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Research report

Absence of gut microbiota influences lipopolysaccharide-induced behavioral changes in mice

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HIGHLIGHTS

- Lack of gut microbiota alters LPS-induced depressive-like behaviors.
- Germ-free mice present impaired increase of TNF and microglial cells activation within the hippocampus after LPS stimulus.
- Animals lacking gut microbiota display an elevated basal level of dorsal raphe activation.

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ABSTRACT

Changes in the microbiota composition of gastrointestinal tract are emerging as potential players in the physiopathology of neuropsychiatric disorders. In the present work we evaluated the relationship between the absence of gut microbiota and neuroinflammatory mechanisms in a murine model of LPS-induced behavioral alterations. Germ-free (GF) or conventional male mice received a single i.p. injection of lipopolysaccharide (LPS i.p.; 0.83 mg/Kg) or PBS, and after 24 h they were tested for depressive-like behaviors (forced swimming test, tail suspension test – TST, or sucrose preference test – SPT). After behavioral evaluation, animals were analyzed for possible changes in neuroplasticity by means of BDNF, NGF and cytokines levels in prefrontal cortex and hippocampus, and the expression of Iba-1 (microglial activation marker) in the hippocampus, and the cellular activity marker, ΔFosB, in the dorsal raphe nucleus. In conventional mice, LPS induced depressive-like behaviors. LPS-induced changes were followed by up-regulation of the expression of TNF and Iba-1 in the hippocampus. The same effects were not observed in GF mice. Behavioral effects of LPS were not observed in GF mice submitted to TST. GF mice present a lower response to the anhedonia-like effect induced by LPS when compared to conventional animals (SPT). There was up-regulation of ΔFosB in the dorsal raphe nucleus in the absence of gut microbiota, events not influenced by LPS treatment. Our results suggest that gut-microbiota interactions influence depressive-like behaviors, raphe nucleus activation and activation of pro-inflammatory mechanisms within the hippocampus.

1. Introduction

According to historical records, in 1845, the French psychiatrist Jean-Étienne Dominique Esquirol stated that mental illness could be epidemic, proposing that psychiatric conditions might appear due to other factors instead of being a disease located primarily in the brain [26]. This view was extended in 1990′s by the immune hypothesis of the psychiatric conditions and recently by the new

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era of "gut feelings", which suggests that the microbiota present in the gut might influence brain functions and emotions [12].

The brain-gut-microbiota axis is composed by the interactions among the central nervous system (CNS), the neuroendocrine and immune systems, the sympathetic and parasympathetic arms of the autonomic nervous system, the enteric nervous system and the intestinal microbiota [21,31]. The interactions among these components are highly complex. While the CNS can influence motor and sensorial gut functions, the gut and its commensal microbiota may modify brain functions through the modulation of the activity of areas responsible for stress response [12].

The modulation of brain-gut-microbiota axis is associated with changes in stress response and behavior. Enteric infections or changes in enteric microbiota are risk factors for irritable bowel syndrome [27,38], a clinical condition with a high prevalence of comorbid anxiety and depressive disorders (50–90%) [4,44]. Accordingly, stress can alter brain-gut axis function and the composition of the gut microbiota when occurs in early life [31] or adulthood Bailey et al. [46].

Some of the most persuasive evidence for a role of bacteria in gut-brain signaling comes from research using germ-free (GF) mice, *i.e.* mice with no exposure to microorganisms. The use of GF animals enables the direct assessment of the role of the microbiota on several aspects of physiology [12]. GF mice presented hyperresponsive hypothalamic-pituitary-adrenal (HPA) axis activity following restraining stress as compared with specific pathogen free (SPF) mice [41]. Moreover, GF mice exhibited reduced basal anxiety-like behavior in comparison with SPF animals, and altered behavior was accompanied by neurochemical changes in the brain [29]. GF mice also displayed memory impairment, providing support for the requirement of commensal gut microbiota in memory functioning. Memory deficits were associated with decrease in hippocampus brain-derived neurotrophic factor (BDNF) and c-Fos [20].

Although considerable advance has occurred in this area, the influence of gut microbiota on brain functions remains poorly understood. The mechanisms underlying the effects of gut microbiota on brain might involve changes in neurotransmitters, HPA axis and inflammatory response [17]. In the current study, we sought to investigate whether gut microbiota influences depressive-like behaviors. Experiments were performed using GF mice, and depressive-like behavior was induced by lipopolysaccharide (LPS) injection. Peripheral administration of LPS is well-known to induce depressive-like behavior in rodents as measured by increased immobility in the forced-swim test or the tail-suspension test and decreased preference for a sweet solution [8].

2. Materials and methods

2.1. Animals

Male Swiss (normal microbiota, according to diet and conditions of the local animal facility, 10 weeks old) and germ-free (GF) mice (Swiss; 10 week-old) kept in the Gnotobiology Animals Facility of the Institute of Biological Sciences—UFMG were used. Animals were housed in 3–5 per cage in a temperature-controlled room (24 °C) with free access to food and water, and 12 h light-dark cycle regulation (lights on at 7am). Sterilized water and food were provided to GF mice and all experimental procedures in these mice were carried out under aseptic conditions to avoid contamination of animals. After being removed from the Gnotobiology Animals Facility, animals were kept sterile in microisolators for 3 days [15,43]. All tests were conducted in the light phase of the cycle (9–15 h), except for the SPT which was performed overnight. This study was conducted in strict accordance with the Brazilian guidelines on animal work and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal ethics committee of the *Universidade Federal de Minas Gerais* (CEUA/UFMG) approved all experimental procedures.

2.2. Behavioral tests

2.2.1. Open field test

this test was conducted in order to control for possible interference of locomotor activity on the FST. Animals were placed in the center of a circular arena with transparent walls (30 cm diameter and 50 cm height), and were allowed to freely move in a 6-min test [7]. Total distance travelled was analyzed by the software ANY-MAZE (Stoelting Co., Wood Dale, IL). After each experiment, the arena was cleaned with a 70% alcohol solution to avoid the influence of smell in the following tests.

2.2.2. Forced swimming test (FST)

FST was used to screen depressive-like behavior. Each animal was placed individually into a transparent glass container filled with 15 cm of water (temperature of 23–25 °C). Total duration of the session was 6 min (the first 2 min of pre-test followed by a 4-min test), and the immobility time, regarded as a measure of behavioral despair/depressive-like behavior, was scored during the last 4 min. All trials were recorded by a video camera, and posteriorly analyzed by an experimenter blind to the treatment/microbiome status.

2.2.3. Tail suspension test (TST)

mice were suspended 50 cm above the floor by their tails (fixed with an adhesive tape) and the immobility time scored during the total time of the test session. Total duration of immobility was measured according to the method proposed by [40] in a 6-min session. The test was recorded by a video camera, and analyzed by an investigator blind to treatment/microbiome status.

2.2.4. Sucrose preference test (SPT)

SPT was carried out in the home cages. This reward-based test is proposed to measure in rodents behaviors related to anhedonia. Briefly, animals were presented in their home cages to two bottles of the same size, color and material, one containing plain drinking water, and the second with a 2% sucrose solution. Test duration was overnight and it was divided in two different sessions. The first session was carried to determine the basal sucrose preference of animals. Only animals that showed basal sucrose preference were used in the experiment. The second session took place 48 h after the basal preference test and 24 h after LPS injection (animals were used as their own controls). In the end of the test the percentage of sucrose preference was calculated by the following formula:

$$[\text{amount of sucrose intake (g)}]/[\text{amount of sucrose intake (g)} + \text{amount of water intake (g)}] \times 100.$$

2.3. Experimental design

2.3.1. Experiment I (Fig. 1A)

GF or conventional mice received a single intra-peritoneal (i.p) injection of lipopolysaccharide (LPS-*Escherichia coli*; stereotype 0111/B4; Sigma Aldrich, Saint Louis, USA) in the dose of 0.83 mg/Kg [18] or vehicle (between 12 and 12:30 pm). The procedure of LPS injection in GF mice (housed in microisolators) was made under aseptic conditions in a laminar flow hood. Twenty hours later, they were submitted to the open filed test in order to evaluate basal locomotor behavior. Four hours later, all animals were exposed to the FST. Immediately after the test, all animals were euthanized under deep anesthesia (Ketamine 60 mg/Kg and Xylazine 80 mg/Kg-i.p.),