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GUT VIROME CHANGES WITH NUTRITION AND METABOLIC DISEASE INFLUENCING COGNITIVE FUNCTION

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SUMMARY

The study of the gut virome and its relationship with illness has emerged in recent years. Some studies have hinted at bacterial microbiome dysbiosis as having a substantial impact on the pathophysiology and development of metabolic diseases such obesity and type 2 diabetes. The gut microorganism ecology interacts with metabolic impairment and could play a role in the systemic traits of those prevalent metabolic diseases. Importantly, the richness and diversity of the gut virome is decreased in adult subjects with obesity. Furthermore, recent research has revealed a link between gut bacteria and cognition. *Caudovirales*, in particular, were associated with enhanced executive function and immediate memory. *Microviridae,* on the other hand, may be detrimental to cognitive function. Siphoviridae and Microviridae counts were associated with specific bacterial microbiome profile in four independent cohorts. Gut bacterial functions and plasma and faecal metabolites run in parallel to bacteriophage counts, integrated in a network that influenced cognition. A kind of dose-response effects of bacteriophages was observed in the human gut microbiome transplanted to mice: the genes that most changed in recipient mice were precisely those involved in memory in a concordant manner with mice cognition. These findings could open up new horizons in the pathophysiology of mental health and neurological diseases arising from the gut.

INTRODUCTION

Cognitive decline is becoming increasingly common as people live longer, and is one of the world's leading public health problems. The prevalence of dementia has risen sharply and is expected to increase by more than 78 million and 139 million by 2050. Obesity, on the other hand, is a metabolic disease of concern due to its exponential upward trend, and has become a serious public health issue in recent decades, with hundreds of millions of subjects showing overweight and obesity (WHO, 2019).

The ineffectiveness of existing approaches and therapies are compelling reasons to discover new ways to understand and treat them. One of the mechanisms that has recently gained strength for addressing and comprehending these disorders is the Gut-Brain Axis (GBA) approach, a bidirectional

Table 1: Cognitive function is divided into six cognitive domains by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), each of which has subdomains

COGNITIVE DOMAINS	SUBDOMAINS	BRAIN AREA
Executive function	Planning, decision making, working memory, responding to feedback, inhibition, and flexibility.	Frontal lobe
Complex attention	Sustained, divided, selective attention and processing speed.	
Social recognition	Recognition of emotions, theory of mind, and Insight.	
Learning and memory	Free and cued recall, recognition memory, semantic and autobiographic, long-term memory, and implicit memory.	Temporal lobe
Language	Objects naming, word-finding, fluency, grammar and syntax and receptive language.	
Perceptual-motor function	Visuoconstructional reasoning and perceptual-motor coordination.	Parietal lobe
	Visual perception	Occipital lobe

communication system that connects the brain (emotional and cognitive processes) with peripheral intestinal functions.

Recent research breakthroughs have discovered the microbiome that influences these relationships. The microbiota influences gut-brain communication via endocrine, (*Fava* et al., 2019), immunological (*Hooper* et al., 2012) and neuroactive pathways (*Socała* et al., 2021). Microbial neurotransmitters (e.g. GABA, catecholamines) and metabolites such as Short Chain Fatty-Acids (SCFAs) (*Topping* et al., 2001; *Koh* et al., 2016), bile acids (2BAs) (*Jones* et al., 2008), and tryptophan (*O' Mahony* et al., 2015) are the most well-known examples of microbial-derived intermediates that communicate from the gut microbiome to the central nervous system (CNS). Although some of these intermediates directly interact with enteroendocrine cells, enterochromaffin cells, and the mucosal immune system to spread bottom-up signalling, others can pass the intestinal barrier and enter systemic circulation, and may even breach the blood-brain barrier (BBB). Microbial signals may also be sent through neurological pathways involving vagal and/or spinal afferents (*Latorre* et al., 2016).

In fact, this new gut-brain approach is rethinking the study of various diseases affecting the central nervous system, from neurodegenerative diseases such as Alzheimer's or Parkinson's, neurodevelopmental disorders such as Attention-Deficit Hyperactivity Disorder (ADHD), Autism spectrum disorder, or even psychiatric diseases such as depression (*Chen* et al., 2021). Previously, these diseases were approached from the perspective of brain mechanisms. However, in recent years, the focus of these diseases has shifted towards the gut and its microbiota, as well as its impact on the brain. Furthermore, not only can the aforementioned disorders influence cognition, but metabolic diseases like obesity also affect

cognitive functioning, especially executive skills and memory (*Arnoriaga-Rodríguez* et al., 2020, 2021). Moreover, cognitive dysfunction has been described as both a cause and a consequence of obesity.

In order to better understand the different cognitive functions, this chapter will provide a brief introduction and description of the cognitive domains according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). Then, the link between the gut and metabolic diseases will be explored. Lastly, current human results relating to cognition and the gut virome will be discussed.

BRAIN STRUCTURE AND COGNITIVE FUNCTIONS

Cognitive functions are processes in the CNS that take place in different areas of the brain, which in turn, are interconnected with each other. CNS integrates data from the entire body and coordinates activities throughout the whole organism.

The Diagnostic and Statistical Manual of Mental Disorders divides cognitive function into six cognitive domains, each of which has subdomains (*American Psychiatric Association*, 2013), which have been acknowledged by the neuropsychological and psychiatric societies.

The brain is divided into four cortical lobes: frontal lobe, temporal lobe, parietal lobe and occipital lobe. These are not only connected to each other, but also to subcortical structures (caudate nucleus, globus pallidus, amygdala, etc.). Despite this interconnectivity between areas, studies of brain damage have revealed which region of the brain is primarily responsible for each cognitive function, as well as emotion and behaviour processes.

The frontal lobe is involved in attention and executive function (working memory, inhibition, self-regulation, organization and planning, and phonemic verbal fluency). Executive function is a combination of cognitive abilities that enable us to manage our behaviour, set goals, and analyse information in order to become as adaptable as possible in our environment. This specific area is involved in psychiatric and movement disorders. The temporal lobe is implicated in language (processing and understanding verbal information and speaking among others) and memory function (storing and retrieving information). The parietal lobe is involved in processes and integrates sensory information, orientation, and visuospatial skills. Finally, the occipital lobe is involved in visuoperception, memory formation, and face recognition (Table 1). A neuropsychologist evaluates cognitive function using particular neurocognitive tests that produce a raw score that is then translated into normative-standard scores according to the test manual.

In the same way that establishing cognitive impairment in patients with neurological diseases is critical, assessing cognitive decline in patients with obesity and/or type 2 diabetes may explain disease maintenance. Therefore, understanding how cognitive impairment has an effect on these metabolic diseases could make it possible to find physical or dietary treatments (*Arnoriaga-Rodríguez* and *Fernández-Real*, 2019).

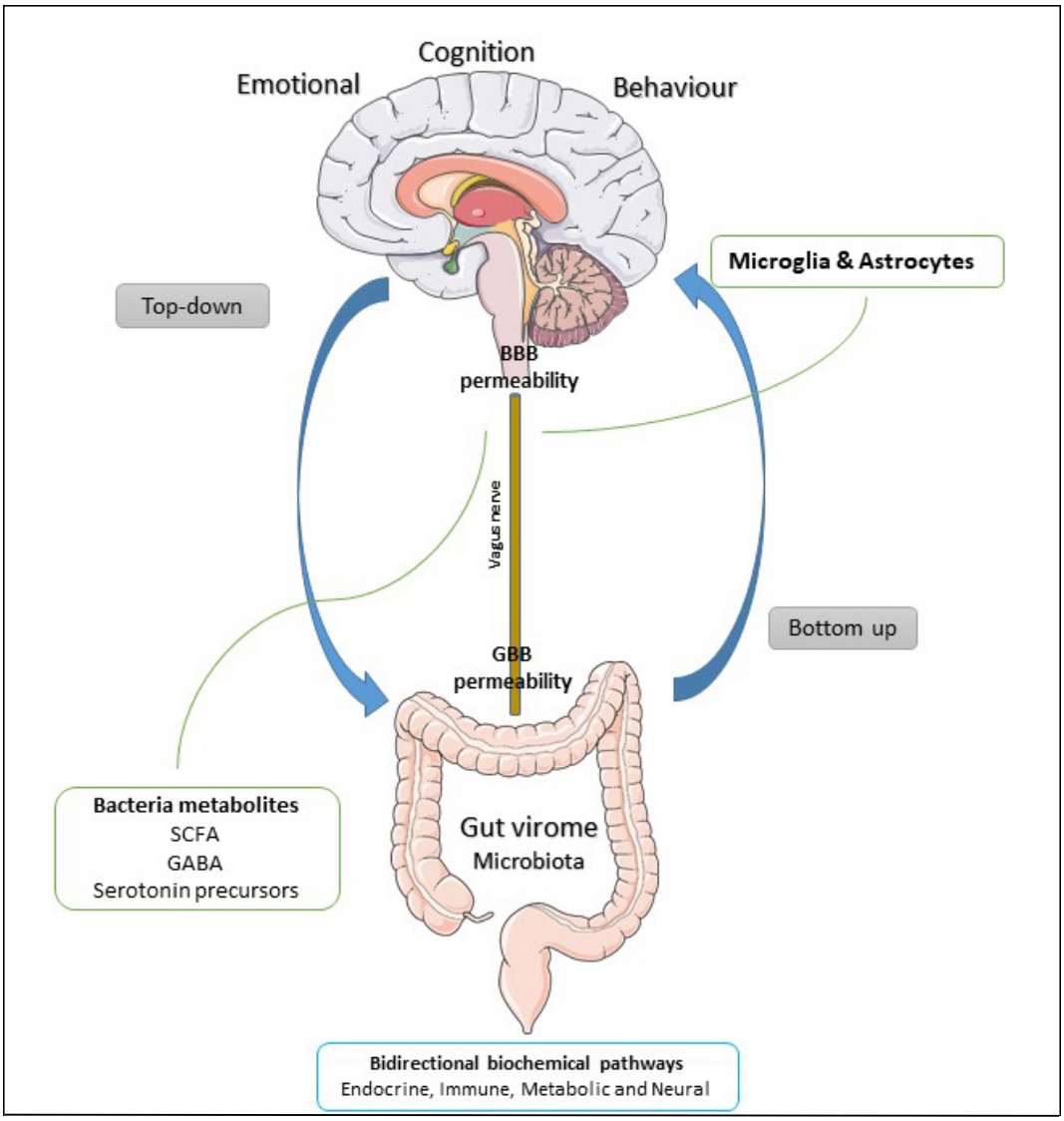

Figure 1: The GUT-BRAIN-AXIS (GBA): The gut microbiome communicates with the Central Nervous System (CNS) predominantly through microbial-derived intermediates, with Short Chain Fatty Acids (SCFAs), secondary Bile Acids (2Bas), and tryptophan metabolites. The GBA can send and receive data messages in both directions: from the brain to the gut and from the gut to the brain.

THE GUT-BRAIN AXIS

The connection between the brain and the intestinal tract is known as the "Gut-Brain Axis" (GBA). The two systems have a strong and reciprocal interaction. The gut and the brain are connected by an information exchange network that encompasses the central nervous, endocrine, metabolic, and immune systems. The GBA has the ability to transfer information in both directions: "top-down" from the brain to the gut and "bottom-up" from the gut to the brain. In addition to the hypothalamic-pituitary-adrenal axis and endocrine

pathways (i.e., intestinal peptides and hormones), there is mounting evidence that bacteria metabolites (e.g. SCFAs, neurotransmitters, and their precursors) affect the levels of related metabolites in the brain via blood circulation, regulating brain functions and cognition. Gut microbiota can also communicate with the brain via the local neurological system (e.g., enteric nerves, vagus nerve), sending very rapid messages to the brain. The gut microbiome communicates with the CNS predominantly through microbial-derived intermediates, with SCFAs, 2BAs, and tryptophan metabolites being the most well-studied examples (*Wikoff* et al., 2009, *Tolhurst* et al., 2012, *Yano* et al., 2015) (Figure 1). However, the interaction between gut viruses, mainly bacteriophages, brain and cognition is fairly unexplored, and only recent studies have shown interactions among the gut phageome, gut bacteriome and cognition (*Mayneris-Perxachs* et al., 2021).

GUT VIROME AND METABOLIC DISEASES

The study of the microbiome and its impact on cognition began with significant discoveries in animal studies (*Braniste* et al., 2014). The gut microbiome is being implicated in a growing number of investigations as a crucial role in the regulation of neurodegenerative processes, cognition modulation, and neurological diseases (*Cryan* et al., 2020; *Morais* et al., 2021).

At the same time, many studies have focused on bacteria (*Morais* et al., 2021). Nevertheless, the gut human viral community has been less studied and recent data reveal that viruses can have a significant impact on physiology and the development of diseases (*Mirzaei* and *Maurice*, 2017; *Keen* and *Dantas*, 2018) through microbiome dysbiosis. There is evidence about the relationship between the human virome and metabolic diseases such as obesity, type 2 diabetes and type 1 diabetes (*Zhao* et al., 2017, *Ma* et al., 2018, *Tetz* et al., 2019, *Vehik* et al., 2019, *Wook* et al., 2019, *Cinek* et al., 2021, *Bikel* et al., 2021, *Hasan* et al., 2021).

Obesity

Alterations of normal microbiota composition are well known to occur in subjects with obesity (*Arnoriaga-Rodríguez* et al., 2020, 2021; *Sandoval-Vargas* et al., 2021). In mice, Bacteria belonging to *Firmicutes* phylum had a positive correlation with total viral content, while *Bacteroidetes* phylum and *Bifidobacterium* genera had a negative correlation with total viral content (*Yadav* et al., 2016). The *Caudovirales* bacteriophages dominated the gut virome (*Biker* et al., 2021, *Yang* et al., 2021). *Escherichia phage, Geobacillus phage*, and *Lactobacillus phage* showed the highest relative abundance among the differential species.

The richness and diversity of the gut virome could vary depending on age and geographic factors. *Bikel* et al., (2021) found that the phage diversity and richness of people with obesity tended to rise in childhood. In contrast, in the adulthood the trend was in the opposite direction: individuals with obesity had lower gut virome richness (Chao1 index) and diversity (Shannon index) than subjects without obesity (*Yang* et al., 2021).

In childhood, the abundance of various phage contigs was linked to gut bacterial taxa as well as anthropometric and biochemical markers in subjects with obesity and metabolic syndrome, including increased serum lipid and glucose levels (*Bikel* et al., 2021). Interestingly, a negative relationship between

BMI, HDL cholesterol and triglyceride levels with the abundance of certain phage contigs was observed.

In comparison with subjects without obesity, 11 virus species were shown to be enriched in subjects with obesity. *Escherichia* phage, *Geobacillus* phage, and *Lactobacillus* phage showed the highest relative abundance among the differential species, showing distinct gut virus abundance and taxonomic compositions (*Yang* et al., 2021).

The gut virome changed after losing weight, either through, diet, exercise or after surgical interventions such gastric bypasses and vertical band gastroplasty (Sandoval-Vargas et al., 2021).

Type 2 Diabetes (T2D)

T2D is a metabolic condition associated with obesity-related insulin resistance with characteristic alterations in the gut microbiota composition (*Larsen* et al., 2010).

Yang et al., (2021) investigated alpha diversity across subjects with and without type 2 diabetes and lean controls. Subjects with obesity and T2DM had a more disrupted gut viral dysbiosis, with reduction in diversity and loss of beneficial viruses, and pathogen conversion of beneficial viruses compared with subjects with obesity alone.

No significant difference was observed in viral Chao1 richness or Shannon's diversity in subjects with obesity without type 2 diabetes when compared with control subjects. On the contrary, subjects with both obesity and T2DM had lower viral richness and diversity and distinct gut viral profiles when compared with lean controls. In comparison to lean controls, 17 viral species were differentially present in subjects with obesity and T2DM. Four viral species (*Micromonas pusilla virus*, *Cellulophaga phage*, *Bacteroides phage*, and *Halovirus*) were found to be elevated in obesity with T2DM, while 13 viral species (*Hokovirus*, *Klosneuvirus*, and *Catovirus*, among others) were found to be decreased. When T2DM patients were compared to patients without T2DM, they found 28 distinct virus species. Finally, these authors also postulated that geographic considerations may be linked to gut virome variation.

Type 1 Diabetes (T1D)

The intestinal microbiota has been linked to the development of autoimmune illnesses, including T1D, according to evidence from murine models. Environmental factors, notably bacteria and viruses, are thought to play a role in the aetiology of T1D (*Faulkner* et al., 2021). Dysbiosis has been linked to disease development in subjects who are at risk for T1D (*Needell* et al., 2016) while SCFAs were increased in patients with T1D. The gut microbiota may play a role in islet destruction (*Brown* et al., 2011). Interestingly, autoimmunity in T1D was associated with changes in bacteriophages and the *Circoviridae* family was linked to a protective effect over autoimmunity. However, the involved mechanisms remain unclear (*Zhao* et al., 2017).

NUTRITION COULD IMPACT THE RELATIONSHIP BETWEEN GUT VIROME AND COGNITION

Recent research has shown that viruses can have a significant impact on the physiology of their bacterial hosts (*Mirzaei* and *Maurice*, 2017; *Keen* and *Dantas*, 2018). Bacteriophages are well known to constitute the most common members of the human virome. Temperate (lysogenic) bacteriophages can

transfer genes to their bacterial hosts, changing their phenotypic and modifying gene expression. Despite this fact, prophages are found in more than 80% of bacterial genomes. As a result, bacteriophages may have a significant impact on bacterial diversity and function, as well as human health (*Mirzaei* and *Maurice*, 2017; *Keen* and *Dantas*, 2018).

A recent study has explored the relationship between gut-resident bacteriophages and the microbiome's structure and metabolism, as well as their effects on cognition (*Mayneris-Perchachs* et al., 2022). The authors found that the presence of, *Caudovirales* bacteriophages in the gut microbiome was associated with improved executive function, specifically, cognitive flexibility and working memory. Specific *Caudovirales* (the former *Siphoviridae* family with the old taxonomy comprising the new *Demerecviridae*, *Drexlerviridae*, and *Siphoviridae* families) levels were positively associated with cognitive flexibility, whilst *Microviridae* counts were negatively associated with this trait.

According to gene and genome analysis of unassembled and assembled data, most of the *Caudovirales* were uncultured and uncharacterized, while others putatively infected predominantly *Lactococcus* spp. and other gut bacteria belonging to *Enterobacteriacaea*, *Firmicutes* (e.g., *Eubacterium rectale*), or *Bacteroidetes*. The gene content and annotation of these *Caudovirales* revealed common gene traits, as those coding for structural proteins (capsid, portal, neck, and tail) and other *Caudovirales* functional proteins (e.g., terminases). For several of these *Caudovirales*, metagenomics assembly resulted in a fragmented genome assembly, particularly for *Lactococcus* viruses, which have been linked to higher performance in central executive processes (*Mayneris-Perchachs* et al., 2022). Within the *Caudovirales*, a strong positive relationship between *Siphoviridae* levels (as per new genome-based taxonomy) and cognitive flexibility was also disclosed. Unlike other *Caudovirales* levels, *Siphoviridae* levels were likewise associated with improved inhibitory control (meaning being less impulsive) and short- and long-term memory, underlining the potential importance of the *Siphoviridae* family in cognitive function.

On the other hand, some the counts of ssDNA *Microviridae* were linked to a worsening of executive function. *Microviridae* levels correlated positively with fat mass, confirming recent findings that showed their rise after a high-fat diet (*Schulfer* et al., 2020). In both unassembled and assembled data, *Microviridae* signature genes and proteins were clearly recognized. Some of them resembled *Escherichia* phage alpha3 and uncultured *Microviridae* seen in the stomach before. Surprisingly, identification of putative hosts revealed that some *Microviridae* infect *Bacteroidetes* (most likely *Alistipes onderdonkii*), and one *Microviridae* virus (contig name c055944) showed a broad host range because CRISPR spacers from *Ruminococcus* spp., *Oscilobacteriales*, and *Lachnospiracea* matched viral protospacers of this virus. *Bacteriophages* may play a crucial role in host health and disease by altering bacterial communities through transposition, induction, and horizontal gene transfer (*Keen* and *Dantas*, 2018). When Mayneris and co-authors (*Mayneris* et al., 2022) looked at the relationships between these bacteriophages and bacterial composition and functionality, Lactic acid bacteria (*Lactobacillales* order), particularly *Streptococcus, Lactobacillus, Lactococcus*, and *Enterococcus* species, were positively associated with specific

Caudovirales levels, while *Bacteroides* species were inversely associated. In fact, all known lactic acid bacteria phages are classified as *Caudovirales*, with the majority of them belonging to the *Siphoviridae* family (*Murphy* et al., 2017). In this study, 40% of the species most associated with certain *Caudovirales* were also associated with cognitive flexibility. *Microviridae* levels, on the other hand, were negatively linked to various *Lactobacillus, Streptococcus*, and *Enterococcus* species, while they were positively linked to *Bacteroides* and *Prevotella* species. In addition, subjects with increased specific *Caudovirales* had better phonemic verbal fluency (specific executive function related to language) and information processing speed (*Mayneris-Perxachs* et al., 2022).

A consistent positive relationship between specific *Caudovirales* levels and *Lactococcus* lactis, as well as several *Lactobacillus* (*L. crispatus, L. plantarum, L. salivarius*, and *Lactobacillus* uc) and *Streptococcus* (*S. mitis, S. salivarius, S. vestibularis, and Streptococcus uc*) species in three out of four cohorts. S. *salivarius* and S. *mitis* are the most common *streptococcal* species in human milk microbiota (*Martin* et al., 2016), whereas *L. lactis* and *Lactobacillus* sp. are commonly employed in dairy product fermentation (*Murphy* et al., 2017). In the human milk microbiota, *S. salivarius* and *S. mitis* are the most common *streptococcal* species (*Martin* et al., 2016). They identified consistent positive relationships between *Caudovirales* levels and the intake of dairy products, as well as with the plasma levels of medium-chain fatty acids, naturally prevalent in dairy fat. The authors also objectived certain *Caudovirales*-linked lactic acid bacteria and dairy products. The *Microviridae* family, on the other hand, showed a negative relationship with medium-chain fatty acids. In this context, it is interesting to mention that the supplementation of mice and humans with medium-chain fatty acids has been demonstrated to promote synaptic plasticity and cognitive performance (*Page* et al., 2009; *Wang* and *Mitchell*, 2016).

Significant correlations between bacterial pathways, bacteriophages, and human host executive functions were also discovered using functional analyses based on the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway. *Caudovirales* levels were found to be substantially linked to folate-mediated one-carbon metabolism (*Mayneris-Perxachs* et al., 2022). Folate metabolism is important for a variety of physiological activities because it provides the 1C units needed for cellular operations (*Ducker* and *Rabinowitz*, 2017). It is also a part of the methionine cycle, which is required for the production of S-adenosylmethionine (SAM), the universal methyl donor in a variety of methylation processes, including DNA methylation. It modulates redox defence by generating antioxidants like taurine and glutathione from cysteine via the trans-sulfuration mechanism. All of these bacterial pathways were positively linked to specific *Caudovirales* levels and negatively with *Microviridae* levels. A link between the metabolism of vitamins B2 and B6 (important cofactors in the folate cycle) and the presence of some *Caudovirales* was also uncovered. The bacterial genes *thyX* and *dut*, both involved in folate-mediated pyrimidine biosynthesis, had the strongest relationships with *Caudovirales* levels, according to functional analyses at the enzyme level. Thymidylate synthase is encoded by the *thyX* gene (*TYMS* in humans). Reduced *TYMS* expression is known to cause a misalignment of DNA synthesis and methylation, which is crucial for neurodevelopment, synaptic plasticity, and

memory (*Heyward* and *Sweatt*, 2015). In addition, a lack of folate-mediated one-carbon metabolism has been linked to neurodegenerative illnesses, which could be caused by a lack of dTMP synthesis and subsequent uracil misincorporation into DNA (*Blount* et al., 1997; *Ducker* and *Rabinowitz*, 2017). Other critical pathways in the central nervous system, such as glutamatergic, GABAergic, dopaminergic, serotonergic synapse, and retrograde endocannabinoid transmission, were similarly negatively related with certain *Caudovirales* levels. Other closely connected bacterial genes were found to be involved in folate-mediated histidine catabolism *(FTCD, FTCD*) and purine biosynthesis (*FTCD, FTCD*) (*purH, purU*) (*Mayneris-Perxachs* et al., 2022).

Finally, many circulating and faecal metabolites were also associated with *Microviridae* and *Caudovirales* levels. Most of these metabolites were directly implicated in one-carbon metabolism: choline, glycine, formate, histidine, and glucose are among the metabolites that feed 1C units to the folate pool, as are related catabolites (urocanate, glutamate, inosine, β-aminoisobutyric acid, and methionine sulfoxide). The most important sources of folate 1C units, choline and glycine (*Ducker* and *Rabinowitz*, 2017), exhibited the closest relationships with *Microviridae* and specific *Caudovirales* levels. Closing the circle, bacterial metabolic pathways for glycine and histidine were also linked to particular *Caudovirales* and *Microviridae* levels. Glycine is produced by the breakdown of dietary choline and serine, which provide carbon units to the 1C-metabolism. Serine can also be made from 3-phosphorylglycerate, which is a glycolysis intermediate. The glycine cleavage system (GCS), which produces a carbon unit for the methylation of tetrahydrofolate, is also a 1C source (THF). Bacteriophage levels were consistently linked to genes involved in the GCS (*gcvH, gcvP, and gcvR*), serine synthesis (serB and serA), and choline transport and catabolism (*serB and serA*) (*sox and opuD*). The GCS transcriptional repressor (gcvR) was associated with the Microviridae family (*Mayneris-Perxachs* et al., 2022), whereas GCS genes exhibited the largest negative correlation with particular *Caudovirales* levels. In both mice and humans, mutations in genes encoding the GCS have been demonstrated to lead to neural tube abnormalities and neurological dysfunction (*Kure* et al., 2006; *Narisawa* et al., 2012).

In order to validate the findings, the authors performed a microbiota transplantation from humans to mice. A dose-response effect based on the specific *Caudovirales* levels in the donor's microbiome was found 4 weeks later: the higher the *Caudovirales* levels, the higher the scores in the novel object recognition test, which is used to assess cognition, particularly immediate memory. Increased *Microviridae* levels in the donor's microbiome, on the other hand, were linked to recipient mice's cognitive impairment. They also investigated whether faecal microbiota transplantation had an effect on the transcriptome of the recipient's prefrontal cortex of mice, involved in executive processes and memory. Of note, 23 and 18 genes were up- and down-regulated, respectively, in response to the donor's specific *Caudovirales* levels, according to RNA sequencing. *Microviridae* levels in donors were linked to up- and down-regulation of 18 and 10 genes, respectively (*Mayneris-Perxachs* et al., 2022) Several of the most up-regulated gene transcripts with increased donor's specific *Caudovirales* levels were well known memory-promoting genes (e.g., *Arc, Fos, Egr2,* and *Btg2)*, whereas those down-regulated (*Ide* and

Ppp1r42) were memory suppressors (*Poon* et al., 2020).

Based on gene ontology analysis, cognition was identified as the most over-represented biological function linked to the donor's unique *Caudovirales* levels (*Mayneris-Perxachs* et al., 2022). In the hippocampus and retrosplenial cortex of adult mice, learning and memory acquisition is known to lead to increased expression of the *IEGs Arc, Fos, Btg2, Sik1, Dusp1, Ier2, and Egr2* (*Peixoto* et al., 2015).

Finally, in this study, the exposure of *Drosophila melanogaster* to lactococcal 936-type bacteriophages led to improved memory retention, changing the expression of memory-related genes in the brain. These findings revealed that thermolabile components in whey powder, including the presence of bacteriophages in this product, could improve memory.

An old paper published in Nature in 1971 showed "*bacterial virus gene expression in human cells*" (*Merrill* et al., 1971). Bacteriophages have long been known to be able to cross the blood-brain barrier (*Frenkel* and *Solomon*, 2002). This raises the question as to whether intrabody bacteriophages can accumulate within the central nervous system or brain, and mediate direct behavioural and neurological effects in mammals. As noted in a seminal paper by Dr Jeremy Barr in 2017, "*practically no research has been done investigating the role and function of native intrabody bacteriophages on the central nervous system and brain*" (*Barr*, 2017). As bacteriophages had been described to bind to β-amyloid and α-synuclein, it was hypothesised that "*it is possible that bacteriophages act as cleaners of the brain" and that "we must consider the possibility of bacteriophage mind control*" (*Barr*, 2017).

Recent findings seem to go far beyond of initial expectations. Not only *Siphoviridae* and *Microviridae* counts were reciprocally associated with executive function (one of the domains of cognition) in two independent cohorts, but also with a specific microbiome profile in four independent cohorts. It was also noted that gut bacterial functions and plasma and faecal metabolites run in parallel to bacteriophage counts, integrated in a network impacting cognition. A kind of dose-response effects of bacteriophages was observed in the human gut microbiome transplanted to mice: the genes that most changed in recipient mice were precisely those involved in memory in a concordant manner with mice cognition (*Mayneris-Perxachs* et al., 2022).

In summary, there is little doubt that the novel findings reported so far are promising to decipher new therapeutic targets through diet and nutrition, focusing on the microbiota and its relationships with body systems. The impact on the central nervous system through treatments for cognitive and memory impairment could include the use of known bacteriophages.

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