Priority Communication

Biological Psychiatry

Obese-type Gut Microbiota Induce Neurobehavioral Changes in the Absence of Obesity

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ABSTRACT

BACKGROUND: The prevalence of mental illness, particularly depression and dementia, is increased by obesity. Here, we test the hypothesis that obesity-associated changes in gut microbiota are intrinsically able to impair neurocognitive behavior in mice.

METHODS: Conventionally housed, nonobese, adult male C57BL/6 mice maintained on a normal chow diet were subjected to a microbiome depletion/transplantation paradigm using microbiota isolated from donors on either a high-fat diet (HFD) or control diet. Following re-colonization, mice were subjected to comprehensive behavioral and biochemical analyses.

RESULTS: The mice given HFD microbiota had significant and selective disruptions in exploratory, cognitive, and stereotypical behavior compared with mice with control diet microbiota in the absence of significant differences in body weight. Sequencing-based phylogenetic analysis confirmed the presence of distinct core microbiota between groups, with alterations in α- and β-diversity, modulation in taxonomic distribution, and statistically significant alterations to metabolically active taxa. HFD microbiota also disrupted markers of intestinal barrier function, increased circulating endotoxin, and increased lymphocyte expression of ionized calcium-binding adapter molecule 1, toll-like receptor 2, and toll-like receptor 4. Finally, evaluation of brain homogenates revealed that HFD-shaped microbiota increased neuroinflammation and disrupted cerebrovascular homeostasis.

CONCLUSIONS: Collectively, these data reinforce the link between gut dysbiosis and neurologic dysfunction and suggest that dietary and/or pharmacologic manipulation of gut microbiota could attenuate the neurologic complications of obesity.

Keywords: Gut dysbiosis, Intestinal permeability, Mental health, Neurobehavior, Neuroinflammation, Obesity, Psychiatric disease

http://dx.doi.org/10.1016/j.biopsych.2014.07.012

The etiology of most neuropsychiatric disorders is likely multifactorial and based on genetic and environmental risk factors (1). One potentially important environmental driver of mental illness is obesity, which dramatically increases risk of depression, dementia, and stroke, and is associated with increased brain pathology and decreased brain function [reviewed in (2)]. For example, functional studies report deficits in learning, memory, and executive function in obese compared with nonobese patients (3,4) and likewise link obesity to enhanced depression and anxiety disorders (5,6). However, there are contradictory reports that dispute these findings (7,8), suggesting that the cause of obesity-associated mental illness is not obesity per se but rather one or more of the variable manifestations of obesity.

One potential site whereby diet-induced obesity could affect physiology is the gut microbiome, as recent advances in 16S ribosomal RNA sequencing and informatics have revealed that modern diets high in fat and sugar trigger robust alterations in the core gut microbiome (9). The human gastrointestinal tract harbors as many as 100 trillion bacteria from up to 1000 distinct species, and this dynamic population of microbes participates in numerous physiologic functions including nutrition/digestion, growth, inflammation, immunity, and protection against pathogens (10–12). Accordingly, the varying combinations of bacteria within individuals have been suggested to underlie variable host susceptibility to illness (13,14), including neuropsychiatric impairment (15,16). For example, specific alterations in colon bacteria are associated with cognitive impairment in patients with hepatic encephalopathy (17), and clinical studies show that oral probiotics decrease anxiety and improve mental outlook (18,19). Furthermore, animal studies have shown that behavior and synaptic plasticity are altered in germ-free mice and that this phenotype is reversed by microbiome colonization (20). The aim of the present study was to test the hypothesis that the obesity-concomitant microbiome undermines behavior even in the absence of obesity. Nonobese, adult male C57BL/6 mice were

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ISSN: 0006-3223

conventionally housed and maintained on chow diet but subjected to a microbiome depletion paradigm followed by adoptive transfer of cecal plus colonic contents collected from donor mice fed either a high-fat diet (HFD) or control diet (CD). Recipient mice were subjected to a battery of neuropsychological tests, followed by sequencing of gut microbiota and thorough biochemical evaluation of intestine, blood, and brain samples.

METHODS AND MATERIALS

Animals and Treatments

The Pennington Biomedical Research Center Institutional Animal Care and Use Committee approved all experimental protocols, which were compliant with National Institutes of Health guidelines. To generate microbiota donor material, 8-week-old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were given regular chow diet (13% fat calories, Purina LabDiet 5001; LabDiet, St. Louis, Missouri) or high-fat diet (60% fat calories, Research Diets D12492; Research Diets, Inc., New Brunswick, New Jersey) for 10 weeks (see Table S1 in Supplement 1 for diet compositions). At the time of microbiota harvest, the high-fat fed mice weighed 37.0 ± 1.7 g and the chow-fed mice weighed 24.5 ± 1.2 g. Mice were euthanized and cecal plus colonic contents were harvested, pooled, and diluted fortyfold (weight:volume) in sterile water. After centrifugation at 800 rpm, the supernatant was aliquoted under sterile conditions for storage at −80°C. Recipient 3-month-old male C57BL/6 mice (Jackson Laboratories) were group-housed under standard laboratory conditions with free access to water and chow diet (Purina LabDiet 5001). Mice were given a cocktail of ampicillin, gentamicin, metronidazole, and neomycin (all at .25 mg/day) and vancomycin (.125 mg/day) once daily for 14 consecutive days by oral gavage (21). Mice were re-colonized 72 hours later via daily oral gavage of donor microbiota (100 μL) for 3 days (22,23). To offset potential founder and/or cage effects (24) and to reinforce the donor microbiota genotype, booster inoculations were given biweekly throughout the study. Body weight and composition were measured regularly, and all mice were euthanized following behavioral testing. Plasma, lymphocytes, intestines, intestinal contents, and brains were collected, with data compiled from 10 animals per group.

Behavioral Testing

All behavioral testing was conducted between 7:00 AM and 1:00 PM and was recorded/analyzed using Any-Maze software (Stoelting Co., Wood Dale, Illinois) for unbiased quantification of body location, orientation, distance, speed, and mobility/immobility. Detailed methods on behavioral assays are provided in Supplement 1. Overall anxiety and exploratory behavior were assessed using elevated plus (25) and open field assays (26). Stereotypical behavior was assessed by quantifying marble burying during a 30-minute trial in a novel cage preloaded with 4 cm of clean bedding and 16 evenly spaced marbles (27) (Figure 1D). Memory was measured using a video-based fear conditioning system (Med-Associates, St. Albans, Vermont) that pairs a unique context (scent and cage) and unconditioned stimulus (auditory tone) with a repeated foot shock (day 1) and then quantifies freezing behavior to the context (day 2) and to the tone (day 3) as measures of memory (28). Behavioral tests were administered in the order listed above over 2 weeks, beginning 3 weeks after the end of antibiotic treatment (Figure 1A). To curtail carryover effects, the elevated plus, open field, and marble-burying assays were conducted during the first week of testing with 48 hours recovery between each task, while fear conditioning was tested the following week (29,30).

16S Metagenomic Sequencing

Fecal samples were collected under aseptic conditions from all mice during the final week of behavioral testing, while cecal samples were collected aseptically at euthanasia. Sequencing and bioinformatics were performed by the Louisiana State University Microbial Genomics Resource Center. DNA was isolated using QIAamp DNA Stool kits (Qiagen, Germantown, Maryland) modified to include a bead-beating step. After DNA isolation, 16S ribosomal DNA hypervariable regions V3 and V4 were polymerase chain reaction amplified using primers with the V3F CCTACGGGAGGCAGCAG and V4R GGACTACHVGGGTWTCTAAT gene-specific sequences, Illumina adaptors, and molecular barcodes as described in Kozich *et al.* (31) to produce 430 base pair (bp) amplicons. Samples were sequenced on an Illumina MiSeq (Illumina, San Diego, California) using V3 sequencing kit (300 bp paired end reads). The forward read files were processed through the UPARSE pipeline (drive5, Tiburon, California) (32), truncating reads to a uniform length of 250 bp, then removing reads with quality scores less than 16. Additional filtering removed reads that appeared only once throughout all samples (singletons) and remaining unique reads were clustered into operational taxonomic units (OTU) at 97% similarity. Chimeric OTUs were removed as identified by UCHIME drive5 run against a gold standard reference database of nonchimeric sequences. Finally, the original filtered reads (before dereplication) were mapped to the OTUs using USEARCH drive5 at 97% identity. QIIME 1.8 (open source, www.qiime.org) was used to pick and align a representative set. The Ribosomal Database Project classifier was used to assign a taxonomic classification to each read in the representative set and a phylogenetic tree was constructed from the representative sequences. Among samples, the minimum read count after filtering was 21,182, with a median read count of 57,537. Relative abundance of each OTU was examined at phylum, class, order, family, genus, and species levels. Alpha (within a community) and beta (between communities) diversity metrics as well as taxonomic community assessments were produced using QIIME 1.8 scripts.

Plasma and Tissue Analyses

Whole blood was collected by cardiac puncture of terminally anesthetized mice into ethylenediaminetetraacetic acid treated tubes, and plasma and lymphocytes were isolated and analyzed immediately or stored at −80°C. Endotoxin levels in plasma were measured using a kinetic limulus amebocyte lysate test (Lonza Group, Limited, Basel, Switzerland). Levels of bioactive lipids and hormones/adipokines were measured as previously reported (33). Lymphocyte, colon, jejunum, and brain (medial prefrontal cortex) samples were homogenized and processed for Western blot with chemiluminescence as