Over the last decade, a large and growing body of evidence reveals a tight relationship between gut microbiota and gastrointestinal disorders, but also infectious diseases, metabolic disorders, immunity, oncology, and neurology. More recently, the “gut brain axis” has emerged as one of the most promising areas in microbiome science. Yet, the exact roles of the gut microbiota in biochemical signaling events between the gut and the central nervous system (CNS) have yet to be clarified.

Furthermore, some questions remain as to how the diet may impact neurodevelopment, mental health, and daily behaviors. From diet to the CNS, the gut microbiota transforms nutriment into metabolites, peptides and lipids, acting as numerous signals for the brain, which, in turn, impacts this microbiota via various signaling pathways as well.

This new scientific booklet, prepared by the Biofortis scientists, provides an overview of the place of the gut microbiome in (I) the gut-brain axis, (II) developmental brain disorders (e.g. autism, schizophrenia), (III) psychiatric illnesses (e.g. anxiety and depression), and finally (IV) neurodegenerative disorders (e.g. Alzheimer’s & Parkinson’s diseases). In addition to a summary of our current knowledge on these topics, we will discuss the hopes they represent for patients, but also the shortcomings that the scientific community still needs to overcome before translating these hopes into reliable clinical solutions. Thus, this booklet illustrates how Biofortis can help scientists design and conduct their future projects on the microbiome in neurology.

I hope you will enjoy your reading.
Microbiome & Neurology

The gut-microbiota-brain axis, or How our mind and our microbes interact

Introduction

The relationship between the gut and the brain was first established in the 19th century when James & Lange suggested that our emotions might originate in our gut. Since then, scientists have studied at length the effect of the central nervous system (CNS) on gastrointestinal (GI) tract functions. However, it was not until the 2000’s that we discovered evidence that the communication between the 2 systems goes both ways. This discovery led to the concept of a bidirectional interaction between the two systems and the name of “gut-brain axis” or “gut-microbiota-brain axis” (GMBA). Despite these advancements, GMBA still remains poorly characterized to date.

It is noticeable that both acute (e.g. viral gastroenteritis) and chronic (e.g. inflammatory bowel disease [IBD]) digestive illnesses are associated with neurological signs, such as decreased social interactions and depression. Conversely, acute (e.g. nausea/vomiting in migraine attacks) and chronic (e.g. constipation/diarrhea in autism) neurological disorders are often associated with digestive disturbances.

Within the digestive tract, the gut microbiota has been linked to multiple physiological brain functions: e.g. feeding & satiety, mating behaviors, social interactions, cognition & memory, personality, mood & fear, or sleep. Conversely, there are numerous publications describing the link between the gut microbiome and brain diseases, some of which will be discussed in this newsletter: e.g. migraines, post-traumatic shock disorder, addiction, epilepsy, brain cancer, stroke.

The components of the gut-microbiota-brain axis (Figure 1)

The GMBA includes various components:
- neuronal elements: the CNS, the ANS (autonomic nervous system), and the ENS (enteric nervous system);
- hormonal elements: the hypothalamus-pituitary-adrenal (HPA) axis, the enteronecrointestinal endocrine cells of the ENS;
- immune elements: the CNS microglial cells, the GI immune cells (some of which are part of the gut-associated lymphoid tissue, GALT);
- microbial elements, i.e. the gut microbiota and its microbial molecules.

Using these different pathways, signaling is exchanged in a bidirectional manner between the CNS and the digestive tract. The “top-down signaling” represents mainly how the brain controls all the gut functions (motility, permeability, secretion). However, the CNS also affects GI inflammation and immunity. The brain also seems to control the growth and the virulence of the gut microbiota. The “down-top signaling” in the GMBA is more relevant to the present review and will, therefore, be discussed into more details.

One of the key elements of the GMBA is the ANS and more specifically the vagus nerve that connects directly the brain to the GI tract. This allows for the former to control the latter, but also to carry some information in the other direction.

The ANS and the CNS also communicate with the ENS, which contains more neurons than the brain, leading to the concept of “second brain”. Note, the gut is the only organ to have its own separate nervous system. In addition to neurons, the ENS includes some enteronecrointestinal cells that link the gut epithelium to the neurons, and also create a link between the neuronal and the endocrine/hormonal components of the GMBA.

The immune component of the GMBA is crucial to the CNS development and activity. The key immune
Figure 1
The components of the gut-microbiota-brain axis
cells in the brain are the microglia. They are key players in the CNS development (including neuronal pruning), its functioning later on, and its protection against infectious agents. Interestingly, the gut microbiota has been shown to influence both microglia maturation and activation, creating a functional link between commensal microbes and neuro-immunity and subsequently neuronal anatomy.

The microbial component of the GMBA communicates with the digestive elements of the neuronal, hormonal, and immune components of the GMBA via various bacterial molecules. Whether all, or just some, of the molecules allow for the gut microbiota to directly affect the central components (CNS neurons, hormonal HPA axis, immune microglia) remains uncertain. Below are some important examples of these bacteria-derived molecules of importance in the GMBA:

- “neurotransmitters” (e.g. GABA, serotonin, dopamine) and neurotransmitter precursors (e.g. tryptophan, tryptamine)
  - endotoxins (e.g. lipopolysaccharide [LPS]) and exotoxins (e.g. wall peptidoglycans)
  - short chain fatty acids (SCFAs, e.g. butyrate, propionate, acetate)
  - prion-like proteins (see the neurodegeneration chapter for more details)
  - biogenic amines (e.g. histamine)

The ENS is the main producer of the very important neurotransmitter, serotonin. The gut microbiota influences their host’s serotonergic system. Some bacteria can even produce some serotonin from tryptophan or modify the expression of its receptors in the GI tract. Bacteria also appear to play a role in the kynurenine pathway by which tryptophan is mainly catabolized, leading to neurotoxic metabolites that play a key role in infection- and inflammation- associated changes in behaviors known as “sickness behavior”. Another important neurotransmitters that some gut bacteria can produce is gama-amino-butyric acid (GABA), the main known inhibitory neurotransmitter, and an important element by which the gut microbiota regulates the ENS and may be the CNS.

Another important type of neuroactive bacterial molecules are the SCFAs. They have been shown to have multiple neurological effects (some beneficial, but also some toxic). Interestingly, SCFAs are also immunoactive on gut immune cells and microglial cells. In addition, SCFAs are known histone deacetylase inhibitors and are also thought to affect ENS and potentially CNS functions via epigenetic effects.

Finally, endotoxins, such as LPS, and exotoxins, such as wall peptidoglycans, in addition to being immunoactive also display some neuroactive properties. It is therefore not clear whether their neural effects are direct or via some neuroimmune activity.

Here are some of the documented effects of the gut microbiota on the brain:

- anatomical effects:
  - neurogenesis & neuron survival
  - myelination
  - blood-brain barrier & gut (ENS)-blood barrier integrity

- functional effects:
  - neuronal firing
  - neurotransmitter signaling (synthesis, metabolism, receptors)
  - blood-brain barrier & gut (ENS)-blood barrier permeability
  - microglia maturation & activation

In 2016, Bauer KC et al 2016 proposed a conceptual framework to study the gut-microbiota-brain axis that rests on 5 hallmarks, among which are:

- “bidirectionality”: the brain affects the gut and its microbiota, which in turn also influence the CNS;
- “indistinguishability”: the interactions between the gut/its microbiota and the CNS are so intricate that the different components of the GMBA appear as one meta-organism;
- “emergence”: new properties appear when different microorganisms are grouped into one ecosystem such as the gut;
- “critical window fluidity”: there are several specific periods in the neurodevelopment (prenatal, perinatal, adolescence, elderly) where the brain will be especially sensitive to the gut and its microbiota, which will be able to leave a long lasting impact on its future anatomy and functioning;
- “homeostasis”: the brain, the gut and its microbiota affect each other’s homeostasis.
The “old friends hypothesis” or “hygiene hypothesis” \(^1\)\(^2\)\(^3\)\(^4\)

Some researchers link the “hygiene hypothesis” of the rise in inflammatory and immune diseases to neurological disorders. Indeed, they hypothesize that reduced exposure to microbes (the “old friends”: commensal microbiota, daily life environmental microbes, ancestors’ infectious agents) increases the risk of neurodevelopmental disorders (e.g. autism, schizophrenia) and psychiatric disorders (e.g. depression, bipolar disorder).

Is there a brain microbiota?

Like with the placental microbiota, the brain microbiome is one of the hottest and most controversial subjects in the field. Over the last few years, bacterial genetic material and proteins have been detected in brain samples (postmortem & surgical) from patients with neurological disorders (multiple sclerosis, amyotrophic lateral sclerosis, HIV, epilepsy, Alzheimer) and matched controls, with some degree of correlation between these bacterial makers and neural disease biomarkers. Similar findings have been shown in preclinical models (mice and primates).\(^23\)\(^24\)\(^25\)

Some researchers believe that these bacteria are a result of contamination during sampling or postmortem. However, in 2018, Roberts et al presented some preliminary work demonstrating the presence of bacteria in both mouse and human (healthy and schizophrenic) brains at the 2018 meeting of the Society for Neuroscience. (Roberts RC et al 2018 - abstract) These bacteria were visualized by electronic microscopy and some of them were intracellular, with variable density depending on the brain area. To test the hypothesis of a possible postmortem contamination, the team observed the brain of mice that were fixed right after death and the brains of germ-free mice: they found the bacteria in the former (same brain and cellular distributions), but not in the latter.

In 2017, Pirs Moir and Tanzi launched “The Brain Microbiome Project” after discovering some evidence that there was a potential link between Alzheimer’s amyloid plaques and a past brain infection\(^2\)\(^7\), but the team has not published any work yet.

Shortcomings in the infatuation of neurology for the gut-microbiota-brain axis\(^1\)\(^4\)\(^5\)\(^7\)\(^9\)\(^12\)\(^28\)\(^29\)\(^30\)

Numerous studies, including on humans, have already looked at gut microbiota profiles and modulation in multiple neurological disorders, some of which will discussed in more detail in the rest of our review. PubMed shows this body of work is rapidly growing, in parallel of the hope in the patient community for relief, or maybe even a cure, one day. Yet, the number of scientific articles and media posts regarding the shortcomings of this scientific body is also growing accordingly.

Some of these scientific flaws are common to all fields of medicine that investigate the microbiota. However, they seem more pronounced in neurology where disorders are usually phenotypically heterogeneous, even within one given diagnosis. Most of the published studies rely on a flawed methodology and/or often claim some unsubstantiated mechanistic speculations based on simple observational results.

Here are listed some flaws common to all microbiota research that also plague the GMBA literature:

- Whether it is in preclinical models or in humans, the scientific community has not been able to agree on what a “healthy”/”normal” microbiome is, likely because it does not exist. This renders complicated, and potentially useless, any study based on comparing the microbiota from diseased individuals to healthy ones.
- Most, if not all, human studies are correlative rather than causative and concern microbiota taxonomy rather than functional activity. This is not an issue when looking for diagnostic biomarkers, but becomes a significant problem when investigating pathogenic mechanisms that could lead to potential therapeutic targets. Yet, many papers tend to speculate on mechanistic implications based on simple observational facts.
- Studying the microbiota in animal models presents some major flaws. Indeed, the gut microbiota, the diet, and the gut anatomy are very different between rodents and humans.
- The gut microbiota is significantly influenced by numerous factors that are not always recorded during studies and/or not always taken into account during data analysis:
  - extrinsic: diet (often source of pro- and pre-biotics that survive gastric conditions), environment, medications and illegal drugs (this latter point is especially important considering the interactions between drugs and microbiota – see following chapter)
  - intrinsic: genetic background, co-morbidities
  - Part of dealing with these confounding factors, beside recording them, is to have a properly matched control group.
- Most studies are conducted on fecal samples, but the microbiota present at the “action site” of the GMBA is likely different.
- Because of important inter- and even intra-individual variations in gut microbiota, studies in that field require large cohorts, which is not always the case.
- Interventional studies take place before some robust therapeutic targets have been identified.
- The available technical tools in the wet and dry steps of microbiota analysis are not optimal yet and offer many sources of inter-study variability.

Here are some aspects that are especially marked in the GMBA literature:

- Animal models in neurology are especially problematic as well:
  - Rodents are very different to humans in terms

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of neurology; e.g. in physiological and stress-induced behaviors (which even vary between strains), in neurodevelopment timeline:

• There is significant lack of adequate animal models in almost all neurological disorders, in terms of clinical phenotype and mechanistics

• When researchers transfer a patient’s gut microbiota into a rodent, there is often a mismatch between the neurodevelopmental stage of the human and the animal.

• A lot of data on the GMBA in neurological disorders come from germ-free mice. Not only is there no such condition in humans, their neurodevelopment is so altered that any neurological observation afterwards is likely confounded by prenatal events.

− Extrinsic and intrinsic confounding factors are an important issue in microbiota research:

  • In some neurological disorders, diet is especially important in microbiota-related studies because a significant proportion of these individuals have abnormal dietary habits (e.g. restricted diets in autism, anorexia or bulimia in depression).

  • Many commonly used neuroactive medicines have been shown to affect the microbiota (see corresponding chapter).

  • Many patients suffering from a neurological disorder abuse illegal drugs, which have been shown to affect the gut microbiota.

  • If some fields have caught up with this fact and have started recording and analyzing them more systematically (e.g. GI diseases, cancer), this is not the case yet in neurology.

  • In addition, a substantial number of studies on microbiota & neurological disorders do not have a proper control group to mitigate the effect of these confounding factors.

− Within one given neurological disorder, the clinical signs vary significantly:

  • This complicates the establishment of robust animal models.

  • This means that robust diagnostic criteria have to be used before recruiting patients and when following their clinical evolution during interventional studies. Yet, for some neurological disorders, there is no consensus on such diagnostic tools.

  • Because of this clinical variability in addition to gut microbiota variations, it is even more important to have large sample sizes. Yet, most published studies to date tend to have relatively small samples.

  • It makes studies based on patients’ self-referral particularly challenging to trust.

Conclusions

With the introduction of a bidirectional gut-microbiome-brain axis, some researchers talk about a paradigm shift in neurology. Indeed, the microbiota obviously plays an important role in neurodevelopment in early life, brain health and disease throughout life, and eventually neurodegeneration. Therefore, microbiota composition and modulation represent a source of hope in neurological disorders, from diagnosis to therapeutic management. The following chapters will summarize these stories. Yet, it is important to acknowledge the flaws of our approach to date and the limits of our present knowledge. For this paradigm shift linking microbiota and neurology to lead to safe, effective, and robust tools in neurology diagnosis and therapy, research will have to rely on multidisciplinary collaborations between experts from various fields (e.g. microbiology, neurology, artificial intelligence, data mining, medicine) and from different work environments (e.g. academia, industry, biotech, governmental agencies, patients’ association).
Gut microbiota & neuropharmacology, A two-way street to be harnessed

The relatively recent collision between the fields of neurology and microbiota represents a paradigm shift for neurology globally, and for the therapeutic management of neurological disorders in particular. Presently, most of these disorders remain without drugs that show a robust efficacy profile without significant side effects. When it comes to neuropharmacology and the gut microbiota, the discussion needs to encompass several angles: the effect of neuroactive drugs on the gut microbiota; the effect of the gut microbiota on neuroactive drugs (especially their pharmacokinetics); and the modulation of the gut microbiota for neurotherapeutic purposes. The former two are linked to the concept of “pharmacomicrobiomics”, and the latter to the concept of “psychobiotics” (or “psychomicrobiotics”).

Neuro-“pharmacomicrobiomics”: a tango between neurological drugs and the gut microbiota

The mutual interactions between the patient’s microbiota and the drugs he/she receives has led to the new field of research called “pharmacomicrobiomics”. Figure 2 summarizes the main pathways by which gut bacteria and neurological drugs could affect each other, based on what is known for drugs used in non-neurological diseases. Indeed, very little is known to date about neuroactive drugs, specifically.

How neuroactive drugs affect the gut microbiota

The analysis of 2 large cohorts showed that medications are the most important factor on total variance in the gut microbiota. Cussotto S et al recently reviewed the literature for the effect on the gut microbiota observed with drugs used in various neurological disorders. There growing evidence is particularly striking for antipsychotics and antidepressants.

How the gut microbiota affects neurological drugs

Some commensal bacteria have been shown to modify the pharmacokinetics of certain drugs via various mechanisms: altered absorption after chemical modification of the drug or because of gut permeability changes by bacteria; bacteria-modulated drug metabolism in the gut mucosa; or bacteria-mediated reactivation of biliary-excreted drug metabolites. These effects on pharmacokinetics further impact the efficacy and the toxicity of the drug. In 2019, Zimmermann M et al evaluated the ability of 76 known commensal bacteria of the human gut to metabolize 271 non-antibiotic oral drugs, including some neurological medications (e.g. antidepressants, Alzheimer’s and Parkinson’s medicines).

One concrete example of the relevance of such gut microbiota-induced alteration in a neuroactive drug pharmacokinetics is the metabolism of the dopamine precursor used in Parkinson’s disease (PD), levodopa. This prodrug is administered orally with an enzyme inhibitor that prevents its inactivation by hosts peripheral enzymes (aromatic amino acid decarboxylase). It then crosses the blood brain barrier (BBB) before being activated to active agent in the CNS (dopamine, that does not cross the BBB and would trigger some side effects peripherally). Despite these inhibitors, less than 60% of levodopa reaches the brain. This peripheral metabolism of the prodrug explains, partially, the great heterogeneity in efficacy and toxicity of levodopa among PD patients. Several studies have demonstrated that some gut bacteria can inactivate levodopa using enzymes that are not affected by the inhibitors usually co-administered to protect the prodrug. Research is ongoing to validate some inhibitors for this bacterial inactivation of levodopa.

In addition, one could imagine that if the gut microbiota does affect the BBB, it could influence the penetration of certain drugs into the CNS. Yet, the sub-field of neuro-pharmacomicrobiomics is in its infancy, so most of its knowledge remains to be discovered.

These bidirectional effects between neurological drugs and the gut microbiota emphasize the importance of recording medications and incorporating them into the biostatistical analyses of any human study looking at the microbiota in neurological disorders. Conversely, patients’ gut microbiota and their diet should be characterized during drug trials in neurology.

“Psychobiotics” – The use of microbiota modulators as neurological therapy

The original definition of a psychobiotic was “a live organism that, when ingested in adequate amounts, produces a health benefit in patients [i.e. a probiotics] suffering from a psychiatric
illness”. It was first extended to include prebiotics (compounds that modify the gut bacteriome’s composition & functions) as potential psychobiotics and to restrict any claim of psychobiotic properties to a specific clinical condition. Psychobiotics are now considered as “any substance that exerts a microbiome-mediated psychological effect” or any “microbiota-based therapy” in a neurological disorder, including prebiotics, probiotics, postbiotics (bacteria-derived molecules or their precursors), fecal microbiota transfer, antibiotics, or even dietary changes (“nutritional psychiatry”).

Presently, the most commonly used bacteria as psychobiotics are Bifidobacteria and Lactobacilli, and the most commonly used prebiotics as psychobiotics are fructans and oligosaccharides. The antibiotics that have been used in the literature to affect neurological disorders in humans are vancomycin (autism) and minocycline (depression).

Modulating the gut microbiota, or mimicking its positive effects, could represent a safer approach to neurological diseases that are presently managed with drugs which often come with significant side effects. To date, however, most of the data come from preclinical studies. Human studies are few, and their results are contradictory. In the case of depression, for instance, there were a sufficient number of human studies to warrant the publication of several systematic reviews and meta-analyses that often highlight the shortcomings of the existing data, without annihilating the fact that pro- and pre-biotics do have a beneficial effect in many patients. It is possible that, at least initially, psychobiotics will be used as adjuvant therapy and used in parallel of the traditional pharmacological drug for that pathology. It is also expected that psychobiotic efficacy will vary significantly between individuals with the same diagnosis and disease severity. Thus, this line of therapy will most likely become part of personalized medicine.

A recent concrete example of Fecal Microbiota Transfer (FMT) used in a neurological disorder successfully is the study conducted by Kang DW et al in 2017 and 2019 in autistic children. Their protocol of oral or rectal FMT over 8 weeks showed some significant improvement in both gastrointestinal and behavioral symptoms that lasted during the 8 week follow-up and were associated with signs of microbiota engraftment of the donor’s microbiota. Interestingly, most of these positive changes were still present 2 years of the original study.

**Concluding remarks**

Accumulating scientific evidence has led to hope that, one day, modulating the microbiota could become a tool in treating neurological disorders. However, it is important to keep in mind that despite its plasticity, the brain might not be able to return to normal anatomically or functionally. Thus, microbiota modulation might more promising when used prophylactically.
Several neurological disorders seem to root from early brain development and are referred to as neurodevelopmental disorders. These pathologies are not “caught”, they are “made” during brain development, based on genetic predispositions, played upon by extrinsic (e.g. diet, medications) and intrinsic factors (e.g. immune events, dysbiosis). Autism is an example of such disorder with an early childhood onset, while the diagnosis of schizophrenia occurs in late adolescence/early adulthood.

The CNS development, from gestation to adolescence, sees some major structural and functional evolutions that require a very finely tuned sequence of intricate events that rely on various cell types. Any deviation from this programmed neurodevelopment can lead to various neurological disorders, with onset from birth to late life. Thus, the “critical window fluidity” is one of Bauer’s 5 landmarks of the GMBA.

Interestingly, the gut microbiota and the CNS go through radical changes during the same postnatal periods (Figure 3).

The principal critical windows in both brain development, but also gut microbiota development are (fig 3):

- the prenatal period, during which the maternal immune activity plays a particularly important role (see below);
- the perinatal period, that is marked by some reshuffle in neuro-anatomy and the colonization of the newborn’s GI tract by commensal bacteria from the mother and the environment;
- the first 2-3 years of life, at the end of which the gut microbiota is thought to be more or less stable and, interestingly, when the child develops the verbal language. Intriguingly, there seems to be a correlation between early childhood infections and exposure to antibiotics and the diagnosis of neurodevelopmental disorders later in life, such as schizophrenia or autism;
- the adolescence, when some significant changes occur in both brain anatomy and functioning, but this period has been seldom studied in terms of microbiota-brain relationships;
- the elderly years when both the gut microbiota and the CNS start deteriorating from aging alone or from pathologies whose risk increases with age (e.g. cancer, neurodegenerative diseases, see corresponding chapter).

Among the different cells that operate during neurodevelopmental programs, microglia have proved to play a central role, orchestrating programmed neuron cell death, neurite formation, synaptogenesis, synaptic pruning, axonal fasciculation, and functional neuronal circuit assembly. Intriguingly, excessive synaptic pruning by microglial cells and/or their excessive activation appear to be implicated in the pathogenesis of neurodevelopmental disorders, with the “microglia hypothesis” of schizophrenia or the “over-pruning hypothesis” of autism.

**Gut-microbiota-brain axis & neurodevelopment**

*What germ-free mice have taught us*

The first evidence of the role of the microbiota in CNS development come from germ-free mice. Indeed, there is a long list of neurodevelopmental deviations in these animals:

- Abnormal brain structures: amygdala and hippocampus morphology, (de-)myelination, microglia numbers, neurogenesis, BBB tight junctions, neurotransmitter receptor density
- Abnormal brain functions: HPA, neurotransmitter metabolism, blood-brain barrier permeability, microglia maturation & activation, neuron survival
- Abnormal behaviors: anxiety, depression, stress, learning, memory, social interactions, motor skills

**Maternal immune activation & neurodevelopmental disorders (fig 3)**

The impact of certain events in the mother’s body during pregnancy has proved to have a significant impact on the brain development of the fetus, with potential pathological consequences later in life (Figure 3).

The “Barker hypothesis” (or “thrifty phenotype hypothesis”) states that prenatal conditions can modify the epigenetic make-up of an individual in a way that will increase his/her chances of survival later in life if similar conditions were to occur, but this could induce a pathological state if different conditions are encountered later in life. The “maternal immune activation” (MIA) model enters within this hypothesis. Indeed, when a significant viral or bacterial infection occurs during pregnancy, the subsequent antimicrobial immune response
is thought to affect the brain development of the fetus (at least partially via microglial activation), leading to behavioral and mood disorders, such as autism or schizophrenia, later in life. This model is supported by both preclinical and epidemiological studies. Other events during pregnancy are thought to impact fetal neurodevelopment: e.g. stress, high-fat diet, medications (including antibiotics and neuroactive drugs, e.g. anti-epileptic valproic acid). Interestingly, the maternal microbiota changes significantly during pregnancy, but the potential direct role of this “pregnancy dysbiosis” on the brain development of the fetus remains unknown. However, whether it is the skin or the gut/vagina maternal microbiota, they will colonize the newborn’s GI tract and will play a role in the postnatal neurodevelopment.

**Gut-microbiota-brain axis & autism**

The autism spectrum disorder (ASD) includes several disorders that can be found in the medical and scientific literature: autism with or without intellectual deficiency, mild to severe autism, regressive-onset autism, Asperger’s syndrome, and pervasive development disorder not otherwise specified (PDD-NOS). ASD is not a psychiatric/psychological disease, but a pervasive and heterogeneous neurodevelopmental disorder associated with structural and functional CNS abnormalities and characterized by a dyad of clinical signs from an early age: (www.who.int)

- “persistent deficits in social communication and social interactions across multiple contexts”
- “restricted, repetitive patterns of behavior, interests, or activities”

This dyad is often associated with various sensory issues, common digestive dysfunctions, and sometimes an intellectual deficit. This syndrome is referred to as “spectrum” because affected individuals show various levels of severity for each given clinical sign associated with the diagnosis.

In developed countries, ASD is estimated to affect up to 1 out of 60 births (with a 3-4 : 1 male to female ratio), which represents over 650,000 individuals in France alone (www.who.int). The average medical expenditures for children and teenagers with ASD are estimated to be 4–6 times greater than for neurotypical children, even more so when recommended behavioral interventions are used. The scientific literature and the media regularly point out to the increase in autism incidence. However, it is likely largely confounded with the improvement in diagnosis (better tools, better trained medical and education communities, better understanding of autism).

Autistic disorders originate from the prenatal period of development, but their exact pathogenesis remains obscure. Some predisposing genetic factors are suspected with genes linked to synaptic formation, microglial functions, transcriptional regulation, and chromatin remodeling have been implicated in ASD. Family and twin studies have shown a 38-83% heritability without identifying any major causative gene, and many mutations identified in autistic individuals are also present in the neurotypical population. Thus, it is likely that a significant portion of the pathogenesis is associated with environmental factors, especially some immune disruptions during pregnancy (see above).

Some postmortem studies and some recent ones involving modern imaging tools (e.g. functional MRI) have shown some structural abnormalities in the brains of autistic individuals. The origin of these GI symptoms in autism remains unclear, but numerous studies have already shown some abnormalities in gut microbiota profiles and SCFA levels in autistic children. There are even several meta-analyses and systematic reviews on the subject with autism being the neuropathology that has received the most attention (with depression) in investigating the role of the gut microbiota. Studies seem to agree on a decreased diversity in fecal microbiota, but not on which taxa are concerned. It is important to note that despite preclinical data indicating the role of gut dysbiosis on neurodevelopment and autistic-like behaviors, we are still not certain whether the gut dysbiosis observed in autistic children is part of the disorder’s pathogenesis or its consequence.

**Gut microbiota dysbiosis in autism**

GI symptoms are common in autistic individuals (4-97%) and often correlate with the severity of the behavioral manifestations. The origin of these GI symptoms in autism remains unclear, but numerous studies have already shown some abnormalities in gut microbiota profiles and SCFA levels in autistic children. There are even several meta-analyses and systematic reviews on the subject with autism being the neuropathology that has received the most attention (with depression) in investigating the role of the gut microbiota. Studies seem to agree on a decreased diversity in fecal microbiota, but not on which taxa are concerned. It is important to note that despite preclinical data indicating the role of gut dysbiosis on neurodevelopment and autistic-like behaviors, we are still not certain whether the gut dysbiosis observed in autistic children is part of the disorder’s pathogenesis or its consequence.

**Gut microbiota modulation in autism management**

It is important to remember that autism is a neurodevelopmental disorder, not a disease. Therefore, it cannot be cured, but its clinical signs can be managed and modulating the gut microbiota is one avenue that has raised some hopes. There have been a sufficient number of studies on the potential efficacy of pre- and probiotics in autism to warrant the publication of a systematic review/meta-analysis, which found no significant GI or behavioral
effects when trials were double-blinded rather than open-label. Another study showed some clinical improvement in both GI and behavioral symptoms while using a non-absorbable oral antibiotic (vancomycin) in regressive autism. Unfortunately, these effects disappear progressively after the drug was discontinued. Another team investigated the therapeutic effects of FMT with improvements still observable 2 years after treatment.

**Gut-microbiota-brain axis & schizophrenia**

Although this is less known, like autism, schizophrenia is not a psychiatric disease, but a heterogeneous neurodevelopmental disorder that is also more appropriately described as a spectrum. The main component of schizophrenia is psychosis, and the alternation of low and high mood episodes. Like with autism, schizophrenia is associated with a decreased life expectancy and a significantly reduced quality of life with deficiencies in social interactions and communication. This is why schizophrenia and autism might be related disorders. However, schizophrenia is less frequent than autism, affecting less than 1% of the population (0.33-0.87%) and the risk in men is only slightly increased compared to women. Similarly to autism, the economic burden of schizophrenia is significant and therapeutic options remain non-optimal. Indeed, antipsychotics are not always effective, and are often associated with significant side effects. In contrast to autism, clinical signs are not present until late adolescence/}

Figure 3

Coevolution of the gut microbiota and the CNS in health and disease
A lot of the work conducted around the gut-microbiota-brain access in neurology takes place in the field of psychiatry and, more specifically, on mood disorders. The most common and studied of these pathologies are depression, anxiety, and bipolar disorder. Interestingly, these neurological diseases are often associated with GI symptoms.

Gut microbiota & depression

Depression is a serious heterogeneous illness that significantly affects every day life and can have severe consequences. According to the World Health Organization (WHO), depression affects over 300 million people worldwide and leads to the suicide of almost 800,000 people every year. (www.who.int) In addition to the individual’s suffering, the burden of depression on modern society keeps increasing, with the WHO predicting that depression will be the first cause of disability in the world by 2030. (www.who.int)

There has been some progress with regard to anti-depressive therapy, but treatments remain inadequate for the most part, with up to 60% of patients responding only partially or not at all. In addition, the lack of robust objective diagnostic tools and suitable antidepressant medication is mainly due to our limited knowledge about the pathogenesis of depression. Interestingly, all the pathways involved in the gut-microbiota-brain axis (e.g. neuro-endocrine, neuro-immune, neuro-metabolic, epigenetics; see 1st chapter) are thought to play a role in the pathogenesis of depression. Over 100 years ago, some scientists had already suspected that bacteria found in our gut could help with depression. Over the past decade, an area of investigation with growing interest has been the potential role of the gut microbiota in depression, but also its modulation as a promising therapeutic option.

Multiple studies demonstrate different gut bacteria and gut bacteria metabolome profiles in depressed patients and animal models of depression, although the dysbiosis profiles vary between articles. In some cases, the abundance of certain bacteria correlated with the severity of the symptoms. It is noteworthy that the few studies that examined the effect of antidepressants on the gut microbiota have shown that most commonly used medications in depression have an effect on gut bacteria, both in vitro and in vivo, making medications significant confounding factors and source of variability in clinical studies (see Cussotto S et al 2019 for a detailed review). Therefore, it is hypothesized that the dysbiotic gut bacteriome observed in depressed patients might be more than a biomarker and could play a role in the pathogenesis of depression. Two preclinical studies even demonstrated that the transfer of the fecal microbiota of depressed patients to rodents induces a depression-like phenotype in the animal that is not observed when transferring the microbiota of healthy humans.

There are different pathways that could implicate gut bacteria in the pathogenesis of depression. Some bacteria that tend to highly metabolize proteins, thus generating potentially higher levels of neurotoxic protein metabolites (e.g. ammonia), are overrepresented in depressed patients. Excessive histone deacetylation has been observed in depression, and histone deacetylation has shown some beneficial effects on depression. Intriguingly, bacteria-derived short-chain fatty acids (SCFAs) are known to be histone deacetylation inhibitors (see chapter 1), but SCFA administration has been associated with inconsistent effects in preclinical models of depression. Another suspected pathway linking the gut microbiota to depression are bacteria-derived vitamins, especially folate and thiamine. In addition, Bifidobacteria tend to be less abundant in depressed individuals and are known to produce high levels of GABA, an important neuromodulator. Various studies have also suggested that gut bacteria-associated neuro-inflammation and redox stress could be part of the pathogenesis of depression. In addition, bacterial endotoxins (e.g. LPS) are known to induce depressive signs in animal models, and are a well-established pathogenic player in “sickness behavior” (i.e. mood changes observed during acute and chronic infections).

This has led to multiple investigations that explored the potential use of pre- and pro- biotics in depression. To date, however, meta-analyses and systematic reviews on the subject struggle to confirm the robust efficacy of such psychobiotics in depression (see 2nd chapter).

Microbiota & anxiety

Anxiety can be present as the main clinical sign of the psychiatric pathology called anxiety disorder, which has an incidence of 3-25% in the general
Anxiety can also be associated with other chronic diseases, such as cancer or heart failure, with an incidence ranging from 1.4 to 70%, depending on the primary condition. Fear-related behaviors are controlled by a complex neuronal communication between the amygdala and the prefrontal cortex. Anxiety is thought to come from a dysregulation of this circuitry. Interestingly, the gut microbiota may affect the anatomy and the activity of the amygdala.

Like in depression, studies have investigated the potential usefulness of psychobiotics in anxiety, but with the similar study design shortcomings and issues of variability between and within cohorts preventing any robust conclusion.

Microbiome & Neurology

Gut microbiota & neurodegeneration, The disorders of our bacteria and brain aging

Neurodegeneration is the process by which nerve cells lose their structure and function, which is physiological during aging, but can sometimes occur in a pathological manner. Such neurodegenerative diseases form a large group of pathologies with the following main representatives: Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis, and multiple sclerosis. The possible implication of the gut microbiota has been investigated, to a more or less extent, in all of these disorders.

Gut microbiota and neurodegeneration

Microbiota & Aging (fig 3)

The research on the relationship between aging and microbiota is relatively recent. Neurodegenerative disorders are, above all, diseases of aging. Interestingly, the gut microbiota in elderly individuals differs significantly from the one observed in younger people, and a diverse gut microbiota is often associated with a healthier aging process. The aged microbiota appears to be similar to what is seen in inflammatory situations. Furthermore, the gut permeability is increased in elderly individuals, which could increase the translocation of gut bacteria and bacterial molecules (e.g. LPS), leading to increased inflammatory markers associated with cognitive and behavioral deteriorations (“inflammaging”). The usual late onset of neurodegenerative disorders allows for small microbiota-mediated pathogenic effects to accumulate. In addition, changes in microbiota and immunity in older individuals might favor these pathogenic events.

Neurodegeneration, a story of proteins and inflammation

The central point in the pathogenesis of neurodegenerative disorders is the aggregation in the brain of a protein that misfolds into a cross-beta-sheet structure and acquires some prion-like properties (neuro-inflammatory effects, inter- and intra-brain transmission). The host’s neurons themselves also synthesize these proteins that participate in physiological synaptic plasticity, melanin synthesis, and microglia function. Some commensal gut bacteria are also capable of synthesizing these proteins that participate in cell adhesion, biofilm formation, tissue colonization, but also infectivity. These host- and bacteria-derived proteins can sometimes accumulate inside the CNS, leading to pathogenic events associated with neurodegenerative disorders.

These protein deposits have been detected in elderly individuals without any neurological symptoms. In addition, the severity of the
neurological signs do not correlate with the amount of protein aggregation.\textsuperscript{13,80} It is therefore likely that other factors than the protein aggregate accumulation participate to the neurodegeneration. Neuro-inflammation is one of these key co-factors. It is possible that gut bacteria play a role in protein-associated neurodegeneration beyond gut bacteria-derived proteins reaching the CNS.\textsuperscript{13,79,80} Indeed, other bacterial (e.g. endotoxins) or bacteria-induced host signals (e.g. mucosal cytokines) also contribute to neurodegenerative diseases and Friedman & Chapman proposed the new term “mapranosis” that refers to a Microbiota-Associated Proteopathy And Neuroinflammation process (“OSIS”).\textsuperscript{13,78} Indeed, the innate immune system recognizes bacterial amyloidogenic proteins as pathogen-associated molecular patterns (PAMPs).\textsuperscript{13} Thus, it was hypothesized that these bacterial proteins prime the immune system in the gut, leading to an enhanced immune response against the corresponding host brain protein (“molecular mimicry” hypothesis).\textsuperscript{13,79} As well as being responsible for protein debris clearance, microglial cells orchestrate neuro-inflammation and protein-specific immunity in the CNS putting them at the center of neurodegeneration pathogenesis.\textsuperscript{8,11,78,79,81} Of specific note for this short review is the fact that microglial cells are sensitive to the microbiota signals within the gut-microbiota-brain axis (see chapter 1).

**Neurodegeneration beyond gut bacteria**

First, although most studies so far have focused on bacteria-derived neurotoxic proteins to date, it is possible that other microorganisms (e.g. fungi, viruses) play a role in neurodegenerative processes.\textsuperscript{82} Second, the gut microbiota is being extensively investigated in neurodegeneration, but there is growing evidence that the oral and nasal flora could be important as well.\textsuperscript{13,83}

**Gut microbiota in Alzheimer’s disease**

AD represents approximately 60-70% of all dementia cases, which are estimated to affect around 50 million people worldwide, with almost 10 million new cases every year. (www.who.int) AD is a chronic disease characterized by a progressive decline in cognitive capacities. As there is currently no available treatment for dementia, it represents a significant decrease in quality of life for both the patient and his/her relatives, and a significant practical and financial burden on society. In 2015, the overall cost of dementia was estimated at over US$800 billion in the US alone.

Aging and some genetic predispositions are thought to play a central role in AD, but its exact etiology remains poorly characterized, which probably explains the failures and shortcomings of clinical trials to date. The neurodegeneration itself in AD is mainly due to the pathogenic aggregation of misfolded β-amyloid and tau proteins in the brain.

**Gut dysbiosis in Alzheimer**

Transgenic mice that serve in AD models have a different gut microbiota to their wild-type counterparts.\textsuperscript{11,13} Similarly, multiple studies have shown an altered gut microbiota in AD patients, with reduced richness and diversity, as well as reversed proportions between various taxa.\textsuperscript{6,78,84} (Furthermore, certain unbalanced diets, high-fat ones in particular, have been associated with an increased risk of developing AD and the effect of these diets on the individual’s gut microbiota is thought to have a causative role in this correlation.\textsuperscript{78})

**The pathogenic role of gut bacteria in Alzheimer**

There are multiple pathways linking commensal bacteria to the pathogenesis of AD. First, various gut bacteria (e.g. E. coli, Streptococcus, Staphylococcus, Salmonella, Klebsiella, Citrobacter, Bacillus) produce some functional amyloid proteins that polymerize into cross-beta-sheets that lead to the formation of protective biofilms.\textsuperscript{13}

Second, the germ-free animals of AD models display an attenuated disease phenotype. A fecal transplant from non-germ-free mice of these models normalized the pathological signs much more efficiently than a fecal transplant from wild-type mice.\textsuperscript{13}

In addition, inflammation plays an important role in AD and most studies on gut microbiota in animal models of AD or AD patients have found a decrease in anti-inflammatory bacteria and an increase in pro-inflammatory ones. The study also showed that levels of >50 bacteria-derived metabolites correlated with an earlier age of onset in AD patients, a faster cognitive decline, and higher biological biomarkers of AD.\textsuperscript{85} More specifically, levels of LPS and E.coli K99 pilli protein are higher in the brain and blood of AD patients.\textsuperscript{86} Furthermore, LPS co-localizes with amyloid plaques in the brain.

Finally, supporting the “molecular mimicry” hypothesis, some anti-β-amyloid antibodies cross-react with extracellular amyloid bacterial proteins.\textsuperscript{11}

**Gut microbiota in Parkinson’s disease**

PD is a slowly progressing neurodegenerative disorder that is characterized by various motor impairments (e.g. resting tremors, muscular rigidity, bradykinesia, gait and balance problems).\textsuperscript{11} (www.parkinson.org) PD is estimated to affect around 1% of the population over 60 worldwide.\textsuperscript{38}

**Parkinson’s disease and GI health**

Prior to the pathognomonic neurological symptoms of PD, patients report having suffered from constipation. GI symptoms then diversify and worsen as the motor signs progress.\textsuperscript{11,13,80} GI dysfunctions are also observed in both chemically- and genetically- induced PD rodent models.\textsuperscript{79} Additionally, the incidence of PD appears to be higher among patients with a diagnosis of chronic inflammatory GI disease (e.g. inflammatory bowel disease) or with non-inflammatory chronic constipation.\textsuperscript{78,80} It is, therefore, hypothesized that the pathogenesis of PD might start in the GI tract.
**Parkinson's disease, another “protein disease”**

PD is characterized by the accumulation of α-synuclein aggregates (called Lewy Bodies) inside dopaminergic neurons of the nigra striatal area of the brain that play an important role in the motor nervous system. Several studies have demonstrated the prion-like properties of α-synuclein: aggregability; transmissibility from one site to another within an individual and from an animal to another; pro-(neuro-) inflammatory activity; pro-neurodegenerative effect; and capacity to increase gut permeability.

**Gut dysbiosis in Parkinson's disease**

First of all, PD's gut seems to be characterized by a certain level of bacterial overgrowth. In addition, multiple studies have identified some differences in gut microbiota profiles in PD patients although with heterogeneous findings, probably because of differences in patients' and methods' parameters. However, all these studies point towards a pro-inflammatory microbiota (e.g. upregulation of genes linked to LPS production), which could promote the translocation of bacteria and/or their metabolites or endotoxins. Interestingly, some neurological signs even appeared positively correlated with the relative abundance of certain taxa in a few studies.

**Commensal bacteria, the missing link in PD pathogenesis?**

Research has shown that a transfer of fecal microbiota from PD patients in a mouse model of PD, compared to the microbiota from healthy individuals, can increase motor abnormalities and induce more severe neuro-inflammation, -synuclein, accumulation, and neurological signs. In addition, -synuclein aggregates have been found in peripheral nervous system in both healthy and PD patients: e.g. enteric nervous system (ENS), olfactory bulbs, vagus nerve, appendix, tonsils. In the early 2000s, Braak et al originally hypothesized that the accumulation of a toxic form of -synuclein might start in these peripheral locations and then would migrate to the CNS during a second phase via the vagus or mesenteric nerves, or the olfactory nucleus. However, the potential protective effect suggested by preclinical models of a vagectomy, an appendectomy, or a tonsillectomy, has led to contradictory results in epidemiological studies.

Intriguingly, the incidence of Helicobacter pylori infections is significantly higher among PD patients and some GI infections are associated with the increased expression of -synuclein in the gut mucosa. Thus, some have hypothesized that -synuclein accumulation might be an immune defense mechanism against pathogens, but it would subsequently and inappropriately migrate to the CNS where it would induce some neuro-inflammation and neurodegeneration in predisposed individuals.

**Gut bacteria and PD drugs**

As discussed in chapter 2, commensal bacteria have been shown to affect the pharmacokinetics and therefore the efficacy and toxicity of the most common PD drug, levodopa.

**Concluding remarks**

The modulation of the microbiota is seen as a promising route of therapy in neurodegenerative diseases. However, as mentioned previously, the brain might not be able to recover from a neurodegeneration advanced enough to be associated with clinical signs. Microbiota modulation might be better used preventively or in the early stages of neurodegenerative disorders.
Our translational Inserm research Unit TENS of Nantes University (The Enteric Nervous System in gut and brain diseases) aims at understanding the role of the gut nervous system (also called 2nd brain) in the pathophysiology of gut and brain diseases. In particular, we characterize, both in patients and animal models, the remodeling of the ENS in both neurodevelopmental (ASD) and neurodegenerative diseases (Parkinson’s disease), determine what are the functional consequences of this remodeling upon gut functions (barrier functions and motility) and finally develop strategies targeting the ENS in order to restore gut functions. In this context, we have initiated research programs aimed at determining more specifically the role of the microbiota or microbiota derived metabolites as a novel contributor to gut brain dysfunctions in disease of interest, using both in vivo (gut and brain functional exploration) and in vitro approaches (as using human intestinal organoids). This research investigating the microbiota-gut-brain axis is performed with strong collaborations with the University Hospital of Nantes, the laboratory LS2N, the Mibiogate consortium and Therassay of Nantes University as well as Hospital Henri Mondor and Biofortis.

Impact of fecal supernatants from Autism Spectrum Disorder patients on the enteric nervous system

Author: Jacques Gonzales, doctorant UMR 1235 TENS - Inserm / Université de Nantes

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in communication, repetitive behaviors and altered social interaction. Recently, alterations in the microbiota-gut-brain axis have been suggested to contribute to ASD pathology. Indeed, ASD patients have a strong comorbidity with gastro-intestinal symptoms (diarrhea, constipation, bloating, abdominal pain), and recent metagenomic analyses have shown changes of intestinal microbiota composition and derived-metabolites concentration in ASD patients. Whether dysbiosis could affect gut functions and the enteric nervous system (ENS) remains unknown.

Human fecal samples from ASD patients and healthy controls (HC) were collected and processed to prepare fecal supernatants (FS) that were applied to ENS cultures. The impact of FS treatment was assessed on the expression level of inflammatory and neural molecules. In addition, several bacterial metabolites (short chain fatty acids and bile acids) were measured in FS and 16S metasequencing of the intestinal microbiota was performed.

After 2 days of treatment of ENS culture, an increase of the inflammatory molecules TNF-α and IL1β mRNA was observed upon treatment with FS from ASD patients. This treatment induced no change in the protein expression of the glial molecule GFAP but induced an increase of the synaptic marker Synapsin-1. The concentration of total bile acids was higher in the FS from ASD patients, which seems reflect an increased level of secondary bile acids produced by the gut microbiota. Moreover, changes in bacterial phylum proportion were observed in ASD patients, with a relative increase of Bacteroidetes and decrease of Firmicutes (Fig.4).

In conclusion, we found that FS from ASD patients alters ENS molecule expression. The factors responsible for these changes remain to be identified. Intestinal microbiota could be a new source of biomarkers and treatment for gastro-intestinal symptoms in ASD.
Christopher U. Missling, PhD, is President and CEO of Anavex Life Sciences Corp, has over 20 years of healthcare industry experience within large pharmaceutical companies and the biotech industry. Prior to joining Anavex, he served as the Chief Financial Officer of Curis and ImmunoGen. In addition, at Aventis (now Sanofi), Christopher’s work is dedicated to finding potential cures for neurodegenerative and neurodevelopmental diseases, like Alzheimer’s disease, and Parkinson’s disease, as well as, Rett syndrome, Fragile X, Angelman’s syndrome, infantile spasms. Dr. Missling is working with his team to advance new potential treatments through clinical trials by involving the respective advocacy groups early on. Dr. Missling has an MS and PhD from the University of Munich in Chemistry and an MBA from Northwestern University Kellogg School of Management and WHU Otto Beisheim School of Management.

Anavex Life Sciences is currently in several clinical studies addressing brain health, ongoing Alzheimer’s disease Phase 2b/3, ongoing Parkinson’s disease dementia Phase 2 and several Phase 2 studies for Rett syndrome.

The study of ‘gut-brain axis’ is providing a growing body of evidence identifying gut microbiota as a critical component of healthy brain function, elevating it to a clinically actionable organ. Nevertheless, very few clinical trials assess the relationship between Alzheimer’s disease and gut microbiota. Anavex Life Sciences investigated within the ANAVEX®-2-73 Alzheimer’s disease Phase 2a study (NCT 02244541) the first microbiota search for biomarkers associated with drug response in Alzheimer’s disease.

The study met its primary endpoint, demonstrating a favorable safety profile, and was subsequently extended by an additional 208 weeks (NCT 02756858), during which patient’s stool collection was performed to analyze gut microbiota. The relationship between available microbiota biomarkers and efficacy outcome measures was investigated and associations between gut microbiota abundances and cognitive outcome were identified. The abundances of two microbiota families, Ruminococcaceae and Porphyromonadaceae, were associated with improved response at week 148 (p<0.01 and p<0.04 respectively). To confirm if ANAVEX®-2-73 may have beneficial homeostatic effect on brain-gut-microbiota axis, the currently ongoing larger clinical Alzheimer’s disease and Parkinson’s disease dementia studies will also assess microbiota data.

Communication between gut microbiota and the brain is a critical component of a healthy brain function. Numerous pre-clinical studies demonstrate beneficial effects of SIGMAR1 agonists on neuroinflammation, including with ANAVEX®-2-73. ANAVEX®-2-73 could potentially normalize neuro-inflammatory processes by several different mechanisms, e.g. reducing microglia over-activation. The effect might potentially be reversal of the microbiota imbalances and might have a homeostatic effect on the brain-gut-microbiota axis. Anavex Life Sciences is committed to continue to work on the potential of harnessing the brain-gut-microbiota axis since gut microbiota is modulating brain morphology and function from birth throughout old age.

According to you, what are nowadays the biggest challenges in gut-microbiota-brain studies?

Communication between gut microbiota and the brain has been shown to be a critical requirement
of a healthy brain function. The reduction in gut microbiota diversity has become one of the hallmarks of aging, and disturbances in its composition are associated with several age-related neurological conditions, including Alzheimer’s disease. These changes in the gut microbiota composition induce increased permeability of the gut barrier and immune activation leading to systemic inflammation, which in turn may impair the blood-brain barrier and promote neuroinflammation, neural injury, and ultimately neurodegeneration. However, the abundance of metagenomic data generated on comparing diseased and healthy subjects can lead to the erroneous claim that a bacterium is causally linked with the protection or the onset of a disease.

The challenge with regulatory approval is that microbiome treatments are potentially a new concept for regulatory authorities, which might take more time, hence a therapeutic or nutritional oral intervention, which might have beneficial effect on gut microbiota might be a preferred intervention.

How do you see the future evolution of the microbiome modulation products in neurological diseases?

Gut microbiota has been implicated in the maturation and modulation of the host immune response. One of the hallmarks of aging comprises of decrease gut microbiota diversity. Disturbances in gut microbiota communities have been linked with several (age-related) neurological conditions, including depression, Alzheimer’s disease, and Parkinson’s disease. More than 100 million years of mammalian–microbial coevolution have shaped a life-long interdependency. In the case of Alzheimer’s disease, disturbances along the brain-gut-microbiota axis, including the central nervous system (CNS) and the enteric nervous system (ENS), contribute to the pathogenesis of Alzheimer’s disease. The gut microbiota is known to upregulate local and systemic inflammation due to lipopolysaccharides (LPS) from pathogenic bacteria and synthesis of pro-inflammatory cytokines. Alterations in the gut microbiota composition may induce increased permeability of the intestinal barrier and the blood-brain barrier further enhancing inflammation at the gut, systemic and CNS levels. Amyloid beta (Aß) formation takes place in the ENS and the CNS. In addition, a large amount of amyloids is secreted by the gut microbiota.

1 Cenci A et al 2016. Presented at World Parkinson Congress
molecules produced by the microbiome; we are not attempting to rebalance the microbiome. We believe that the study of human physiology should now integrate gut microbiota in is paradigm. For instance, human metabolism can no longer be studied by just looking human genes but also by looking its metagenome. Over hundreds of thousands of years human beings and our ancestors have co-evolved with microorganisms within our digestive tracts and other tissues even including our skin. We have identified gut microbiota metabolites that are associated with disease mechanisms and we are developing these as drugs to delay the progression of neurodegeneration.

Working on the microbiome help us to realize how much gut microbiota physiology is an important component of the success of new therapeutics. We now know it can be involved in the degradation of current symptom-treating drugs and side effects. Metagenomics and metabolomics are not only methodologies that can be utilized in discovery but also at later stage in preclinical or even in clinical development. Gut microbiota physiology investigations may even be part of the regulatory toxicology in the future.

Stellate Therapeutics has a portfolio of microbiome-derived small molecules that we screen for potential therapeutic indications. Our in-house pipeline today consists of several molecules to treat multiple neurodegenerative diseases. Our lead molecule, STL-101 is in preclinical development for Parkinson’s disease and we have launched proof-of-concept experiments in other neurodegenerative indications including an orphan disease. We have additional research programs through collaborations including with academic labs. We believe that historically, the poor predictivity of animal models of Parkinson’s disease, often based on genetic alterations associated with the disease, have impeded the discovery of disease modifying therapies. Working with an endogenous molecule produced by our own gut microbiota enables us to de-risk the development in a way by practicing clinical observational studies in healthy subjects and patients in order to validate our pathophysiological scenario and therapeutic strategy. Following on, we select animal models to demonstrate the therapeutic effect and to support our future clinical studies in humans.

According to you, what are nowadays the biggest challenges in gut-microbiota-brain studies (especially scientific insight, from biomarker to therapy, animal model quality…)?

There is huge unmet need in the field of neurodegeneration and we all agree that the biggest challenge is to find therapies that are disease-modifying as opposed to those now that just treat symptoms. Beyond that, we believe that effective treatments should be given early, even before symptoms arise, so I think a big challenge is to find early stage biomarkers for pre-symptomatic identification. Also, finding a relevant cohort of patients to treat is essential to demonstrate clinical efficacy – this challenge is shared with many others in drug development. But perhaps the biggest challenge is also an opportunity: to develop new strategies to develop endogenous drugs originating from gut microbiota. We are reversing the canonical drug development pathway that has in the past focused on insufficient animal models in order to select drugs that go into the clinic. Instead, human observational studies could be pivotal to demonstrate of the efficacy of our portfolio of small molecules so that they are de-risked for the clinic. Moreover, there is still huge unmet medical need when it comes to neurodegenerative diseases but we believe that the acceleration of the understanding of the biological pathways associated with neurodegeneration combined with personalized medicine will pave the route for the discovery of successful therapies. Our company is based in two leading geographies for research in the microbiome and neuroscience, Paris and New York. We are residents of JLABS @ NYC, an initiative of Johnson and Johnson Innovation and are enjoying participating in the surge of growth in the biotechnology community in New York City.

How do you see the future evolution of the microbiome modulation products in neurological diseases?

We believe that microbiome modulation is just the tip of the iceberg of human microbiome therapeutics. Understanding the biology behind microbiota gut-brain axis may help to find a wide array of therapeutic strategies adapted to each patients and disease stage. By focusing on small molecules produced by our own gut microbiome, we are bringing forward many potential drugs that are de-risked for toxicology.
Pr Ted Dinan is an Irish MD psychiatrist, Professor of Psychiatry at University College Cork (Ireland). His background is a PhD in clinical pharmacology. He has always focused his research on the stress biology, being firstly chair of Clinical Neuroscience and Professor of Psychological Medicine at St. Bartholomew’s Hospital, London. He was back in Ireland 16 years ago and he started collaboration with microbiologists about microbiota and neurology research. His main research interest has become on the role of the gut microbiota in influencing brain function and development. His research was supported by several fundings from the SFI (science foundation of Ireland). Ted Dinan continue to receive patients in clinics.

What are your main projects in neurology research. Do they include gastrointestinal health, the gut-brain axis, and/or the microbiota? If yes can you tell us more about them?

My project are focused on the gut microbiota, especially in studying how it affects brain function & stress related disorders such as depression. I work with international and multidisciplinary experts bringing a strength experiences in different fields (psychiatrists, psychologists, nutritionists, microbiology, pharmacologists, neurophysiologists). My main Collaborators are Pr John Cryan (Professor & Chair, Dept. of Anatomy & Neuroscience, University College Cork), Catherine Stanton (expert in nutrition, and Research Professor at UCC and Pr Clarke (expert in neuropharmacology, at University College Cork).

My primary interest is to understand the impact of the gut microbiota on stress response, the routes of communication between the gut microbiota and the brain (especially via the vagus nerve, SCFAs, bacteria-produced tryptophan).

I work with patients as well as in rodent models. We observed a decreased in diversity in the gut microbiota, associated with depression. Moreover, FMT of depressed patient feces to rats induced a depressed behavior and an increased in peripheral inflammation and tryptophan metabolism (not with FMT from healthy subjects). Moreover, we observed that psychiatric drugs affect the gut microbiota (e.g. olanzapine, that induced a massive weight gain and that is thought to be mediated via the gut microbiota - the lithium also generated significant changes in gut microbiota).

Moreover, we explore the Impact of diet in psychiatry clinical signs (eg. the Mediterranean diet modulate the antidepressant effects is thought to be mediated by the gut microbiota, this discoveries give some possible nutritional interventions such as adjuvants in antidepressant therapy. Moreover, we found evidence that the effects of exercises on mood could be mediated via its effect on the gut microbiota.

According to you, what are nowadays the biggest challenges in gut-microbiota-brain research (especially your scientific insight, from biomarker to therapy, animal model quality…)?

I believe that the main challenge before moving the field forward is to better understand how the microbiota affects the brain, more in depth; our knowledge so far is relatively superficial. In particular, significant challenges in bioinformatics and mathematical tools need to be addressed, considering the complexity of the microbiota data analysis. We also lack of appropriate animal models in psychiatric disorders. It is very important to identify ‘Confounding factors’. It is very important to track diet’s information during clinical studies on microbiota & psychiatric diseases as well as other habits that could change their symptoms and could influence the data. Data are difficult to interpret because patients with same diagnosis and diseases severity might have very different diets (e.g. anorexia vs bulimia). It is therefore important to group patients based on their diet.

Another challenge is to determine how to translate the biomarker to therapy. One difficulty with depression is that available biomarkers are not reliable. This renders clinical studies more difficult because it is hard to ensure the appropriate diagnosis tools (purely symptomatic rather than biomarker-based) and the objective measure of its severity (heterogeneity of the study population). There is a proliferation of publication of less robust studies, especially with such a trendy subject like gut-microbiota-brain axis. This muddies the ‘water of science’ in that field, scientifically damaging the research in this field. One improvement is to involve multidisciplinarity of the teams in terms of research field, but also the richness of public-private collaborations.
How do you see the future evolution of the use of the microbiota in neurology diseases (as a diagnosis biomarker and/or as a therapy strategy)? Primarily therapeutic applications are promising approaches:

- probiotics and Antibiotics have been used in depression for a very long time

- forsee the use of psychobiotics in a variety of disorders, especially depression, within a relatively short period of time, at least for milder forms of depression/anxiety where patients tend to hesitate to take antidepressants, but would probably accept to take some probiotics; forsee this as a significant advance in neurology.

However which doses? There is no dose-response curve possible. We don’t even know where to start with and this is a serious question to overcome. What will be best? one bacteria or a cocktail? Besides, we need to insure major toxic issues that are not expected with commensal bacteria. This will fasten the process of commercialization, may be 5 years is realistic. Concerning the use of gut microbiota as a diagnostic biomarker in depression, I think we are far from this.

Do you discuss pre/probiotics, FMT, or specific diets with your patients? If so, could you share your opinion on these strategies for microbiota modulation?

Yes I do. I recommend a fruit/veggie diet with less fermented food and fish and red meat in depressed patients. It is based on an ongoing study on the effect of dietary manipulations on depression symptoms. For example, the ‘smiles study’ is on patients with depression and we investigate the social support or Mediterranean diet with no changes on their medical prescriptions. We latter showed that the treatments were more effective within a few weeks only. Moreover, I recommend regular aerobic exercise which I think to act directly on the brain but also to act on the brain via the microbiota. I also recommend trying pre- or probiotics even in more severe depression, or to combine biotics with exercise and diet changes to help patients who are not antidepressant responders in order to get them start to be responding.

Do you have any other comments on this subject to share with us?

An open question remains about what is the best between pre- and pro- biotics? There is a lack of objective data to answer the question at this stage, but I hypothesize that prebiotics might be more beneficial as they can affect more than one bacteria population.
HIGHLIGHTS 2019

SCIENTIFIC CONGRESS PARTICIPATION

December 2018
- BacTouBac
- Biofit

January 2019
- Microbiome Drug Development
- Gen2bio

March 2019
- Pharmabiotics Event (PRI)
- Journée AFCRO

April 2019
- In Cosmetics (talk)
- Journée POLEPHARMA du microbiomique (roundtable)

May 2019
- Microbiome Probiotics Series (poster)

June 2019
- Animal health - Kisaco
- MibioGate (Best poster award)

July 2019
- JOBM
- Kisaco Animal Microbiome

September 2019
- IASP Nantes 19 (talk)
- Nutriform Business Day (talk)

October 2019
- The Nestlé Skin Health SHIELD 2nd Skin Microbiome Summit

November 2019
- MIBIOC
- BIO-EUROPE Hambourg
- Société Française de Toxicologie 2019 - (talk)
- Microbiome Movement - Human Nutrition - Boston
- European Microbiome Congress - Kisako - London

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