CHALLENGE 5

ABSTRACT

Brain function is influenced by internal inputs from many parts of the body, including chemicals in the blood and bacteria in the gut. The gut microbiota is a fundamental component of the body that can be transferred across generations and contribute to the unique features of the human phenotype influencing both health and disease. Deciphering the controlling mechanisms of microbiome-body-brain interactions may help in identifying new molecular targets to prevent and/or treat a range of psychiatric and neurologic disorders as well as their physical comorbidities. Here we provide an update on the functioning of the gut microbiome-body-brain axis and outline open scientific challenges and future research directions.

KEYWORDS

microbiome | microbiota-gut-brain axis | lifestyle | dietary patterns | brain disease | comorbidities

CHALLENGE 5

BODY-BRAIN-MICROBIOME INTERACTION

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1. INTRODUCTION AND GENERAL DESCRIPTION

Multicellular organisms have co-evolved with complex communities of microorganisms (microbiota) and their genomes (metagenome), collectively referred to as microbiomes (Marchesi and Ravel, 2015). Host-microbe symbiotic relationships benefit both organisms. The human gut microbiome is constituted mainly by representatives of Bacteria, but also includes Archaea, lower and higher Eukarya and viruses. The gut microbiome orchestrates an array of bodily and brain functions (metabolic, immune, endocrine, neural, etc.) through interactions with the host and the environment (diets, antibiotics, stress, etc.) with profound impact on human physiology and health maintenance (Sanz et al., 2018). Alterations of the gut microbiome (dysbiosis) can contribute to disease susceptibility and pathogenesis as reported, initially, for physical disorders and, more recently, for neurological and psychiatric conditions including autism, depression, Alzheimer's disease and Parkinson's disease (Cenit et al., 2017; Dinan et al., 2019).

The influence of the gut microbiome on the bidirectional crosstalk between the gut and the brain, the so-called "gut-brain axis" is a relatively new research field with multiple applications in health and disease (Dinan and Cryan, 2017). This axis is regulated through hormonal, immunological and neural pathways, and represents a route through which the gut microbiome influences neurodevelopmental processes and brain function (Agustí et al., 2018). Emerging evidence supports causal effects of the gut microbiome on

cognitive functions as well as on social, eating and emotional behaviors, including depression and anxiety-like behavior (De Palma et al., 2015; Dinan and Cryan, 2017, Agustí et al., 2018).

These effects are believed to be mediated through distinct mechanisms, including modifications in factors regulating synaptic plasticity and neural function (e.g., brain-derived neurotrophic factor and neurotransmitters) and through the regulation of endocrine and inflammatory pathways. These effects driven by microbially-produced dietary metabolites as well as by microbial stimuli (lipopolysaccharide, lipoteichoic acids, etc.) of non-dietary nature. Despite the mounting evidence on the significance of these microbial products, the mechanisms and molecular mediators of the complex interactions are far from being fully understood.

Physical disorders contribute to the risk of developing mental conditions and viceversa, indicating that our mind and our body are deeply interconnected. This interconnection accounts for the development of comorbidities, which complicates diagnosis and management. Indeed, the co-existence of different disorders poses a major social challenge, as clinical practice fundamentally addresses individual disorders. Modifiable dietary and lifestyle factors (physical activity, stress, drugs, social behavior, etc.) are known to influence brain and body functions. Specifically, unhealthy dietary habits have been identified as major risk factors for the development of physical and mental disorders (GBD, 2017). Accordingly the adoption of healthy dietary and behavioral habits has the potential to play an essential role in health promotion and in mitigating the drivers of disease vulnerability. The diet is also a major determinant of gut microbiota composition and function (Portune et al., 2017). Consequently, dietary health-effects could theoretically be mediated and optimized as a function of an individual's gut microbiome and its response to the diet (Sanz et al., 2018).

Elucidating the biological and molecular bases of the complex systemic communication between the brain, body and gut microbiome as well as their interactions with diet and lifestyle might open new diagnostic, preventive and therapeutic horizons for highly prevalent physical and mental conditions that are often comorbid.

Here we summarize the main challenges in the field to be addressed in the upcoming years:

1. Disentangling the mechanisms underlying body-brain-microbiome interactions and their consequences on health and disease.
2. Developing microbiome-based therapies and predictive tools for improving treatment and management of psychiatric and neurologic disorders and associated comorbidities.
3. Personalizing lifestyle and nutritional strategies for effective disease prevention as a necessary step towards reducing the societal and economic burden of of non-communicable mental and physical disorders.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The impact of addressing these ultimate goals will apply horizontally and transversally across disciplines, from basic science and to health and nutritional applications, and across sectors, from industry to health care and nutritional professionals to associations and society. At the institutional level, this will promote interactions between the three global areas of the CSIC: Life, Matter and Society.

Scientific impact

The integration of the gut microbiome and lifestyle factors for investigating the communication between the body and the brain will provide a new conceptual framework to understand biology and medicine. This broad, multi-disciplinary approach and vision will enable us to extend our knowledge into the roots and pathophysiological mechanisms underlying both mental and physical disorders and identify shared risk factors and molecular pathways which, in turn, could translate into common solutions. This research field has great potential to provide new directions for improving prediction (early diagnosis/prognosis) and disease management (treatment and prevention) applied to mental and physical co-morbidities.

Furthermore, the gut microbiome-brain axis is considered as a paradigm shift in neuroscience and mental health. Considering that CSIC researchers have been central to this shift, this research field is strategic to project CSIC values and strengthen its already highly competitive international position in this area.

Economic impact

The knowledge generated will lead to a number of applications from more accurate predictive and diagnostic tools (algorithms and biomarkers) for early

disease detection to more effective preventive and therapeutic strategies, all based on the integration of the individual microbiome and dietary variables and applying holistic approaches that target the body, the brain and the lifestyle of the subject. These advances will boost innovative capacities, especially of the health, biotech and food industries by providing unique solutions. The results of this research approach will broaden the focus in terms of diagnosis and also in terms of drug discovery and development. Opportunities will likewise emerge for further development of personalized lifestyle and dietary strategies for disease prevention. Indeed, the human microbiome market represents a great opportunity as it is steadily growing and is expected to reach USD 899.1 million by 2025 from USD 506.5 million in 2022, with an annual growth rate of 21.1% during the period 2022–2025.

Societal impact

Mental and physical disorders are a major economic and societal burden, particularly when they are comorbid, which complicates diagnosis and treatment. This research line will have a positive impact on clinical diagnosis and therapy and also in self-management of health through dietary and lifestyle strategies. Our approach will contribute to reducing the socio-economic disease burden and ensure the sustainability of the health care system. It can also have other social consequences by reducing disease stigmatization and inequalities (job losses and limited professional opportunities) and favoring social integration and cohesion, all essential pillars of the sustainable development agenda of the United Nations for 2030 and of the EU priority policies.

3. KEY CHALLENGING POINTS

3.1. Disentangling the mechanisms underlying body-brain-microbiome interactions

Understanding the mechanisms governing the connection between the microbiome, the body and the brain is essential in identifying new molecular targets and common therapeutic solutions for mental and physical comorbidities. This interaction occurs through neural, endocrine and immune pathways through which gut microbes and dietary factors influence the brain and different body functions (Agustí et al., 2018; Dinan and Cryan, 2017). Dissecting the contribution of these different routes is particularly challenging, considering the local connections between intestinal immune and enteroendocrine cells and the enteric nervous system, and the links between the enteric, autonomic and central nervous systems and systemic immunity.

The different routes of microbiome-body-brain communication that need to be further explored include the following:

- Neural pathway: The gut is innervated by the enteric nervous system (ENS), which communicates with the central nervous system (CNS) through the parasympathetic (e.g., via the vagal nerve) and sympathetic branch of the autonomic nervous system (ANS). The vagal nerve is one of the most important neural pathways mediating the bidirectional communication between gut and brain (Forsythe et al., 2014), while the connection with the sympathetic nervous system remains largely unknown. More research is needed to elucidate how gut bacteria and bacterially-produced factors activate the vagal afferents that transmit the signals from the gut to the brain and the neural circuitry underlying the effects of vagal stimulation on brain, behavior and body functions. These new means of interaction between the gut microbiota and the sympathetic nervous system and how they influence the gut-brain-body communication and function remain to be explored. Gut bacteria are also known to influence the host production of neurotransmitters and contribute directly to the synthesis of neuroactive molecules (Dinan and Cryan., 2017). Additional efforts are required to fully determine whether the production of neuroactive molecules by the gut microbiota influences the functioning of the ENS and the CNS, and the biological consequences of this.
- Enteroendocrine pathway: Enteroendocrine cells (EECs) are specialized cells of the gastrointestinal tract that produce neuroendocrine molecules with numerous primary functions for example in energy metabolism (appetite, insulin signaling), and that vary in response to gut microbiota-diet interactions. Emerging evidence indicates that EECs also modulate ENS activity and express innate immune receptors, pointing to additional roles in neural and immune signaling. Understanding the mechanisms whereby the stimulation of EECs (through contact with bacterially-produced factors) impact the immune system and the downstream effects on brain and peripheral organ functions represent a major challenge in the field. Also, we need to unravel the paracrine signalling between EECs and neurons of ENS, which may act as a sensorial channel in the bidirectional communication between the gut and the CNS.
- Immune and hypothalamic pituitary adrenal pathways: The immune system interacts directly with the ENS, the ANS and the hypothalamic

pituitary adrenal (HPA) axis and plays a key regulatory role in the gut-brain axis (Foster et al., 2017). Interestingly, functional adrenergic receptors and glucocorticoid receptors are expressed in immunocompetent cells, which suggest new types of interactions for exploration. Further research is needed to gain better understanding into how the gut microbiota and the diet modulate immune signaling through interactions with the ENS and the sympathetic arm of the ANS, and to uncover the downstream effects of the HPA axis on brain and body functions. This understanding could help, for instance, in identifying strategies intended to modulate the gut microbiota to increase our resilience to chronic stress, a robust risk factor for the onset of both cardiometabolic and psychiatric conditions, via the regulation of HPA-immune crosstalk.

The establishment of new experimental models is also critical to classify interactions between the different organs and systems and to facilitate the identification of possible preventive/therapeutic targets. Advances in the development of in vitro models such as 2D and 3D cell cultures, based on established cell lines, as well as in ex vivo organoid-like structures (e.g. the 3D Brain Model), derived from animal and human tissue samples, have been instrumental in reproducing the complexity of the interactions between different intestinal or brain cell types. Nonetheless, these systems still have a number of limitations; for example, they do not fully recapitulate the intestinal oxygen gradient required for co-cultivation of microbes and for maintaining their stability and dynamics (Jalili-Firoozinezhad et al., 2019). Models to investigate multi-organ interactions remain very elementary. A multidisciplinary approach is needed to further develop this area including experts in neuroscience, microbiology, bioengineering and bioinformatics. This could be the case for the development of new *in vitro* models, such a microfluidic gut-on-a chip models can faithfully mimic the intestinal environment, including the complexity and diversity of microbial populations and of different epithelial and immune cell types, and their maintenance to explore long-term dynamic host-microbiome interactions. Also, new multi-organ/body-on-a-chip models will help to explore inter-organ interactions *in vitro* (Harjes, 2019), including those occurring through the gut-microbiome brain axis (Raimondi et al., 2019). These models will serve as discovery platforms enabling large-scale screening of potential therapeutic molecules and bioactive agents (intestinal bacteria and products thereof) with higher predictive potential before moving on to costlier *in vivo* models.

Advanced approaches that enable monitoring of real-time bidirectional communication between the body, the brain and the gut microbiome *in vivo* will also be critical to fully understand the mode of action of biological and environmental variables affecting our health status, and to validate effector molecules/bioactive agents as lead candidates for therapeutic trials. The development of *in vivo* models will also present specific challenges, such as the design of microdialysis probes that enable the prolonged, automatized and more comprehensive monitoring of brain activity under other body site stimuli. The application of optogenetic techniques to *in vivo* models coupled with microbiome-related assessments will be extremely useful to progress in the understanding of the underpinning mechanisms that govern the brain-microbiome communication and functions at a cellular level.

3.2. Developing microbiome-based therapies and predictive tools

Considering the limited efficacy of current therapies—medical or psychological—for psychiatric and neurologic disorders and, in particular, the difficulties in managing mental and physical comorbid conditions, the identification of novel mediators and moderators of these disorders will provide new opportunities for improving their management and reducing their high societal burden. Of these, the gut microbiome—through its connection to the brain and peripheral tissues—represents a tractable target to manage disease (Kashyap et al., 2017). The use of classical probiotics (bifidobacteria, lactobacilli, etc.) and other strategies directed to the gut ecosystem (e.g. prebiotic fibres, etc.) as well as fecal transplants demonstrated the potential of these strategies to ameliorate or intercept the disease development in experimental models. A better understanding of the specific bacterial consortia (beyond those classically used as probiotics) offering health benefits as well as derived metabolites/molecular mediators of such effects is critical for the development of rational and more efficacious microbiome-based therapies (Romaní-Pérez et al., 2017).

To advance the development of microbiome-based therapies, key challenges include (i) proving and validating causality between specific bacteria/bacterial consortia and health outcomes in robust models; (ii) leveraging existing bioinformatic tools and combinatorial chemistry for the discovery of structurally new microbiome-produced metabolites/molecules and their targets as candidates for new therapeutics; (iii) replicating and scaling-up of intestinal bacterial cultures to ensure safe microbiota enrichment and replacement; and (iv) developing miniaturized delivery systems of microbiome-based

products targeting specific organs and functions. The assessment of the therapeutic action of microbiome-based strategies in humans will also benefit from new technologies —for example, advances could be foreseen from the use of brain imaging technologies and wearable sensors that detect brain activity, to facilitate the assessment of real-time feelings like mood and emotion. This, combined with *omics*-based technologies for monitoring gut microbiome activity and body functions, could be of much help to obtain information about the gut microbiota-brain axis function and effects on interventions in mental and physical health.

When considering the potential of the microbiome to better inform therapeutics, it will be critical to gain a deeper understanding of microbiome-drug interactions and their consequences. Evidence suggests that a large number of non-antibiotic drugs (up to 24% of human drugs) might have an impact on key bacterial species of the intestinal microbiota, with possible downstream effects on human health (Maier et al., 2018). In turn, the gut microbiome might be involved in the primary or secondary biotransformation of drugs through its enzymatic machinery or through host-micro co-metabolic processes, influencing the pharmacokinetics, efficacy and side effects of drugs (Turnbaugh, 2018). This seems to be the case for antipsychotics, which have a significant ability to inhibit commensal intestinal bacteria and this might be part of the side effects or the mechanism of action (Maier et al., 2018). Human studies show that —for example, the medication (levodopa) for Parkinson's disease can be metabolized by gut microbiota, potentially reducing drug availability and causing side effects (Maini Rekdal et al., 2019). This evidence might be critical to predict efficacy and side effects as a function an individual's microbiome as well as for drug repurposing. The future development of any new potential therapeutic drug will have to consider the complex metabolic interactions between the host and the microbiome. In the light of the current evidence, there is a need to address the following aspects: (i) integrate the microbiome as an additional biological variable in pharmacokinetic and pharmacodynamic studies for fine-tuning dose-response and side-effect assessments and (ii) identifying new pharmacological uses for existing drugs (drug repurposing) or therapeutic effects mediated by the gut microbiome.

Taken together with other variables, information from the microbiome could also serve for early disease detection, prognosis and prediction of response to therapy. Specially the development of more accurate predictive tools is essential to move from reactive care to disease prevention and positive medicine.

This is especially needed for the management of comorbid conditions since, until now, diseases have been investigated and clinically addressed as individual entities. The discovery of modifiable factors that help maintain body-brain homeostasis and contribute to health promotion and resilience against disease (understood as an active process) is also an essential aspect to progress towards disease prevention as addressed by this challenge. Of these factors, the gut microbiome is considered as one of the missing pieces that could help explain our resilience or vulnerability to mental and physical conditions and, at the same time, represents a preventive target.

To advance the development of microbiome-informed predictive tools and biomarkers of health status and early disease detection, we need to progress towards the so called "human phenomic science" (FitzGerald et al., 2018) based on longitudinally deeply phenotyped subjects. This implies the integration of not only clinical endpoints but also environmental modifiable factors, like diet, lifestyle and psychosocial stress, as well as big data generated by advanced technologies, including brain imaging and multi-omics readouts (metagenomics/transcriptomics, metabolomics, etc.), which would reflect the outcomes of body-brain-microbiome interactions with the environment. This would help to attain a more comprehensive understanding of the mediators and moderators that determine our health trajectory and leverage information from larger-scale but less phenotyped epidemiological studies. These advances are key to (i) identify robust drivers of the inter-individual variability and disease susceptibility, (ii) validate biomarkers for early detection of departure from "normality", (iii) develop friendly use prototypes for biomarker detection and computational models/algorithms that help to predict an individual's health trajectory. Furthermore, this basic information on modifiable disease risk factors is essential for the design of personalized cost-effective preventive measures based on changes in diet and lifestyle, as described in the next section.

3.3. Personalizing lifestyle and nutritional strategies for effective disease prevention

Considering that suboptimal diets are responsible for more deaths than any other risks globally, including tobacco, and that ~7 million deaths and 255 million disability-adjusted life-years were attributable to unhealthy diets in 2017 (GBD, 2017), dietary changes are clearly key to reduce societal and economic disease burdens. Unhealthy dietary patterns and a sedentary lifestyle are major contributors to non-communicable diseases, varying from

cardio-metabolic to psychiatric disorders. Indeed, adherence to the Mediterranean Diet and diets rich in fiber have shown promising benefits in both cardio-metabolic and mental disorders, like depression (Dinan et al., 2019). The role of specific essential nutrients (e.g. polyunsaturated fatty acids, vitamins and minerals) in mental health is also well-established. Nonetheless, the effectiveness of dietary and lifestyle changes for ameliorating or reducing the risk of these disorders remains, in some cases, unclear, partly due to the large variability of the individual response. In turn, diet is instrumental for modulating the structure and function of the human gut microbiota, as well as for altering the type and abundance of bacterial metabolites and bacterial-host co-metabolic products, with a potential impact on metabolic and mental health (e.g., short chain fatty acids, neuroactive compounds, etc.). Yet, an understanding of the influence of the microbiome on dietary health effects remains limited to precisely inform dietary recommendations (Sanz et al., 2018).

Physical exercise is also a key lifestyle intervention with preventive and therapeutic potential. Its benefits and the mechanisms through which it exerts its effects are well documented. Focused on the brain and mental health, exercise can be antidepressant, anxiolytic and can improve cognition, improve mitochondrial function of neural cells, and even increase neurogenesis (Llorens-Martin et al., 2006; Lopez-Atalaya et al., 2011; Llorens-Martin et al., 2011). Nonetheless, an important challenge that remains is to understand why some exercise practitioners do not benefit and how exercise routines could be individually tailored according to a person's hormetic characteristics. Hormesis consists of the presentation of beneficial effects for the brain by the practice of a certain amount of physical exercise (mainly duration and intensity) up to a limit after which, potentially negative effects accumulate. There is no definitive evidence on how the amount of physical exercise affects each person in their cognitive ability or mood, due to the limited available tests and their intrinsic difficulties. Current knowledge is generally based on the net neurobiological evidence obtained from laboratory models, which lack the subjective components obtained in human studies.

We need to progress towards personalized nutrition and lifestyle strategies (physical exercise) through the integration of all biological variables into algorithms that predict the individual responses to dietary change and to physical exercise with the support of more robust assessment tools. We also need to better understand differences in sensitivity to dietary effects, considering different developmental stages, age and the overlapping comorbidities, which

are of key relevance. New efforts are needed to develop microbiome-directed foods tailored to the individual. Overall, this will increase the efficacy of dietary and lifestyle measures, empower citizens to take control of their own health, and contribute to disease prevention in the long-term. The development of new tools and devices to achieve this goal is thus very important.

Overall, much evidence on the role of the microbiome in the regulation of the gut-brain axis and body functions has emerged in the past few years. A truly multidisciplinary approach is required to address current and upcoming challenges in the field such as how the gut microbiota can be modulated by lifestyle and dietary habits to influence physical and mental health, and to elucidate the microbiome-mediated mechanisms influencing brain development and impacting both brain and body functions. This research may uncover new opportunities for improved diagnostic, preventive and therapeutic approaches for a number of mental disorders (e.g., anxiety, depression) and their physical comorbidities.

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