MICROBIOME

Young microbiota rejuvenates the aging brain

The gut microbiota controls immunity and brain function, but its role in cognitive aging is unclear. Boehme et al. found that fecal microbiota transplantation from young into aged mice attenuated cognitive impairments and reversed differences in hippocampal metabolites, and some aspects of peripheral and brain immunity.

Rochellys Diaz Heijtz, Ayoze Gonzalez-Santana and Jon D. Laman

Globally, the aging population (≥ 65 years) is projected to increase to unprecedented levels by 2050. Cognitive decline among the aging population is a major challenge to their health and wellbeing. It is increasingly recognized, however, that the aging brain is more malleable and plastic than previously believed. A rapidly expanding body of literature has identified the gut microbiota — trillions of indigenous microorganisms such as bacteria, fungi and viruses — as a key regulator of host immunity, metabolism and brain health[1]. In parallel with the age-related decline in immune system functions, the gut microbiota undergoes dramatic changes in composition and function during normal aging, which are associated with inflammaging and cognitive decline[2,3]. These findings raise the intriguing possibility that manipulation of the gut microbiota could counteract or even reverse aging-associated cognitive and behavioral impairments.

In this issue of *Nature Aging*, Boehme et al.[4] provide the first experimental evidence in mammals that fecal microbiota transplantation (FMT) from young into old mice can help to restore aging-associated immune and neurocognitive impairments (Fig. 1). The authors conducted FMT from either young adult (3–4 months) or old (19–20 months) donor mice into aged recipient mice, referred to as aged yFMT and aged oFMT, respectively. Notably, they adopted a holistic, multi-level analysis approach to assess the response to yFMT in the aged host, encompassing peripheral and brain immunity to more complex behavioral functions.

Boehme et al.[4] discovered that innate and adaptive immunity in the gut-associated mesenteric lymph nodes (MLN) of old mice is particularly sensitive to modulation by yFMT. For instance, yFMT reversed the aging-induced increase in early-activated CD8$^+$ T cells and CD103$^+$ dendritic cells, as well as decreased expression of the activation marker CD11b on Ly6C^{hi} monocytes (Fig. 1a). Conversely, yFMT failed to reverse the aging-associated reduction in circulating levels of cytokines, such as IL-5 and IFN-γ. These observations suggest that yFMT into an aged host selectively modulates peripheral immunity — that is, immune tissues in close proximity to the gut — thus restoring some aspects of peripheral immunity. However, it will be important to determine whether yFMT can modulate other immune headquarters of the aged host, such as the thymus, bone marrow and spleen.

The hippocampus is a key brain region involved in emotions, learning and memory, and is particularly susceptible to aging. Several morphological and functional alterations in the aging hippocampus are associated with age-related cognitive decline (for example, oxidative stress and neuroinflammation) as well as reduced neurogenesis and synaptic plasticity[5]. Microglia, the brain's resident macrophages, are the principal cell type mediating neuroinflammatory and maintenance processes. Over the last decade, microglia have emerged as important contributors to central nervous system development, homeostasis and function, and their dysregulation implicated in cognitive decline during normal aging and neurodegenerative diseases[6]. Consistent with previous studies linking gut microbiota to microglia morphology and function[7], Boehme et al. demonstrated that age-associated microglia soma enlargement was reversed by yFMT (Fig. 1b). Moreover, hippocampal transcriptomic analysis revealed that expression of six microglia sensome genes were altered by aging and reversed by yFMT, namely *Trem2*, *Dap12*, *C1qb*, *Gpr84*, *Fcgr2b* and *Tlr13*. Many of these molecules are associated with cognitive decline in mouse models of neurogenerative disease, including Alzheimer's disease. Moreover, defective functions of Triggering

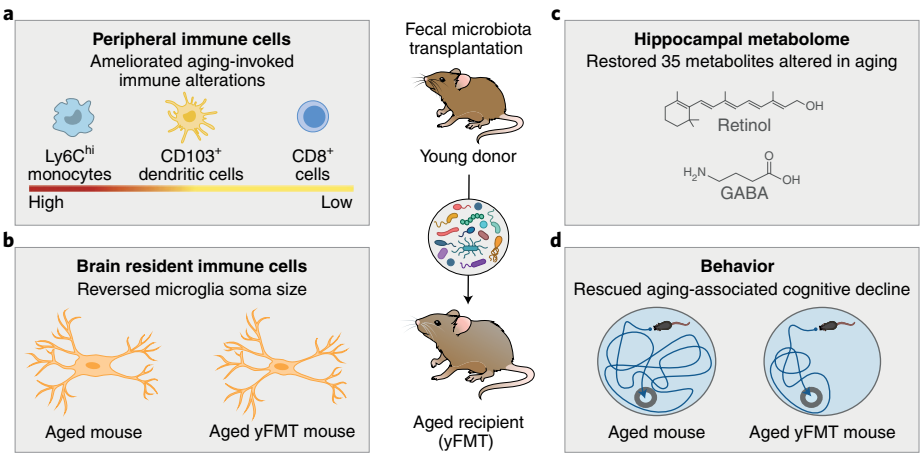

Fig. 1 | Fecal microbiota transplantation from yFMT to aged mice ameliorates aging-associated immune and neurocognitive impairments. a, Aging triggers a decrease of the activation marker CD11b on Ly6C^{hi} monocytes in MLNs, which was reversed by yFMT into aged mice. Furthermore, fecal microbiota from young mice transplanted into aged mice rescued the increase in number of early activated CD8$^+$ T cells and CD103$^+$ dendritic cells in MLN. **b**,**c**, yFMT reversed age-related enlargement in microglia soma size (**b**) and restored altered hippocampal metabolites (**c**). **d**, Aging-associated cognitive decline was rescued by yFMT.

receptor expressed on myeloid cells 2 (TREM2)–DNAX-activating protein of 12 kDa (DAP12) complex play a central role in the pathogenesis of several human diseases, including Alzheimer's disease and other neurodegenerative disorders[8]. GPR84 is particularly intriguing since it has been an orphan receptor for two decades and its functions remain under debate, but contributions to inflammation and macrophage phagocytosis appear well-established[9]. Studies have shown that microglia manifest exquisite phenotypical heterogeneity across different regions in the mammalian brain, and selective regional sensitivities to aging[10]. Therefore, it will be important to tease out which neural circuits (for example, cortical, cerebellar, hippocampal and basal ganglia networks) are more sensitive to modulation by yFMT in the aged host, and their relationship with cognitive decline.

Boehme et al.[4] also investigated the impact of yFMT on the aged hippocampal metabolome. The authors identified 35 dysregulated metabolites in the hippocampus of old mice that were restored by yFMT. These metabolites were enriched in six pathways, predominantly related to amino acid metabolism and aminoacyl transfer RNA biosynthesis, which are essential for proper brain function. One such amino acid restored towards pre-age levels by yFMT is arginine, which is linked to the nitric oxide pathway and neurodegeneration. Moreover, the authors found that dysregulated levels of gamma aminobutyric acid (GABA), N-glycolylneuraminate and vitamin A (retinol) were restored by yFMT (Fig. 1c). Dietary vitamin A supplementation rescues alterations in gut microbiota composition and recognition memory deficits induced by high-fat and high-sugar diet exposure[11], suggesting that vitamin A deficiency may mediate the beneficial effects of yFMT in the aged host. These findings also raise many questions: are the 35 metabolites sensitive to yFMT synthesized within the brain or transferred from the gut to brain? Can these metabolites, when given alone or in combination, reverse morphological and functional alterations in the aging hippocampus? This would facilitate clinical applications, as many obstacles limit long-term FMT therapy.

At the behavioral level, Boehme et al.[4] investigated whether yFMT could rescue age-related cognitive impairments using a comprehensive behavioral test battery, including the Morris water maze (MWM) task for hippocampal-dependent long-term spatial memory. Fascinatingly, aged mice that received yFMT showed improvement in aging-associated impairments in long-term spatial memory (Fig. 1d). However, this does not appear to be related to changes in neurogenesis, since yFMT did not improve reduced survival of newborn hippocampal neurons in aged mice. On the other hand, the GABA level and other metabolites in the hippocampus positively correlated with the mean distance traveled by the mouse to reach the platform in the MWM test. It is therefore likely that yFMT-induced changes in hippocampal metabolites contribute to improved cognitive performance, but this needs to be explored in future studies. One of the most striking differences observed in aged yFMT mice was in the elevated plus maze (EPM), which measures anxiety-like behavior. Although the authors did not find an age effect on anxiety-like behavior per se, yFMT dramatically increased the time aged mice spent in the open arms of the EPM. These observations may have important clinical implications, since anxiety disorders are highly prevalent among older adults and associated with poor quality of life and cognitive impairments[12].

The encouraging results reported by Boehme et al.[4] suggest that FMT from young individuals could be a potential therapeutic approach for the treatment of aging-associated cognitive decline and anxiety disorders, which could move rapidly to clinical testing. In clinical practice, heterologous FMT (where feces from a healthy donor are transplanted into a recipient patient) is a highly successful and cost-effective therapy for the treatment of recurrent *Clostridioides difficile* infection. However, a critical concern about heterologous FMT is the long-term safety of the procedure and potential exposure to life-threatening infectious diseases. Autologous FMT therapy (that is, banking self-stool when healthy to later rebalance gut microbial communities) has been proposed as a more optimal approach, especially for high-risk populations such as elderly people. Given the link between inflammaging and cognitive decline, it would be important to design and implement high-throughput functional assays (for example, using in vitro human microglia cell models) to identify the anti-inflammatory potential of fecal samples, as part of the screening process for FMT intervention in elderly people, or test probiotics and postbiotics identified by FMT.

Boehme et al.[4] also identified 20 bacteria genera significantly altered in aged mice following yFMT. Certain genera, such as *Enterococcus*, were reduced in aged mice but transitioned towards young mouse abundance following yFMT. Some *Enterococcus* species are already used as probiotics to improve animal health. Therefore, future studies should use more in-depth analysis — for example, shotgun metagenomics sequencing — to identify specific bacterial species modulated in the aged host microbial ecosystem by yFMT. Similarly, it would be interesting to explore whether defined bacterial consortia are sufficient to rejuvenate the aging brain. Emerging evidence implicates gut-derived microbial products as novel regulators of the microbiota–gut–brain axis, thus raising the question whether these bacterial molecules, such as peptidoglycans, may be sufficient to modulate the aged host immune system and brain[13]. Therefore, it will be important to determine whether similar results could be obtained with heat-treated fecal samples from a young donor.

Finally, an important point not addressed by Boehme et al.[4] is the potential impact of sex-specific factors in the response to yFMT in the aged brain. In animal studies, many effects of the gut microbiota on the brain are sex-specific[14], but the mechanisms are poorly understood. A recent large cohort study suggests that aging women have a faster decline in global cognition and executive function than men, but not in memory[15]. Therefore, in addition to hippocampus-related functions, it will be important to explore the potential beneficial effects of yFMT on executive functions in old males versus females in animal models.

In summary, the data presented by Boehme et al.[4] show that fecal microbiota transplantation from young into aged recipient mice can restore cognitive behavioral impairments as well as some aspects of peripheral and brain immunity. ❐

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Published online: 9 August 2021
https://doi.org/10.1038/s43587-021-00100-z

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Acknowledgements

The Diaz Heijtz Laboratory is supported by grants from the Swedish Research Council, the Swedish Brain Foundation, the Olle Engkvist Byggmästare Foundation, the Frimurare Barnhus and the European Community (SmartAge project). Work in the group of J.L. is supported by the Dutch MS Research Foundation and the Zabawas Foundation.

Competing interests

The authors declare no competing interests.