microbiota after radiation exposure (Table 1), and given the aforementioned relationship between the GI microbiota, neuroinflammation, and the CNS, it is plausible to hypothesize that the cognitive changes observed after treatment with radiotherapy are mediated by radiation-induced shifts in the composition and function of the GI microbiota.

2.3 *Human Exposure to High LET Radiation*

Other than hadron therapy for cancer (e.g., radiotherapy with protons, carbon ions, etc.), there are few instances where humans would be exposed to high LET radiation on Earth, but

with modern advancements in space travel and humankind's sights set on Mars, it is a relevant factor to consider. On a flight to Mars, humans would be exiting the Earth's magnetosphere, and therefore escaping the protection it provides from potentially harmful high LET radiation. It is approximated that one would be exposed to roughly 1.3 mGy of radiation per day outside of the Earth's magnetosphere, and that the total dose of a return mission to Mars could add up to 0.5 Gy (*81*). This radiation dose would be coming from solar particle events (SPEs), which involves high dose rate exposures to protons, as well as galactic cosmic radiation (GCR), which can include iron, silicon, oxygen, carbon, and helium ions that are extremely energetic and can't be easily shielded by existing materials used during space travel.

Acute and chronic tissue damage may arise from the effects of charged, highly energetic particles crossing through the spacecraft itself and penetrating human tissue (*82*). This could have many possible effects on the human body, including changes in the immune system. Astronauts could have impaired immune responses in space, and therefore increased risk of infections (*83*). Accordingly, mice exposed to SPE radiation exhibited transient increases in proinflammatory cytokine levels including IFN-alpha, IL-6, and TNF-alpha (*84*). Radiation also resulted in gram negative bacteria breakdown and an increase in circulating immune stimulatory bacterial lipopolysaccharide (LPS) and the type I acute phase protein, LPS binding protein.

2.4 *GI Dysbiosis Caused by Spaceflight Factors*

The spaceflight environment includes several factors that can influence the GI microbiome, including microgravity and radiation. Experiments performed on the International Space Station (ISS) are in low earth orbit (LEO), and while they do include radiation exposure, mostly in the form of protons, they are still protected by the Earth's magnetosphere. As such, the results are primarily due to the effects of microgravity. Ritchie *et al*. analyzed the GI microbiota

in female mice who had embarked on a thirteen-day long spaceflight (*71*). They found that the flight mice harbored more Bacteriodetes and Clostridiales than controls, and less *Firmicutes* and Lactobacillales than controls. Although not significant, there was also a reduced species richness in the flight mice in comparison to the ground control mice (*71*). Another study observed reduced length of villi and depth of crypts in rats that had been flown to space (*85*). Due to these changes in intestinal morphology, mucin production was decreased by the intestinal epithelial cells. No significant intestinal injury or inflammatory infiltration was found as a result of short-term exposure to the space environment, but this would not necessarily hold true for longer duration missions outside of the Earth's magnetosphere.

Just like low LET radiation for radiotherapy can cause dysbiosis in the GI microbiome of patients, high LET radiation encountered outside of the Earth's magnetosphere can do the same (Table 1). In support of this phenomenon are experiments performed using particle accelerators, with radiation as the only variable (not including microgravity). Casero *et al*. found an increase in the amount of *Akkermansia muciniphila* in the GI tract of mice following whole-body exposure to ^{16}O ions (*81*). Since *A. muciniphila* is a mucin-degrading bacterium thought to be important in maintaining the integrity of the mucosal epithelial barrier, this finding could indicate that high LET radiation causes intestinal injury and leakiness of the GI epithelium. However, the long-term consequences of this increase in *A. muciniphila* are uncertain.

In the same study, which included 0, 0.1, 0.25, and 1 Gy doses of ^{16}O ions, marked increases in alpha diversity were observed between 10 and 30 days post-radiation exposure as well as significant decreases in beta diversity, regardless of dose level (*81*). In terms of metabolites, there was an increase in phosphatidic acid (PA) and other lyso-PA metabolites. This is notable since these metabolites can activate pro-inflammatory mTOR signaling directly. There

was also downregulation of carbohydrate digestion and absorption (*81*). Overall, it is evident that exposure to high LET radiation can alter the composition of the GI microbiome, and therefore induce downstream metabolomic, immune, and morphologic changes.

2.5 *Cognitive Effects of Cosmic Radiation*

Just as low-LET radiation has been shown to have cognitive effects, high LET radiation can do the same. A study by Mange *et al*. exposed rats to whole-body 5 and 25 cGy doses of ^{16}O ions, and then performed social odor memory recognition testing on the subjects (*86*). One-month post-irradiation, both the 5 and 25 cGy groups exhibited memory deficits for the recall of social odors. However, six months post-irradiation, only the 25 cGy group showed deficits while the 5 cGy group did not (*86*). Therefore, the lower dose radiation group was able to recover, while the higher dose group had long term cognitive damage. Jones *et al*. also observed deficits in social odor recognition memory at both 1 and 10 cGy of ^{16}O ions, with the 1 cGy group developing impairments later on, while the 10 cGy group displayed consistent deficits (*87*). Another study by Krukowski *et al*. also exposed rats to ^{16}O radiation, but in doses of 25 and 40 cGy. The 25 cGy rats had social odor recognition memory deficits at 4 months post-exposure, while the 40 Gy group did not (*88*). In both cases, though perhaps within a certain window of dosage, it is evident that this type of heavy ion exposure is capable of producing long-term memory deficits.

Following radiation exposure, there are many changes in the brain that occur. For example, various studies have investigated how different forms of cosmic radiation affect hippocampal neurogenesis. Hellstrom *et al*. found that hippocampal neurogenesis was reduced to five percent of the level of the control group in Wistar rats nine weeks post-radiation exposure (*89*). In another study, mice were exposed to helium ions, the second most prevalent form of

radiation in space. The mice exhibited significant hippocampal and cortical-based issues that lasted one year post-exposure, including deficits in memory and cognitive flexibility, reduced rates of fear extinction, and increased anxiety and depressive-like behavior (*82*). Finally, there were increased amounts of activated microglia, indicating neuronal inflammation. While these changes could be due to radiation exposure alone, it is possible that they are connected to alterations in the GI microbiome. For example, while Casero *et al*. used high LET radiation and found changes in the GI microbiome composition, the studies by Hellstrom *et al*. and Parihar *et al*. also used high LET radiation and this resulted in cognitive effects (*81, 82, 89*). Overall, like low LET radiation, it is evident that high LET radiation is also capable of inducing cognitive issues and molecular imbalances in the brain, and we hypothesize that this may be mediated at least in part by the effects of high LET on the GI microbiome (Figure 1).

2.6 *Comparison of Changes in the Gut and Brain Post-High LET Radiation*

When comparing the literature regarding changes in the GI microbiome and changes in the CNS following high LET radiation, there is little overlap. Studies have utilized similar doses of high LET radiation in ranges that would be relevant for long-term space travel. However, while CNS changes have been observed at more long-term time points, from at least one month up to one year, microbiome changes have mostly been studied at more acute time points, as early as 24 hours post-exposure up until 6 weeks (*71, 72, 74, 81, 82, 86-89*). There is also a general dearth of scientific literature specifically studying changes in the GI microbiome following high LET radiation. Evidence of peripheral low LET radiation causing changes to the CNS exists, but there is not much literature regarding the opposite situation of head-only irradiation causing microbiome alterations (*75*). Additionally, this situation has only been studied using low LET radiation. Overall, more work is definitely needed in this area to specifically examine the gut-

brain axis following exposure to low and high LET radiation. Studying changes in the CNS at earlier time points following radiation exposure and changes in the GI microbiome at later time points as well as efforts that study both changes in the GI microbiome and cognitive function in the same subjects post-exposure would provide a more holistic view of the situation and allow more mechanistic questions and connections to develop.

3. Interventions for Microbiome Manipulation

Given further evidence linking the GI microbiota to the effects of radiation on cognition, the next consideration should be ways to manipulate the GI microbiome in order to prevent cognitive deficits and/or improve mental health of humans affected by radiation. There are several possible ways to accomplish this, which will be discussed in this section.

3.1 *Antibiotics*

One possible side effect of radiation is the translocation of GI bacteria across the intestinal mucosa due to increased GI leakiness (*90*). This can result in sepsis and even death. Therefore, one suggested intervention for this issue is antibiotics. In the event of acute radiation syndrome, for example, administration of antibiotics is part of the standard treatment plan (*91, 92*). Preferably, this antibiotic would be one that specifically targets only the species of bacteria that have a proclivity to translocate after exposure to radiation so as not to eliminate commensal bacteria, since this could result in dysbiosis and other detrimental effects. Antibiotics could perhaps be administered before radiation exposure as a prophylactic effort.

3.2 *Probiotics and Prebiotics*

Another strategy that can be used to help shape the composition of the GI microbiome is administration of probiotics. Probiotics are defined as live bacteria that are ingested and are

intended to colonize the gastrointestinal tract of the consumer. Prebiotics, on the other hand, are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health (*93*). Both types of supplements can be easily administered to patients in pill form.

Prebiotics are usually oligosaccharides, particularly fructans and galactans, acting as a nutrient source for microbial populations in the GI tract (*94*). Another class of prebiotics includes polyphenolic compounds such as anthocyanins, flavonoids, tannins, and lignins. These compounds can be transformed into bioactive metabolites by microbes in the GI tract, where they can they be used to modulate the microbial composition (*95*).

In terms of probiotics, certain species of bacteria have been found to be very beneficial for the GI microbiome, such as *Lactobacillus* and *Bifidobacterium*. For example, *Lactobacillus* has been found to stimulate macrophages and/or dendritic cells in the Peyer's patches in the small intestine to release cytokines, including regulatory cytokines IL-4 and IL-10 (*83*). *Lactobacillus rhamnosus* reduced elevated levels of TNF-alpha in feces, therefore reducing inflammation in the gastrointestinal tract (*96*). Oral administration of *Lactobacilli* and *Bifidobacterium* increases systemic and mucosal IgA response to antigens (*96*), enforcing the point that the GI microbiome has an important role in shaping the host's immune system.

Both pre- and probiotics can have beneficial effects on the GI tract, including antimicrobial peptide production, increased mucus production by Goblet cells, SCFA production, antioxidant effects, barrier enhancement, and prevention of pathogenic biofilm formation (*97-101*). Both types of supplements have also been shown to induce cognitive changes via the gut-brain axis. Schmidt *et al*. found that prebiotics (specifically Bimuno®-galactooligosaccharides) modulate emotional attention performance (*102*). Probiotics (fermented milk product with a

cocktail of four probiotic bacterial strains) were found to alter functional brain activity in women while performing emotional attention tasks (*103*). Alterations in the GI microbiome have also been linked to depression, anxiety, and age-related cognitive decline (*104*). Therefore, manipulating the GI microbiome through use of pre- and probiotics has the potential to treat many of the cognitive changes seen in individuals exposed to different forms of radiation.

3.3 *Postbiotics*

Since the metabolites produced by the microbiota in the gastrointestinal tract have direct effects on the body, another method to alter or restore the microbiome's metabolic function is to administer the products that the desired microbes would make without altering the microbiome at all. Postbiotics, defined as non-viable products of GI microbiota that exert biological activities in the host, can serve this purpose. For example, while some bacterial species produce antioxidants to help protect against radiation damage, consuming antioxidants before or after radiation has been shown to confer some level of radioprotection (*83*). Other examples of postbiotics would be administration of SCFAs such as butyrates, which can have beneficial cognitive effects as previously discussed. Overall, directly administering the desired metabolites that would have been produced by radiation-altered microbiota in the GI tract can be an effective method of achieving the same end result.

3.4 *Fecal Microbiota Transplant (FMT)*

A final emergent method of GI microbiome manipulation is fecal microbiota transplant (FMT). Although this phenomenon has been traced back to as early as the fourth century, the first FMTs recorded in more modern times were used in the latter half of the twentieth century to treat recurrent *Clostridium difficile* infection (*105*). By administering fecal matter from a healthy donor to a sick patient, either via the upper or lower gastrointestinal tract, it is possible to re-

diversify the infected patient's microbiome and provide a "colonization resistance" that protects them from future infections (*105*).

FMT has been tested to resolve a wide range of gastrointestinal and non-gastrointestinal disorders, not limited to but including inflammatory bowel disease, irritable bowel disease, idiopathic constipation, non-alcoholic fatty liver disease, sclerosing cholangitis, idiopathic thrombocytopenic purpura, multiple sclerosis, myoclonus dystonia, Parkinson's disease, obesity, insulin resistance, metabolic syndrome, chronic fatigue syndrome, autism, stress-related behavior and anxiety (*105*). Due to its wide range of applications, it is likely that in the near future there will be a wide selection of products with differing microbiota profiles and purposes commercially available, for example in the form of lyophilized materials and capsules.

Given a confirmed association between radiation exposure and perturbation to the gut-brain axis, FMT treatments could potentially be used, for example, to alleviate symptoms of radiation therapy for cancer patients or as a countermeasure for astronauts on long-duration missions. Indeed, Cui *et al*. found that the GI microbiota of male and female mice differed, and the difference was associated with the subjects' susceptibility to radiation-induced toxicity. FMT from sex-matched healthy donor stool immediately following radiation exposure increased the survival rate of the animals (*106*).

Conclusions

The field of gut-brain axis research is still evolving, and certainly an open area for active research. The effects of radiation on this axis are even less understood. Various time courses after radiation exposure remain to be studied, especially the longer-term effects of radiation on the gut-brain axis. Next, the specific microbiota as well as the microbial metabolites involved in

these interactions need to be further teased out; particularly, whether there are specific microbiota that either contribute to or ameliorate different neurocognitive pathophysiologies induced by radiation exposure. Furthermore, it is unclear which microbe-produced metabolites circulate systemically, and thus may act directly on the brain.

Despite the remaining gaps of knowledge in the field, it is increasingly evident that a connection between the gut and brain exists. It is also apparent that exposure to different types of radiation can exert detrimental effects on the GI microbiome, consequently altering its composition, and therefore potentially the messages it sends to the brain. Further understanding of the mechanistic link between radiation exposure and the gut-brain-axis will be important to the development of therapeutic and/or prevention strategies such as targeted antibiotics or pre/pro/postbiotic use.

Acknowledgements

We would like to gratefully acknowledge Space@Hopkins (to C.B.J., C.M.D., and K.S.S.) and NASA 80NSSC18K1080 (to C.M.D.) for research support, as well as the Pathobiology Graduate Program at the Johns Hopkins University School of Medicine.

References

1. Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, et al., Strains, functions and dynamics in the expanded Human Microbiome Project. Nature 2017; 550, 61.

2. Kim YK, Shin C, The Microbiota-Gut-Brain Axis in Neuropsychiatric Disorders: Pathophysiological Mechanisms and Novel Treatments. Curr Neuropharmacol 2018; 16, 559-73.

3. Dinan TG, Cryan JF, The microbiome-gut-brain axis in health and disease. Gastroenterology clinics of North America 2017; 46, 77-89.

4. Mörkl S, Wagner-Skacel J, Lahousen T, Lackner S, Holasek SJ, Bengesser SA, et al., The role of nutrition and the gut-brain axis in psychiatry: A review of the literature. Neuropsychobiology 2018.

5. Liu L, Zhu G, Gut-brain axis and mood disorder. Frontiers in psychiatry 2018; 9, 223-23.

6. Nemani K, Hosseini Ghomi R, McCormick B, Fan X, Schizophrenia and the gut–brain axis. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2015; 56, 155-60.

7. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al., Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. Nature 2016; 534, 213.

8. Moser G, Fournier C, Peter J, Intestinal microbiome-gut-brain axis and irritable bowel syndrome. Wiener medizinische Wochenschrift 2018; 168, 62-66.

9. Rothschild LJ, Microbes and radiation. In: Seckbach J editor. Enigmatic Microorganisms and Life in Extreme Environments. Place Springer Netherlands: Springer Netherlands; 1999.

10. Report No. 180 – Management of Exposure to Ionizing Radiation: Radiation Protection Guidance for the United States. Place National Council on Radiation Protection and Measurements National Council on Radiation Protection and Measurements 2018.

11. Sherwin E, Sandhu KV, Dinan TG, Cryan JF, May the Force Be With You: The Light and Dark Sides of the Microbiota-Gut-Brain Axis in Neuropsychiatry. CNS Drugs 2016; 30, 1019-41.

12. Berthoud HR, Neuhuber WL, Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 2000; 85, 1-17.

13. Breit S, Kupferberg A, Rogler G, Hasler G, Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders. Front Psychiatry 2018; 9, 44.

14. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al., Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America 2011; 108, 16050-5.

15. Klarer M, Arnold M, Gunther L, Winter C, Langhans W, Meyer U, Gut vagal afferents differentially modulate innate anxiety and learned fear. J Neurosci 2014; 34, 7067-76.

16. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al., The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 2011; 141, 599-609, 09 e1-3.

17. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al., The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 2011; 23, 1132-9.

18. Tanida M, Yamano T, Maeda K, Okumura N, Fukushima Y, Nagai K, Effects of intraduodenal injection of Lactobacillus johnsonii La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci Lett 2005; 389, 109-14.

19. Dopkins N, Nagarkatti PS, Nagarkatti M, The role of gut microbiome and associated metabolome in the regulation of neuroinflammation in multiple sclerosis and its implications in attenuating chronic inflammation in other inflammatory and autoimmune disorders. Immunology 2018; 154, 178-85.

20. van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, et al., Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol 2018; 596, 4923-44.

21. Cani PD, Plovier H, Van Hul M, Geurts L, Delzenne NM, Druart C, et al., Endocannabinoids--at the crossroads between the gut microbiota and host metabolism. Nat Rev Endocrinol 2016; 12, 133-43.

22. Butler MI, Cryan JF, Dinan TG, Man and the Microbiome: A New Theory of Everything? Annu Rev Clin Psychol 2019; 15, 371-98.

23. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al., Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. 2013; 110, 9066-71.

24. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al., A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med 2017; 23, 107-13.

25. Hawkins MN, Horvath TL, Cannabis in fat: high hopes to treat obesity. J Clin Invest 2017; 127, 3918-20.

26. Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology 2017; 112, 399-412.

27. Gershon MD, Tack J, The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 2007; 132, 397-414.

28. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al., Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015; 161, 264-76.

29. Valladares R, Bojilova L, Potts AH, Cameron E, Gardner C, Lorca G, et al., Lactobacillus johnsonii inhibits indoleamine 2,3-dioxygenase and alters tryptophan metabolite levels in BioBreeding rats. FASEB J 2013; 27, 1711-20.

30. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ, Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci 2012; 13, 465-77.

31. McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, Crawley JN, Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes Brain Behav 2008; 7, 152-63.

32. Steiner L, Gold M, Mengel D, Dodel R, Bach JP, The endogenous alpha7 nicotinic acetylcholine receptor antagonist kynurenic acid modulates amyloid-beta-induced inflammation in BV-2 microglial cells. J Neurol Sci 2014; 344, 94-9.

33. Albuquerque EX, Schwarcz R, Kynurenic acid as an antagonist of alpha7 nicotinic acetylcholine receptors in the brain: facts and challenges. Biochem Pharmacol 2013; 85, 1027-32.

34. Samavati R, Zador F, Szucs E, Tuka B, Martos D, Veres G, et al., Kynurenic acid and its analogue can alter the opioid receptor G-protein signaling after acute treatment via NMDA receptor in rat cortex and striatum. J Neurol Sci 2017; 376, 63-70.

35. Mittal R, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, et al., Neurotransmitters: The critical modulators regulating gut-brain axis. Journal of cellular physiology 2017; 232, 2359-72.

36. Kuley E, Balikci E, Ozogul I, Gokdogan S, Ozogul F, Stimulation of cadaverine production by foodborne pathogens in the presence of Lactobacillus, Lactococcus, and Streptococcus spp. J Food Sci 2012; 77, M650-8.

37. Liu WH, Chuang HL, Huang YT, Wu CC, Chou GT, Wang S, et al., Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. Behav Brain Res 2016; 298, 202-9.

38. Luczynski P, McVey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF, Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. The international journal of neuropsychopharmacology 2016; 19.

39. Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, et al., Histamine derived from probiotic Lactobacillus reuteri suppresses TNF via modulation of PKA and ERK signaling. PLoS One 2012; 7, e31951.

40. Watkins LR, Maier SF, Goehler LE, Cytokine-to-brain communication: a review & analysis of alternative mechanisms. Life Sci 1995; 57, 1011-26.

41. Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N, Lyte M, Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni. Brain Behav Immun 2005; 19, 334-44.

42. D'Mello C, Ronaghan N, Zaheer R, Dicay M, Le T, MacNaughton WK, et al., Probiotics Improve Inflammation-Associated Sickness Behavior by Altering Communication between the Peripheral Immune System and the Brain. J Neurosci 2015; 35, 10821-30.

43. Gacias M, Gaspari S, Santos PM, Tamburini S, Andrade M, Zhang F, et al., Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. Elife 2016; 5.

44. Hoban AE, Stilling RM, Ryan FJ, Shanahan F, Dinan TG, Claesson MJ, et al., Regulation of prefrontal cortex myelination by the microbiota. Transl Psychiatry 2016; 6, e774.

45. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK, The Central Nervous System and the Gut Microbiome. Cell 2016; 167, 915-32.

46. Ogbonnaya ES, Clarke G, Shanahan F, Dinan TG, Cryan JF, O'Leary OF, Adult Hippocampal Neurogenesis Is Regulated by the Microbiome. Biol Psychiatry 2015; 78, e7-9.

47. Dickerson F, Severance E, Yolken R, The microbiome, immunity, and schizophrenia and bipolar disorder. Brain Behav Immun 2017; 62, 46-52.

48. Yunes RA, Poluektova EU, Dyachkova MS, Klimina KM, Kovtun AS, Averina OV, et al., GABA production and structure of gadB/gadC genes in Lactobacillus and Bifidobacterium strains from human microbiota. Anaerobe 2016; 42, 197-204.

49. Li H, Qiu T, Huang G, Cao Y, Production of gamma-aminobutyric acid by Lactobacillus brevis NCL912 using fed-batch fermentation. Microb Cell Fact 2010; 9, 85.

50. Bender L, Childhood schizophrenia. Psychiatr Q 1953; 27, 663-81.

51. Severance EG, Yolken RH, Eaton WW, Autoimmune diseases, gastrointestinal disorders and the microbiome in schizophrenia: more than a gut feeling. Schizophr Res 2016; 176, 23-35.

52. Peitl V, Stefanovic M, Karlovic D, Depressive symptoms in schizophrenia and dopamine and serotonin gene polymorphisms. Prog Neuropsychopharmacol Biol Psychiatry 2017; 77, 209-15.

53. Xia X, Ding M, Xuan JF, Xing JX, Pang H, Wang BJ, et al., Polymorphisms in the human serotonin receptor 1B (HTR1B) gene are associated with schizophrenia: a case control study. BMC Psychiatry 2018; 18, 303.

54. Neufeld K-AM, Kang N, Bienenstock J, Foster JA, Effects of intestinal microbiota on anxiety-like behavior. Communicative & integrative biology 2011; 4, 492-94.

55. Lyte M, Varcoe JJ, Bailey MT, Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. Physiol Behav 1998; 65, 63-8.

56. Baranyi A, Amouzadeh-Ghadikolai O, von Lewinski D, Breitenecker RJ, Rothenhausler HB, Robier C, et al., Revisiting the tryptophan-serotonin deficiency and the inflammatory hypotheses of major depression in a biopsychosocial approach. PeerJ 2017; 5, e3968.

57. Duan KM, Ma JH, Wang SY, Huang Z, Zhou Y, Yu H, The role of tryptophan metabolism in postpartum depression. Metab Brain Dis 2018; 33, 647-60.

58. Dell'Osso L, Carmassi C, Mucci F, Marazziti D, Depression, Serotonin and Tryptophan. Curr Pharm Des 2016; 22, 949-54.

59. Roager HM, Licht TR, Microbial tryptophan catabolites in health and disease. Nature Communications 2018; 9, 3294.

60. Kelly JR, Borre Y, C OB, Patterson E, El Aidy S, Deane J, et al., Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res 2016; 82, 109-18.

61. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, Bolte E, et al., Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 2002; 35, S6-S16.

62. Song Y, Liu C, Finegold SM, Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol 2004; 70, 6459-65.

63. Williams BL, Hornig M, Parekh T, Lipkin WI, Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio 2012; 3.

64. Parracho HM, Bingham MO, Gibson GR, McCartney AL, Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol 2005; 54, 987-91.

65. Martirosian G, Ekiel A, Aptekorz M, Wiechula B, Kazek B, Jankowska-Steifer E, et al., Fecal lactoferrin and Clostridium spp. in stools of autistic children. Anaerobe 2011; 17, 43-5.

66. Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, et al., Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. BMC Microbiol 2014; 14, 189.

67. Danzker M, Kessaris ND, Laughlin JS, Absorbed dose and linear energy transfer in radiation experiments. Radiology 1959; 72, 51-61.

68. Grasty R, Applications of gamma radiation in remote sensing. Remote sensing for environmental sciences. Place Springer: Springer; 1976.

69. Lavrinienko A, Mappes T, Tukalenko E, Mousseau TA, Moller AP, Knight R, et al., Environmental radiation alters the gut microbiome of the bank vole Myodes glareolus. ISME J 2018; 12, 2801-06.

70. Goudarzi M, Mak TD, Jacobs JP, Moon B-H, Strawn SJ, Braun J, et al., An integrated multi-omic approach to assess radiation injury on the host-microbiome axis. Radiation Research 2016; 186, 219-34.

71. Ritchie LE, Taddeo SS, Weeks BR, Lima F, Bloomfield SA, Azcarate-Peril MA, et al., Space Environmental Factor Impacts upon Murine Colon Microbiota and Mucosal Homeostasis. PLoS One 2015; 10, e0125792.

72. Gerassy-Vainberg S, Blatt A, Danin-Poleg Y, Gershovich K, Sabo E, Nevelsky A, et al., Radiation induces proinflammatory dysbiosis: transmission of inflammatory susceptibility by host cytokine induction. Gut 2018; 67, 97-107.

73. Yamanouchi K, Tsujiguchi T, Sakamoto Y, Ito K, Short-term follow-up of intestinal flora in radiation-exposed mice. J Radiat Res 2019; 60, 328-32.

74. Nam YD, Kim HJ, Seo JG, Kang SW, Bae JW, Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. PLoS One 2013; 8, e82659.

75. Feiock C, Yagi M, Maidman A, Rendahl A, Hui S, Seelig D, Central Nervous System Injury - A Newly Observed Bystander Effect of Radiation. PLoS One 2016; 11, e0163233.

76. Jordan KR, Loman BR, Bailey MT, Pyter LM, Gut microbiota-immune-brain interactions in chemotherapy-associated behavioral comorbidities. Cancer 2018.

77. Maass SW, Roorda C, Berendsen AJ, Verhaak PF, de Bock GH, The prevalence of long-term symptoms of depression and anxiety after breast cancer treatment: A systematic review. Maturitas 2015; 82, 100-8.

78. Shibayama O, Yoshiuchi K, Inagaki M, Matsuoka Y, Yoshikawa E, Sugawara Y, et al., Association between adjuvant regional radiotherapy and cognitive function in breast cancer patients treated with conservation therapy. Cancer Med 2014; 3, 702-9.

79. Feng LR, Espina A, Saligan LN, Association of Fatigue Intensification with Cognitive Impairment during Radiation Therapy for Prostate Cancer. Oncology 2018; 94, 363-72.

80. Cui M, Xiao H, Li Y, Dong J, Luo D, Li H, et al., Total abdominal irradiation exposure impairs cognitive function involving miR-34a-5p/BDNF axis. Biochim Biophys Acta Mol Basis Dis 2017; 1863, 2333-41.

81. Casero D, Gill K, Sridharan V, Koturbash I, Nelson G, Hauer-Jensen M, et al., Space-type radiation induces multimodal responses in the mouse gut microbiome and metabolome. Microbiome 2017; 5, 105.

82. Parihar VK, Maroso M, Syage A, Allen BD, Angulo MC, Soltesz I, et al., Persistent nature of alterations in cognition and neuronal circuit excitability after exposure to simulated cosmic radiation in mice. Exp Neurol 2018; 305, 44-55.

83. Cervantes JL, Hong BY, Dysbiosis and Immune Dysregulation in Outer Space. Int Rev Immunol 2016; 35, 67-82.

84. Zhou Y, Ni H, Li M, Sanzari JK, Diffenderfer ES, Lin L, et al., Effect of solar particle event radiation and hindlimb suspension on gastrointestinal tract bacterial translocation and immune activation. PLOS ONE 2012; 7, e44329.

85. Shi J, Wang Y, He J, Li P, Jin R, Wang K, et al., Intestinal microbiota contributes to colonic epithelial changes in simulated microgravity mouse model. FASEB J 2017; 31, 3695-709.

86. Mange A, Cao Y, Zhang S, Hienz RD, Davis CM, Whole-Body Oxygen ((16)O) Ion-Exposure-Induced Impairments in Social Odor Recognition Memory in Rats are Dose and Time Dependent. Radiat Res 2018; 189, 292-99.

87. Jones CB, Mange A, Granata L, Johnson B, Hienz RD, Davis CM, Short and Long-Term Changes in Social Odor Recognition and Plasma Cytokine Levels Following Oxygen ((16)O) Ion Radiation Exposure. Int J Mol Sci 2019; 20.

88. Krukowski K, Jones T, Campbell-Beachler M, Nelson G, Rosi S, Peripheral T Cells as a Biomarker for Oxygen-Ion-Radiation-Induced Social Impairments. Radiat Res 2018; 190, 186-93.

89. Hellstrom NA, Bjork-Eriksson T, Blomgren K, Kuhn HG, Differential recovery of neural stem cells in the subventricular zone and dentate gyrus after ionizing radiation. Stem Cells 2009; 27, 634-41.

90. Packey CD, Ciorba MA, Microbial influences on the small intestinal response to radiation injury. Curr Opin Gastroenterol 2010; 26, 88-94.

91. Dainiak N, Medical management of acute radiation syndrome and associated infections in a high-casualty incident. J Radiat Res 2018; 59, ii54-ii64.

92. Guinan EC, Barbon CM, Kalish LA, Parmar K, Kutok J, Mancuso CJ, et al., Bactericidal/permeability-increasing protein (rBPI21) and fluoroquinolone mitigate radiation-induced bone marrow aplasia and death. Sci Transl Med 2011; 3, 110ra18.

93. Valcheva R, Dieleman LA, Prebiotics: Definition and protective mechanisms. Best Pract Res Clin Gastroenterol 2016; 30, 27-37.

94. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al., Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017; 14, 491-502.

95. Cardona F, Andres-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuno MI, Benefits of polyphenols on gut microbiota and implications in human health. J Nutr Biochem 2013; 24, 1415-22.

96. Majamaa H, Isolauri E, Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997; 99, 179-85.

97. Celebioglu HU, Svensson B, Dietary Nutrients, Proteomes, and Adhesion of Probiotic Lactobacilli to Mucin and Host Epithelial Cells. Microorganisms 2018; 6.

98. Chen HL, Wang CH, Kuo YW, Tsai CH, Antioxidative and hepatoprotective effects of fructo-oligosaccharide in d-galactose-treated Balb/cJ mice. Br J Nutr 2011; 105, 805-9.

99. Guilloteau P, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Van Immerseel F, From the gut to the peripheral tissues: the multiple effects of butyrate. Nutr Res Rev 2010; 23, 366-84.

100. Kanjan P, Hongpattarakere T, Prebiotic efficacy and mechanism of inulin combined with inulin-degrading Lactobacillus paracasei I321 in competition with Salmonella. Carbohydr Polym 2017; 169, 236-44.

101. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA, Probiotics inhibit enteropathogenic E. coli adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol 1999; 276, G941-50.

102. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW, Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology (Berl) 2015; 232, 1793-801.

103. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al., Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 2013; 144, 1394-401, 401 e1-4.

104. Kuo PH, Chung YE, Moody microbiome: Challenges and chances. J Formos Med Assoc 2018.

105. Brandt LJ, Fecal Microbiota Transplant: Respice, Adspice, Prospice. J Clin Gastroenterol 2015; 49 Suppl 1, S65-8.

106. Cui M, Xiao H, Li Y, Zhou L, Zhao S, Luo D, et al., Faecal microbiota transplantation protects against radiation-induced toxicity. EMBO Mol Med 2017; 9, 448-61.

107. Lam V, Moulder JE, Salzman NH, Dubinsky EA, Andersen GL, Baker JE, Intestinal microbiota as novel biomarkers of prior radiation exposure. Radiat Res 2012; 177, 573-83.

108. Kim YS, Kim J, Park SJ, High-throughput 16S rRNA gene sequencing reveals alterations of mouse intestinal microbiota after radiotherapy. Anaerobe 2015; 33, 1-7.

109. Liu X, Zhou Y, Wang S, Guan H, Hu S, Huang R, et al., Impact of Low-dose Ionising Radiation on the Composition of the Gut Microbiota of Mice. Toxicol Sci 2019.

110. Carbonero F, Mayta-Apaza AC, Yu JZ, Lindeblad M, Lyubimov A, Neri F, et al., A comparative analysis of gut microbiota disturbances in the Gottingen minipig and rhesus macaque models of acute radiation syndrome following bioequivalent radiation exposures. Radiat Environ Biophys 2018; 57, 419-26.

111. Garrett-Bakelman FE, Darshi M, Green SJ, Gur RC, Lin L, Macias BR, et al., The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight. Science 2019; 364.

112. Lencner AA, Lencner CP, Mikelsaar ME, Tjuri ME, Toom MA, Valjaots ME, et al., [The quantitative composition of the intestinal lactoflora before and after space flights of different lengths]. Nahrung 1984; 28, 607-13.

Figure Legends

Figure 1. Potential mechanisms whereby low- and high-LET radiation may influence the gut-brain axis. Radiation exposure may cause dysbiosis of the intestinal microbiota, resulting in altered metabolite production and/or inflammation. These changes may further lead to cognitive impairments via signaling pathways that exist between the GI tract and the brain. Radiation-induced changes to the GI microbiota may be prevented via use of targeted-antibiotics, pre/pro/postbiotics, or fecal microbiota transplant (FMT) prior to or after radiation exposure.

Table 1. Summary of Studies Examining Effects of Radiation on Microbiota

Radiation Type	Subjects	Dose	Effects	Reference
Low-LET	Female C57BL/6J Mice	Internal rectal brachytherapy (Ir-192 source)	*Akkermansia muciniphila* increased and Firmicutes decreased in mice exposed to radiation	Gerassy-Vainberg (*72*)
	Female C57BL/6J Mice	Whole body X-ray radiation (2 and 4 Gy)	Immediate decrease in *Lactobacillus*, increase in *Bifidobacterium* in 4 Gy group	Yamanouchi (*73*)
	Female Humans	Pelvic fractionated X-ray radiation (50.4 Gy 5x/week for 5 weeks)	GI microbiota change maximally by 1-3 months post-irradiation	Nam (*74*)
	Male Sprague-Dawley Rats	Whole body fractionated low-LET gamma radiation (^{137}Cs, 3 Gy)	Increased Lactobacillales and decreased Clostridiales and Firmicutes	Ritchie (*71*)
	Male C57BL/6 mice	0, 5, or 12 Gy X-rays	Increased abundance of *Lactobacillaceae*, *Staphylococcaceae*, and *Bacteroidaceae* families, decrease of *Lachnospiraceae* and *Ruminococcaceae* families	Goudarzi (*70*)
	Male Wistar rats	10 Gy acute, or 18 Gy fractionated	Increased Bacteriodales, Lactobacillaceae, and Streptococcaceae, decrease of 47 Clostridiaceae members	Lam (*107*)
	Male C57BL/6 mice	8 Gy acute dose of gamma irradiation (^{60}Co source)	Increased *Altipes* in large intestine and decreased in small intestine, and increased *Corynebacterium* in small intestine. Decreased *Prevotella* in large intestine	Kim (*108*)
	Male BALB/c mice	Gamma irradiation (0.5 Gy acute, 0.5 Gy fractionated)	*Clostridium*, *Helicobacter*, and *Oscilibacter* increased, *Bacteroides* and *Barnesiella* decreased	Liu (*109*)

	Gottingen Minipigs and Chinese Rhesus macaques	1.9 Gy (minipigs) and 6.8 Gy (macaques), photon irradiation source	Increase in Firmicutes and decrease in Bacteroidetes and Proteobacteria in minipigs, enrichment of Bacteriodetes in macaques	Carbonero (*110*)
	Bank vole *Myodes glareolus*	Environmental radiation in Chernobyl exclusion zone (30.1 Sv/hr and 0.25 μSv/hr)	Increase in Firmicutes:Bacteriodetes ratio	Lavrinienko (*69*)
High-LET	Female BALBc/ByJ	Whole body acute ^{28}Si radiation (0.05 Gy), along with partial load bearing (1/6 G)	Increase in Bacteroidetes and decrease in Firmicutes	Ritchie (*71*)
	Male C57BL/6J Mice	Whole body acute ^{16}O radiation (0.1, 0.25, and 1 Gy)	Increase in *Akkermansia muciniphila*	Casero (*81*)
Space Flight	Male Human	340-day long spaceflight	Increased Firmicutes:Bacteriodetes ratio in flight	Garrett-Bakelman (*111*)
	Female C57BL/6J Mice	13-day long spaceflight	Flight mice had more Bacteriodetes and Clostridiales; and less Firmicutes and Lactobacillales than controls	Ritchie (*71*)
	Humans	Spaceflights of varying lengths	Alterations in *Lactobacillus* species	Lencner (*112*)