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Potential Roles of the Gut-Brain Axis in the Cognitive Evolution of Primates

Yumiko Yamazaki and Atsushi Iriki
Laboratory for Symbolic Cognitive Development, RIKEN BDR
yumiko.yamazaki@riken.jp

The gastrointestinal tract has been proved to influence behavior through the interactions with the brain by several pathways including symbiotic microbiota. Animals forage on prey using their cognitive and motor skills, and the gastrointestinal tract subsequently engages in nutritional intake together with the residential microbiota. The maintenance of adequate nutritional conditions enables the learning of new skills and the acquisition of cognitive abilities for further adaptation to different environments. The relationship among the brain, gut, and microbiota, the so-called "brain-gut axis", formed during the evolution of each species. Using nonhuman primate species as models, we are analyzing the relationships between unique foraging behavior and gut microbiota, both of which adapted to the specific environmental conditions of their habitats. By doing so, the historical steps of human civilization can be viewed from new perspectives involving behavioral acquisition through interaction between the gut and the brain.

Se ha demostrado que el tracto gastrointestinal influye en el comportamiento mediante las interacciones con el cerebro a través de varias vías, incluyendo la microbiota simbiótica. Los animales se alimentan de sus presas utilizando sus habilidades cognitivas y motoras. Posteriormente el tracto gastrointestinal se encarga de la absorción nutricional junto con la microbiota residente en el organismo. El mantenimiento de unas condiciones nutricionales adecuadas permite el aprendizaje de nuevas habilidades y la adquisición de habilidades cognitivas para una mayor adaptación a diferentes entornos. La relación entre el cerebro, intestino y la microbiota, el llamado "eje cerebro-intestino", se formó durante la evolución de cada especie. Usando especies de primates no humanos como modelos, analizamos las relaciones entre el comportamiento de alimentación único y la microbiota intestinal, ambos adaptados a las condiciones ambientales específicas de sus hábitats. Al hacerlo, los pasos históricos de la civilización humana se pueden ver desde nuevas perspectivas que involucran la adquisición del comportamiento a través de la interacción entre el intestino y el cerebro.

The Gut-Brain Axis Regulates Behavior

The brain is obviously the locus of adaptive and effective behavior. Recent findings, however, show that our behavior is influenced by other organs that were not previously thought to be involved in brain function (e.g., Dum et al., 2016, 2019; Levinthal & Strick, 2012). Among the organs, the gut is one of the most important for digesting food items and absorbing nutrients necessary for maintenance of the host body, including the brain. In the gut, we have microbes that colonize us soon after birth and play an important role in nutritional intake.

In addition to these functions, the gut and cohabiting "microbiota" (a concept for the community of microbes as a whole) have pathways to communicate with the brain in several manners, both directly and indirectly (Collins et al., 2012). For example, the vagus nerve has been known to communicate with the brain (Bonaz et al., 2018), and a recent study described novel enteroendocrine cells in the small intestine rapidly signaling sensory stimuli to the brain stem (Kaelberer et al., 2018). Oral intake of a species of bacteria, *Lactobacillus reuteri*, improved the microbiota composition and social behavior of mice previously showing dysbiosis (Buffington et al., 2016), and it had similar positive effects on symptoms of a mouse model of autistic spectrum disorder through stimulation of the vagus nerve by the bacteria, inducing an increase in oxytocin levels and modulation of the reward system (Sgritta et al., 2018). Compared to the specific pathogen-free (SPF) population, germ-free mice showed altered performance, such as an exaggerated hippocampal-pituitary adrenal (HPA) response to mild stress (Sudo et al., 2004), increased motor activity, and reduced anxiety (Diaz Heijtz et al., 2011), all of which were reversed after colonization of microbes in the gut.

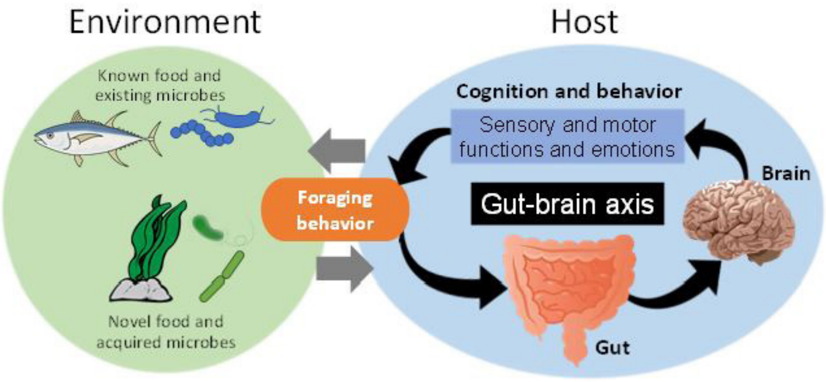
Although many of the studies listed above were of rodent models, interactions between the brain and gut in various aspects, the so-called gut-brain axis, is expected to also be important for primate species to develop various behaviors requiring motor, cognitive, emotional, and sensory functions. Among these sets of behaviors, foraging would be a good example to investigate the gut-brain axis because successful foraging enables individuals to take in nutrients necessary for maintenance of the host body, resulting in normal development of brain functions as a species.

Foraging behavior in each species shows unique characteristics that have been developed through interactions between organisms and the environment over a long period of time. Because environmental conditions are quite variable, acquired foraging behavior must be adjusted and modified. To change behavior in an adaptive manner, one must have excellent 1) sensory organs to process the environmental conditions, 2) motor organs to execute responses to obtain food items, 3) sensorimotor ability to coordinate these organs, and 4) cognitive ability to remember the results of the executed behavior and to integrate all information throughout the course of the interactions.

Species meeting these conditions may have been able to survive in their ecological niches over the course of their evolution. In turn, environmental microbiota helped them develop their own microbe communities in and on their bodies. These can be transferred across generations through social transmission by families and social units (Moeller et la., 2016; Yatsunenko et al., 2012). This microbiome community influences brain development by supplying neurotransmitters and metabolites, and the developed brain can learn new behaviors to adapt to variable environmental conditions efficiently. Thus, foraging is one of the key behavioral sets that can mediate the body, the brain and its functions, and the environment of each organism (Figure 11.1).

Figure 11.1.

Foraging is the Key Behavior at the Interface between the Host and the Environment.



Note. On the one hand, inside the host body, the gut is responsible for brain development through nutritional intake and the reception of various signals from cells and microbiota. The developed brain enables the host to exhibit cognitive, sensory, motor, and emotional functions, allowing for enhanced foraging behavior to be acquired. Meanwhile, food items eaten by the host contain microbes that are integrated into the gastrointestinal microbiota of the host. Through the acquired ability of foraging, the host can take in novel food items, and novel microbes contained within are transferred to the host's gut and become commensal, which in turn supports additional brain function to be exhibited.

To address the question of how behavioral characteristics related to foraging customs unique to each species have been formed with the help of the gut microbiome, we focus on two primate species: common marmosets originally from Brazil and long-tailed macaques living in Thailand.

Impact of Food Intake on the Gut Microbiome in Marmosets

Common marmosets are a species of New World monkey and have been widely used in various fields of biology and medical sciences for decades by breeding them in captivity ('t Hart et al., 2012). There are many types of complete foods commercially available for marmosets, but additional food items are highly recommended in order to supplement vitamins and minerals and stimulate appetite (e.g., Bairrão Ruivo, 2017; National Research Council, 2003; Rensing & Oerke, 2005). While captive marmosets accept most food items, including fruits, vegetables, milk products, and produce, control of balanced food intake for captive marmosets is essential not only for maintaining their bodily health condition, but also for full expression of brain function, including perception, motor control, and cognition. It is necessary to think about food resources for marmosets obtained in wild habitats because they must have developed anatomical and physiological characteristics for digesting those food items over the course of their evolution.

The habitat of wild marmosets is in the northeastern part of Brazil, spreading into areas where food resources are not abundant, especially in the dry season (Arruda et al., 2019). They are omnivorous and eat fruits, insects, small reptiles, eggs, etc. (Rylands & de Faria, 1993; Stevenson & Rylands, 1988). As a species of the genus Callithrix, common marmosets are distinct in that they are highly exudativorous, relying heavily on gum or tree exudates, which are relatively stable food resources throughout the year (Ferrari, 1993). They have anatomical characteristics adapted as gummivores, such as dentition for tree gouging (Resing et al., 2005) and large intestines for fermentation of gum by commensal microbes (Ferrari et al., 1993 for similar characteristics in a species of genus Callithrix). Insects are important protein resources, accounting for 30–70% of their diet (National Research Council, 2003). They eat various insects, such as grasshoppers, crickets, cicadas, and cockroaches (Stevenson & Rylands, 1988).

Role of Intestinal Microbes in Common Marmosets

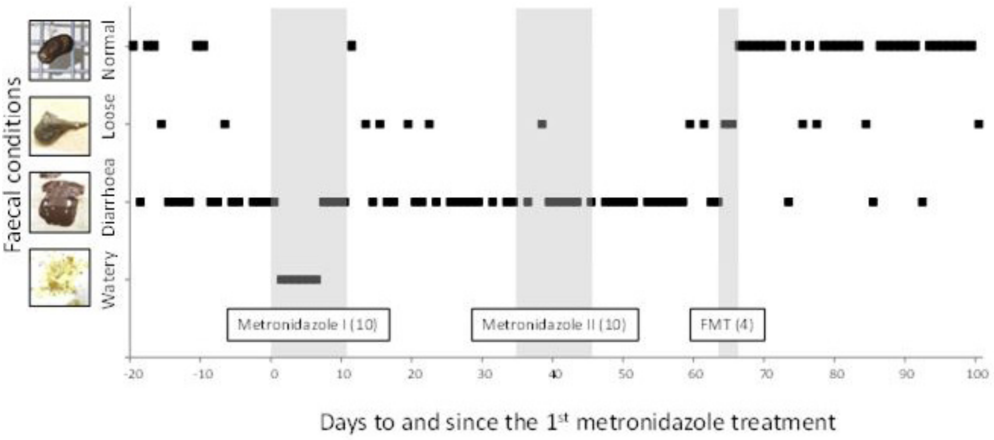
How does the microbiome interact with the host body system in common marmosets? Lewis et al. (1987) examined the microbiota of individuals with a disease that was unique to captive marmosets known as "marmoset wasting syndrome" (MWS) or "wasting marmoset syndrome" (WMS). MWS has been a well-known health problem endemic to captive marmoset colonies for several decades (Cabana et al., 2018; Shimwell et al., 1979; Tucker, 1984). The syndrome is quite problematic because a specific cause has not yet been determined, but affected animals show various symptoms, such as diarrhoea, anorexia, and anemia, which can become fatal (Chalmers et al., 1983; Juan-Salles et al., 2003). Of the several suggested causes for the syndrome, malnutrition might be one of the important factors (Cabana et al., 2018; Shimwell 1979). Lewis (1987) compared the microbiota of fecal samples from individuals with and without MWS and revealed differences in the abundance of only anaerobic, not aerobic, bacteria. Shigeno et al. (2018) reported that the group with a higher rate of chronic diarrhoea had a lower proportion of Bifidobacterium than the other group, but there was no significant difference between the groups in terms of microbiota diversity. These studies clearly indicate that captive marmosets with diseases would have different microbiota compositions from those without diseases.

Distribution of Microbiota Considerably Affects the Bodily State of Common Marmosets

Our case study (Yamazaki et al., 2017) clearly showed striking changes in the composition of microbiota on the whole body. We had a male common marmoset who experienced chronic diarrhoea after antibiotic treatments, experiencing severe weight loss despite his normal appetite. We found that the antigen of *Clostridioides (C.) difficile* and its toxin were positive, but could not confirm the presence of protozoa and gastrointestinal parasites from his fecal samples. We first administered metronidazole orally because it is the first-choice antibiotic for *C. difficile* infections in both humans and small animals (Johnson-Delaney, 1994). Although the medicine was effective in suppressing the symptoms of diarrhoea to some degree the first time, it was no longer effective when he showed recurrence of the symptoms. Then, we decided to administer an oral fecal microbiota transplantation (FMT) by feeding him fresh feces from healthy individuals of the colony mixed with the marmoset complete food that he used to eat. FMT was chosen because it is an emergent treatment for diarrhoea caused by various agents (e.g., van Nood et al., 2013) and a study reported immediate, successful recovery from recurrent *C. difficile* infectious disease (Kahn et al., 2012). A few days of treatment had a clear effect on the symptoms of severe diarrhoea, and the test for *C. difficile* became negative for both toxins and antigens. The symptoms never recurred again after the treatment and he regained weight to the level before the disease. To our surprise, after the treatment, the marmoset also recovered from sinusitis and bilateral dacryocystitis, which were concurrently evident with the severe diarrhoea. These conditions also did not recur. The implication of the evidence is that dysbiosis of microbiota in intestinal

Figure 11.2.

Results of Several Treatments for Chronic Diarrhoea Caused by C. difficile Infection in a Captive Marmoset.



Note. The "0" on the horizontal axis indicates the first day of the first treatment using metronidazole, which is the first-line drug for the disease. The feces was divided into four categories based on the appearance—normal, loose, diarrhoea, and watery—as represented in the pictures next to the vertical axis. Shaded vertical lines show the two periods of metronidazole treatment and the fecal microbiota transplantation. The numbers next to the names of the treatments indicate the period in days. Adapted fromYamazaki et al. (2017) with permission.

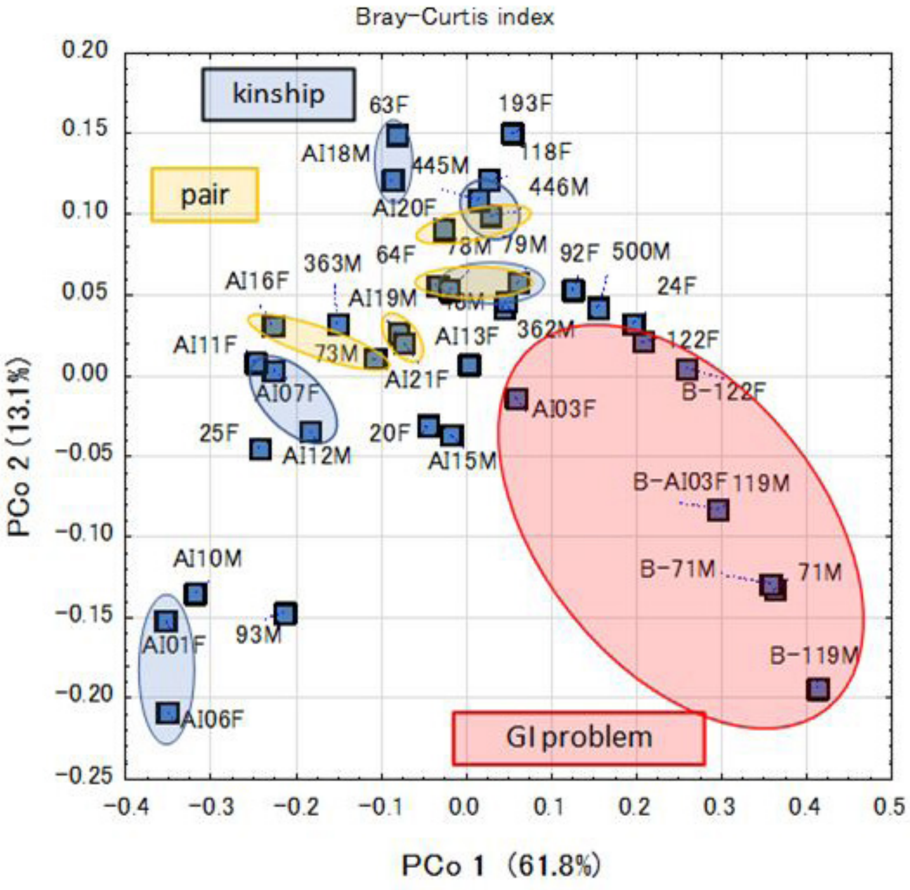
tracts—atypical increase in *C. difficile* with toxin related to preceding antibiotic treatment—induced severe symptoms quite similar to those of human patients and the marmoset recovered only through treatment aimed at inducing symbiosis of the intestinal environment, not through antibiotic treatment. Recovery from sinusitis as a byproduct of the treatment suggests that symbiosis of microbiota in the gut of marmosets has a tremendous role in immunity regulating the whole body.(Figure 11.2)

Characterizing Microbiota of Captive Marmosets

The microbiota of captive marmosets differs in each facility, as they are influenced by breeding conditions and food regimens. As a result, we tried to determine the characteristics unique to our own colony. We conducted 16S rRNA sequencing on fecal samples to analyze possible factors affecting the similarity of intestinal microbes of each individual. Figure 11.3 shows the principal coordinates analysis using the Bray-Curtis index. Because microbes are vertically transmitted from mother to offspring, some individuals were closely located in the graph, as grouped by blue circles. Additionally, pairs had similar microbe distributions, as shown by yellow circles. These observations are in accordance with observations in other primate species where social units and sociability determined microbiota similarity (humans: Yatsunenko et al., 2012; chimpanzees: Moeller et al., 2016). Moreover, we found that individuals showing chronic gastrointestinal problems (such as vomiting and bowel distension) were also closely distributed, as grouped by the red circle. Thus, other than vertical transmission from mothers, several acquired factors, such as cohabitation and gastrointestinal diseases, could modify microbiota in captive common marmosets.

Figure 11.3.

Individual Distribution of Microbiota Similarity in our Colony Using the Bray-Curtis Index.



Note. Blue circles indicate individuals with kinship ties. Yellow circles indicate pairs of individuals. Individuals in the red circle share gastrointestinal problems, such as vomiting and bowel distension, with no kinship or pair relationships.

We found that acquired factors influenced the microbiota composition in captive marmosets. Acquired factors must interact with existing microbes in the intestinal tract. Thus, to clarify the interaction, we focused on the insect-eating habits of marmosets in the wild population. While wild marmosets eat many insects every day, as discussed above, captive marmosets do not necessarily eat insects in the facilities, although some guidelines recommend providing them with insects together with complete commercial food and produce (vegetables and fruits) (Bairrão Ruivo, 2003; National Research Council, 2003). Although insects seem to play important nutritional roles in their health, the unique impact of insects on the physiological functions of marmosets has not been clarified. Thus, we aimed to determine the effect of insect feeding on captive marmosets by analyzing the microbiome extracted from fecal samples (Yamazaki et al., 2019).

We had two groups, Group Insect Feeding and Group Control, which differed in the number of insects (crickets and mealworms) provided in the seven-day intervention period. By analyzing microbes and transcripts taken from feces sampled before, during, and after the intervention using the total RNA-seq technique (Elekwachi et al., 2017; Li et al., 2016), we found that the intervention made distinct changes in the composition of the microbiome in Group Insect feeding. We also found changes in transcripts coincident with those in microbes. Our preliminary experiment suggested that food items that are similar to those originally eaten in wild habitats can dramatically change the composition of microbiota. Although the functional significance of the observed changes after insect-feeding on the whole body remain to be clarified, the results suggest that information concerning the feeding ecology in wild habitats is important to maintain the health of captive marmosets by developing food regimens appropriate for the microbiota. By using techniques for preserving fecal samples and analyzing the microbiome and transcriptome developed in the study, future studies will reveal relationships between the microbiome and other variables, such as physical (body weight, dentition, measurements of body parts, etc.) and behavioral (sociality, anxiety, vocalization, etc.) traits of wild populations of the common marmoset, and contribute to our understanding of the evolutionary history of interaction between host and microbes.

Development of Novel Technology for Foraging Found in a Limited Population of Wild Macaques in Thailand

Humans use different kinds of tools for various purposes, some of which nonhuman animals have never used (Asano, 1994; Yamazaki et al., 2009). Stone hammers, however, are also used by some nonhuman animals (e.g., Haslam et al., 2019; Inoue-Nakamura & Matsuzawa, 1997; Moura & Lee, 2004) and can be examined from multiple perspectives: How the technology evolves (a view from anthropology); how the underlying cognitive abilities develop (behavioral and developmental psychology); how sensory-motor control and brain structures change (neuroscience); how new food resources contribute to host metabolic functions (physiology and microbiology); and how prey respond to novel foraging pressures (ethology and evolutionary biology).

Ancient hominins started using tools related to foraging and food-processing approximately 3.3 MY ago (Harmand et al., 2015) and stone hammers have been found from many archaeological sites worldwide. In nonhuman primates, evidence of stone hammers is scattered in various sites on several continents. For example, chimpanzees (*Pan troglodytes*) living in Guinea use stone hammers together with anvils to aid in food-processing (Inoue-Nakamura & Matsuzawa, 1997). Nutcracker-use in bearded capuchin monkeys (*Sapajus libidinosus*) is spreading in several areas in Brazil, which might be related to terrestrial habits of the populations, rather than food scarcity in the regions (Ottoni & Izar, 2008).

Stone-tool use in long-tailed macaques was reported for the first time on an island in Thailand in 2007 (Malaivijitnond et al., 2007). They use stone hammers for processing oysters, snails, sea almonds, and palm nuts (Falótico et al., 2017; Gumert et al., 2012; Proffitt et al., 2018). It is noteworthy that only the monkeys living on the islands show stone tool use (Bunlungsup et al., 2015), although monkeys living inland can access the same food items. The tool-users proved to have a common genetic origin (Bunlungsup et al., 2015). These findings lead to many further questions, such as who was the first to start using stone tools, why stone tools are limited to the islands, how the skill spread to groups beyond the family, and so on. From the perspective of the gut-brain axis in the evolutionary history of primates, we can assume that the shared microbiome among the populations on the islands helped to enhance the learning of stone tool-use within groups (Moeller et la., 2016; Yatsunenko et al., 2012). By using stone tools repeatedly, microbes that had originally been novel to the monkeys would have transferred from processed food items and become commensal to their gastrointestinal tracts. These microbes created a new ecosystem in their gut, resulting in special internal factors supporting sustained learning of tool use. While this hypothesis is speculative, it is testable through analysis of the microbiome of tool-users, comparison with microbiomes of non-tool-users, and detection of the functional significance of observed differences in the microbiome.

Conclusion

The nonhuman primate models discussed above provide ideas to examine the gut-brain axis along an evolutionary timeline: Specifically, how foraging strategies and techniques construct unique host bodies and microbiomes, resulting in the development of various kinds of psychological functions. By studying the current relationships between the behavior and microbiome of these species, we could restructure the evolutionary history of the adaptation of a given species in a given niche. This approach would also shed light on the evolution of human behavior, both ancient and modern, from the perspective of how they attempt to maintain their biological activities through foraging and to establish systems for building communities by inventing and spreading technological skills.

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