Cell Reports Medicine

CellPress
OPEN ACCESS

Commentary

How nutrients and natural products act on the brain: Beyond pharmacology

Aurelie de Rus Jacquet,[1,2,3,*] Sophie Layé,[4,5] and Frédéric Calon[1,2,5,6]
[1]Neurosciences Axis, Centre de Recherche du CHU de Québec – Université Laval, Québec, QC G1V 4G2, Canada
[2]Institute of Nutrition and Functional Foods, Université Laval, Québec, QC G1V 0A6, Canada
[3]Department of Psychiatry and Neurosciences, Faculty of Medicine, Université Laval, Québec, QC G1V 0A6, Canada
[4]Université de Bordeaux, INRAE, Bordeaux INP, NutriNeuro, UMR 1286, Bordeaux, France
[5]OptiNutriBrain – Laboratoire International Associé, Québec, QC G1V 0A6, Canada
[6]Faculty of Pharmacy, Université Laval, Québec, QC G1V 0A6, Canada
*Correspondence: aurelie.jacquet@crchudequebec.ulaval.ca
https://doi.org/10.1016/j.xcrm.2023.101243

Understanding how natural products promote brain health is key to designing diverse strategies to improve the lives of people with, or at risk of developing, neurodegenerative disorders. The mechanisms of action involved and recent technological progress are discussed.

The tangled tree of phytomolecules and metabolites

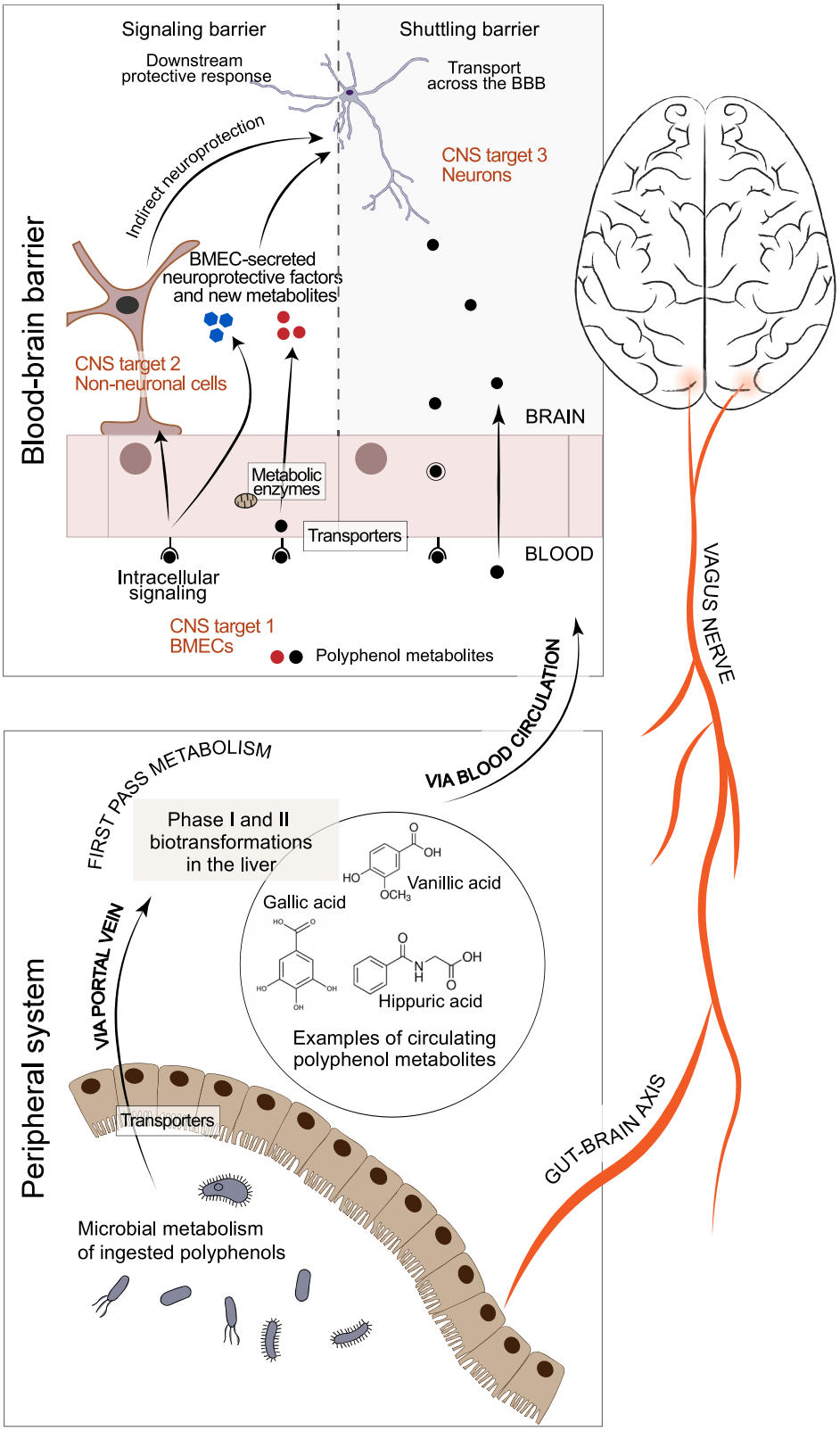
Knowingly or not, each and every one of us depends on plants for survival, from the food we eat, the air we breathe, to the clothes we wear. Throughout their lives, humans will interact with hundreds of botanical species, and they will rely on the nutritive and medicinal properties of thousands of natural compounds, such as the large family of polyphenols–more than 8,000 different chemical structures are known to date. To appreciate the medical and scientific importance of phytomolecules, we must put them into context. Health Canada defines natural health products as "naturally occurring substances that are used to restore or maintain good health." However, their potential may be greater than currently appreciated. The plant kingdom, similarly to the animal kingdom, is constantly exposed to environmental stressors. Therefore, plants have evolved to produce thousands of chemically diverse secondary metabolites with potent biological activities, such as antibiotics, antiparasitics, and other biochemicals that repel predators. They also manufacture phytochemical compounds to promote cellular health and survival in situations of environmental stress. In the context of neurodegenerative diseases, it is the latter that we attempt to leverage for neuroprotective purposes. These protective molecules retain their pro-health biological properties when ingested by mammalian organisms, but they undergo additional chemical modifications by the gut microbiota before crossing the epithelial barrier to reach the circulation. These molecules are further modified by first-pass metabolism in the liver and phase II enzymatic conversion (Figure 1). The resulting bioavailable metabolites form a large new pool of bioactive compounds and, together with their parent compounds, further enrich the diversity of plant-derived phytomolecules. These complex metabolic patterns and the resulting molecular diversity support a conceptual framework whereby the individual or synergistic actions of metabolites modulate central and peripheral biological targets to sustain brain health.

Biodistribution of phytomolecules and their metabolites: Where are the cellular targets?

The health benefits of bioactive molecules depend on their ability to reach target cells, tissues, or organs in sufficient concentrations. In the context of neurodegenerative diseases, the primary objective is to increase survival of vulnerable neuronal populations to stop or slow disease progression. Puzzling observations show that dietary interventions result in measurable neuroprotective outcomes in animal and clinical studies despite low apparent brain bioavailability.[1] The concept that bioactive polyphenols and other phytochemicals may not necessarily need to reach the brain tissue to induce neuroprotective benefits remains incredibly relevant and–for the most part–unanswered. Circulating metabolites may affect the central nervous system (CNS) via several mechanisms, from crossing the blood-brain barrier (BBB) and directly acting on the brain parenchyma to modulating the function of peripheral targets that will indirectly benefit brain health[2] (Figure 1).

Proposed target 1: Brain microvascular endothelial cells

Brain microvascular endothelial cells (BMECs) are unique gatekeepers of the cerebrovascular system that help maintain brain homeostasis and are key components of the neurovascular unit (NVU). Capable of integrating complex environmental cues, they form a dense vascular network that irrigates the brain parenchyma. The biological function of these highly specialized cells is driven by their key positioning at the interface of the blood and the brain, thus acquiring a dual luminal vs. abluminal polarity and accompanying molecular characteristics.[3] For example, polarized membrane lipid composition, localization of receptors and transporters, release of signaling proteins, and response to extracellular cues differ between the luminal and abluminal sides,[3] suggesting that blood-brain communication is tightly controlled by the endothelium. Circulating natural compounds and their metabolites naturally come into contact with the vasculature, and one could argue that downstream pro-health effects may result from a BMEC-mediated response triggered by these molecules instead of a direct action



(legend on next page)

on CNS target cells, as recently shown with insulin.[4] Physiological barriers are living cellular units and signaling hubs at the interface of tissues and peripheral circulation. Fascinating new studies revealed the high connectivity existing between cells forming the endothelium, which are organized into synchronized cell clusters that respond to biochemical stimuli and rapidly propagate local signals via network connections.[5] These signals can be endogenous, like circulating hormones, neurotransmitters, or immunomodulating factors, but we propose that they could also be exogenous molecules such as natural compounds. BMECs are equipped to interact with and process these exogenous factors, as demonstrated by their expression of metabolizing enzymes (e.g., cytochrome P450 family), which suggests an active interplay between circulating factors and the brain vasculature. Overall, much research remains to be accomplished to shed light on how phytomolecules may act on BMECs to improve hallmarks of cerebrovascular health and trigger the release of neuroprotective molecules into the parenchyma.

Proposed target 2: Cells of the brain parenchyma

A small subset of phytomolecules and their derivatives has the ability to cross the BBB and reach target cells such as neurons and glia. While the specific mechanisms by which these bioactive compounds enter the brain remain to be fully explored, small phenolic metabolites with hydrophobic functional groups and displaying low hydrogen-bonding potency may cross the BBB without specialized transport mechanisms.[6] However, observations regarding the possible stereoselectivity of natural compounds from the polyphenol family (e.g., epicatechin vs. catechin) suggest that some of these molecules may also interact with specialized endothelial transporters. The cellular membranes of BMECs harbor a diversity of uptake and efflux transporters, such as solute carriers (SLCs) and ABC transporters (e.g., p-glycoprotein) that regulate entry of circulating molecules. While the role of the BBB as a functional barrier separating the blood and the brain is conserved among mammals, brain microvessels display species-specific transcriptomic profiles,[7] and new human-based *in vitro* models may better model conditions specific to human diseases. Brain molecular atlases revealed the unique signature of various regions involved in neurodegenerative disorders, and different nutrients may have specific tropisms toward defined brain regions.[8] While this field of investigation is still at its infancy, better understanding the mechanisms of entry of natural products and metabolites into the brain parenchyma, identifying their tropism toward neuronal and glial targets, and defining the potential impact of regional differences would further support these molecules as sources of neuroprotective agents.

Proposed target 3: Peripheral organs

Paradigm-shifting discoveries on the dynamics of blood-brain exchanges have recently emerged, and the realization that the peripheral system acts as a significant modulator of brain health is gaining momentum.[9] It is also increasingly recognized that neurodegenerative, inflammatory, and cardiometabolic disorders share many risk factors and pathophysiological pathways. In this context, it is reasonable to suspect that beneficial phytomolecules and their metabolites induce protective effects on peripheral organs more readily accessible than the brain, and by altering the composition of the systemic circulation, this could further translate into neuroprotective outcomes. The gut-microbiota-brain axis is among the peripheral elements that could play a protective role and possesses intriguing properties. In addition to being the first and only organ to interact with the complete pool of ingested phytomolecules, the gut has a privileged mode of communication with the brain via afferent spinal and efferent vagal nerves (Figure 1). Moreover, the gut microbiome produces neuromodulating molecules, mediates stress responses, releases factors into the systemic circulation, and, depending on the nature of its resident microorganisms, is associated with distinct behavioral outcomes.[10] Since food intake is a significant factor regulating the composition of the gut microbiome, beneficial dietary compounds could induce neuroprotective effects by reshaping the ecosystem of this peripheral organ.[10] Similarly, dietary interventions aiming at improving cardiometabolic health are currently being investigated in various neurodegenerative disorders.

Methodological considerations to study the benefits of natural products in neurodegenerative diseases

Randomized clinical trials

Randomized clinical trials (RCTs) are the gold standards to make health recommendations. However, when exploring the benefits of natural health products, we must distinguish between dietary trials involving complex food matrices and purified phytocompounds with defined biochemical activities. Over the years, population-based association and cohort studies have convincingly shown a relationship between brain health and nutrition; however, translation into practice has been hampered by challenges in determining causal relationships between dietary interventions and health outcomes. In contrast to RCTs evaluating the effect of a single molecule, dietary interventions are uniquely affected by confounding factors such as the participants' baseline dietary practices, inherent variations in the nutritional value of food products, individual genetic variations in metabolic responses, and long-term trial durations that reduce compliance to the defined dietary intervention, which are all difficult to control with a placebo group.[11] Nonetheless, efforts aimed at the identification of dietary biomarkers associated with improved brain health could lead the way to rigorous mechanistic experimentation in clinical settings. Once identified, these biomarkers would allow the

Figure 1. Overview of the potential mechanisms involved in the neuroprotective benefits of plant-derived compounds

Ingested compounds are metabolized by the gut microbiome. The parent molecules are retained in the gut, where they can modulate the microbiota-gut-brain axis by communicating through the vagus nerve. The metabolites are transported to the liver via the portal vein, where they undergo first-pass metabolism and enter the systemic circulation to reach the brain and peripheral organs. Metabolites could interact with BMECs through transporters, receptors, and metabolizing enzymes that remain to be confirmed and fully characterized. Abbreviations: BMEC, brain microvascular endothelial cell; CNS, central nervous system.

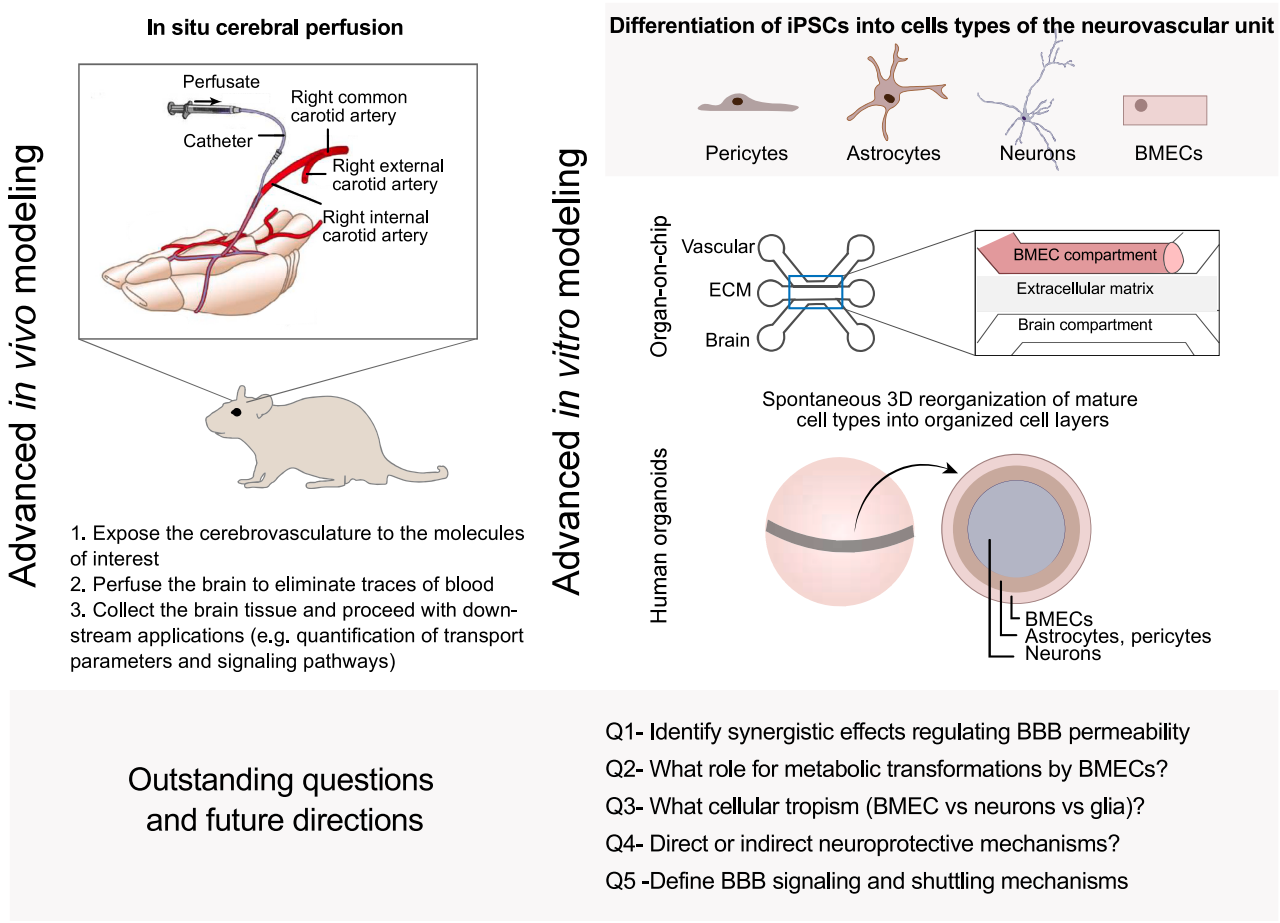


Figure 2. Advanced methodological approaches to study the blood-brain barrier

Advanced experimental approaches used to investigate the BBB *in vitro* and *in vivo* will enable to pinpoint the specific mechanisms involved in brain distribution and neuroprotective benefits of natural health products. Abbreviations: 3D, three dimensional; BMEC, brain microvascular endothelial cell; ECM, extracellular matrix.

implementation of depletion-repletion paradigms to validate a direct cause-effect relationship between nutrients and the health outcome under investigation.[12] Difficulties in securing intellectual property have also been a strong deterrent to the full development of unpatentable interventions.[13] While these factors may appear as challenges to conduct pricy clinical trials, they actually offer a wealth of opportunities to modulate clinically relevant disease phenotypes, such as the gut-microbiome-brain axis, and pursue personalized nutritional recommendations to promote brain health. Still, many RCTs have been conducted in the last 15 years, suggesting active efforts to improve the well-being of patients through plant-based natural health products.[1,2]

In vivo *models*

Prior to conducting clinical trials, animal models are key to establishing natural product oral bioavailability, i.e., prediction of absorption, distribution, metabolism, and excretion along with pharmacokinetic (PK) parameters.[2] An important aspect to consider for disease-modifying studies involving neurodegenerative disorders is the ability of compounds to cross the BBB to reach neuronal and glial targets. Considering the range of targets and mechanisms of action detailed above, it becomes essential to determine whether a molecule of interest enters the brain parenchyma to reach the minimum effective concentration (MEC) established for this compound. If the brain MEC is not achieved despite clear neuroprotective benefits, biological targets are likely to fall into one of the categories discussed earlier and indirectly improve brain health. To inform on potential neuroprotective mechanisms and CNS penetration, advanced *in vivo* methodological approaches such as intracarotid *in situ* cerebral perfusion enable the quantification of kinetic variables including brain uptake and permeability constants. This technique was developed in the 1980s, but it has been underused despite major advantages over other approaches. In this model, the right common carotid artery is catheterized after ligation of the external branch and perfused with the molecule(s) of interest, which directly reach the brain vasculature and bypass the peripheral metabolism to maintain the perfused molecule(s) in their native forms (Figure 2). As a result, transport

from the blood to the brain via the BBB can be efficiently quantified while requiring only a small number of animals to complete the study.[4] This direct measure of brain penetration is more informative compared with other approaches that rely on surrogate measures of compound permeability, for example in the cerebrospinal fluid. These BBB-specific techniques are key add-ons to more standard approaches to determine the PK and biodistribution of natural or pharmaceutical compounds. In essence, animal studies of natural product distribution and brain permeability are necessary to inform on the mechanisms by which bioactive molecules improve brain health and function. However, most animals have feeding habits and nutrient needs that can be very different from humans, and interspecies differences in metabolism and endothelial cell molecular composition and function could confound *in vivo* findings and limit translation of the results. Therefore, complementary *in vitro* studies using human-derived cells could be implemented.

In vitro *models*

Advances in microfluidic and organoid technologies have recently allowed the generation of BBB-in-a-dish platforms that recapitulate tissue-level organization and enable the deep investigation of cellular and molecular mechanisms underlying neuroprotection (Figure 2).[14] These approaches leverage the differentiation of human iPSCs into BBB-related cell types or utilize human primary cells to reconstruct the three-dimensional organization and blood-brain compartmentalization of this multicellular unit. These models have the distinct advantages of being composed of human cells originating from healthy donors or people with the neurodegenerative disease of interest, being able to include biological sex as a variable, and being suitable to screening experiments. In contrast to two-dimensional models, microfluidic channels mimic capillary shear stress, a mechanical pleiotropic regulator of BMEC function. Together with glial/neuronal signals originating from the brain compartment, laminar flow promotes physiological BMEC polarization and reorganization into a single-layer three-dimensional vessel with luminal and abluminal surfaces. Therefore, BBB chips are ingenious tools to study signaling at the BBB, in particular how natural compounds and metabolites may cross the endothelial layer or propagate signals from within the vasculature. For instance, studies can estimate the mechanisms of brain penetration by measuring the passage of bioactive molecules across the endothelial barrier via specialized transporters and evaluate how synergistic effects between molecules may promote transport across the BBB. The next key consideration is to define the tropism of natural compounds toward specific cell type(s). While drug discovery for neurodegenerative disorders has mostly focused on neuronal targets, accumulating evidence suggests that glial cells and cellular dysfunctions at the neuro-glia-vascular unit could contribute to neuronal loss.[14,15] In this context, natural products may be at an advantage, as they have been shown to modulate multiple protective pathways in both neuronal and non-neuronal targets. Furthermore, the discovery of new cellular subtypes, particularly at the BBB, is a welcome reminder of the diverse ecosystem in which beneficial compounds may exert their biological properties. However, differences in molecular and functional profiles between *in vitro* cultures and their *in vivo* counterparts need to be considered, as changes/adaptations to the *in vitro* microenvironment could alter native cellular responses.

Ethics, diversity, equity, and inclusion considerations

From an ethical standpoint, natural products and synthetic drugs should be valued equally when searching for neuroprotective molecules. The chemical complexity of natural molecules is often greater than that of synthetic compounds, and they can be manipulated by medicinal chemists to improve desirable biophysical properties. Importantly, botanical sources of phytomolecules and nutrients are found globally from sustainable sources and are not confined within cutting-edge laboratories, thus potentially allowing a greater access of medicinal resources to the global population. Greater collaborations between neuroscientists, pharmacists, and ethnopharmacologists are one way by which we can produce impactful biomedical scientific discoveries. Better understanding the properties of natural products originating from the diet or pharmacopeia of populations generally underrepresented in the sciences could not only unveil new horizons in our search for neuroprotective candidates but also promote ideals of diversity, equity, and inclusion. The North American and European patent systems have greatly favored the discovery of new synthetic scaffolds over botanical sources of natural products, and this legislative ecosystem may explain the relative neglect of phytochemicals in the second half of the 20th century. In contrast, the blooming multi-billion-dollar market of natural supplements is clear evidence that people long for non-medical approaches to health. We postulate that natural health products are not meant to replace synthetic drugs or become "alternative medicines." Instead, they provide a diverse arsenal of molecules that can work together with synthetic medicines.

Concluding remarks

The complexity of how nutrients and natural products act on the brain calls for the development of innovative conceptual and methodological approaches that consider that several tissues and targets could be affected by the beneficial compound(s). The study of neuroprotective molecules is further complexified by the restrictive BBB, but the vascular network is a complex biological system integrating and disseminating signals from the blood to the brain, and it should be envisioned as a potential key player in mediating CNS benefits. The controversy of where neuroprotective natural compounds act to improve brain health is a reminder of the complexity of body-brain interactions, and it questions conceptual frameworks that tend to uncouple peripheral and central biology.

ACKNOWLEDGMENTS

A.d.R.J. is supported by the Parkinson's Foundation (PF-Launch-937655). S.L. is supported by INRAE (AlimH), Université de Bordeaux, Eranet-Neuron Gut2Behave (ANR-19-NEUR-0003-03), JPND SOLID (ANR-21-JPW2-0004-05), and Nouvelle Région Aquitaine ExoMarquAge (13059720–13062120). F.C. is a Fonds de recherche du Québec-Santé research scholar and is supported by the Canadian Institutes of Health Research. This work was also support by internal funds from INAF to A.d.R.J. and F.C.

DECLARATION OF INTERESTS

F.C. is co-inventor on a provisional patent application: CA3002753A (World, PCT: WO2017072219A1).

REFERENCES

1. Scarmeas, N., Anastasiou, C.A., and Yannakoulia, M. (2018). Nutrition and prevention of cognitive impairment. Lancet Neurol. *17*, 1006–1015. https://doi.org/10.1016/S1474-4422(18)30338-7.
2. Leclerc, M., Dudonné, S., and Calon, F. (2021). Can Natural Products Exert Neuroprotection without Crossing the Blood-Brain Barrier? Int. J. Mol. Sci. *22*, 3356. https://doi.org/10.3390/ijms22073356.
3. Worzfeld, T., and Schwaninger, M. (2016). Apicobasal polarity of brain endothelial cells. J. Cereb. Blood Flow Metab. *36*, 340–362. https://doi.org/10.1177/0271678X15608644.
4. Leclerc, M., Bourassa, P., Tremblay, C., Caron, V., Sugère, C., Emond, V., Bennett, D.A., and Calon, F. (2023). Cerebrovascular insulin receptors are defective in Alzheimer's disease. Brain *146*, 75–90. https://doi.org/10.1093/brain/awac309.
5. Lee, M.D., Buckley, C., Zhang, X., Louhivuori, L., Uhlén, P., Wilson, C., and McCarron, J.G. (2022). Small-world connectivity dictates collective endothelial cell signaling. Proc. Natl. Acad. Sci. USA *119*. e2118927119. https://doi.org/10.1073/pnas.2118927119.
6. Velásquez-Jiménez, D., Corella-Salazar, D.A., Zuñiga-Martínez, B.S., Domínguez-Avila, J.A., Montiel-Herrera, M., Salazar-López, N.J., Rodrigo-Garcia, J., Villegas-Ochoa, M.A., and González-Aguilar, G.A. (2021). Phenolic compounds that cross the blood-brain barrier exert positive health effects as central nervous system antioxidants. Food Funct. *12*, 10356–10369. https://doi.org/10.1039/d1fo02017j.
7. Song, H.W., Foreman, K.L., Gastfriend, B.D., Kuo, J.S., Palecek, S.P., and Shusta, E.V. (2020). Transcriptomic comparison of human and mouse brain microvessels. Sci. Rep. *10*, 12358. https://doi.org/10.1038/s41598-020-69096-7.
8. Lauriola, V., Brickman, A.M., Sloan, R.P., and Small, S.A. (2023). Anatomical biology guides a search for nutrients for the aging brain. Mol. Aspects Med. *89*, 101154. https://doi.org/10.1016/j.mam.2022.101154.
9. Pluvinage, J.V., and Wyss-Coray, T. (2020). Systemic factors as mediators of brain homeostasis, ageing and neurodegeneration. Nat. Rev. Neurosci. *21*, 93–102. https://doi.org/10.1038/s41583-019-0255-9.
10. Liu, L., Huh, J.R., and Shah, K. (2022). Microbiota and the gut-brain-axis: Implications for new therapeutic design in the CNS. EBioMedicine *77*, 103908. https://doi.org/10.1016/j.ebiom.2022.103908.
11. Mirmiran, P., Bahadoran, Z., and Gaeini, Z. (2021). Common Limitations and Challenges of Dietary Clinical Trials for Translation into Clinical Practices. Int. J. Endocrinol. Metab. *19*, e108170. https://doi.org/10.5812/ijem.108170.
12. Brickman, A.M., Yeung, L.K., Alschuler, D.M., Ottaviani, J.I., Kuhnle, G.G.C., Sloan, R.P., Luttmann-Gibson, H., Copeland, T., Schroeter, H., Sesso, H.D., et al. (2023). Dietary flavanols restore hippocampal-dependent memory in older adults with lower diet quality and lower habitual flavanol consumption. Proc. Natl. Acad. Sci. USA *120*. e2216932120. https://doi.org/10.1073/pnas.2216932120.
13. Calon, F. (2006). Nonpatentable drugs and the cost of our ignorance. CMAJ (Can. Med. Assoc. J.) *174*, 483–484. https://doi.org/10.1503/cmaj.050663.
14. de Rus Jacquet, A., Alpaugh, M., Denis, H.L., Tancredi, J.L., Boutin, M., Decaestecker, J., Beauparlant, C., Herrmann, L., Saint-Pierre, M., Parent, M., et al. (2023). The contribution of inflammatory astrocytes to BBB impairments in a brain-chip model of Parkinson's disease. Nat. Commun. *14*, 3651. https://doi.org/10.1038/s41467-023-39038-8.
15. de Rus Jacquet, A., Tancredi, J.L., Lemire, A.L., DeSantis, M.C., Li, W.-P., and O'Shea, E.K. (2021). The LRRK2 G2019S mutation alters astrocyte-to-neuron communication via extracellular vesicles and induces neuron atrophy in a human iPSC-derived model of Parkinson's disease. Elife *10*, e73062. https://doi.org/10.7554/eLife.73062.