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Plastic brains and gastrointestinal strains: The microbiota-gut-brain axis as a modulator of cellular plasticity and cognitive function (Commentary on Darch et al., 2021)

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The last decade has witnessed rapid advances in DNA sequencing technologies and bioinformatic approaches, leading to fast and cost-effective phylogenetic identification of microbial communities and gut microbiota in particular. These breakthroughs have been revolutionary for different fields, from ecology and agriculture to biological and medical sciences. Remarkably, the communication between these trillions of microorganisms, the gut they inhabit, and the brain, has been found to be both extensive and dynamically bidirectional. The microbiota-gut-brain axis has been associated with mechanisms mediating neurodevelopment, behavior, cognition, and many neurological and psychiatric disorders (Cryan *et al.*, 2019). This extra level of complexity has been revealed with respect to brain function and dysfunction and fits into a broader conceptualisation of the centrality of bidirectional brain-body interactions in health and disease. Does this mean that more neuroscientists should consider the role of the microbiota-gut-brain axis in their studies of brain structure and function? A new study in this issue of EJN adds weight to this argument, particularly for those who study learning, memory and associated cellular plasticity.

Darch *et al*. (2021) provided new insights into the role of the microbiome as a potential modulator of synaptic plasticity in the hippocampus of adult mice. They evaluated the electrophysiological properties of hippocampal slices from germ-free (GF) adult mice (C57/Bl6 wild-types at 8-12 weeks of age), raised in the absence of microbiota, in comparison with their conventionally (Conv) raised counterparts. While the GF mice showed normal basal synaptic excitability and pre-synaptic function, post-synaptic long-term potentiation (LTP) was

altered in GF male (but not female) mice, demonstrating sex-dependent changes in synaptic plasticity in the absence of microbiota (keeping in mind that GF mice lack microbes in all parts of their body, not just the gut). However, the hippocampal CA1 neurons of these GF mice showed enhanced dendritic input-output coupling (both sexes), suggesting a compensatory hyperexcitability. In summary, Darch and colleagues uncover a sex-specific alteration in dendritic signaling and associated synaptic plasticity in the hippocampus of mice induced by the absence of microbiota (Darch *et al.*, 2021).

These findings have implications for how we might understand the regulation of synaptic plasticity and associated learning and memory mechanisms. These new results suggest a role of the microbiome in modulating aspects of hippocampal plasticity, and LTP in particular. This is not totally surprising since direct and indirect modulation of the gut microbiome (by far the most abundant microbiome in mice and humans) has been already linked with cellular plasticity, learning, memory and other aspects of cognition (Davidson *et al.*, 2018; Cryan *et al.*, 2019). Interestingly, the gut microbiome has been even suggested to be a contributor to individual differences in cognition, with evolutionary consequences that benefit both host and microbial populations (Davidson *et al.*, 2018). Furthermore, this capacity for gut microbiota to modulate such aspects of neural cell function and cognition has the potential to provide a missing link between the effects of environmental interventions such as exercise, diet and stress, on brain health and disease (Gubert *et al.*, 2020). Such environmental factors have been shown to modulate gut microbiota, as well as have impacts on the brain, and therefore it is possible that some such environmental stimuli could modify brain health and disease via the microbiota-gut-brain axis (Gubert *et al.*, 2020).

While Darch *et al*. (2021) didn't reveal specific molecular and cellular mechanisms involved in the effect of the lack of gut microbiota on hippocampal cellular plasticity, they found a sexual dysmorphism in their observed changes, with males being more affected. This sexual dimorphism raises new questions, including the potential role of sex hormones and associated receptor signaling, that should drive further studies. The investigation of sex-related differences, or sexual dimorphism, in the context of the gut microbiome, is a relatively new field, but it is growing rapidly. Sex seems to affect gut microbiota composition over the entire lifespan while affecting species diversity and, importantly, disease susceptibility (Valeri & Endres, 2021).

Sex differences have also been previously described for various aspects of synaptic plasticity, including evidence for hormones acting directly and indirectly (Hyer *et al.*, 2018). Therefore, the present results of Darch and colleagues could be followed up by investigating correlations with, and manipulations of, the estrous cycle of the female mice, since it was demonstrated that the levels of steroids in hippocampus fluctuate across the female cycle and sex can alter synaptic plasticity with implications for learning and memory (Hojo & Kawato, 2018). On the other hand, in male hippocampus, testosterone levels also appear to modulate LTP and dendritic sprouting (Skucas *et al.*, 2013). Therefore, sex hormones and neurosteroids, and their potential modulation of the microbiota-gut-brain axis, could provide a link between the recent findings of Darch and colleagues and sexual dimorphism of hippocampal plasticity and associated cognitive processes.

There are other questions raised by this new study (Darch *et al.*, 2021). The authors showed sexually dimorphic impacts of microbial absence on hippocampal LTP, but within the

'yin and yang' of synaptic plasticity, it is unknown whether long-term depression (LTD) was also affected in the GF mice. As discussed above, future studies should carefully measure the estrous cycle in female animals to establish whether any changes in brain function (and dysfunction) are correlated with sex hormone fluctuations. GF mice constitute a highly artificial construct, as no humans (except perhaps for very rare cases of individuals with severe immune disorders who must live in sterile 'bubbles', albeit with their endogenous microbiota intact) exist under such extreme conditions. Therefore, it would be of great interest to know whether oral administration of a cocktail of non-absorbable antibiotics, to greatly deplete the gut microbiota, could have similar impacts on the animals' brains, when delivered either during specific periods of development, or in adulthood. This would also address the possibility that the brain changes in GF mice are not only due to the absence of gut microbiota but also other microbiota (which are also absent in GF animals) and associated systemic changes which are also known to modulate brain function (Pluvinage & Wyss-Coray, 2020). Furthermore, the findings in this new article (Darch *et al.*, 2021) could be followed up with manipulations, including prebiotics, probiotics and fecal-matter transfer, to establish what aspects of the missing microbiota in the germ-free mice, and which intermediate cellular and molecular mechanisms, led to the reported hippocampal changes.

Another question not yet addressed is whether microbiota contribute to age-related decline in these aspects of hippocampal plasticity. It was recently reported that fecal-matter transplant from aged mice into young recipient mice was able to transfer cognitive impairment, and that this modulated cognition was associated with altered hippocampal proteins involved in synaptic plasticity (D'Amato *et al.*, 2020). However, in addition to the need to replicate and

extend such surprising findings, there are substantial remaining questions regarding mechanisms mediating the hippocampal changes reported in germ-free mice. How does the microbiome normally regulate these aspects of hippocampal function? Does microbial modulation of this aspect of brain function occur during development or adulthood? Is it via microbiota-derived molecules such as short-chain fatty acids (SCFAs) that can travel from the gut to the brain (via the bloodstream) and signal via specific receptors? Or does it depend more on communication via the vagal nerve, or perhaps other intermediaries such as the immune system?

There is much still to be understood about the microbiota-gut-brain axis, in health and disease. Furthermore, there is an urgent need to better understand the relationship between such findings in laboratory animals, including rodents, and the human microbiome and its complex bidirectional relationship with nervous system development, structure and function. If we can better understand the biological mechanisms at molecular, cellular and systems levels, it may lead to novel therapeutic approaches for a wide range of devastating neurological and psychiatric disorders (**Figure 1**).

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Conflict of interest statement

CG and AJH have no relevant financial disclosures or conflicts of interest to declare.

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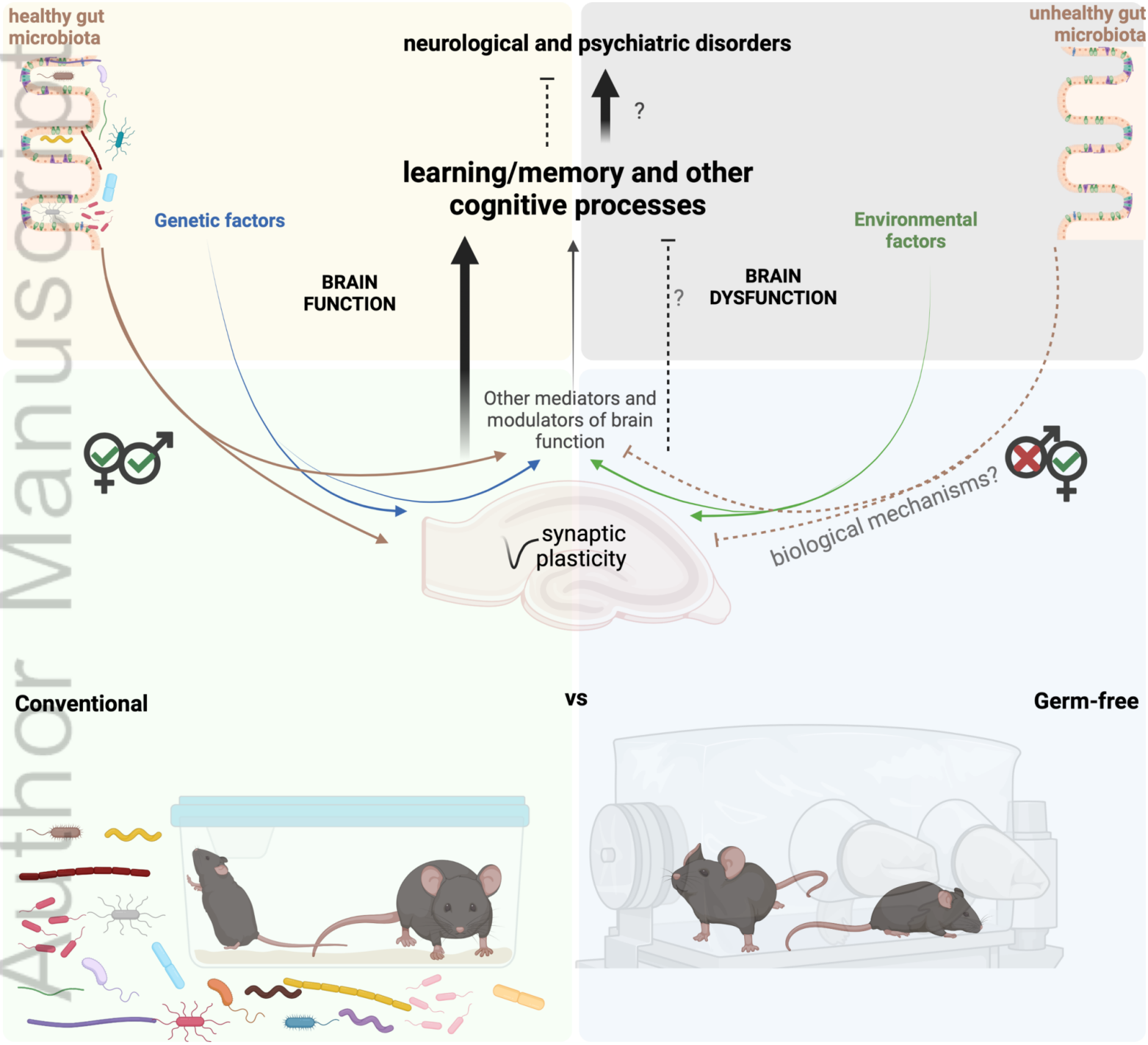
Legend

Figure 1. A schematic diagram illustrating how the absence of microbiota in germ-free mice could alter hippocampal synaptic plasticity and related aspects of brain function, and dysfunction. Darch and colleagues analysed hippocampal plasticity in germ-free adult mice (C57/Bl6 wild-types at 8-12 weeks of age), raised in the absence of microbiota, in comparison with their conventionally raised counterparts. They found alteration in synaptic plasticity in the hippocampus of mice induced by the absence of a microbiome, with males being more affected (Darch *et al*., 2021). While these investigators didn't reveal specific biological mechanisms involved in the effect of the lack of gut microbiota on hippocampal cellular plasticity, or the sex-specificity for this effect, this study points to the microbiota-gut-brain axis as having a central role in brain function and dysfunction. If an unhealthy gut microbiome is able to impact hippocampal plasticity, that could in turn lead to an impairment in cognition (e.g. learning and memory), and this could be involved in the pathogenesis of relevant neurological and psychiatric disorders. Therefore, if we can better understand the biological mechanisms at molecular, cellular and systems levels, it may lead to a range of novel therapeutic approaches for these devastating disorders of the brain, and body.



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