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Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease

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Emge JR, Huynh K, Miller EN, Kaur M, Reardon C, Barrett KE, Gareau MG. Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 310: G989–G998, 2016. First published April 7, 2016; doi:10.1152/ajpgi.00086.2016.—Anxiety, depression, and altered memory are associated with intestinal diseases, including inflammatory bowel disease (IBD). Understanding the link between these behavioral changes and IBD is important clinically since concomitant mood disorders often increase a patient's risk of requiring surgery and developing secondary functional gastrointestinal diseases. Anxiety-like behavior (light/dark box test) and recognition memory (novel object recognition task) were determined at the peak and during resolution of inflammation in the dextran sodium sulfate (DSS) mouse model of acute colitis. DSS (5 days) was administered via drinking water followed by 3 or 9 days of normal drinking water to assess behavior during active or resolving inflammation, respectively. Disease (weight, colon length, and histology) was assessed and the composition of the gut microbiota was characterized by using qPCR on fecal pellet DNA. In a subset of mice, pretreatment with probiotics was started 1 wk prior to commencing DSS. During active inflammation (8 days), mice demonstrated impaired recognition memory and exhibited anxiety-like behavior vs. controls. These behavioral defects were normalized by 14 days post-DSS. Shifts in the composition of the gut microbiota were evident during active inflammation, notably as decreases in lactobacilli and segmented filamentous bacteria, which were also reversed once the disease had resolved. Administration of probiotics could prevent the behavioral defects seen in acute DSS. Taken together, our findings indicate that changes in mood and behavior are present during acute inflammation in murine IBD and associated with dysbiosis and that these outcomes can be prevented by the administration of probiotics.

anxiety; cognition; colitis; microbiota; stress

IT IS WELL ESTABLISHED THAT the brain communicates with the gastrointestinal tract in a complex, bidirectional relationship referred to as the gut-brain axis. Increasing numbers of studies are emerging demonstrating how the microbes within the intestinal tract can modulate intestinal physiology, consequently influencing the brain, mood, and behavior in both health (6, 22) and disease (4, 14). The pathways by which this bidirectional communication is established remain to be fully elucidated and likely involve a combination of neural, endocrine, and immune factors.

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Inflammatory bowel diseases (IBDs) are relapsing and re-mitting diseases of the gastrointestinal tract with etiologies that are not yet fully understood. A combination of factors, including an overt immune response (10), dysbiosis (35), genetic susceptibility (8), and unknown environmental factors (1) are thought to lead to disease. Numerous mouse models of IBD exist, each mimicking different aspects of disease. The dextran sodium sulfate (DSS) model in mice uses a mucosal irritant administered via the drinking water in either an acute or a chronic setting. DSS administration results in pathology similar to that seen in patients with ulcerative colitis (UC), including diarrhea, bloody stools, mucosal ulcers, and weight loss (29). DSS-induced colitis has distinct advantages for the study of the microbiota-gut-brain axis over other models because administration is stress free, in contrast to colorectal instillation required in other models (e.g., dinitrobenzene sulfonic acid (DNBS) and 2,4,6-trinitrobenzenesulfonic acid (TNBS)). In addition, it allows for the study of resolution of inflammation in contrast to most genetic (e.g., IL-10 knockout) and cell-based models (e.g., CD45RBhi transfer) in which disease progresses without improvement (20, 27). Despite the benefits of use of DSS in our study, it also has an important limitation in that it lacks a requirement of lymphocytes for disease development in contrast to human disease (7). Nevertheless, it serves as a useful model of the study of the innate immune response in colonic disease.

IBDs have a strong association with behavioral abnormalities in patients suffering from active or resolved intestinal inflammation (12). The exact mechanism by which abnormal behaviors and IBD correlate with one another is currently unclear. Feelings of hopelessness and anxiety are correlated with higher Crohn's disease (CD) activity index scores, and a strong positive correlation was seen between depression and CD activity (23). Furthermore, IBD patients suffering from psychiatric illness have a decreased chance of entering remission compared with those who do not show psychiatric symptoms, and patients demonstrating behaviors characteristic of depressed individuals show a more severe illness over a longer period of time (34). Adolescent IBD patients are particularly vulnerable to behavioral symptoms and have been demonstrated to display mild cognitive problems, particularly with verbal memory (12). Cognitive dysfunction is also a common side effect of depression, which may be the main diagnosis in many patients (12). Taken together, the clinical findings described suggest a role for gut-brain communication in IBD severity.

Behavior in IBD has increasingly been studied in mouse models. In a chronic murine DSS model, anxiety-like behavior

was demonstrated via the step-down latency test, which could be ameliorated by administration of a *Bifidobacterium*-containing probiotic via a vagally mediated pathway (3). Another recent study demonstrated that mice subjected to acute low-dose DSS display altered stress-associated behaviors compared with controls (32). Despite these findings, other behavioral changes in acute DSS colitis, including cognitive function in particular, have yet to be assessed. Therefore, our aim was to better understand the microbiota-gut-brain axis, specifically cognitive function, in the context of acute IBD. These studies might help elucidate the basis of behavioral abnormalities seen in patients, potentially providing strategies for future therapies.

MATERIALS AND METHODS

Animals. Male and female C57BL/6 mice (6–8 wk of age; 40 male and 40 female) were used for all experiments. Mice were originally obtained from Jackson Laboratory but bred in house. Entire cages of mice were subjected to each treatment, given the need to administer compounds in the drinking water. Cages were randomly selected for each treatment group to generate *n* numbers of 10–12 for behavioral tests, 8–10 for microbiota analysis, and 4–6 for immunohistochemical analysis based on power calculations [$\alpha = 0.05$; power $(1 - \beta) = 0.8$]. Since we did not see effects of potential sexual dimorphism in pilot studies, male and female mice were randomly assigned to each group. Mice were housed in cages lined with chip bedding with free access to food and water. Animals were kept in a specific pathogen-free vivarium, and behavioral testing was performed in a biosafety cabinet in a dedicated room within the laboratory. All procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee at UC San Diego and UC Davis.

Novel object recognition task. After a 1-h habituation in individual cages, mice underwent the novel object recognition (NOR) task composed of three phases in the following sequence: training, resting, and testing as described previously (14, 33). Mice were videotaped for 5 min, and videos were analyzed for the number of times the mice approached, or sniffed, each object. Results were expressed as an exploration ratio. A value of 50% indicated that the mouse investigated both objects equally whereas a higher value indicated that the mouse investigated the new object more than the old object (14). The mouse was considered to have investigated an object if its nose came within 1–2 cm of it. Mice were excluded from analysis if they did not pass the training phase (i.e., they had a preference for an object over another) or if they did not approach the objects during the testing phase.

Light/dark box test. To measure anxiety-like behavior, mice were placed in a light/dark box for 10 min, and behavior was video recorded for analysis as previously described (14, 33). Mice demonstrating a higher degree of anxiety-like behavior spend more time in the dark portion of the box and less time in the light portion of the box, compared with their less anxious counterparts. The total amount of time the mouse spent in the light box, reflecting anxiety, and the frequency of transitions between the two compartments, reflecting

activity, were assessed (14). Mice were excluded if they did not enter the dark box.

Study design. At 6–8 wk of age, mice were provided 3% DSS (molecular weight 36,000–50,000; MP Biomedical, Solon, OH; no. 160110) in drinking water ad libitum for 5 days and replaced with normal drinking water for the remainder of the study. Control mice were given normal drinking water throughout the study. All mice were weighed daily and water consumption was monitored. Mice were tested for behavior and samples were collected at either 8 or 14 days post DSS, corresponding to active disease or resolution of colonic inflammation, respectively.

On the day of testing, mice were transferred in their original cages to a biosafety cabinet and allowed to acclimatize for 60 min. At this point, mice did not have access to food and water until euthanasia, for a total of ~2–3 h. After acclimatization, mice were subjected to the light/dark box test. Immediately after completion of light/dark box testing, mice were placed in clean individual cages and allowed to habituate for 60 min. After habituation, mice underwent NOR testing. Immediately after completion of behavioral testing, mice were euthanized by CO₂ inhalation and cervical dislocation. Preliminary studies in our group have demonstrated that concurrent behavioral testing does not influence the result of each test (14, 33). To maintain consistency of disease progression and resolution, we wanted to test all animals on the same day post-DSS.

At the time of euthanasia, colons and brains were isolated and fixed in formalin. Fecal samples were collected for DNA isolation. It is an important technical note that feces could not be collected from some animals owing to occurrence of diarrhea and weight loss following administration of DSS.

Histology. Distal colons were collected and fixed in formalin. Samples were embedded in paraffin, and 5- μ m sections were cut onto polarized glass slides. Sections were processed for hematoxylin and eosin staining and scored for histological damage (31).

Microbiota: qPCR. Colonic fecal samples were collected at euthanasia and frozen at -80°C . Bacterial DNA from stool was extracted by using a kit (Qiagen) following the manufacturer's instructions. Isolated DNA was amplified via qPCR using SYBR and primer sets (Table 1), targeting 16S rRNA of bacterial species (14, 30) including *Eubacteria* (housekeeping gene), *Eubacterium rectale*, segmented filamentous bacteria (SFB), *Bacillus*, *Lactobacillus*, *Enterobacteriaceae*, *Bacteroides*, and *Firmicutes* to compare overall colonization patterns between DSS and control groups as performed previously (14, 33). These primers were previously validated to exclude cross-reactivity (2, 30). The qPCR conditions consisted of 1 cycle for 2 min at 50°C , 1 cycle for 10 min at 95°C , and 40 cycles of 15 s at 95°C followed by 1 min at 60°C . A melt curve was used to ensure quality control. Results are presented as percentage expression of each species relative to total *Eubacteria*.

Probiotics. A mixture of two species of *Lactobacillus* was administered to a subset of mice via a gavage needle into the oral cavity starting 1 wk prior to onset of DSS and continued until euthanasia on day 8 post-DSS. The commercially available probiotic containing *Lactobacillus rhamnosus* (R0011) and *L. helveticus* (R0052) (Lacido-

Table 1. qPCR primers for assessment of the microbiota

| Target | Forward (5'-3') | Reverse (5'-3') |
|--|-------------------------|-------------------------|
| <i>Eubacteria</i> | ACTCCTACGGGAGGCAGCAGT | ATTACCGCGGCTGTGTCG |
| <i>Bacillus</i> | GCGGCGTGCCATAATACATGC | CTTCATCACTACGCGGGCGT |
| <i>Bacteroides</i> | GAGAGGAAGGTGCCCCAC | CGCTACTTGGCTGGTTCAG |
| <i>Enterobacteriaceae</i> | GTGCCAGCMGCCGCGGTAA | GCCTCAAGGGCACAACTCCAAG |
| <i>Lactobacillus/Lactococcus</i> | AGCAGTAGGGAATCTTCCA | CACCGCTACACATGGAG |
| <i>Segmented filamentous bacteria</i> | GACGCTGAGGCATGAGAGCAT | GACGGCAGGATTGTTATTCA |
| <i>Eubacterium rectale/Clostridium coccoides</i> | ACTCCTACGGGAGGCAGC | GCTTCTTAGTCAGGTACCGTCAT |
| <i>L. rhamnosus</i> R0011 | ACTCCAAGAGCATTACTCCTCCG | TGAATATGCCGGATCTAAGTCCA |

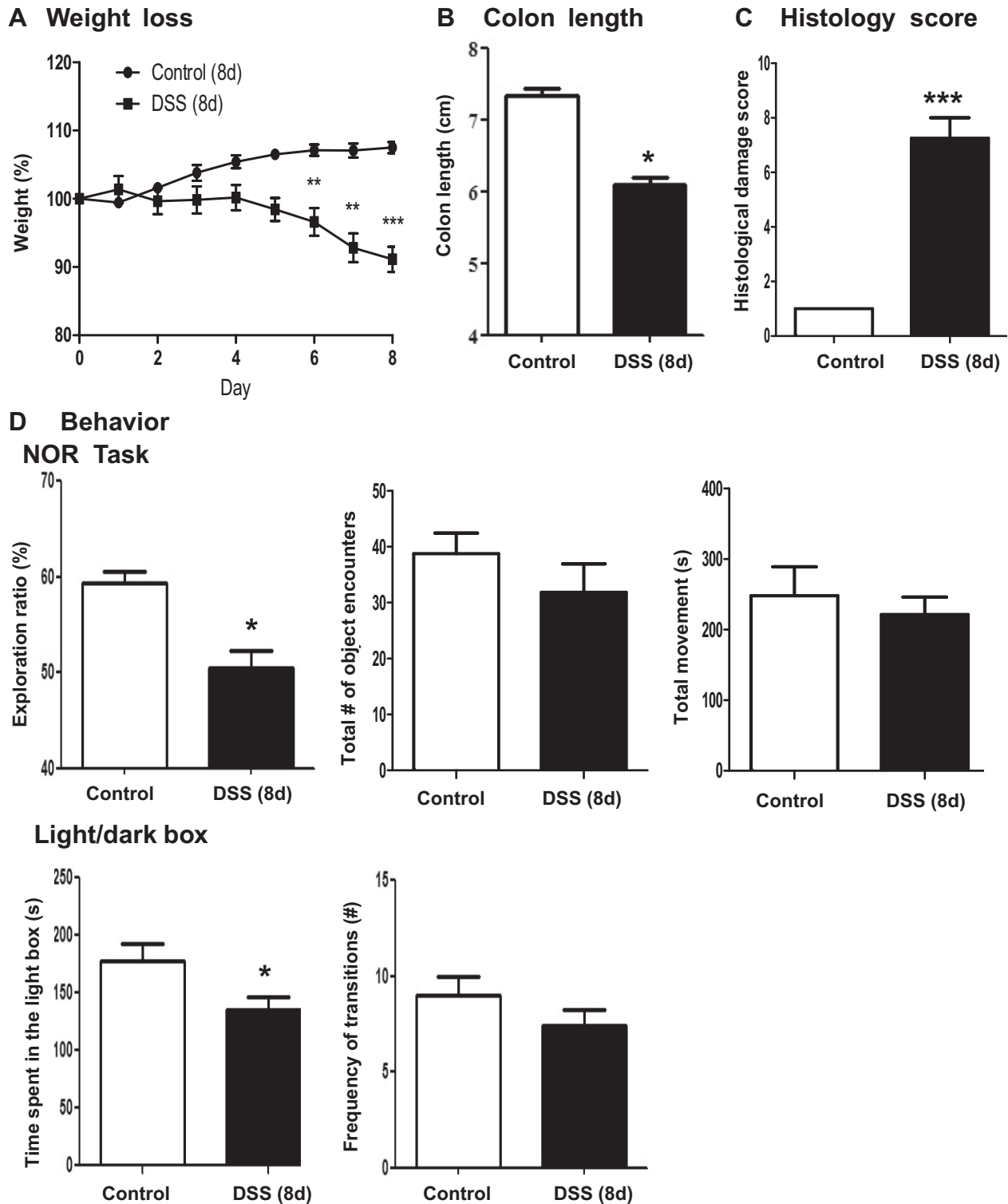


Fig. 1. DSS causes colonic disease and changes in behavior at 8 days post-DSS. DSS administration resulted in weight loss starting at 5 days and continuing until 8 days post-DSS (A). Weight loss was accompanied by colonic shortening (B) and increased histological disease scoring (C). At 8 days post-DSS, mice demonstrated a significantly lower exploration ratio compared with controls, whereas overall mobility was not affected [total no. of object encounters or total movement (s)] (D). DSS administration also resulted in decreased time spent in the light box compared with controls, without affecting the number of transitions between the light and dark portions of the box (D). * $P < 0.05$ Student's *t*-test; $n = 10$ –12 mice/group.

fil, Lallemand Health Solutions, Montreal, QC, Canada) was suspended daily in Luria-Bertani (LB) broth immediately prior to administration (200 μ l of 10^{10} CFU/ml). Lacidofil has previously been shown to ameliorate stress-induced memory defects in *Citrobacter rodentium*-infected mice (14). In contrast to our previous studies (14, 19), probiotics could not be added to the drinking water because of the confounding presence of DSS in the water. LB was used as a palatable vehicle. As a placebo control, maltodextrin was dissolved in LB broth and administered as described above.

Immunohistochemistry. Brains were isolated, fixed in 10% formalin for 48 h, embedded in paraffin, and cut (5 μ m) onto glass slides as described previously (33). Briefly, rehydrated sections were subjected to heat-induced epitope retrieval (citrate buffer), blocked (Bloxall, Vector Laboratories; 5% normal goat serum, Jackson Immune Research), and exposed to primary antibody (anti-c-Fos; 1:200, Abcam) overnight. Detection was performed (Vectastain Elite ABC kit) and samples were developed (ImmPACT-DAB; Vector). Quantification of c-Fos was performed with ImageJ by an observer who was blinded to the experimental conditions (K. Huynh). Briefly, images of the CA1 were taken by light microscopy at $\times 10$ magnification. Total numbers of c-Fos-positive cells were counted and compared with total number of cells in the image; numbers were normalized to percent of control.

For each sample, left and right hippocampus sections were analyzed and averaged from one to two serial sections.

Statistics. Results are expressed as means \pm SE. Statistically significant differences were assessed by Student's *t*-test or ANOVA followed by a Newman-Keuls post hoc test as appropriate. Two-way ANOVA was used to assess a DSS vs. treatment effect where appropriate. All statistical analysis was performed with GraphPad (San Diego, CA) and $P < 0.05$ was considered significant.

RESULTS

DSS administration in mice results in colitis. As expected, DSS administration to mice via the drinking water (3% wt/vol) resulted in maximal weight loss 8 days post-DSS (Fig. 1A). Correspondingly, by day 8 post-DSS, colon lengths were significantly shorter in the DSS group compared with controls (Fig. 1B). Histological analysis at 8 days post-DSS demonstrated colonic inflammation (Fig. 1C) with increased histological damage scores. Colons from the DSS group showed neutrophilic infiltration, increased vascularization, and edema, which were absent in controls.

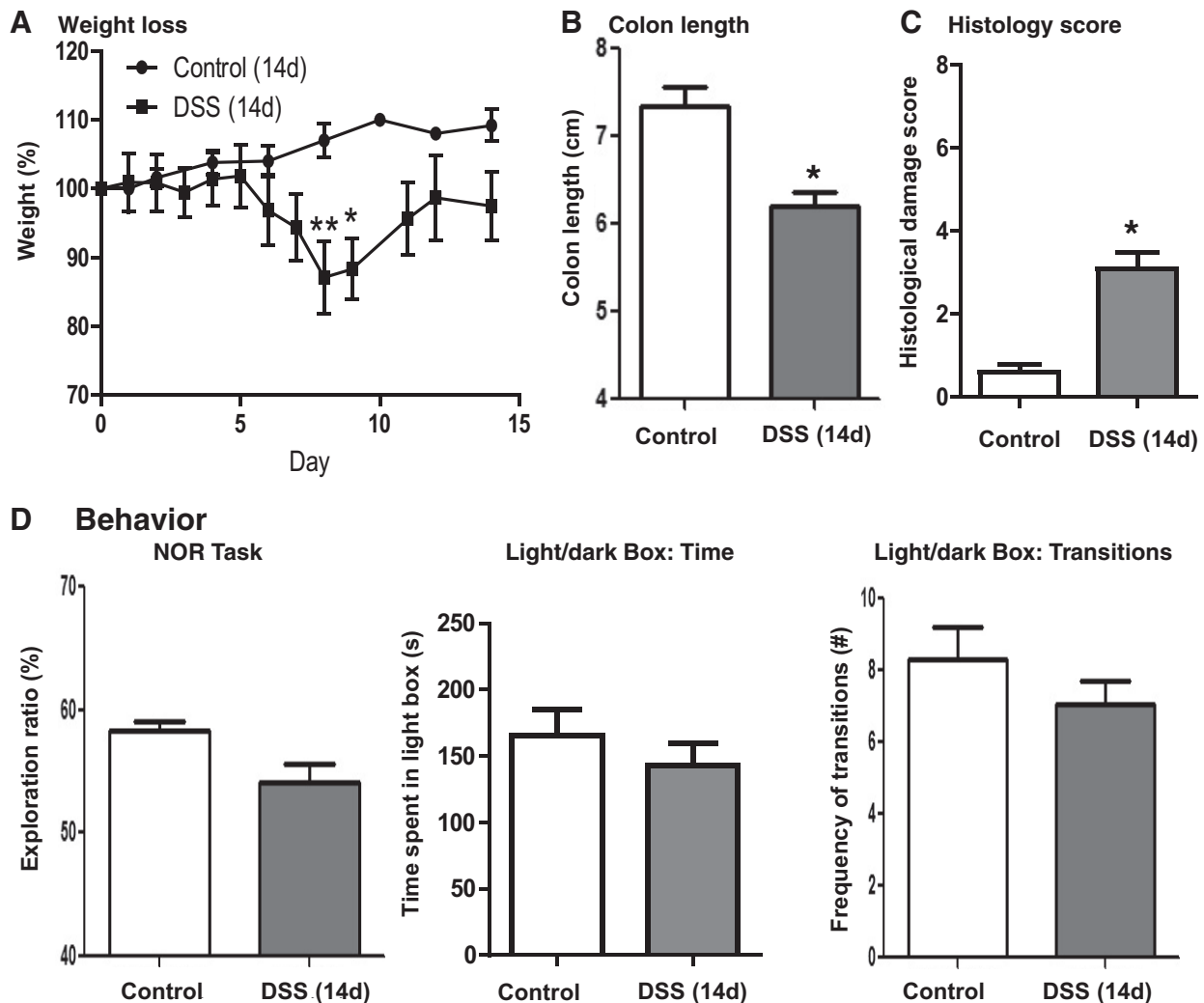


Fig. 2. Amelioration of colonic inflammation reverses changes in behavior. By 14 days post-DSS, weight loss in DSS-treated mice is restored (A). Colonic shortening was observed (B), and histology scores elevated in mice receiving DSS (C), although ameliorated from those recorded 8 days post-DSS (Fig. 1, B and C). Behavior, including recognition memory and anxiety, was normalized following amelioration of DSS-induced colitis (D). * $P < 0.05$ Student's *t*-test; $n = 10$ –12 mice/group.

DSS-induced colitis results in memory impairment and anxiety-like behavior without affecting mobility or exploratory behavior at the peak of inflammation. Recognition memory was assessed by the NOR task and calculated as the frequency of exploration of a novel vs. a known object. DSS-treated mice demonstrated impairment in recognition memory compared with controls as indicated by a significantly lower exploration ratio (Fig. 1D) at 8 days post-DSS.

Anxiety-like behavior was assessed by use of the light/dark box. At 8 days post-DSS, treated mice demonstrated anxiety-like behavior as indicated by a decrease in the total

time spent in the light box vs. the dark box compared with controls (Fig. 1D).

The total number of encounters with objects and the total amount of activity in the NOR task [total movement (s)] was quantified, with no effect of DSS on both these parameters (Fig. 1D, middle and right, NOR task). In addition, the number of times mice transitioned between the light and dark portions of the light/dark box was calculated as an indicator of overall exploratory behavior. The frequency of transitions was not affected at the peak of colonic inflammation caused by DSS compared with controls (Fig. 1D, right, light/dark box).

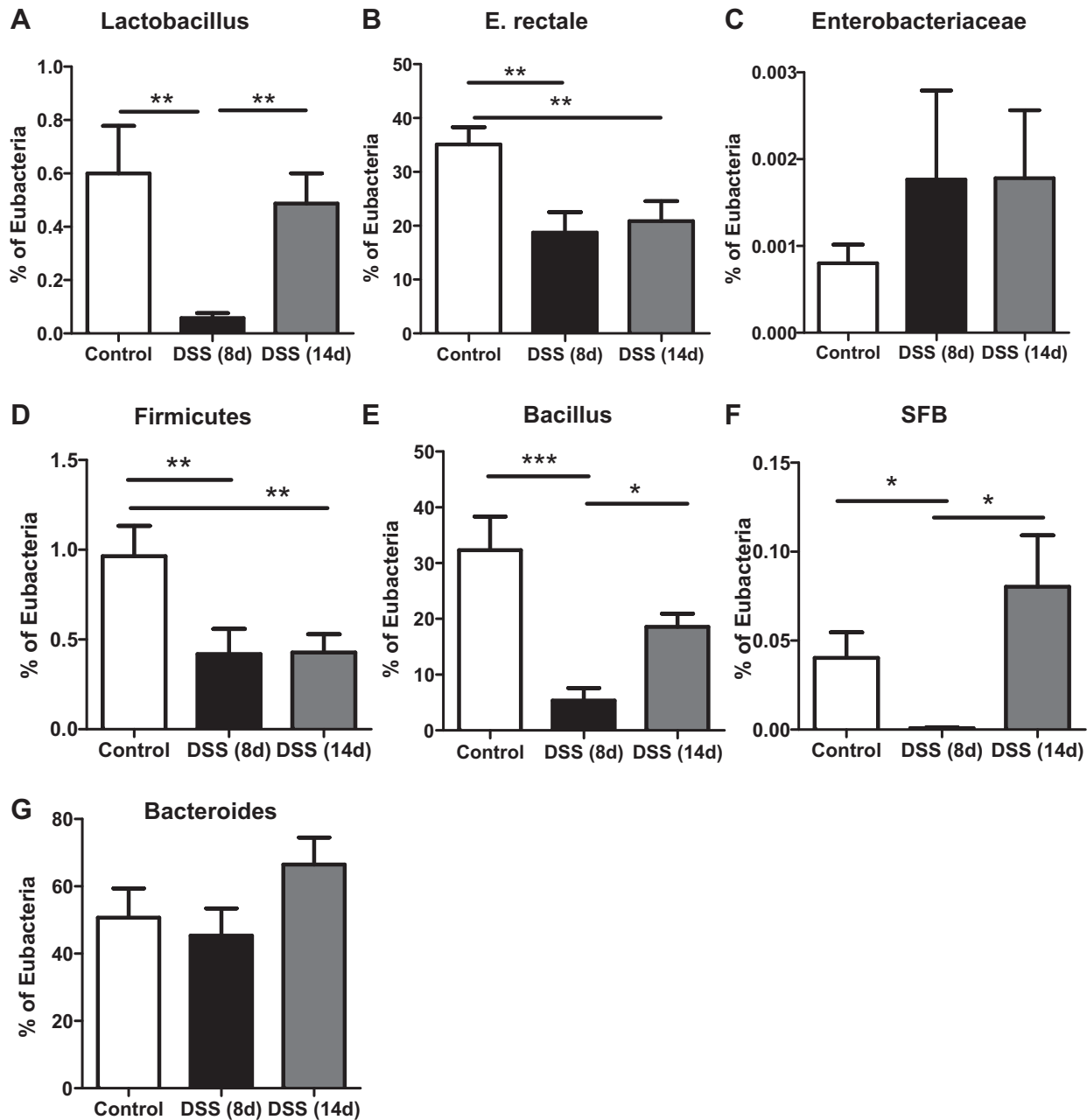


Fig. 3. Alterations in the composition of the gut microbiota in DSS-treated mice. The proportion of *Lactobacillus* (A), *E. rectale* (B), *Bacillus* (E), and SFB (F) was significantly decreased 8 days after the administration of DSS compared with controls. The shifts in *Lactobacillus* (A), *E. rectale* (B), *Bacillus* (E), and SFB (F) were reversed by 14 days post-DSS. Levels of *Enterobacteriaceae* (D) and *Bacteroides* (G) were unaffected by DSS administration. The proportion of *Firmicutes* was not impacted at 8 days post-DSS; however, a significant decrease in *Firmicutes* occurred by 14 days post-DSS (G). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the relevant control by ANOVA; $n = 8 - 12$.

Changes in behavior following DSS-induced colitis are normalized as colitis resolves. Weight loss following development of DSS-induced colitis began to reverse by 9 days post-DSS and normal weight gain then continued until euthanasia on day 14 post-DSS, corresponding to ongoing resolution of inflammation (Fig. 2A). However, despite the gain in weight in DSS-treated mice, colon length (Fig. 2B) and histology scores (Fig. 2C) remained significantly different from controls, although improved compared with disease observed at 8 days, suggesting ongoing recovery from disease. In contrast, behavior was no longer impacted at 14 days post-DSS, with recognition memory and anxiety-like behavior similar to those observed in control mice (Fig. 2D).

DSS administration causes shifts in the composition of gut microbiota. The composition of the microbiota was assessed at both 8 and 14 days post-DSS by qPCR for the 16S-rRNA gene and compared with control mice. The proportion of *Lactobacillus*, *E. rectale*, *Firmicutes*, *Bacillus*, and SFB (Fig. 3) were significantly decreased in the 8 days post-DSS group compared with controls. These losses in *Lactobacillus*, *Bacillus*, and SFB were normalized by 14 days post-DSS (Fig. 3, A, E, and F). In contrast, the representation of neither *Enterobacteriaceae* nor *Bacteroides* was impacted by DSS (Fig. 3, C and G). Finally, the decrease in the proportion of *E. rectale* and *Firmicutes* was not restored by 14 days post-DSS (Fig. 3, B and D).

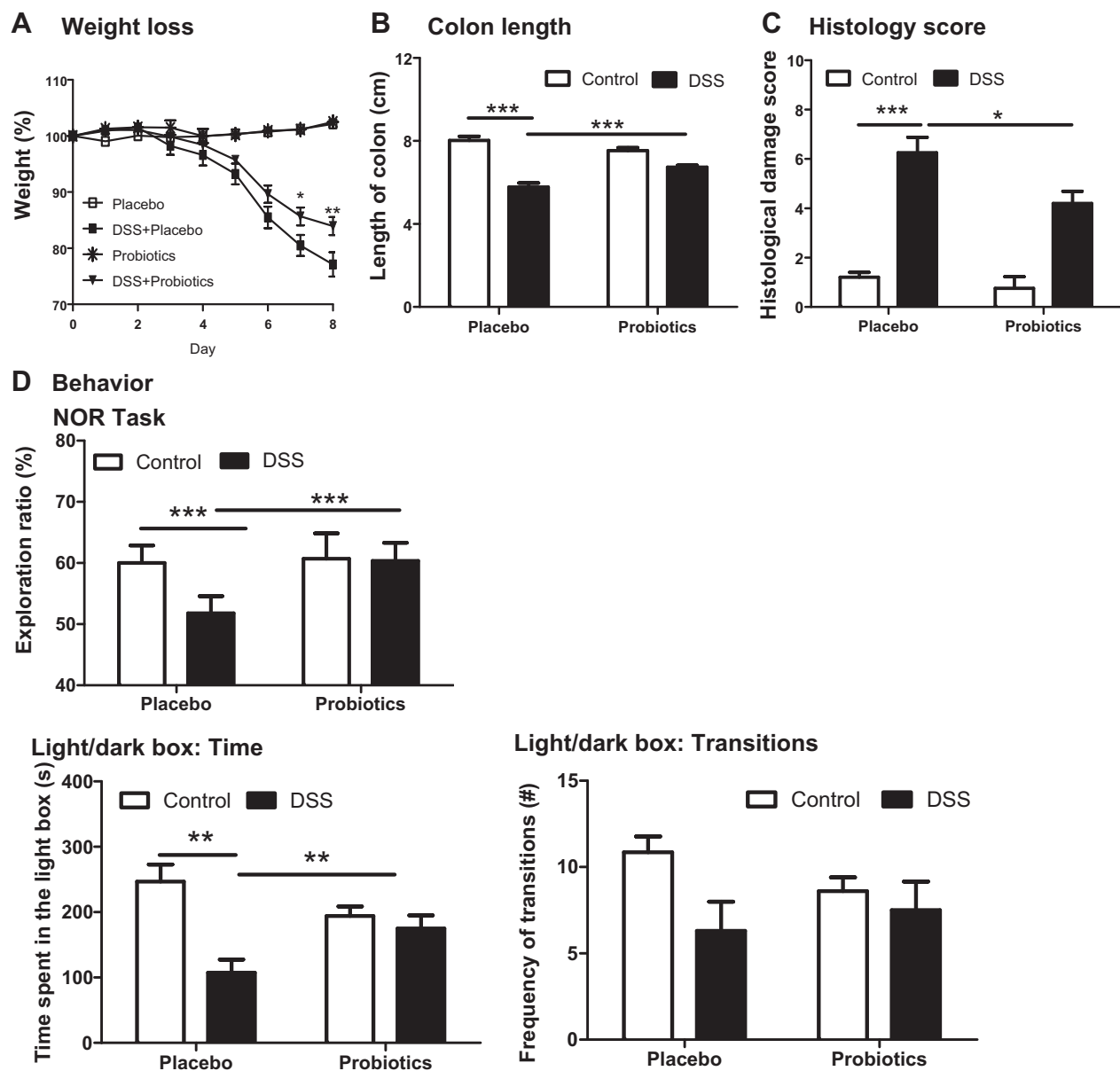


Fig. 4. Probiotics ameliorate DSS-induced colonic disease and changes in behavior. Probiotics ameliorated DSS-induced disease as indicated by reduced weight loss (A), increased colon length (B), and decreased histological disease activity score (C) compared with those receiving placebo. The DSS-induced impairments in both recognition memory and anxiety-like behavior were improved with probiotic administration (D). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the relevant control by 2-way ANOVA; $n = 9-12$.

Probiotic administration diminishes colonic disease, ameliorates behavioral deficits, and restores the composition of the microbiota following induction of DSS colitis. Administration of probiotics starting 1 wk prior to DSS protected against DSS-induced weight loss compared with mice treated with placebo (Fig. 4A). Similarly, probiotics prevented colonic shortening observed at 8 days post-DSS (Fig. 4B) and ameliorated histological damage (Fig. 4C) compared with the placebo-treated group. We demonstrated a DSS-induced and a probiotic-induced effect (2-way ANOVA) for both parameters.

Administration of probiotics significantly improved deficits in recognition memory and diminished anxiety-like behavior following induction of colitis compared with the placebo-treated group (Fig. 4D), illustrating a disease and treatment effect (2-way ANOVA) for both behaviors. Exploratory be-

havior, quantified by transitions between the light and dark box, was not affected by probiotic administration (Fig. 4D). There was no effect of probiotics alone on behavior (1 way-ANOVA).

The influence of probiotics or placebo on the gut microbiota was assessed at 8 days post DSS by qPCR. Probiotic administration in DSS-treated mice resulted in an increase in *Lactobacillus*, *E. rectale*, *Bacillus*, and SFB compared with the DSS group that received placebo (Fig. 5, A, B, E, and F; demonstrated by 2-way ANOVA). No effect of probiotics was seen on levels of *Enterobacteriaceae*, *Firmicutes*, and *Bacteroides* (Fig. 5, C, D, and G). Probiotics alone caused a significant decrease in *Bacillus* and a significant increase in SFB (Fig. 5, E and F; 1-way ANOVA). Using specific primers for *L. rhamnosus* strain R0011 (the strain composing 95% of the

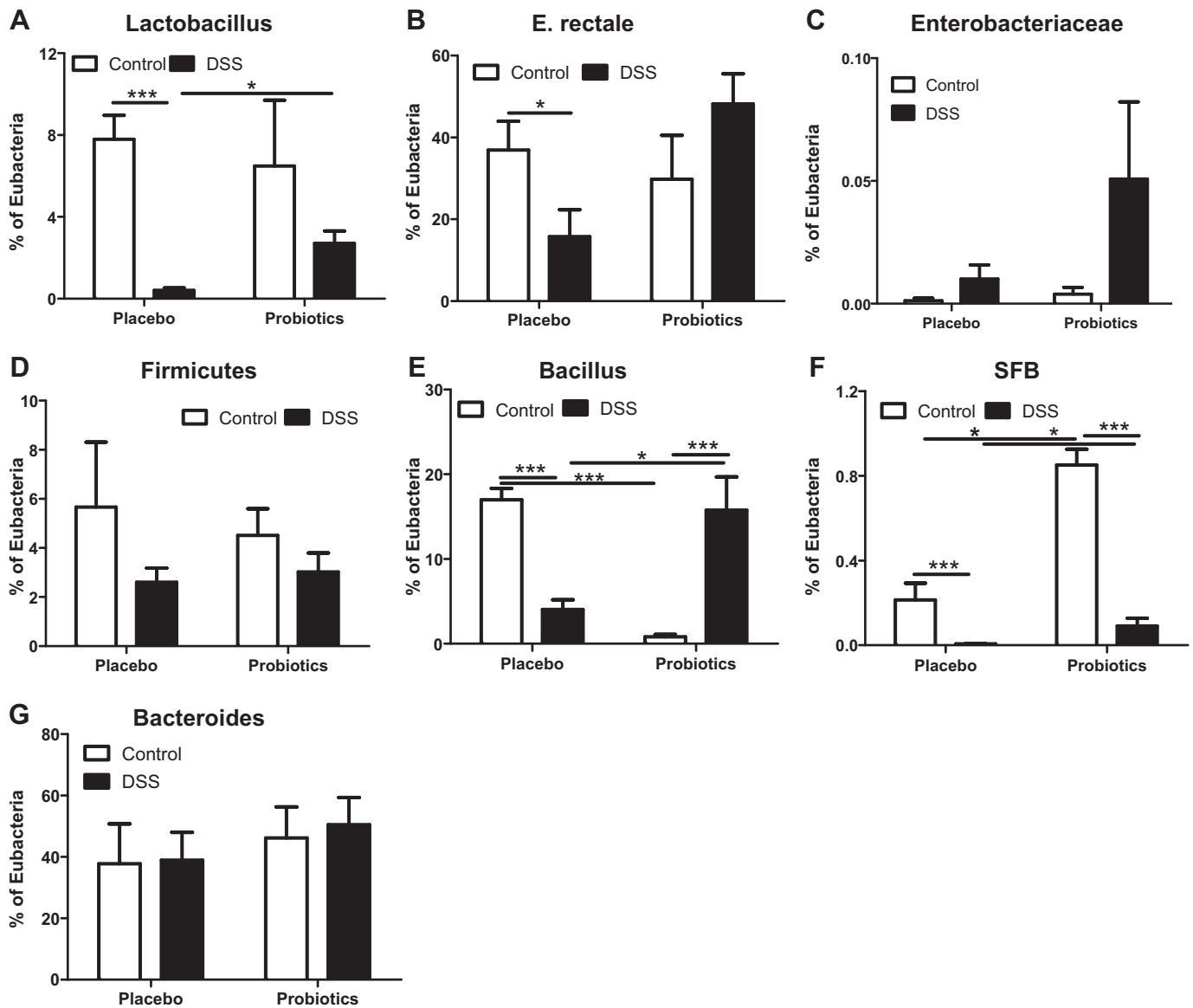


Fig. 5. Probiotic administration ameliorated DSS-induced dysbiosis. The reductions in *Lactobacillus* (A), *E. rectale* (B), and *Bacillus* (E) were reversed by probiotic administration in DSS-treated mice compared with the DSS group that received placebo. Levels of SFB (F) were increased by probiotic administration but could not be restored following DSS administration. The proportion of *Enterobacteriaceae* (C), *Firmicutes* (D), and *Bacteroides* (G) were not impacted by DSS administration with probiotics nor with placebo compared with controls. * $P < 0.05$, *** $P < 0.001$ compared with the relevant control by 2-way ANOVA; $n = 6-10$.

probiotic cocktail), we were able to demonstrate the presence of the probiotics in the fecal microbiota ($0.04 \pm 0.01\%$ of total Eubacteria [$n = 3$]) from those mice that were treated with probiotics but not those administered placebo, indicating that colonization with the probiotic took place, at least transiently.

Finally, we assessed c-Fos expression in the CA1 region of the hippocampus to assess neuronal activation in response to colonic inflammation (14, 32, 33). Hippocampal activation has been implicated in development of long-term memory (9). Moreover decreased c-Fos expression has been correlated with deficits in recognition memory (33). DSS-induced colitis led to a significant decrease in the number of c-Fos-positive cells in the CA1 region of the hippocampus compared with placebo-treated mice (Fig. 6). Administration of probiotics restored c-Fos expression compared with placebo.

DISCUSSION

This study demonstrates that acute DSS-induced colitis in mice is associated with impaired recognition memory and anxiety-like behavior. These behavioral deficits were transient in nature, as they were normalized when colonic inflammation abated at 14 days post-DSS. Changes in the composition of the gut microbiota were also present, coinciding with the changes in behavior, and partially restored by day 14. Administration of probiotics starting 1 wk prior to DSS initiation and continued until euthanasia prevented the changes in behavior elicited by DSS, in part by modulating the microbiota and hippocampal c-Fos expression. Taken together, these findings suggest that colonic inflammation results in shifts in the gut microbiota, which, in turn, are associated with deficits in mood and behavior.

The NOR task was used to assess cognitive function in mice (11), by testing the ability to remember a familiar vs. a novel object. DSS administration resulted in impairment of recognition memory at 8 days post-DSS, which normalized by 14 days post-DSS. Previous studies have shown that shifts in the composition of the gut microbiota, as demonstrated by terminal restriction length polymorphisms in IL-10 knockout model of colitis, correlate with altered memory as shown by the Barnes maze (25). Our group has also previously demonstrated that infection with the murine enteric bacterial pathogen *C. rodentium*

results in stress-induced memory impairment as measured by the NOR task (14). Given that colonic inflammation - such as that seen in IL-10^{-/-} mice, *C. rodentium* infection, and DSS-induced colitis is correlated with memory deficits, inflammation may serve as a common trigger for the altered cognitive function observed in these models. This hypothesis is further substantiated by recent evidence that chronic colitis leads to reduced hippocampal neurogenesis (36). Taken together, these findings suggest that colonic inflammation may lead to reduced hippocampal neurogenesis and cognitive deficits.

Anxiety-like behavior was assessed in mice by the light/dark box test (5). DSS administration resulted in anxiety-like behavior at 8 days post-DSS, as indicated by a decreased time spent in the light box, which normalized by 14 days post-DSS. Similar to our findings, chronic DSS administration has previously been shown to result in anxiety-like behavior as measured by the latency to step-down test (3). As an indicator of overall activity level and locomotor behavior, the frequency of transitions between the light and dark portions of the box was recorded and compared between control and experimental groups. In addition, total encounters with both objects and total movement time were both calculated during the NOR task to assess overall activity between the groups. Because the frequency of transitions between the light/dark box, the total number of encounters with objects, and overall movement in the arena between the two groups were similar, overall activity levels likely are not impaired by colonic disease and weight loss seen in DSS-induced colitis. This supports the notion that the deficit in recognition memory and anxiety-like behavior we observed was not simply resulting from sickness behavior in the DSS mice, whereby sick mice would be expected to be less active. This suggests that the presence of cognitive deficits and anxiety-like behavior in an acute murine model of IBD mimics findings observed in patients.

Recently, evidence for a role for the microbiota in mediating anxiety-like behavior and cognitive function has emerged, although it is conflicting. GF mice demonstrate either anxiety-like behavior (17) or anxiety-like behavior (24) compared with colonized control mice, depending on the strain. Similarly, recognition memory was impaired in GF mice (14), suggesting a role for the microbiota in regulating this function. Dysbiosis has been demonstrated to occur following induction of DSS colitis (15) with amelioration following probiotic administration (22), prompting us to assess in our cohort whether this dysbiosis was transient, and recovered following initiation of resolution of inflammation. Using qPCR to study the overall composition of the gut microbiota, we demonstrated that DSS-induced colitis led to temporary changes in the microbiota that were, in part, restored by 14 days post-DSS. Despite these changes, we cannot exclude a role for DSS itself in affecting the microbiota at day 8 post-DSS. Previous work by our group using a *C. rodentium* infection model demonstrated that normalizing the gut microbiota by administration of probiotics could rescue the behavioral alterations and cognitive deficits caused by infection (14). In our present studies, we demonstrated that shifts in the composition of the gut microbiota in DSS-induced colitis broadly coincided with behavioral changes. This was particularly true for changes in *Lactobacillus* and SFB, which are known to be important in the context of colonic inflammation. Despite these interesting findings, follow-up studies using fecal microbiota transplant or cohous-

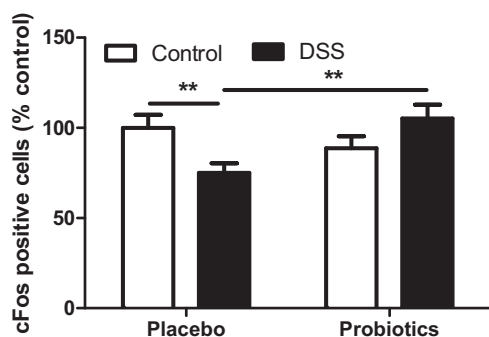


Fig. 6. Decreased hippocampal c-Fos expression in the CA1 region following DSS was prevented by probiotic administration. Administration of DSS (+ placebo) resulted in a significant decrease in the number of c-Fos-positive neurons in the CA1 region of the hippocampus. This decrease was prevented by daily administration of probiotics starting 1 wk prior to DSS administration. ** $P < 0.01$ compared with the relevant control by Student's *t*-test; $n = 5-6$; data presented as a % of placebo control.

ing strategies to normalize the microbiota would be required to demonstrate causality. In addition, in-depth analysis of the microbiota by 16S sequencing is also warranted in future studies.

Many species of *Lactobacillus* are classified as probiotic organisms that are beneficial to the host. In our study, a decrease in *Lactobacillus* at 8 days post-DSS was observed, which returned to normal by 14 days post-DSS. Many members of the *Lactobacillus* genus are capable of metabolizing lactose to lactic acid and other products. Metabolic by-products of *Lactobacillus* are postulated to influence memory function. A recent study showed that one product in particular, Calpis sour milk whey, a fermented milk product produced with *L. helveticus*, improves memory as indicated by the NOR task in mice (26). *Lactobacillus* consumption has also been shown to increase GABA expression in the brain, an effect not seen in vagotomized mice (6). Consequently, the vagus nerve may serve as an important conduit in the brain-gut axis responsible for communicating the underlying shift in microbiota to the brain. Therefore, the decrease in *Lactobacillus* species observed at 8 days post-DSS, but not at 14 days post-DSS, may in part explain the impact of the microbiota on behavior.

Similarly to *Lactobacillus*, a decrease in SFB was also observed at 8 days post-DSS, which was restored with the reduction of inflammation at 14 days post-DSS. SFB have recently been shown to play a primary role in the induction of T helper 17 (Th17) cell responses (18, 21) that are important in inflammation, particularly in IBD (13). SFB are also critical to the maintenance of gut homeostasis because they serve a protective role against pathogenic bacteria, such as *C. rodentium* (18). Therefore, in this study, the decrease in SFB colonization at 8 days may be playing a role in modulating behavior in our DSS mice, with recovery by 14 days post-DSS, although a precise mechanism for this potential regulation is not currently known.

Administration of probiotics increasingly is being used as a means to modify the composition of the gut microbiota beneficially in both health (6) and disease (3, 14). Different probiotic formulations have proven effective in limiting disease in mouse models of IBD, including DSS (16, 28). Given the association of the microbiota with DSS-induced behavioral changes, the effect of probiotics was assessed. A combination of *L. rhamnosus* and *L. helveticus* not only reduced the severity of colitis but also corrected defects in recognition memory and anxiety-like behavior observed at 8 days post-DSS. In addition, in a related study, we demonstrated that immune-deficient Rag1 mice displayed behavioral abnormalities similar to those induced by DSS, including impaired recognition memory and anxiety-like behavior, and that these were ameliorated following administration of probiotics (33). As seen in the present study, administration of *Lactobacillus*-containing probiotics modified the gut microbiota and modulated hippocampal c-Fos expression, suggesting a common beneficial pathway in regulating behavior. Interestingly, as also seen in our Rag1 mice (33), administration of probiotics did not significantly increase the overall abundance of *Lactobacillus*, compared with placebo, perhaps in part due to a prebiotic effect of the maltodextrin (33). We did, however, isolate the specific strains of probiotics by qPCR, confirming their presence in the feces. Together, these findings demonstrate the beneficial effects of probiotic administration on the microbiota-gut-brain axis in the

context of IBD and provide a rationale for the use of probiotics to ameliorate behavioral deficits seen in patients.

In conclusion, mice subjected to DSS-induced acute colitis demonstrate changes in behavior, including anxiety-like behavior and cognitive deficits, which may result from shifts in the composition of the gut microbiota due to colonic inflammation. Administration of probiotics normalized changes in behavior, which broadly correlated with restoration of the microbiota. Overall this suggests that, in acute inflammation, changes to the microbiota may modulate behavior, including cognitive function and anxiety-like behavior. Elucidating the full impact of colitis-associated dysbiosis may provide a key to combating behavioral comorbidities in patients suffering from IBD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.R.E., K.H., C.R., K.E.B., and M.G.G. conception and design of research; J.R.E., K.H., E.N.M., M.K., C.R., and M.G.G. performed experiments; J.R.E., K.H., E.N.M., M.K., C.R., and M.G.G. analyzed data; J.R.E., K.H., and M.G.G. interpreted results of experiments; J.R.E., K.H., and M.G.G. prepared figures; J.R.E., K.H., and M.G.G. drafted manuscript; J.R.E., K.H., K.E.B., and M.G.G. edited and revised manuscript; J.R.E., K.H., K.E.B., and M.G.G. approved final version of manuscript.

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