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Research report

Bifidobacteria modulate cognitive processes in an anxious mouse strain

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HIGHLIGHTS

- *B. longum 1714* improves cognition in BALB/c mice.
- *B. breve 1205* had little or no positive effects on memory.
- Neither of the bacteria had an effect on visceral sensitivity.
- The effects of bacteria on cognition are strain-dependent.

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ABSTRACT

Increasing evidence suggests that a brain–gut–microbiome axis exists, which has the potential to play a major role in modulating behaviour. However, the role of this axis in cognition remains relatively unexplored. Probiotics, which are commensal bacteria offering potential health benefit, have been shown to decrease anxiety, depression and visceral pain-related behaviours. In this study, we investigate the potential of two *Bifidobacteria* strains to modulate cognitive processes and visceral pain sensitivity. Adult male BALB/c mice were fed daily for 11 weeks with *B. longum 1714*, *B. breve 1205* or vehicle treatment. Starting at week 4, animals were behaviourally assessed in a battery of tests relevant to different aspects of cognition, as well as locomotor activity and visceral pain. In the object recognition test, *B. longum 1714*-fed mice discriminated between the two objects faster than all other groups and *B. breve 1205*-fed mice discriminated faster than vehicle animals. In the Barnes maze, *B. longum 1714*-treated mice made fewer errors than other groups, suggesting a better learning. In the fear conditioning, *B. longum 1714*-treated group also showed better learning and memory, yet presenting the same extinction learning profile as controls. None of the treatments affected visceral sensitivity. Altogether, these data suggest that *B. longum 1714* had a positive impact on cognition and also that the effects of individual *Bifidobacteria* strains do not generalise across the species. Clinical validation of the effects of probiotics on cognition is now warranted.

1. Introduction

Increasing evidence suggests that a brain–gut–microbiome axis exists and that it plays a key-role in regulating emotional functions, brain and behaviour [1–3]. Notably, disruption of the microbiota has been linked to gastrointestinal (GI) disorders following antibiotic treatment or infection [4–7], as well as stress-related disorders and alterations in behaviour [8]. Indeed, mice allowed to grow up in a germ-free environment showed altered anxiety behaviour [9–11], impaired stress axis [12] and deficits in sociability and social cognition [13]. These mice also displayed changes in the serotonergic system [14] and in brain-derived neurotrophic factor (BDNF)

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expression, one of the key molecules involved in memory functions [15,16]. Moreover, BDNF expression was also increased following enteric microbiota manipulation in healthy rats through prebiotics feeding [17]. Gut bacterial infection also induced increased anxiety [18,19] and such infection followed by stress, could induce memory impairments [20]. Conversely, social stress, or stress early in life, can also alter the enteric microbiota [21–23]. Thus, regulating the enteric microbiota may be an interesting strategy for targeting new treatments for cognitive deficits, either related to stress [24–26] or to neurodegenerative disorders such as Alzheimer's disease for which there is still no satisfactory treatment [27].

Probiotics, which are commensal bacteria offering potential health benefit to the host, when provided in adequate amount, actively interact with the endogenous microbiota [28]. Among these bacteria, certain *Lactobacilli* and *Bifidobacteria* spp have been shown to improve gut health, as well as mood disorders and stress-induced alterations such as impaired colonic microbiota [26,29]. Some *Lactobacilli* strains also normalised corticosterone release, reversed stress-induced colonic alterations [30] and improved anxiety associated with chronic fatigue syndrome [31]. Moreover, we have recently shown that *L. rhamnosus* was able to improve the naturally anxious phenotype of healthy BALB/c mice by decreasing their anxiety [32] and it has been shown that a *B. longum* decreased anxiety in both healthy and DSS-induced colitis AKR mice or mice infected with *T. muris* [33]. Thus it is of high relevance to investigate whether gut bacteria would also improve cognition. To this aim, Gareau and colleagues [20] found that the stress-induced cognitive impairments induced by gut bacterial infections could be reversed by ingestion of probiotics.

Bifidobacteria spp, which are amongst the main components of human and animal GI tracts, are of high health benefit to the host and are used as beneficial food supplements in dairy products [34–36]. *B. infantis 35624* was shown to have potential therapeutic effects on GI disorders and associated symptoms [37,38], as well as depression [39,40]. Also, *B. breve NCIMB 702258* showed a therapeutic potential for inflammatory and neurodegenerative diseases via its modulation of fatty acids composition [41], whereas *B. breve 6330* positively modulated BDNF expression in the hippocampus [42]. Moreover, *B. longum NCC3001* decreased the anxiety of both healthy and DSS-induced colitis AKR mice [33].

We have recently shown that two different *Bifidobacteria* strains, *B. longum 1714* and *B. breve 1205*, improved the anxious phenotype of BALB/c mice by reducing their anxiety [43]. Interestingly, the pattern of behavioural effects induced by both strains was different. Indeed, *B. longum 1714* reduced stress and anxiety of mice in the stress-induced hyperthermia and marble burying tests and reduced the latency to the anxiogenic inner zone of the open field, whilst also reducing depression-like parameter in the tail suspension test. On the contrary, *B. breve 1205* reduced rather various forms of anxiety solely by having a positive effect in the marble burying test and the elevated plus maze. However, it is unclear if either or both of the bacteria can also modify cognitive processes. As a result, we assessed in this study the effects of *B. longum 1714* and *B. breve 1205* on various aspects of cognition. Importantly, cognitive deficits have been associated with a myriad of diseases, and notably with the functional gastro-intestinal disorder irritable bowel syndrome (IBS) [44,45]. Probiotics, and especially *Bifidobacteria* spp, have shown particular efficiency against one of the core symptoms of IBS, visceral pain [37,46,47]. This latter has been shown in our laboratory to be associated with the activation of different regions of the prefrontal cortex and amygdala [48], which we suspected to be positively modulated by the two *Bifidobacteria* strains we are testing here following a study on anxiety [43]. Therefore in the present study, we also assessed the effects of *B. longum 1714* and *B. breve 1205* on visceral sensitivity.

2. Material and methods

2.1. Animals

Forty-eight male BALB/cOlaHsd (BALB/c) mice, 7–8 weeks old (Harlan Laboratories, UK), were used and remained housed in groups of 4 in plexiglas cages (33 cm × 15 cm × 13 cm, L × H × W) under standard controlled laboratory conditions (22 ± 1 °C, humidity 55 ± 5%) on a 12-h light/dark cycle (lights on 7.30 a.m.). Mice were provided with standard laboratory diet and water *ad libitum* throughout. Animals were housed in a separated room from other animals and treatments groups were separated from each other to avoid cross contamination. For each treatment group, mice were issued from 3 different litters. All mice were evenly distributed regarding treatment groups, order of feeding, order of testing, day and time of testing. The sex of the mice was specifically chosen to compare with our previous studies in BALB/c mice investigating the effects of the same *Bifidobacteria* strains [43] and of other potential probiotics [32]. BALB/c mice were chosen for their innate anxiety [49], as they are fundamental to model stress-related disorders, and their associated impaired cognitive processes [32,50–53] and as they have been used to characterise the in vivo effects of probiotics [54]. All experiments were conducted in accordance with the European Directive 86/609/EEC, the Recommendation 2007/526/65/EC and approved by the Animal Experimentation Ethics Committee of University College Cork.

2.2. Bacteria treatment

B. longum 1714 and *B. breve 1205* were kindly donated by Alimentary Health Ltd. (Cork, Ireland) from freeze-dried stocks (−80 °C). Bacteria were reconstituted in sterile phosphate buffered saline (PBS) so that the final concentration ingested by mice was 1×10^9 CFU mL^{-1}. This dose was selected based on previous studies showing a reduction in visceral pain following treatment with *B. infantis 35624* [37]. Vehicle-treated animals received PBS only. All treatments were given orally.

2.3. Study design

The experiment design is presented in Fig. 1. After a 5-day habituation to the animal facility, mice were fed daily (6–7 p.m.) with *B. longum 1714*, *B. breve 1205* or vehicle treatment, using sterile gavage needles, for 11 weeks. Bodyweight was monitored throughout. Behavioural testing was conducted (Fig. 1) from week 4 onward, from the least to the most stressful task [55], including resting days between tests. Animals were tested one at a time in a counterbalanced fashion regarding cage, treatment and time of the day and under the same conditions with an experimenter blind to conditions. All apparatus were cleaned between animals with 70% ethanol to remove odours. Starting at 10–11-week old, mice ($n = 12$ per group) were tested in a battery of cognitive tasks, the object recognition test for short term/episodic memory, the Barnes maze for spatial learning and memory and the fear conditioning for Pavlovian conditioning, memory and extinction. Locomotor activity was also assessed in the two first tests. At 17-week old (10-week feeding), animals underwent colorectal distension test (CRD) for visceral sensitivity. All animals were sacrificed 5–7 days following last test (18-week old, 11-week feeding) and blood was collected for measure of basal corticosterone levels in the plasma.

2.4. Behavioural testing

2.4.1. Object recognition

This test presents the advantages of not requiring an extensive training or aversive conditions (fearful environment, food