Brain, Behavior, and Immunity 94 (2021) 4–5

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Viewpoint

From gut to brain: Microbiota depletion in mice as a tool to explore causality

Sofia Cussotto [a], Bruno Aouizerate [a,b], Lucile Capuron [a,*]

[a] University of Bordeaux, INRAE, Bordeaux INP, NutriNeuro, UMR 1286, Bordeaux, France
[b] CH Charles Perrens, Pôle de Psychiatrie Générale et Universitaire, Centre de référence régional des pathologies anxieuses et dépressives, Bordeaux, France

ARTICLE INFO

Keywords:
Microbiota
Gut-brain communication
Antibiotic treatment
Cognitive function
Mice

The gut microbiota is receiving increasing attention for its involvement in brain function, spanning from cognition to stress response known to occupy a pivotal position in mental health. With the field rapidly expanding, more mechanistic insights are required in order to move beyond associations and examine causal relationships between gut microbiota composition and brain function (Cryan et al., 2019). The paper by Fröhlich and colleagues, that has won the 2020 BBI Impact Award (assigned to the paper published in 2016/2017 that has received most citations in 2018), reports the effects of a short-term antibiotic-induced microbiota depletion on cognitive function in mice (Fröhlich et al., 2016). Following an intragastric administration of an antibiotic mixture, the authors performed a systematic, comprehensive and multi-level assessment of: i) gut microbiota composition, ii) metabolomics patterns in both the colon and plasma, iii) expression of neuronal signaling molecules in different brain areas, and iv) cognitive performance. The authors described a strong antibiotic-mediated effect on microbial composition and cognitive behavior coupled with abnormal levels of peripheral metabolites and functional changes in neural signaling pathways in the brain.

Germ-free (GF) mice, which are born and raised in sterile conditions, have been instrumental in studying the causal involvement of the gut microbiota in cognitive function. However, they represent a highly artificial model with intrinsic alterations in behavior and neuronal circuits (Heijtz et al., 2011). Short-term antibiotic administration is therefore an appealing alternative, less intrusive and closer to the human setting, nevertheless carrying possible limitations. A recurrent methodological concern that accompanies antibiotic administration involves the pharmacokinetic properties of the antibiotics chosen, as those with high oral bioavailability can enter the circulation and potentially cross the blood–brain barrier (BBB) causing, in turn, a direct central effect (Tochitani et al., 2016). Among the antibiotics used by Fröhlich and colleagues (ampicillin, bacitracin, meropenem, neomycin, vancomycin), only ampicillin is absorbed to some extent from the human gut. Thus, to exclude the risk of an ampicillin-mediated direct central effects, the authors quantified its levels in the brain. They found them to be below the detection limit, suggesting that the cognitive impairment (disruption of novel object recognition memory) observed in antibiotic-treated mice more likely resulted from gut dysbiosis rather than from a systemic antibiotic response.

Antibiotic administration caused a task-dependent effect, inducing a reduction in memory index (time spent exploring the novel object *vs* known object) in the novel object recognition test (NORT), whilst not affecting spatial learning and memory in the Barnes maze test. These results suggest that gut dysbiosis does not have a generalized effect on cognitive function, but that certain aspects of cognition may be more susceptible to antibiotic-induced alterations in gut microbiota than others. The question arises regarding the possibility to modify the behavioral outcome by changing the composition of the antibiotic mixture and/or route of administration, thus targeting a different range of gut bacteria (or depleting selectively some specific taxa). This important issue opens up an interesting research avenue for further investigation into the influence of specific gut bacteria on distinct

* Corresponding author at: INRAE 1286, Laboratory of Nutrition and Integrative Neurobiology (NutriNeuro), University of Bordeaux, 146 rue Léo Saignat, F-33076 Bordeaux, France.
E-mail address: lucile.capuron@inrae.fr (L. Capuron).

https://doi.org/10.1016/j.bbi.2021.02.029
Received 24 February 2021; Accepted 25 February 2021
Available online 1 March 2021

components of cognition.

The authors moved on to explore the possible mechanisms involved in the gut-to-brain communication and likely to mediate cognitive alterations. To this end, several cognition-relevant signaling pathways were explored into specific brain areas. Interestingly, the hippocampal and hypothalamic expression of brain-derived neurotrophic factor (BDNF) was decreased and correlated with reduced memory index in the NORT in antibiotic-treated mice. In addition, NPY showed an increased expression in the amygdala and hypothalamus of antibiotic-treated mice, an observation that parallels earlier findings in GF mice (Schéle et al., 2013). Similarly, antibiotic-treated mice exhibited increased expression of the serotonin transporter SLC6A4 and the ionotropic glutamate NMDA receptor N2B subunit in the amygdala. Lastly, circulating levels of corticosterone were elevated in antibiotic-treated mice. Given that that prolonged exposure to corticosterone was found to dramatically impact brain connectivity, neurotransmitters systems, and cognitive function (McEwen 2002, Lupien et al., 2009), the authors hypothesized that increased corticosterone could play a major role in mediating cognitive deficits in antibiotic-treated mice. An increase in circulating corticosterone could, in turn, contribute to the down-regulation of BDNF in the hippocampus and hypothalamus (Naert et al., 2015). Noteworthy, the antibiotic administration did not affect concentrations of central and peripheral cytokines, hence the authors excluded inflammation as potential mechanism responsible for gut-to-brain related cognitive alterations.

What emerges from the paper here discussed is that antibiotic administration represents a useful pharmacological tool to investigate the causal relationships between changes in gut microbiota, brain function and behavior. Not only the administration of an antibiotic mixture offers chronological flexibility (i.e. the microbiota depletion can be performed acutely or chronically at any stage of the animal's lifespan), but it also permits to titrate the dose of antibiotics and to mimic as closely as possible the clinical setting in humans. Once the experimental model is refined and verified, understanding how gut microbes influence brain function requires a better mechanistic effort into exploring all the possible mediators involved in gut-to-brain communication. The intriguing findings from Fröhlich and colleagues should be considered as a starting point for a translational investigation into the role of gut microbiota in human cognition.

References

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., et al., 2019. The microbiota-gut-brain axis, Physiol. Rev. 99(4), 1877–2013.

Fröhlich, E.E., Farzi, A., Mayerhofer, R., Reichmann, F., Jačan, A., Wagner, B., et al., 2016. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav. Immun. 56, 140–155.

Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., et al., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. 108(7), 3047–3052.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10 (6), 434–445.

McEwen, B., 2002. Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. Neurobiol Aging 23 (5), 921–939.

Naert, G., Zussy, C., Tran Van Ba, C., Chevallier, N., Tang, Y.-P., Maurice, T., Givalois, L., 2015. Involvement of endogenous brain-derived neurotrophic factor in hypothalamic-pituitary-adrenal axis activity. J Neuroendocrinol 27 (11), 850–860.

Schéle, E., Grahnemo, L., Anesten, F., Hallén, A., Bäckhed, F., Jansson, J.O., 2013. The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. Endocrinology 154 (10), 3643–3651.

Tochitani, S., Ikeno, T., Ito, T., Sakurai, A., Yamauchi, T., Matsuzaki, H., 2016. Administration of non-absorbable antibiotics to pregnant mice to perturb the maternal gut microbiota is associated with alterations in offspring behavior. PLoS One 11 (1), e0138293.