Chapter 18
Neuroimaging the Microbiome-Gut–Brain Axis

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Abstract The brain is the most complex organ in the human body, interacting with every other major organ system to continuously maintain homeostasis. Thus it is not surprising that the brain also interacts with our microbiota, the trillions of bacteria and other organisms inhabiting the ecosystem of the human being. As we gather knowledge about the way that our microbiota interact with their local environments, there is also increasing interest in their communication with the brain.

Abbreviations

BOLD	Blood oxygen level dependent
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
HPA	Hypothalamic-pituitary-adrenal
MGBA	Microbe-gut-brain axis
MRI	Magnetic resonance imaging
PET	Positron emission tomography

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Introduction

The brain is the most complex organ in the human body, interacting with every other major organ system to continuously maintain homeostasis. Thus it is not surprising that the brain also interacts with our microbiota, the trillions of bacteria and other organisms inhabiting the ecosystem of the human being. As we gather knowledge about the way that our microbiota interact with their local environments, there is also increasing interest in their communication with the brain.

Brain-Gut Communication

Bidirectional communication between the brain and gut has been well described (Fig. 18.1) [1–4]. The brain communicates with the gut via the autonomic nervous system (particularly the vagus nerve) and the hypothalamic-pituitary adrenal axis. Descending monoaminergic pathways also act on the dorsal horn and can regulate gut-related sensations. Gastrointestinal motility, secretion, local blood flow, and immune regulation are modulated by the brain, generating stereotypic patterns of gut response which are context specific, such as the classic gastrointestinal stress response of nausea and/or fecal urgency. Thus the local environment of gastrointestinal microbes is continuously adjusted by central influences. These interactions provide a partial explanation for the differences in gut bacterial populations between healthy persons and those with gastrointestinal illness [5–7] or prolonged psychological stress [8]. Similarly, preclinical studies have identified altered fecal bacteria after experimental pre and post-natal stress [9–12].

Completing the bidirectional loop, the brain receives afferent input from the gut, likely from a variety of pathways, as described below. With a surface area far exceeding that of the skin, the gut is the largest interface between the body and the external environment, and contains the body's most numerous population of microbes. The gut also has a vast immune system and complex nervous system through which the microbiota can communicate with the brain. Biologically active compounds such as serotonin, histamine [13], catecholamines [14], gamma-aminobutyric acid (GABA) [15], and others can be produced in various amounts by specific bacteria. Additionally, organisms can stimulate the release of these compounds by gut enterochromaffin cells, leading to central signaling and clinically apparent symptoms [16]. An example of this is the central nausea induced at the nucleus tractus solitarius after rotavirus-stimulated gastrointestinal serotonin release [17]. An alternate pathway by which information may reach the brain from the gut is via neurochemicals secreted into the portal venous system, as is seen in hepatic encephalopathy [18, 19].

The vagus nerve has been shown to be essential in some but not all preclinical studies of microbe-brain interactions and likely plays a key role in the microbe-gut-brain axis (MGBA) in humans [20, 21]. Interoceptive (internal) signals of body

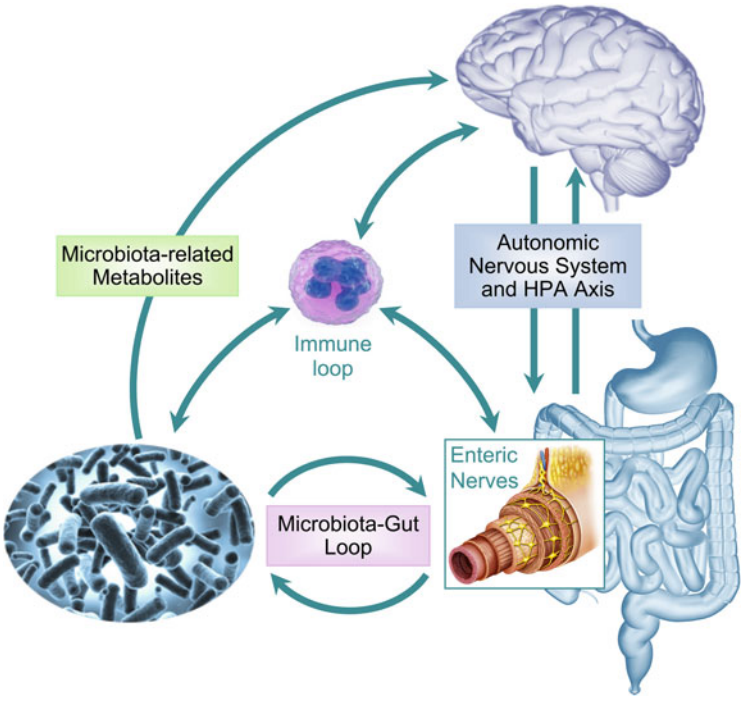

Fig. 18.1 The microbiota-gut-brain axis (MGBA). The traditional gut-brain axis consists of the brain with bidirectional connections to the enteric nervous system of gastrointestinal tract via the autonomic nervous system (sympathetic and parasympathetic branches) and hypothalamic-pituitary-adrenal (HPA) axis. Here the expanded MGBA network is shown. The gastrointestinal microbiota communicate with the brain via enteric nervous system and via metabolic products. The immune system interacts with each member of the MBGA bidirectionally

state are relayed from vagal and spinal afferent nerves to the brain stem and then for further processing in higher cortical centers [22, 23]. It has been proposed that interoceptive input has relevance beyond merely reporting the homeostatic "status" of the body. In the model proposed by Craig and others, interoceptive signals appear to be integrated with emotional and cognitive input primarily in the anterior insula. This combined input is used continuously to create a sense of momentary "self" which can be consciously interpreted as happy, sad, healthy, ill, etc. [24, 25]. Since visceral feedback from the gut and other body sites contributes to our conscious state of wellbeing, it then follows that the gut's luminal organisms also have the opportunity to influence mood states like anxiety or depression [26, 27]. Given the difficulty of gaining access to the cellular workings of the brain in humans, neuroimaging has emerged as a tool to increase our understanding of the MGBA. In the section below, several of the key imaging modalities will be reviewed and their integration into analyses of the MGBA will be discussed.

Neuroimaging in Humans

Functional Neuroimaging Techniques

One of the most common research techniques used to image changes in brain function between groups or after a treatment intervention is functional magnetic resonance imaging (fMRI). This technique is non-invasive, safe, and easy to perform. Functional MRI measures changes in the percentage of oxygenated versus deoxygenated hemoglobin, taking advantage of the differing magnetic properties of the molecules. During an experimental task, when a brain region is more active compared to a baseline or control task, blood flow increases and thus a higher proportion of oxygenated hemoglobin is observed in that area. This change in the regional magnetic properties is measured as the blood oxygen level dependent (BOLD) signal by the scanner and provides an indirect measurement of a change in brain activity. Functional MRI has fairly good spatial resolution of 2–4 mm but does not have the precision of post-mortem studies in animals. Functional MRI has been used successfully to identify differences in brain function in gastrointestinal disease states, such as irritable bowel syndrome and inflammatory bowel disease, as well as in healthy people before and after chronic ingestion of probiotics [28–30].

The other common mode of functional neuroimaging is Positron Emission Tomography (PET). Radiolabeled chemicals are injected into the blood stream and PET measures the emissions regionally throughout the brain. PET has the advantage of measuring physiologic processes more directly via the use of radiolabeled ligands; however it has the drawback of being more invasive and requires radiation exposure. Radioligand PET can be used to explore baseline interactions between regional brain distribution of a variety of signalling systems (including dopamine [31, 32], serotonin [33], substance P/neurokinin-1 [34, 35]) with gut microbiome and metabolomic profiles, as well as assess pre- to post-intervention changes in the MGBA after intervention with specific probiotics. While PET imaging is more invasive and difficult to perform, it has the advantage over fMRI of isolating specific biological processes or pathways for measurement.

The Functional Imaging of the Gut-Brain Axis

The brain-gut axis has been examined using fMRI and PET in humans, particularly in the setting of evoked pain, or anticipation to pain in the esophagus and distal colon. Alterations in resting brain function have also been described in patients with functional gastrointestinal disorders, which are believed to involve brain-gut axis dysfunction [36–38]. Whether these resting brain signal changes represent ongoing gastrointestinal input to the brain or persistent changes in the function of neural circuitry due to chronic disease is not yet known.

Functional MRI has been extensively used to observe changes in brain response after a treatment intervention, most commonly using pharmaceuticals or behavioral interventions, but little has been done to image the effects of antibiotics, probiotics, or dietary interventions in humans [39–41]. Only one study to date has described functional brain changes in response to a probiotic intervention [29]. In this study healthy, normal weight women without any gastrointestinal symptoms, pain or psychiatric disorder, were randomized to treatment with a probiotic, a placebo dairy product or no treatment. The response to an emotional attention task was measured with fMRI before and after the treatment period and the probiotic group showed reductions in response to the emotional task, suggestive of reduced vigilance to negative emotional stimuli. This difference in brain activity was not correlated to any subject reports of mood or gastrointestinal symptoms. Evaluation of the microbiota in that study confirmed that the experimental probiotic could be identified in the stool of the probiotic ingesting subjects but did not show group specific changes in the overall architecture of the microbiota. This is consistent with other studies and suggests that microbial metabolites rather than overall microbial configuration may be the salient result of probiotic ingestion [42]. This initial study suggests that subtle changes in the gut contents can lead to measureable changes in brain function, even in the absence of a conscious awareness of the change. Future studies, which may be able to use microbiome composition, along with metabolomic and metagenomic measurements from stool to correlate with brain function at baseline or after a probiotic intervention, will lead to a better understanding of how the MGBA can be modulated in health and disease.

Structural Neuroimaging

In addition to functional neuroimaging, advances in MR imaging of gray and white matter structure have proven valuable in describing group differences in psychiatric illness and chronic pain syndromes compared to healthy populations. Differences in both white matter and gray matter have been identified in irritable bowel syndrome and functional dyspepsia, both of which are considered to be disorders of the brain-gut axis and which likely are accompanied by alterations in the gut microbiota [43–51]. High resolution structural brain images can be used to produce global (whole-brain), regional, and voxel-level indices of gray matter density and volume as well as cortical thickness, surface area and mean curvature (Fig. 18.2). Network analysis from graph theory has recently been applied to gray matter morphometry to demonstrate alterations in regional topology, providing strong evidence for extensive structural reorganization of cortical and subcortical regions previously implicated in altered brain responses to visceral pain stimuli and their expectation [43]. The biological substrate underlying grey matter changes may involve increased or decreased glial cells, changes in dendritic spines or synapses or less likely, neural degeneration. Gray matter has been shown to remain quite plastic even during adulthood [53–55]. The effects of peripheral factors such as the

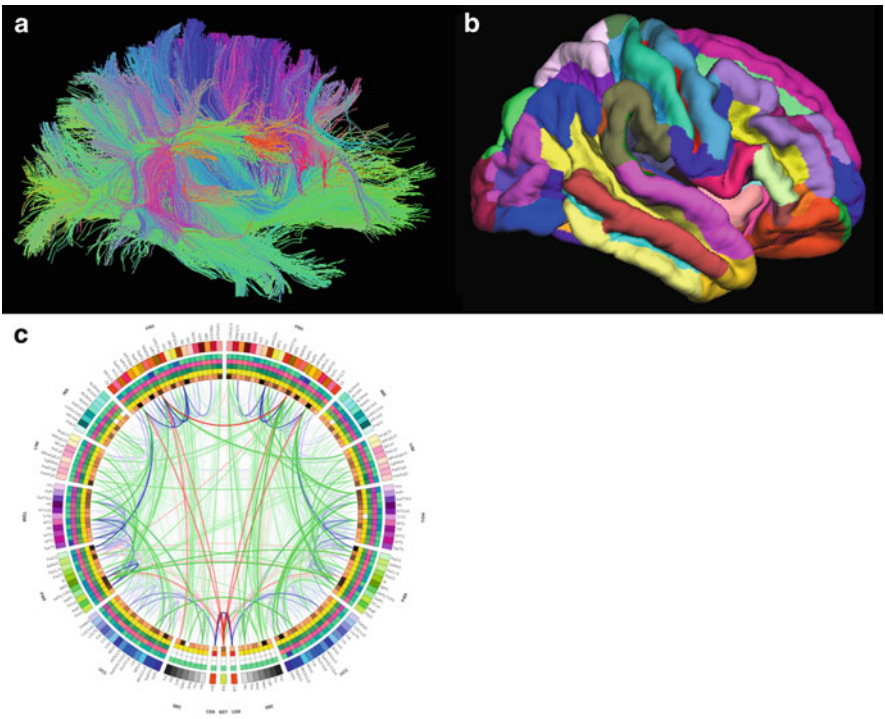

Fig. 18.2 Multimodal neuroimaging. (**a**) White matter tracts in the brain can be visualized with diffusion tensor imaging (DTI). (**b**) The gray matter structure can be viewed with magnetic resonance imaging (MRI) and parcellated into structural or functional regions, measuring characteristic features including volume, cortical thickness and regional curvature. (**c**) Visualization subcortical and cortical brain architecture is depicted using a 'connectogram' [52]. The outer ring shows the brain regions represented by location. The next inner four rings depict the gray matter volume, surface area, cortical thickness, and degree of connectivity. Connectivity between regions was determined using DTI and probabilistic tractography. The color of the links represents the distribution of fractional anisotropy. The number of fiber tracks between regions is represented by the transparency of the line

microbiota on gray matter structure is likely most profound during development, and has been shown in rodent models [56]. However, given that alterations in brain function and behavioral symptom changes occur in response to probiotic interventions in adults, it is likely that structural changes will follow.

Another MRI-based modality of assessing brain structure is diffusion tensor imaging (DTI), which allows the evaluation of white matter integrity and anatomy. DTI can assess the connectivity between gray matter regions via white matter tracts, measuring the fiber pathways that support functional networks. Two main types of DTI analyses are frequently performed [57]. In the first, white matter tract integrity is measured, most commonly expressed as fractional anisotropy (FA), although additional measurements, such as radial or mean diffusivity are also used. This technique assesses the diffusivity of water in the brain tissue. Water molecules

unconstrained by cellular architecture, such as in the CSF, freely move in all directions (isotropic) and thus have a FA value of 0. However, water molecules in dense, parallel white matter tracts containing axons are constrained and have high FA values. Decreases in the FA of white matter tracts can indicate decreased axonal number, myelin integrity, or axonal cytoskeleton integrity. The other DTI analysis method, tractography, allows quantification of fiber density between brain regions, and is commonly used to describe limited or whole brain networks.

It has yet to be clearly defined whether the differences in brain structure in disorders of the brain-gut axis are a result of the chronic condition or a predisposing factor, though there is a great likelihood that both pathways occur. Associations between brain structure and microbiota profiles have not yet been described but provide an opportunity to better understand the interactions between the luminal contents and the brain.

Neuroimaging in Animals

Imaging the brain in animals is also achieved with MRI and PET, as well as more direct radiotracer studies. Rodent fMRI and PET provide fair spatial and temporal resolution but require restraint and/or sedation of the animal to avoid movement, which may confound the interpretation of the functional results. Autoradiography allows neuroimaging in non-sedated, nonrestrained animals. A radiotracer is injected and after the experiment the animal is sacrificed and the brain is cryosectioned to identify regional tracer uptake, allowing a very detailed view of the involved neural circuitry [58]. Using animal imaging in parallel with modulation of the microbiota is likely to inform human studies as animal studies allow for the control of more variables and ability to perform post-mortem studies of the brain.

Incorporation of Behavioral and Gastrointestinal Measurements to Neuroimaging Studies

Preclinical studies have been useful in identifying potential behavioral and peripheral measures that are of particular relevance in examining the MGBA. Modulation of gastrointestinal flora in rodents by using specific bacterial strains, antibiotics, or by using germ-free animals has shown associations with anxiety-like behavior across multiple paradigms [20, 21, 56, 59, 60]. Rodent models of anxiety-like behavior are well developed and show responses to pharmacological agents, such as selective serotonin reuptake inhibitors, indicating the presence of relevant shared core neural circuitry with humans. In humans, measures of anxiety and depression including clinical diagnosis, trait measures and psychological symptoms correlate

with brain structure and function [61–63]. Similar to the findings in rodent models, the ingestion of a *Bifidobacterium* and *Lactobacillius* containing probiotic in healthy humans showed diminished psychological symptoms, including anxiety symptoms in a placebo controlled randomized clinical trial [64]. The central mechanisms through which these symptoms change can be probed with neuroimaging, using symptom measures as covariates. In addition to looking at the interactions between psychological symptoms and brain function when modulating the microbiota in clinical trials, additional gastrointestinal measures such as intestinal permeability, immune activation, motility and visceral sensitivity will be useful in better elucidating gut to brain communication.

Evaluating the MGBA in the Era of Big Data

The ability to analyze the large datasets produced by neuroimaging studies and microbiota profiling has been advancing rapidly [65]. While studies evaluating effects of single organisms or probiotic consortia on the brain will continue to be of great interest; the emerging use of systems biology approaches to the understanding of the relationship between complex structural and functional neural networks and the microbiome is likely to advance our understanding of the MGBA tremendously [66]. Both the microbiome and the brain act within integrated networks for which classical hypothesis driven analytic approaches are not ideal. Agnostically applied multivariate analysis techniques are being used to identify neural networks to develop biomarkers of complex diseases, such as chronic pain, anxiety and depression. These approaches can be utilized to combine complex imaging datasets with genomic, metagenomic and metabolomic data to study the interaction between neural and microbial networks [67]. Since current evidence suggests that the gastrointestinal microflora are likely to play a role in the development and persistence of these disorders, it will be important to look at the interactions between brain phenotypes and the gut microbiome.

Limitations in Neuroimaging of the MGBA

In both the imaging of animal and human MGBA there are a number of limitations. In animals, we have the ability to meticulously manage the presence or absence of specific microorganisms, we are able to image the brain in both direct and indirect ways, and we can observe the effects of various environmental pressures on the developing animal. However, we are faced with the difficulty of translating the relevance of behavior from rodent models to humans, and must deal with the clear differences in the brain between species. As stated by Craig, “A rat is not a monkey is not a human” [68]. He and others [69] have described the difficulties of the bench to clinical translation with a particular focus on interoception and pain processing,

but similar arguments can be made for the study of the stress response, emotion and cognition. If an animal model, as Craig describes in the case of the rodent, lacks the anterior insular cortex, the site in which our subjective sense of physical wellbeing may arise, and if the basic pathways through which the visceral afferents communicate with emotional and cognitive centers vary, then our animal models of complex phenomena must be interpreted with caution.

In humans on the other hand, we have great limitations in our ability to study all three branches of the MGBA precisely. Our access to the gut is limited and most data samples are collected non-invasively, via the stool. This allows us to examine the gut microbiome in broad strokes, but does not differentiate between the luminal and mucosal environment, much less local microenvironments or regional differences throughout the gut [70, 71]. In humans the effects of diet, medications, and external stressors on microbiota content, gastrointestinal motility and immune function are difficult to account for even in the most carefully controlled experiments. Additionally, it is likely that many of the MGBA pathways affected by the microbiota are established early in life, while the brain has its most rapid and dramatic remodeling [72]. Despite these concerns, the combination of human and animal imaging, using a translational or reverse-translational model [73–75] may prove to be the most effective and flexible strategy in evaluating the role of the gut microbiome in brain function, mood and cognition.

Conclusion

Neuroimaging of the MGBA is in its infancy but will clearly be an important modality on the road to understanding the role of microbes in many aspects of health and disease. The current focus on disorders of gastrointestinal disease, such as inflammatory or function bowel diseases, is already shifting to the study of anxiety and depression, metabolic diseases and neurologic disease. With this shift, incorporation of neuroimaging techniques will allow us to measure the rich connectivity between three complex systems: the microbiota, gut and brain.

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