Biological Psychiatry

Archival Report

Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice

Aurelijus Burokas, Silvia Arboleya, Rachel D. Moloney, Veronica L. Peterson, Kiera Murphy, Gerard Clarke, Catherine Stanton, Timothy G. Dinan, and John F. Cryan

ABSTRACT

BACKGROUND: The realization that the microbiota-gut-brain axis plays a critical role in health and disease, including neuropsychiatric disorders, is rapidly advancing. Nurturing a beneficial gut microbiome with prebiotics, such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), is an appealing but underinvestigated microbiota manipulation. Here we tested whether chronic prebiotic treatment modifies behavior across domains relevant to anxiety, depression, cognition, stress response, and social behavior.

METHODS: C57BL/6J male mice were administered FOS, GOS, or a combination of FOS+GOS for 3 weeks prior to testing. Plasma corticosterone, microbiota composition, and cecal short-chain fatty acids were measured. In addition, FOS+GOS- or water-treated mice were also exposed to chronic psychosocial stress, and behavior, immune, and microbiota parameters were assessed.

RESULTS: Chronic prebiotic FOS+GOS treatment exhibited both antidepressant and anxiolytic effects. Moreover, the administration of GOS and the FOS+GOS combination reduced stress-induced corticosterone release. Prebiotics modified specific gene expression in the hippocampus and hypothalamus. Regarding short-chain fatty acid concentrations, prebiotic administration increased cecal acetate and propionate and reduced isobutyrate concentrations, changes that correlated significantly with the positive effects seen on behavior. Moreover, FOS+GOS reduced chronic stress-induced elevations in corticosterone and proinflammatory cytokine levels and depression-like and anxiety-like behavior in addition to normalizing the effects of stress on the microbiota.

CONCLUSIONS: Taken together, these data strongly suggest a beneficial role of prebiotic treatment for stress-related behaviors. These findings strengthen the evidence base supporting therapeutic targeting of the gut microbiota for brain-gut axis disorders, opening new avenues in the field of nutritional neuropsychopharmacology.

Keywords: Animal behavior, Anxiety, Microbiota-gut-brain axis, Prebiotics, SCFAs, Stress

http://dx.doi.org/10.1016/j.biopsych.2016.12.031

Increasing evidence suggests that the microbiota-gut-brain axis plays a key role in regulating brain functions, particularly emotional processing and behavior (1,2). Indeed, the microbiota plays an important role in neurodevelopment, leading to alterations in gene expression in critical brain regions and resulting in perturbation to the programming of normal social and cognitive behaviors in mice (3–6). The gut microbiota has principally been exploited to yield positive effects on brain health via probiotics, with various bifidobacteria and lactobacilli strains shown to have anxiolytic and procognitive effects in both rodents (7–10) and humans (11–14). Although single- or multistrain probiotics have shown potential to modify behavior, they also are limited by their ability to have relatively narrow spectrum effects on the microbiome. Moreover, given that they are live biotherapeutics, there are formulation and storage issues to consider.

An alternative but underinvestigated strategy to target the microbiome is via dietary prebiotics. These are defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thereby conferring benefits on host health (15). Unabsorbed/undigested carbohydrates in the small intestine are fermented by the gut microbiota in the large bowel, producing their main end products, short-chain fatty acids (SCFAs) and lactic acid (16), which may have multiple effects, including the modulation of enteroendocrine serotonin secretion (17).

Fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are soluble fibers extensively used as prebiotics that

SEE COMMENTARY ON PAGE 456

 ISSN: 0006-3223

are traditionally associated with the stimulation of beneficial bacteria such as bifidobacteria and lactobacilli, among other gut members (18). Many beneficial effects on the gut and immune system have been associated with prebiotic use (19,20). It has previously been shown that the prebiotic sialyllactose is able to diminish stress-induced alterations in colonic mucosa-associated microbiota community structure, anxiety-like behavior, and immature neuron cell numbers irrespective of immune or endocrine functionality in mice (21). Furthermore, oligosaccharides increased brain-derived neurotrophic factor expression and *N*-methyl-D-aspartate receptor signaling in rats (22). In a clinical setting, human subjects supplemented with GOS presented suppression of the neuroendocrine stress response and an increase in the processing of positive versus negative attentional vigilance, showing an early anxiolytic-like profile (23). However, the central nervous system (CNS) effects of prebiotic administration have not been extensively explored, and the links to a behavioral repertoire require extensive elaboration.

In the current study, we investigated whether administration of the prebiotics FOS and GOS, alone or in combination, affects behavior—specifically anxiety, depression-like behavior, cognition, and social behavior—in parallel with associated changes in discrete brain regions, gut microbiota composition and SCFAs produced, and endocrinology. Moreover, we assessed the impact of the combination prebiotic treatment on chronic psychosocial stress-induced changes in behavior, hypothalamic-pituitary-adrenal axis, immune system, and microbiota.

METHODS AND MATERIALS

Animals

In this study male C57BL/6J mice (n = 69; Harlan, Cambridgeshire, UK; 7 weeks of age on arrival) were used. (More details can be found in the Supplement.) All experiments were conducted in accordance with European Directive 86/609/EEC, Recommendation 2007/526/65/EC, and approved by the Animal Experimentation Ethics Committee of University College Cork.

Prebiotic Administration

Mice were administered the prebiotics (Healy Group, Dublin, Ireland) FOS, GOS, a combination of FOS and GOS (dissolved in drinking water for 0.3–0.4 g/mouse/day), or water during all of the studies. Duration of treatment was chosen based on previous studies in rodents showing behavioral and neurochemical effects following 2 to 3 weeks of treatment with prebiotics (21,22,24,25).

Anxiety-like Behavior

Anxiety-like behavior was assessed using the open field, defensive marble burying and elevated plus maze and stress-induced hyperthermia as previously described (7) and detailed in the Supplement. The experimental design is presented in Figure 1.

Depression-Related Behavior

Anhedonia was assessed using the female urine sniffing test (26), and antidepressant sensitive behaviors were assessed with the tail suspension and forced swim tests as previously detailed (7,27) (see Supplement).

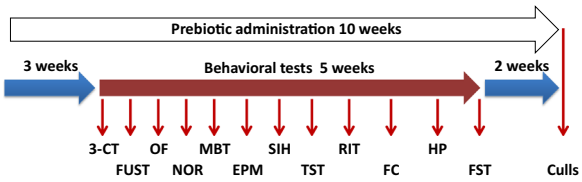

Figure 1. Experimental schedule of study 1 during the 10 weeks. Behavioral testing was conducted starting with the least stressful test to the most stressful test. Except for stress-induced hyperthermia, animals were brought to the experimental room 30 minutes prior to testing, which occurred between 8 AM and 4 PM (between 8 AM and 12 noon for the forced swim test). Briefly, 40 adult male mice (n = 10 per group) had a battery of different behavioral tests during 5 weeks. Week 4: 3-CT, three-chamber test; FUST, female urine sniffing test; OF, open field; NOR, novel object recognition test. Week 5: MBT, marble burying test; EPM, elevated plus maze; SIH, stress-induced hyperthermia. Week 6: TST, tail suspension test; RIT, resident-intruder test. Week 7: FC, fear conditioning. Week 8: HP, hot plate; FST, forced swim test and blood collection. Week 10: animals are culled and tissue is collected.

Social Behavior

Sociability was assessed by the three-chambered social approach task (28,29) and the resident-intruder test (30) with minor modifications (see Supplement).

Cognition

Cognitive function was assessed using the novel object recognition test (27,31) and fear conditioning paradigm, which allows differentiating between context and context/cue-related behavioral responses in the same setting (9), with nociception assessed by the hot plate test to ensure specificity (see Supplement).

Corticosterone, Tryptophan, and Neurotransmitter Levels

Plasma corticosterone and tryptophan levels, as well as brain neurotransmitter, were measured as previously described (32) and detailed in the Supplement.

Social Defeat/Overcrowding Procedure Followed by Social Interaction Test

Chronic unpredictable social stress was carried out as previously described (26). Deficits in social interaction have been one of the most robust manifestations of chronic social defeat-induced anxiety in rodents (see Supplement).

Spleen Cytokine Assay

Spleens were collected immediately following sacrifice and cultured as previously described (33) (see Supplement).

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted using the *mir*Vana microRNA Isolation Kit (Ambion/Life Technologies, Paisley, UK) and DNase treated (see Supplement).