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MINERVA: the technological project to boost research on the microbiota-gut-brain axis in chronic neurodegenerative disorders

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Abstract Chronic neurodegenerative disorders including dementias, as Alzheimer's disease (AD) and movement disorders, as Parkinson's disease (PD) are strongly increasing worldwide. Up to now, very little is known about their biochemical mechanism and no effective therapeutic strategies are available to face brain functionality deterioration. A very intriguing hypothesis actively under investigation is referred as "microbiota-gut-brain axis". It suggests that human gut microbial community might impact central nervous system functionality through a bidirectional interaction that potentially might play also a key role in neurodegenerative disorders. Here we present a novel technological project, named MINERVA supported by the European Research Council (ERC) Programme, that aims, by using an innovative bioengineering approach, at evaluating microbiota impact on brain functionality. MINERVA goal is to develop a cutting edge technological platform, based on organ on chip microfluidic device, to model the main players of the microbiota-gut-brain axis. The final aim of MINERVA is to investigate the role of microbiota on brain functionality in physiological but also in pathological neurodegenerative conditions in order to improve the knowledge in the field and open the way to potential novel therapeutic microbiota-based approach for neurodegenerative disorders.

MICROBIOTA-GUT-BRAIN AXIS: A FOCUSED OVERVIEW

Alzheimer's (AD) and Parkinson's (PD) disease are severe chronic neurodegenerative pathologies. ADfeatures memory loss, followed by behavioral changes and impairment in everyday life. PD is characterized by movement disturbances, but it is not unusual the presence of dementia (1). An extremely interesting hypothesis is that the intestinal microflora (collectively named "microbiota") is a key player in chronic neurodegeneration. A relation between gut microbiota and brain, referred to as "microbiota-gut-brain axis", was firstly hypothesized more than 100 years ago by the Russian embryologist Elie Metchnikoff: he surmised that a healthy microbiota could help counteracting aging and that some bacterial strains (what we call today "probiotics") found in sour milk and yogurt would increase longevity (2). Literature shows that Metchnikoff's findings have been recently re-evaluated, becoming a new, exciting hypothesis in neuroscience: up to now, the papers on a possible role of gut microbiota on brain or AD/PD increased impressively, going from 8 in 2008 to 842 in September 2017, with 258 articles published in 2016 only (Table 1; source: PubMed). Many proposals dealing with the microbiota have been granted in the last 6 years: in 2011 and in 2012, the Michael J. Fox Foundation (USA) funded two grants on gut microbiota effect on PD, while "MYNEWGUT" was financed in Europe and it deals with diet, microbiota and its impact on brain development. The Human Microbiome Project-HMP (USA) was designed to sample, determine and quantify all human-associated microbiota; MetaHIT in Europe, is focused on intestinal microbiota; Eldermet in Ireland, is a national project associated with the large European project "NU-AGE" (the latter centered on the elderly) aimed at assessing the association between gut microbiota, food and health in the elderly. Very recently, the H2020 EU Programme granted the "AD-GUT" project, where for the firsttime microbiota composition will be manipulated in AD patients to evaluate how this affect the diagnosis and progression of the disease.

Year	Microbiota gut brain (MGB) *(number of publications)*	MGB and Alzheimer's disease *(number of publications)*	MGB and Parkinson's disease *(number of publications)*
2008	4	0	0
2009	8	0	0
2010	16	0	0
2011	30	0	0
2012	31	0	0
2013	67	2	0
2014	142	3	0
2015	174	7	7
2016	258	11	11
2017 (September)	229	21	23

Table 1. Number of published articles on the microbiota-gut-brain axis in the last 10 years, with a focus on Alzheimer's disease and Parkinson's disease. (source: PubMed; keywords: "microbiota gut brain"; "microbiota gut brain Alzheimer's disease"; "microbiota gut brain Parkinson's disease".

Note: the sum of published article/year exceeds the single search from 2008 to 2017 (842, 37 and 38, respectively) as some papers in the single year search are counted twice (year of e-pub and printed version).

NEURODEGENERATIVE DISORDERS AND MICROBIOTA IN LITERATURE

We have already several studies addressing the role of gut microbiota in (AD) or (PD) disease. For example, Bhattacharjee et al that described a number of evidences supporting microbiota-AD relationship (3). In addition, Wang et al reported a role of the intestinal microbiota in the protective activities of polyphenols in AD (4), and a similar conclusion was drawn in a mouse model of neurodegeneration (SAMP8 mice) (5). A direct action of microbiota on mechanisms involved in AD was reported by Minter et al, that suggested in mice a regulation by the gut microbiota community of host innate immunity mechanisms, with an impact on inflammation and amyloidosis (6). Many other researchers reported a link between gut microbiota and AD in animal models (7-10). A very important finding came from a seminal work in human patients, where it was reported an increase of a pro-inflammatory microbiota in patients with cognitive impairment and brain amyloidosis (11). As for PD, researchers have already found a different microbiota composition in PD patients in comparison to controls, (12-14), while PD animal models point to a role of microbiota in clinical signs, as reported by Sampson et al, whose findings suggested that gut bacteria regulate movement disorders (15).

Disease	Animal models	Clinical studies	*In vitro* cell based models
Alzheimer's Disease	-Transgenic / Germ free mice mice (6-8) Invertebrate models (Drosophila, C. elegans) (9)	-AD patients compared to healthy controls (11)	*In vitro* model of amylod formation (4)
Parkinson's Disease	- Transgenic / Germ free mice (15)	-PD patients compared to healthy controls (12,13)	

Table 2. Examples of current models to address the role of gut microbiota in Alzheimer's and Parkinson's neurodegenerative disease.

CURRENT AND INNOVATIVE MODELS TO STUDY THE IMPACT OF MICROBIOTA ON BRAIN

Currently the main experimental findings on microbiota-gut-brain axis impact on brain functionality, and in particular on AD/PD, rely mostly on *in vivo* assessments in rodent models, that allow to investigate the whole-body response to selected stimuli. Some of them have been used in particular to asses microbiota role in neurodegenerative disorders such as AD and PD (Table 2). However, they have several limitations: (a) rodent's microbiota is very different from the human one, (b) even if we transplant human microbiota in rodents, residual murine or environmental contamination is a major issue, and (c) they are expensive, lab-intensive and do not allow a strict control on microbiota composition once inoculated. Many papers are also based on *in vitro* studies, but they are performed with standard techniques that rely on 2D static immortalized cell models and their treatment with bacterial-derived molecules, a model that is very far from the real in vivo situation. In this setting, the availability of valuable innovative technological tools to consolidate the potential mechanisms by which the microbiota impacts the brain functionality might greatly help in overcoming the current limitations of *in vitro* and *in vivo* tools (16). Actually, some interesting dynamic digestion model that involves microbiota component and reliably reproduce some key features of the human digestive tract are available, in particular: the Simulator of Human Intestinal Microbial Ecosystem (SHIME™ and M-SHIME™) (17); the TNO Gastro-Intestinal Model (TIM) (18); HUMIX (19). However, the study of the impact of gut microbiota modifications on brain functionality is far from being their aim.

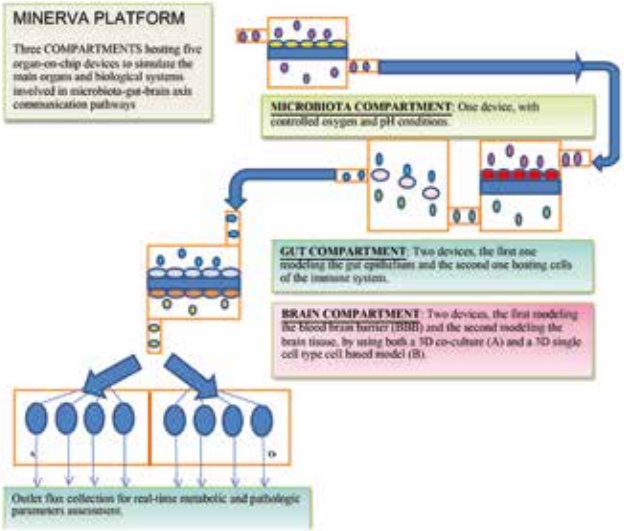

Figure 1. MINERVA platform: it is based on three main compartments, representing the microbiota, the gut, and the brain that hosts five optically accessible, organ-on-chip microfluidic devices. Each device hosts advanced in vitro cell models to model the main players of the microbiota-gut-brain axis: the microbiota; the gut epithelium; the immune system; the blood brain barrier; the brain.

MINERVA: AN ERC TECHNOLOGICAL PROJECT TO EVALUATE MICROBIOTA IMPACT ON BRAIN FUNCTIONALITY

Recently, an ERC Consolidator project named "Microbiota-Gut-Brain engineered axis to evaluate microbiota impact on brain functionality" (acronym: MINERVA, ERC-CoG-2016 - Proposal 724734), was funded and aims at designing the first complete "microbiota-gut-brain" axis physical model by using a bioengineering approach. This innovative platform relies on three compartments, based on state-of-the-art miniaturized, optically accessible microfluidic devices, hydraulically connected to reproduce *in vitro* the microbiota-gut-brain axis connection (Figure 1). In the MINERVA platform, human microbiota will be cultured in the "Microbiota-compartment" and the secreted molecules (the so called "secretome") transported to the "Gut-compartment" where gut epithelial cells and cells from the immune system will metabolize them as occurs *in vivo*. The resulting modified secretome will reach the "Brain-compartment", built up by a complete blood-brain barrier *in vitro* model followed by two exhaustive 3D human brain cell models, featuring the three main populations of brain cells: neurons, astrocytes and microglia. In the first model, the cells will be co-cultured, to recapitulate brain cell-to-cell contacts; in the second model,

each cell type will be cultured individually to let explore the microbiota's effect on each cell type. MINERVA platform will be functionally and biologically validated using an approach at increasing complexity, to finally assess neural cell response once exposed to human complete microbiota from (a) healthy donors, and (b) Alzheimer's disease (AD) patients. MINERVA has the potential to fill the gap existing in current tools in the field of microbiota-gut-brain axis at the boundaries between *in vitro* and *in vivo* models, to deep our knowledge in the field and suggest innovative strategies to be tested after the approval of clinical studies enrolling AD/PD cases that ultimately will demonstrated the impact of microbiota control in neurodegeneration.

CONCLUSIONS

The fundamental concept of an interaction of gut microbiota with the nervous system has been revisiting in recent years: the main novelty is the search for a causal link between microbiota composition or metabolism and the molecular determinants of pathologies as Alzheimer's or Parkinson's disease, mostly considered up to now as based on brain autonomous mechanisms. We have very limited knowledge in this field but studies and models are under development. This hopefully will boost the perspective of a clinical translation, as the microbiota-brain relation would represent a breakthrough also from the therapeutic point of view: human microbiota composition is dynamic and tunable by diet and probiotics, a very common tool in medicine, for instance in association with antibiotics therapy.
It is out of doubt that the challenge is complex and only a multidisciplinary approach based on fruitful discussion among neuroscience, immunology, gastroenterology and bioengineering will be successful and MINERVA might represent a first challenge on this way.

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