



HHS Public Access

Author manuscript

Eur Radiol. Author manuscript; available in PMC 2023 June 01.

Published in final edited form as:

Eur Radiol. 2022 June ; 32(6): 3683–3692. doi:10.1007/s00330-021-08486-5.

Structural and functional neuroimaging of the effects of the gut microbiome

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Abstract

Interactions between intestinal microbiota and the central nervous system profoundly influence brain structure and function. Over the past 15 years, intense research efforts have uncovered the significant association between gut microbial dysbiosis and neurologic, neurodegenerative, and psychiatric disorders; however, our understanding of the effect of gut microbiota on quantitative neuroimaging measures of brain microstructure and function remains limited. Many current gut microbiome studies specifically focus on discovering correlations between specific microbes and neurologic disease states that, while important, leave critical mechanistic questions unanswered. To address this significant gap in knowledge, quantitative structural and functional brain imaging has emerged as a vital bridge and as the next step in understanding how the gut microbiome influences the brain. In this review, we examine the current state-of-the art, raise awareness of this important topic, and aim to highlight immense new opportunities – in both research and clinical imaging – for the imaging community in this emerging field of study. Our review also highlights the potential for preclinical imaging of germ-free and gnotobiotic models to significantly advance our understanding of the causal mechanisms by which the gut microbiome alters neural microstructure and function.

Keywords

gut microbiome; gut-brain axis; magnetic resonance imaging; fMRI; diffusion tensor imaging

INTRODUCTION

Over the past two decades, surging interest in gut microbiome (GM) research has uncovered the GM's expansive role in human health and disease [1, 2]. As with nearly all major organ systems, the central nervous system (CNS) is no less susceptible to changes in the GM. Facilitated by metabolic, endocrine, and immune actions, the gut-brain axis (GBA) is now understood to represent a robust bidirectional relationship between the GM and the CNS, in which secreted bacterial products like short chain fatty acids, bioactive molecules, or neuroactive gut bacterial metabolites influence neuronal structure and function [3] and link the emotional and cognitive centers of the brain with peripheral systems. Emerging evidence has highlighted the GM's ability to shape complex social and emotional behaviors in both mice and humans [3–5] and has brought to light the role of the GM in neurologic and neuropsychiatric health throughout the lifespan.

Research into the GBA has revealed that perturbations of the GM are associated with a wide variety of CNS diseases (Figure 1). These include psychiatric illnesses such as schizophrenia, anxiety, depression, and obesity [6–8]; neurologic disorders like autism spectrum disorder (ASD), Parkinson's disease, and Alzheimer's disease [9–11]; and neurocognitive dysfunction in intestinal diseases like irritable bowel syndrome (IBS) [12]. Spurred by technologic, computational, and methodological advances over the past decade, a growing number of GM studies have begun to leverage metagenomic approaches to analyze GMs and have uncovered distinct associations between neurologic disease states and specific strains of bacteria. While early metagenomic studies were typically restricted to preclinical models [13–15], the vast majority of reports have turned their attention to human clinical samples in an attempt to identify bacterial species that may be directly associated with specific disease states [16–20].

While more of these GM-disease associations are readily being discovered [21–24], studies investigating the causal role of the GM in disease remain limited. The few pioneering studies include reports such as Erny *et al* [25] who demonstrate that germ-free (GF) mice and antibiotic treatment of specific-pathogen free mice had malformed microglia, disrupted circulating microglial maturation factors, and changes in genes associated with microglial maturation and work from Sharon and colleagues [26], who showed that fecal matter transplantation of patients with autism spectrum disorder (ASD) into mice promotes altered ASD-like behavior in mice with concomitant changes in neuronal excitability. Causal and mechanistically oriented studies such as these are critical to advancing the field and, though preclinical in nature, collectively represent the first steps towards linking the GM, gut microbial dysbiosis, and specific bacterial genera and species to neurologic and neuropsychiatric illness.

As these first vital steps towards understanding the causal relationship between the GM and neurologic health begin to take place, neuroimaging tools, such as magnetic resonance imaging (MRI), rapidly emerges as an essential bridge towards clinically translating our emerging understanding of the GBA to the clinic. Neuroimaging techniques can provide unique insights into GBA-associated psychiatric conditions and neurobiological disorders [27–30]. Quantitative neuroimaging data can also be extracted and mined to detect

patterns in functional or structural changes associated with specific disease states [31]. As new causal and mechanistic links between the GM and CNS are made, associated neuroimaging biomarkers will also be critical for translating these insights into improved clinical diagnostics and subsequent therapeutic monitoring. In the era of multi-omics and big data, the combination of radiologic outputs and microbial metagenomic data heralds untapped potential for both the development and subsequent clinical application of novel GM-neuroimaging biomarkers (Figure 1) [32]. In this review, we highlight and discuss the current technologies, roles, and applications of neuroimaging in studies of the GBA and then explore new directions, opportunities, and the clinical translational potential of neuroimaging in studies of the GBA.

Neuroimaging the Gut-Brain-Axis

Functional Neuroimaging—Functional MRI (fMRI) is an imaging technique that measures brain activity by detecting changes associated with blood flow under the hypothesis that cerebral blood flow levels and neuronal activation are tightly coupled [33]. Thus, using blood-oxygen-level-dependent (BOLD) imaging, the hemodynamic activity of specific brain regions can be assessed by detection of relative levels of oxyhemoglobin and deoxyhemoglobin based on their magnetic susceptibility. Alterations in neural activity can be evoked either by asking or training a subject to perform a task (task-based) or spontaneously when the subject is at rest (resting state) [34–37]. In both instances, resting state (rsfMRI) and task-based fMRI studies have uncovered significant alterations in brain function and connectivity due to alterations in the gut microbiome. In one example, GM perturbations were correlated with differences in functional connectivity (FC) in a study of 22 patients with amnesic mild cognitive impairment and found that differences in GM composition was negatively associated with activity of the cerebellar vermis [38]. Similarly, Labus et al [39] conducted a study of 65 patients with irritable bowel syndrome (IBS) and 21 healthy patients and found associations between GM variations, gastrointestinal (GI) sensorimotor function, and FC. rsfMRI has also been used to detect effects on the brain following the consumption of probiotics and after treatment with antibiotics. These novel studies represent an emerging strategy to assess the effect of specific gut microbiota on neural function and structure. In one such study Tillisch, et al [5] studied the effects of consuming fermented milk products and probiotics in 12 healthy women and discovered altered activity within a periaqueductal, gray-seeded network following treatment. In another study, 45 healthy volunteers consuming probiotics had differences in FC compared to control subjects in the default mode network (DMN), salience network, visual network, and middle and superior frontal gyrus network [40]. In addition to consumption studies, systems-based investigations have also facilitated the discovery of further complex associations between the gut microbiome and functional connectivity in the brain. rsfMRI scans of 39 1-year-old infants were analyzed and correlated to both stool microbiome analysis and cognitive testing at 1 and 2 years of age and found that GM diversity and cognitive outcomes at 2 years were associated with increased FC between the supplementary motor area and the inferior parietal lobule [41]. Another study of 99 female patients with IBS found higher connectivity in the left DMN to the left basal ganglia and from the right somatosensory network to the right basal ganglia to develop a brain-gut interactome map [42]. As described

by the previous studies, the use of rsfMRI for GBA research provides an explanation for how the GM may be affecting resting state neural activity.

Complementing resting state approaches, task-based fMRI can provide novel insights into the functional organization and influence of gut microbiota on brain regions that are activated during specific cognitive demands or actions. For example, in the aforementioned Tillisch et al [5] consumption study, a standardized emotional attention task demonstrated decreased BOLD signal in the primary viscerosensory and somatosensory cortices when compared to a control subjects. Another study by Tillisch et al [43] administered an emotion induction task to 40 healthy woman and found an increased negative affect and decreased hippocampal BOLD response to negative images in women with greater abundances of *Prevotella*. In another study, 44 probiotic-treated IBS patients were given a task presenting fearful and happy faces, resulting in reduced engagement of the amygdala and frontal and temporal cortices as well as heightened engagement of the occipital regions in response to fear stimuli relative to control subjects; however, no compositional differences in the GM were noted [44]. A similar study by Bagga et. al [45] of 45 healthy women treated with probiotics resulted in a subtle change in GM composition and improved behavioral measures of positive affect and memory performance. Additionally, differences in the activity of cingulum, precuneus, inferior parietal lobule, thalamus, and parahippocampal gyrus was observed during an emotional decision-making task and an emotional recognition memory task [45]. These latter studies demonstrate the utility of task-based fMRI by establishing a foundation for a mechanism as to how GM alterations may be influencing responsive neural activity and its associated behavior.

Structural Neuroimaging: Voxel-based Morphometry—Voxel-based morphometry (VBM) is a computational technique of MRI data that can be used to characterize neuroanatomical variations in brain tissue on a voxel-by-voxel basis [46]. Typically using T1-weighted volumetric imaging, VBM performs statistical tests across all voxels in the image to identify brain volume differences between groups. These measurements contribute to our understanding of neural organization and in combination with GM data, can describe GM-induced volumetric changes. For example, in a study of 89 1-year-olds who were clustered by specific GM abundance, T1 weighted-imaging (T1WI) showed GM-specific grey matter volume differences in the right superior occipital gyrus and after one-year, additional GM-specific volume differences in the left and right caudate nucleus [47]. Similarly, in another study of 29 IBS patients clustered by GM composition, GM-dependent brain volumes were identified with increases in brain volume in sensory regions and decreased volumes in insular and prefrontal cortices, as well as varied cortical thickness and surface area in the insula, right globus pallidus, and motor cortex [48].

With the establishment of strong associations between GM composition and brain structure and function, new questions arise regarding the biological mechanisms mediating these observed changes. However, to characterize the complexity of GM-induced structural changes, extensive experimental control is required. Preclinical animal models provide a degree of experimental control not currently accessible in human studies. This control enables higher resolution and confidence when determining the effects of the GM on brain structure. For example, Lu et al [49] scanned specific pathogen-free (SPF) and

germ-free (GF) mice and used T1WI to show regional expansion of the olfactory bulbs and prefrontal cortex in GF mice. Alongside behavioral correlates associated with these brain regions, they demonstrated that the presence of commensal bacteria is necessary for normal neural morphologic development. Another preclinical study which performed T1WI and T2WI neuroimaging of rhesus macaques discovered that nursing-associated increases in Proteobacteria was correlated with decreases in total brain volume, specifically of cortical grey matter volume in both hemispheres [50]. Unfortunately, methodological variations in VBM such as spatial transformations or smoothing procedures can affect results in a manner that may be indistinguishable from variation caused by biological differences [51]. Notably, VBM techniques lack the resolution necessary to identify microstructural changes that may be occurring both at a gastrointestinal or CNS level. Though limited in quantity, these studies use volume-based morphometric imaging techniques to detect gross structural changes occurring in the brain and thus the adaptation of these MR techniques to GBA research provides much needed insight into GM-induced structural variations of grey matter.

Structural Neuroimaging: Quantitative Diffusion Weighted Imaging—The development of VBM and other morphometric analyses unlocked the ability to quantitatively characterize neuroanatomy and, more importantly, to quantify changes occurring in the brain in the disease state. A conspicuous shortcoming of morphometric approaches, such as VBM, is the non-specificity of the gross structural changes observed. To garner greater insight and specificity, new techniques such as diffusion weighted imaging (DWI) were developed [52]. DWI techniques such as diffusion tensor imaging (DTI) measure the rate and pattern of water diffusion in biological tissues. As water diffusion in tissue is influenced by the biophysical organization of the local tissue environment, differences in water diffusivity allow for inferences to be made of tissue microstructure [53]. The first and most extensively used model to relate the microstructural environment of the brain to water diffusion is the diffusion tensor model, which provides measures of fractional anisotropy (FA) and mean diffusivity (MD) that have been interpreted as microstructural indices of white matter structural integrity [52, 54]. Notably, several of the studies previously discussed have called for DTI as a necessary next step in describing the overall mechanism underlying functional abnormalities [47, 55] while others have incorporated DTI into their multimodal approaches. For example, in the Lu et al study [49], DTI was used to observe increased FA in the fimbria, anterior commissure, corpus callosum, optic tract, internal capsule, and the periventricular white matter in SPF mice at earlier developmental stages. The study from Bagga et al [45] used DTI as a complement to their functional analysis to demonstrate no significant differences in FA or mean diffusivity (MD) when compared to baseline before treatment.

These studies use DTI as a complementary modality. However, there are other studies using DTI as their primary imaging technique. A recent study of the GBA used human-to-mouse fecal matter transplant to colonize GF mice with the GM of patients with Attention-Deficit Hyperactivity Disorder (ADHD). DTI analysis revealed decreased FA in right and left hippocampus, and in the right internal capsule and right optic tract, while identifying increased MD in the right hippocampus and fornix, and increased radial diffusivity (RD) in corpus callosum and bilateral hippocampus and right internal capsule when comparing

ADHD to control subject gut transplanted animals [56]. An earlier study of 20 patients with obesity revealed that men with obesity had decreased GM diversity and those with a relative increased abundance of Actinobacteria were associated with increased FA in the amygdala and thalamus [57]. More recently, our group has reported diet-dependent FA increases in the left frontal neocortex as well as identified extensive areas of diet-dependent decreases in RD, axial diffusivity, and MD within rats [58]. Importantly, we were able to identify specific bacterial populations that can be linked to diffusion tensor measurements in ROIs. Although DTI is proving to be an extremely useful modality, its use is currently confined to the brain and has yet to provide the high enough resolution for the spinal cord and even less for peripheral nervous tissue. Additionally, care must be taken when analyzing DTI data (*i.e.* preprocessing assumptions, poor tractographic interpretation, overprocessing [59]). Still DTI provides tremendous insight into imaging endophenotypes across a range of neurologic and psychiatric diseases and, with high sensitivity, can further our understanding of the modulatory influence that the GM has on neural microstructure.

Metabolic Neuroimaging: Positron Emission Tomography—Positron emission tomography (PET) is a neuroimaging modality that implements the use of radiotracers to visualize and quantify physiologic function and disease. Recently, Giron et al [60] described the potential utility of PET in GBA research, listing various radiotracers being used to label bacteria and bacterial metabolites in addition to common metabolic radiotracers such as [18F]-fluorodeoxyglucose (FDG), a radiolabeled glucose analog. While the aforementioned imaging modalities provide structural information, PET provides cellular-level information which cannot be ascertained from MRI, establishing PET's role in diagnostic and prognostic assessment.

While others have used PET for peripheral imaging in relation to the GM [61], the current application of PET for neuroimaging the GBA is extremely sparse. One such study used PET to investigate the amyloid pathology in 89 patients with varied cognitive performance. Using [18F]-florbetpir, a radiotracer binding amyloid plaques, they found correlations between specific GM metabolites and regions (frontal, anterior cingulate, precuneus cortex) found to have increased amyloid load as measured via [18F]-florbetpir PET [62]. Another PET study used a mouse model to characterize the bacterial composition of high fat diet dams and correlated this to memory and exploratory behavior dysfunction in the offspring while additionally demonstrating that intranasal insulin administration lowered cerebral glucose metabolism in the thalamus, hypothalamus, hippocampus, amygdala, globus pallidum, striatum, frontal, medial, sensory, and temporal cortex, and cerebellum of the control animals, measured via 18F-FDG PET [63].

The number of studies using PET for neuroimaging the GBA still remains small. While this may be due to the field's infancy and current lack of neuroimaging incorporation, the utility of PET is evident. Unfortunately, the use of radioactive tools presents a concern for specific patient populations like pregnant individuals and pediatric populations. Additionally, PET's inability to account for anatomical or structural data results in information that cannot be spatially correlated to specific neural regions, highlighting another limitation of PET-only studies.

CONCLUSION

Neuroimaging of the gut brain axis will be pivotal for expanding our knowledge of the temporal and spatial impact of the gut microbiota on brain structure and function, as well as for realizing translational innovations in the clinic and at the bedside. While early preclinical studies established the fundamental role of the GM in neurologic and neuropsychiatric health, current studies are focused on determining what specific GM populations are associated with disease, particularly in humans [3, 64]. Although these GM compositional associations do not provide a complete mechanistic framework to determine how the GM impacts the brain, they do set the stage for future more hypothesis-driven investigations to better understand the precise role and manner by which gut microbiota contribute to neurologic health throughout the lifespan.

The studies discussed in this review highlight the utility of neuroimaging to assess the functional, structural, and metabolic neural alterations associated with GM variations. It should be noted that the discussed imaging modalities do not encompass the entirety of techniques currently utilized to describe the actions of the GBA. Other studies use macromolecule proton fraction mapping [49], magnetic resonance spectroscopy [65–67] and R2* MRI [68] to investigate these differences. Yet, to understand the causal relationships within these GM-induced neural changes, greater experimental control is necessary to account for the variability and undiscovered factors of the GM. While many GBA neuroimaging studies have focused on humans with relatively small sample sizes (Table 1), preclinical models offer an accessible means to extend our understanding of the mechanisms by which the GM impacts the brain with rigorous experimental controls and with higher-powered imaging studies. Germ-free and gnotobiotic animal models are being used to examine the role of GM populations in health and disease [15, 25, 26, 56, 69–71]. Adapting neuroimaging techniques to these models can provide longitudinal insights in a non-invasive manner. And, while limited, some have begun to use neuroimaging to reveal the functional and structural neuromodulatory effects of specific non-pathologic or disease-associated GM populations in these preclinical models [49]. In conjunction with behavioral and GM compositional correlates [7, 8, 49, 72–75], neuroimaging modalities can serve as an exciting complementary tool to reveal the underlying causal mechanisms by which the gut microbiome impacts the CNS in both health and disease.

Abbreviations:

GM	gut microbiome
CNS	central nervous system
GBA	gut-brain axis
ASD	autism spectrum disorder
IBS	irritable bowel syndrome
GF	germ-free

FC functional connectivity

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Key points:

1. Alterations to the gut microbiome can significantly influence brain structure and function in health and disease.
2. Quantitative neuroimaging can help elucidate the effect of gut microbiota on the brain and with future translational advances, neuroimaging will be critical for both diagnostic assessment and therapeutic monitoring.

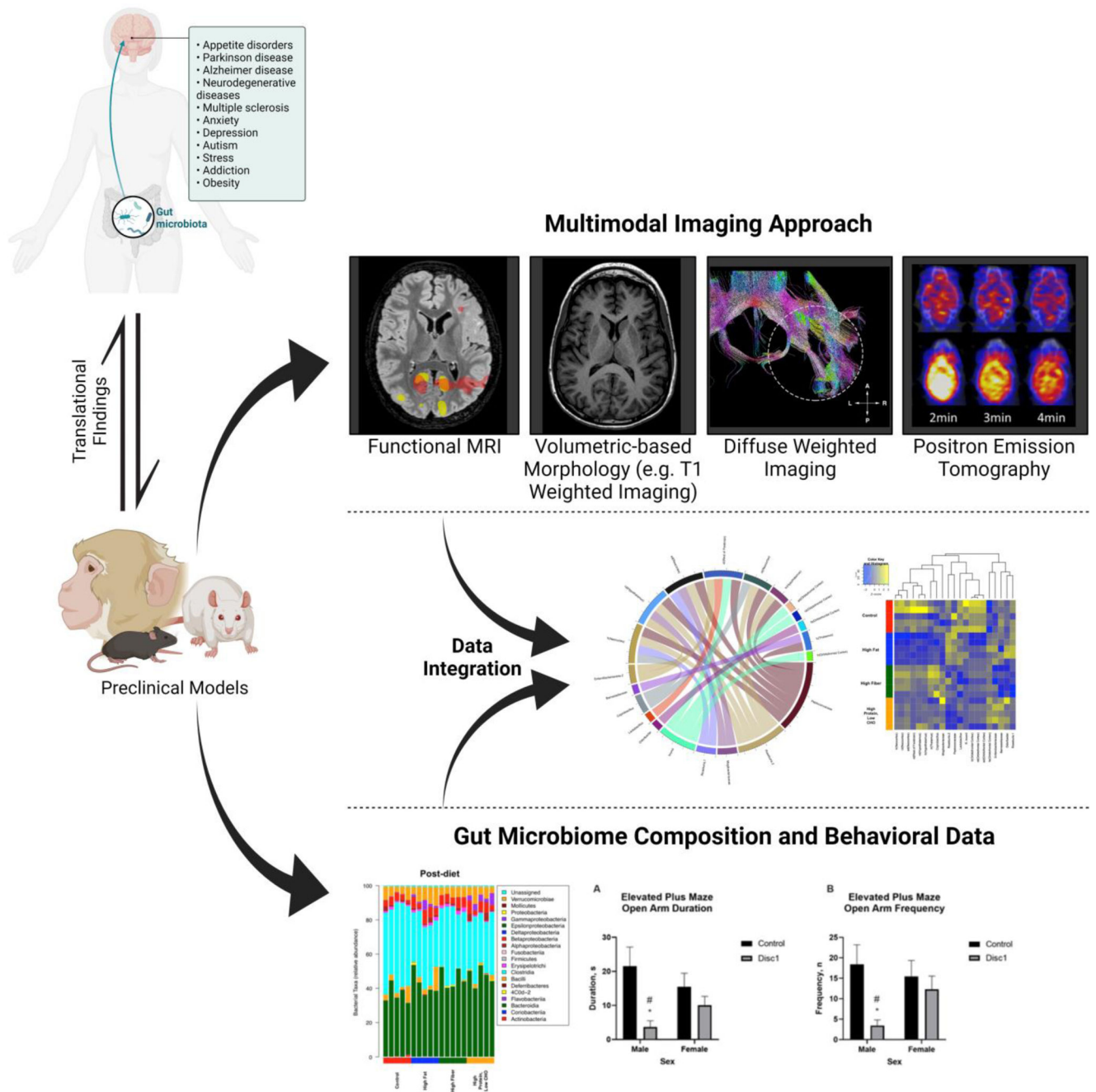


Figure 1: Multimodal imaging approach and gut microbiome composition and behavioral data

TABLE 1:

Summary of neuroimaging studies of the gut-brain axis

Species	Imaging Modality	Methodology Altering GM	Outcome	Reference
Human	rsfMRI	None – prospective study	GM composition was negatively associated with activity of the cerebellar vermis	[38]
Human	rsfMRI	None – prospective study	GM composition was associated with GI sensorimotor function and FC	[39]
Human	rsfMRI task-based fMRI	Consumption study	Consumption of fermented milk products resulted in altered activity within a periaqueductal, gray-seeded network; decreased BOLD in primary viscerosensory and somatosensory cortices	[5]
Human	rsfMRI	Consumption study	Consumption of probiotics resulted in FC differences in the DMN, salience network, visual network, and middle and superior frontal gyrus network	[40]
Human	rsfMRI	None – prospective study	GM composition and cognitive outcomes in 2-year-olds was associated with FC between the supplementary motor area and the inferior parietal lobule	[41]
Human	rsfMRI	None – prospective study	Patients with IBS were associated with higher FC in the left DMN to left basal ganglia and from the right somatosensory network to the right basal ganglia	[42]
Human	Task-based fMRI	None – prospective study	<i>Prevotella</i> abundance was associated with decreased hippocampal BOLD response to negative images	[43]
Human	Task-based fMRI	Consumption study	Probiotic treatment of IBS patients shown emotional faces showed a reduced engagement of the amygdala and frontal and temporal cortices, and heightened engagement of the occipital regions	[44]
Human	Task-based fMRI, DTI	Consumption study	Probiotic consumption resulted in a subtle GM composition change and in response to tasks, differences in the activity of the cingulum, precuneus, inferior parietal lobule, thalamus, and parahippocampal gyrus were observed; no differences in FA or MD were observed	[45]
Human	Structural brain imaging	None – prospective study	GM-dependent grey matter volume differences in the right superior occipital gyrus were noted and after a year, GM-specific differences were observed in the left and right caudate nucleus	[47]
Human	Structural brain imaging	None – prospective study	GM-specific groups of patients with IBS showed increased volumes in sensory regions and decreased volumes in insula, right globus pallidus, and motor cortex	[48]
Human	DTI	None – prospective study	Decreased GM diversity in patients with obesity and an abundance of <i>Actinobacteria</i> was positively correlated with FA in the amygdala and thalamus	[57]
Human	18F-Florbetapir Amyloid PET	None – prospective study	Increased amyloid uptake in the frontal, anterior cingulate and precuneus cortex was associated with an increase in bacterial byproducts in plasma	[62]
Mouse	Structural brain imaging, DTI	Germ-free mice	GF mice showed regional expansion of the olfactory bulbs and prefrontal cortex; FA was increased in the fimbria, anterior commissure, corpus callosum, optic tract, internal capsule, and the periventricular white matter	[49]
Mouse	DTI	Fecal matter transplant to germ-free mice	Fecal matter transplant from patients with ADHD to GF mice showed decreased FA in the right and left hippocampus and in the right internal capsule and right optic tract, and increased MD in the right hippocampus and fornix	[56]
Mouse	18F-FDG PET-CT	High fat diet consumption	Maternal high-fat diet consumption resulted in brain insulin resistance and reduction of memory and exploratory behavior in offspring with an age-dependent change in bacterial composition	[63]
Rat	DTI	None – prospective study	Diet-dependent FA increases were observed in the left frontal neocortex	[58]

Species	Imaging Modality	Methodology Altering GM	Outcome	Reference
Rhesus macaques	Structural brain imaging	Transition to solid food in developing macaques	Nursing-associated increases in <i>Proteobacteria</i> was negatively correlated with total brain volume, specifically cortical grey matter of both hemispheres	[50]

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