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Influence of Gut Microbes on the Brain-Gut Axis

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Summary

Psychological stress and its morbidity are often encountered in functional gut disorders and this emphasizes the importance of the brain-gut axis in regulating gastrointestinal physiology.[1,2] The bidirectional interaction between the gut and the brain is vital in maintaining homeostasis but the exact mechanism on how both communicate remains elusive. Evidence from animal studies suggests a possible role of intestinal microbiota to influence central control in this brain-gut axis.[3,4]

The current study by Gareau et al[5] in the Gut 2011 issue provided a direct link for the first time between enteric infection and impaired learning and memory in the presence of psychological stress. The memory impairment persisted even after bacterial clearance and resolution of intestinal pathology. In addition, the study demonstrated the beneficial effect of probiotics in preventing stress-induced memory deficits, normalizing alteration in the hypothalamic-pituitary-adrenal (HPA) axis and colonic injury. This effect was associated, in part, with the restoration of hippocampus brain-derived neurotropic factor (BDNF) and c-Fos expression in the context of normalized microbiota. Germ-free mice also displayed an absence of memory, providing support for the requirement of commensal gut microbiota in memory.

In this study, female mice at 5-6 weeks were sourced commercially from 2 sites (Charles River, Canada and Taconic Farms, New York, USA) but both populations of mice behaved similarly according to the author (data not shown). Germ-free mice were contained for 72-96 hours before study to normalize the stress from shipping. The mice were infected with *Citrobacter rodentium* (strain DBS100, Dr David Schauer, Massachusetts Institute of Technology, MA, USA) administered by oral gavage and were tested for behavior at 10 days and 30 days after infection, respectively.[6] *C. rodentium* is a gram-negative bacterium that causes transient colitis in mice including attaching and effacing lesions and colonic epithelial cell hyperplasia.[7] A subset of mice was treated with probiotics (Lacidofil, Montreal, Canada) or placebo daily via their drinking water (orogastric gavage induces stress) starting 1 week before infection. Psychological stress was induced using water avoidance stress,[8] behavioral testing included the novel object test,[9] while the T-maze test[10] was used for dorsal hippocampus memory function (non-spatial and working memory) and light/dark box test[11] for anxiety. After behavioral testing, the mice were sacrificed. Blood was collected for serum corticosterone to measure HPA activation, colon tissue for histology to assess for colonic pathology, intra-colonic fecal samples for assessment of bacterial DNA and brain tissue to assess for intensity of BDNF and c-Fos in the CA1 region of the hippocampus.

C.rodentium infection in mice was not associated with anxiety behavior whether stressed or not. This was in contrast with another study showing an anxiety response after 8 hours of infection.[12]

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However the current study assessed for anxiety after 10 days which was at the peak of host response to the pathogen. Infected mice demonstrated water avoidance stress-induced memory impairment which was maintained until 30 days after infection, a time period where bacteria should clear and colonic lesions resolved. Probiotics when administered to mice, prevented stress-induced memory deficits with reduction in hippocampus BDNF and c-Fos, reduced serum corticosterone, ameliorated colonic injuries and normalized changes in intestinal microbiota. To confirm the role of microbiota in mediating memory, behavior was studied in germ-free mice. No anxiety was observed in the germ-free mice but memory deficits were evident and this was associated with decrease in hippocampus BDNF and c-Fos.

Comment

The microbiota-gut-brain axis is an exciting and emerging concept which suggests an important link and interaction between gut microbes and the brain. The evidence came from an indirect clinical observation of a beneficial effect of using antibiotic and prebiotic in hepatic encephalopathy[13] and a multitude of animal studies which indicate a central involvement by the peripheral enteric infection.[3,4] The question is how does the gut microbes communicate with the brain and which area of the brain is involved?

This study by Gareau et al[5] demonstrated for the first time that enteric infection resulted in stress-induced memory deficits and this was accompanied by decreased neuronal activation assessed by lower BDNF expression and c-fos staining in the hippocampus. This was consistent with study by Li et al[14] who showed that diet-induced changes in gut bacteria were temporally associated with changes in cognitive function. The memory deficits can be prevented with the administration of probiotics. Stress alone is not sufficient to cause memory impairment and this was shown in a study by Schaaf et al[15] where the authors showed that with the Morris water maze training, the corticosterone increased, while the hippocampus BDNF did not change.

The memory deficit at 30 days of infection with stress was a novel and unexpected finding. At this point, the pathogen should have cleared including colonic injuries with the exception of enteroendocrine cell signaling.[16] This suggested that the HPA axis is primed so that mild stressor exposure is sufficient to elicit memory impairment. This may well parallel with the finding of stress and post-infectious irritable bowel syndrome (IBS) in humans. Probiotics were shown to ameliorate HPA axis activation in addition to improving colonic lesions. Probiotics to some extent were also shown to counterbalance the dysbiosis induced by *C.rodentium*. Both mechanisms may well partly explain the beneficial effects seen with probiotics in IBS.[17]

The study was well conducted with novel results and provided important knowledge and evidence to the concept of microbiota-gut-brain axis. Some limitations are worth mentioning. Firstly, whether animal studies can be translated to human disease remain to be elucidated. Secondly, the germfree mice used in the study are different from colonized mice in many aspects. For example, in a study by Hooper and Macpherson,[18] colonization of germ-free mice with a commensal bacteria upregulated multiple genes with different functions. This suggests that the differences in brain function between germfree mice and colonized mice may be a reflection of maturation of mucosal and systemic changes rather than direct effects on the brain. Thirdly, female mice were used in the current study, because higher frequency of IBS was observed in female than male mice. There was evidence that male mice may express different stress response and different activation of brain areas compared to female.[19,20] Therefore, studies should also be conducted in male mice to assess gender differences.

In conclusion, the microbiota-gut-brain axis is a valid and interesting concept. Alterations in the composition of gut microbes can exert a measureable impact on animal behavior especially coupled with stress. Normalization of the microbiota with the use of probiotics can prevent behavioral abnormalities.

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