Relationships Between Diet-Related Changes in the Gut Microbiome and Cognitive Flexibility

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Title: RELATIONSHIPS BETWEEN DIET-RELATED CHANGES IN THE GUT MICROBIOME AND COGNITIVE FLEXIBILITY

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Abbreviations:

ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
LSD	least significant difference
NMDA	N-methyl-D-aspartate
NW	northwest
OTU	operational taxanomic units
SE	southeast

Abstract

Western diets are high in fat and sucrose and can influence behavior and gut microbiota. There is growing evidence that altering the microbiome can influence the brain and behavior. This study was designed to determine whether diet-induced changes in the gut microbiota could contribute to alterations in anxiety, memory or cognitive flexibility. Two month old, male C57BL/6 mice were randomly assigned high fat (42% fat, 43% carbohydrate (CHO), high sucrose (12% fat, 70% CHO (primarily sucrose) or normal chow (13% kcal fat, 62% CHO) diets. Fecal microbiome analysis, step-down latency, novel object and novel location tasks were performed prior to and two weeks after diet change. Water maze testing for long- and short-term memory and cognitive flexibility was conducted during weeks 5-6 post-diet change. Some similarities in alterations in the microbiome were seen in both the high fat and high sucrose diets (e.g., increased Clostridiales), as compared to the normal diet, but the percentage decreases in Bacteroidales were greater in the high sucrose diet mice. Lactobacillales was only significantly increased in the high sucrose diet group and Erysipelotrichales was only significantly affected by the high fat diet. The high sucrose diet group was significantly impaired in early development of a spatial bias for long-term memory, short-term memory and reversal training, compared to mice on normal diet. An increased focus on the former platform position was seen in both high sucrose and high fat groups during the reversal probe trials. There was no significant effect of diet on step-down, exploration or novel recognitions. Higher percentages of Clostridiales and lower expression of Bacteroidales in high-energy diets were related to the poorer cognitive flexibility in the reversal trials. These results suggest that changes in the microbiome may contribute to cognitive changes associated with eating a Western diet.

Keywords: Executive function, intestinal microbiota, Western diet, Clostridiales, Bacteroidales, sucrose

The Western diet contributes to many chronic, diet-related illnesses in the United States, including the obesity epidemic (Cordain et al., 2005). Western diets are typically high in fat and simple carbohydrates (Cordain et al., 2005). Higher intake of fats and refined sugars are associated with deficits in cognitive flexibility and hippocampal-dependent memory in humans (Kalmijn, 2000, Francis and Stevenson, 2011) and an increase in the incidence of Alzheimer's disease (Pasinetti, 2002). There is evidence of a vicious cycle of hippocampal damage associated with a Western diet, followed by increased energy intake (Kanoski and Davidson, 2011, Kanoski, 2012). It is not clear whether these effects are due to direct or indirect influences of alternative energy sources on the brain.

Diets high in fat and/or sugar alter the microbiome of the gut (Li et al., 2009, Turnbaugh et al., 2009). Western diets in rodents are associated with increases in microbes in the phylum Firmacutes and decreases in Bacteriodetes (Turnbaugh et al., 2009, Ohland et al., 2013, Daulatzai, 2014, Patterson et al., 2014). Obese mice with a leptin mutation and obese people also show similar alterations in prevalence of these microbial phyla, compared to their lean counterparts (Ley et al., 2005, Ley et al., 2006b).

The gut microbiome in humans consists of approximately 100 trillion microorganisms (Ley et al., 2006a). Firmacutes and Bacteriodetes make up the majority of this population in the mouse and human gut (Ley et al., 2005, Ley et al., 2006a, Ley et al., 2008, Turnbaugh et al., 2009). Firmacutes are predominantly gram-positive bacteria, and include three classes: Bacilli, Clostridia and Erysipelotrichia (Ludwig et al., 2009). Bacteriodetes are gram-negative, anaerobic, rod-shaped bacteria, found in the environment and guts of animals (Eckburg et al., 2005). In recent years it has become increasingly clear that the intestinal bacteria impact main functions in the body, including maturation of the immune system and metabolic processes. In fact, the

effect of the composition of the microbiome in obesity is a good example of the importance of the bi-directional communication between the host and the resident microflora.

There is increasing evidence for an influence of the microbiome on the brain and behavior (Collins et al., 2012). Probiotics and/or antimicrobials can alter memory, anxiety and long-term potentiation and hippocampal and amydala levels of brain-derived neurotrophic factor (BDNF) in animals (Bercik et al., 2011, Collins et al., 2012, Davari et al., 2013, Hsiao et al., 2013). Fermented milk with a probiotic reduced activation of brain regions associated with emotional responses in humans (Tillisch et al., 2013). The probiotic contained primarily bacteria from the Fimicutes; Bacilli; Lactobacillales order (Ludwig et al., 2009, Tillisch et al., 2013). A lean ground beef diet promoted greater bacterial diversity in the intestine and improved working and reference memory (Li et al., 2009). The current study was designed to determine whether specific changes within the microbiome could account for alterations in cognitive functions. High-energy diets were used to manipulate the gut microbiome (Li et al., 2009, Turnbaugh et al., 2009).

2. EXPERIMENTAL PROCEDURES

2.1. Animals

Eighteen male C57BL/6J mice (8 weeks of age) were purchased from JAX Laboratories (Bar Harbor, ME). Mice were housed at Oregon State University's animal facilities under a 12/12 hour light/dark cycle with food and water available on an *ad libitum* basis. Three siblings were housed together in each cage for the first 2 weeks on a control chow diet (normal; Table 1). The mice were rotated between uncleansed cages for 2 weeks, until all the mice had been exposed similarly, in order to establish uniform microbiome baseline. Mice were then individually housed and, within each trio, were randomly assigned to a 42% fat diet (high fat), 70% carbohydrate diet (66% sucrose; high sucrose) or normal diet group (Table 1). Fecal collections for

microbiome analysis and step-down latency and novel object and novel location tasks were performed prior to the diet change and were repeated two weeks after the diet change. Water maze testing for long- and short-term memory and cognitive flexibility was conducted during weeks 5 and 6 post-diet change.

2.2 Microbiome analysis

2.2.1. DNA extraction and 16S sequencing.

Genomic DNA was extracted from approximately 100 mg of fecal sample using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions with the following changes: during the step 2 vortex step manual homogenztion with a sterile pestle was performed to break up the sample, in step 3 the samples were heated to 94 degrees for 5 minutes, and step 6 vortexing was done for 4 minutes. All samples were diluted to 3ng/ul and 5 ul of template went into each DNA amplification. Custom Lib-L Forward Primer A primers with unique MID barcodes, and Custom Lib-L Primer B primers with no MID, were designed to amplify the V3 and V4 regions of the 16S rRNA bacterial gene from each individual sample for sequencing.

Forward Primer Lib-L Primer A CCATCTCATCCCTGCGTGTCTCCGAC***T CAG***	**MID** ACACGACGACT	**Sequence Specific - LH 16SF** GTGCCAGCMGCCGCGGTAA
Reverse Primer Lib-L Primer B CCTATCCCCTGTGTGCCTTGGCAGTC***T CAG***	**No MID**	**Sequence Specific - LH 16S R** GAGCTGACGACARCCATGCA

The barcoded 16S amplicons were sequenced using the Roche 454 GS Jr sequencing platform at the Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR). A total of 36 mouse fecal microbiota samples were sequenced and analyzed. We obtained a total of 196,297 reads from the run with a mean of 5453 reads per sample.

2.2.2. Analysis of 16S rRNA sequence.

The raw sequence data were demultiplexed, quality filtered and analyzed further using the QIIME software for comparison and analysis of microbial communities (Caporaso et al., 2010). The sequences were clustered into operational taxanomic units (OTUs) at 97% sequence similarity using the uclust method against the Greengenes database (McDonald et al., 2012). The constructed OTU tables were then used to determine the relative percentage of each bacteria for each sample. The phylogeny based UniFrac distance metric as part of the QIIME package was then used to investigate the differences in the microbial communites.

2.3. Behavioral testing

2.3.1. Step-down latency

The step-down latency task was conducted, as described by Anisman and coworkers (Anisman et al., 2001), one week prior to the diet change and two weeks after the diet change. Briefly, mice were placed on a platform (12.5 X 8.5 cm) elevated 4 cm from a metal surgical table in a dimly lit room. The latency to step down off of the platform (all four paws on the table) was recorded. Three trials (5 minute maximum) were conducted with a 1-minute inter-trial interval. Two different rooms were used and a block design was utilized for animals to be tested in different rooms pre- versus post-diet change.

2.3.2 Novel object/location recognition

Novel object recognition and change of object location recognition tasks were based on procedures used by Hohmann, et al. (Hohmann et al., 2007), with some modifications. The arenas consisted of plastic boxes (12"W X 18"D X 10"H). The objects and arenas were made of glass or plastic and were cleaned with 70% ethanol between sessions.

There were a total of 8 sessions over 3 days. Each session was 5 minutes in duration.

Session 1: habituation to an empty arena, 7 minute break, Session 2: exploration of 5 objects in a training location pattern, 1 hour break, Session 3: Novel object test (1 of the 5 objects was replaced by a novel object), 23 hour break, Session 4: Novel object test (another of the 5 objects was replaced by a new object), 7 minute break, Sessions 5 & 6: Continued training of location pattern, 1 hour break, Session 7: Two objects were moved to new locations, 23 hour break, Session 8: Two other objects were moved to new location. The habituation trial was also used to assess open field behavior; the trial was tracked and analyzed for % time in center (area defined as 10 cm from each wall), exploration distance traveled and speed with the use of SMART tracking system (San Diego Instruments, San Diego, CA). For novel object and location tasks, exploration time for each object was obtained by manual timing for Sessions 2-4 and 6-8 and was expressed as a percent of the total exploration time for a single object. The explorations times of the 2 moved objects were averaged to give a mean exploration time per object.

2.3.3 Morris water maze - Memory and cognitive flexibility

Mice were acclimated to the water maze for 2 days prior to place training, which consisted of each animal swimming for 60 seconds in the tank without the platform and then being trained to remain on the platform for 30 seconds each day. Spatial long- and delayed short-term memory, cognitive flexibility and associative memory were assessed using the Morris water maze, as described previously with some modifications (Magnusson et al., 2003b, Das et al., 2012b). Briefly, following acclimation, mice underwent 4 days of long-term spatial memory testing and one day of reversal task, in order to assess cognitive flexibility (Das et al., 2012b). The mice underwent place training for long-term memory for 8 trials per day for 4 days, with a 2-minute inter-trial interval. The platform remained in the same quadrant (SE) for all the place platform trials. During the trials for place training, the mice were placed in the tank, facing the wall of the tank, and were allowed to search for the platform for 60 seconds. They remained on the

platform for 30 seconds before being removed to their cages for 2 minutes. Entry points were along the wall in the middle of the quadrants that did not contain the platform and were varied between trials within each day. There was a 90 minute break after the first 4 place trials of each day and a one hour break after the 8th trial of each day, followed by a probe trial, which was designed to assess the animal's memory for the platform location (Gallagher et al., 1993). The platform was not present during the probe trial and the animal was allowed to search for 30 seconds. Reversal trials were used to assess cognitive flexibility (Das et al., 2012b). One day following long-term memory training, the platform was moved to the opposite quadrant (NW) and 8 reversal trials and one reversal probe trial were conducted as described above.

A delayed short-term spatial memory task was then performed (Magnusson et al., 2003a). The task consisted of two daily sessions of 4 place trials each for 4 days. The platform position changed for each session. Sessions consisted of a naïve trial, followed by a trial after a 10-minute delay and 2 more trials at 30-second delays (not used for evaluation). Trials consisted of 60 seconds maximum in the water searching for the platform and 30 seconds on the platform. Following the delayed short-term spatial memory task, an associative (control) memory task (Das et al., 2012a) was employed to test motivation, visual acuity, and physical ability for memory testing.

2.4. Data Analysis

Bacterial orders and genera with averages of <.01 percent within all diet groups and sequences that couldn't be assigned to a phylum were eliminated for further analysis. The percent of microbiome was recalculated as a percentage of the remaining taxa. Body weight, food consumption and percent of microbiome were analyzed by two-way ANOVA, followed by post-hoc analysis with Tukey's correction for multiple comparisons (treating all bacterial comparisons as one family) with Prism software (Graphpad Software, Inc., www.graphpad.com).

Data for water maze testing were analyzed as described earlier with a few modifications

(Das and Magnusson, 2008). Briefly, the distance of the animal from the platform was measured every 0.2 second by the computer for the whole duration of the trial. Cumulative proximity was calculated by adding together the distance calculated at each 0.2-second interval. Correction for start position was performed using a macro in Excel software (Microsoft Corp., Seattle). A cumulative proximity measurement for the ideal path was calculated by this macro, with the use of starting position, average swim speed and platform position. This cumulative proximity measure for the ideal path was subtracted from the cumulative proximity score for the whole track to obtain the corrected cumulative proximity scores for the place, reversal, short-term memory and cued control trials. For the probe trials, the corrected cumulative proximity score for the trial was divided by the corrected sample number to obtain a corrected average proximity score. Statistical analysis for behavioral testing was performed by repeated measures ANOVA (diet X trial (X session)) or (diet X Pre- vs. Post-diet), followed by Fisher's protected LSD where applicable, with the use of Statview software (SAS Institute, Inc., Cary, N.C.). For novel object recognition, performance compared to a chance level of 20% exploration was analyzed with one-sample t-tests. Correlational analysis was performed using linear regression analysis with Prism software. A p-value cutoff of .01 was used to determine significance.

3. RESULTS

3.1 Body weight and food consumption

The effects of the high-energy diets on weight gain and food consumption were monitored throughout the experiment. There was a significant main effect of Time ($F(5,75)=66$, $p < .0001$) and a significant Time X Diet interaction ($F(10,75)=7.5$, $p<.0001$), but no main effect of Diet ($F(2,15)=.56$, $p=.58$) on body weight (Fig. 1A). The mice on the high fat diet weighed significantly more than those on the high sucrose diet ($p<.01$), but only at 5 weeks post-diet change (Fig. 1A). There was a significant main effect of Diet ($F(2,15)=8.5$, $p=.004$) and Time

(F(4,60)=54, p <.0001) on changes in body weight from before the diet change (Pre-diet; Fig. 1B). Mice on the high fat diet gained more weight after the diet change than both mice on normal (p<.05) and high sucrose (p<.01) diets when data was collapsed across weighings (Fig. 1B). There was a significant main effect of Diet (F(2,15)=31, p<.0001) and Time (F(4,60)=7.2, p <.0001) on average daily food consumption (kcal/day; Fig. 1C). Mice that remained on the chow diet (Normal) consumed more than both those on the high fat and high sucrose diets (p<.0001; Fig. 1C) when data was collapsed across weighings.

3.2 Microbiome changes

Feces were examined prior to the diet change in order to assess the efficacy of the cage rotations for normalizing the microbiome between diet assignment groups. There was a significant main effect of Bacterial Order (F (9,150) = 968.6, p<.0001), but no significant interaction between Pre-diet Assignment (Fig. 2; A,B, & C) and Bacterial Order (F (18,150) = .06, p>.99) in percent of the microbiome represented by each order before the diet change (Fig. 2 (Pre-diet change)). High-energy diets were used in this study as a method to alter the gut microbiome (Li et al., 2009, Turnbaugh et al., 2009). In order to verify the effects of diet on the microbiome, fecal 16S RNA was also examined 2 weeks after the diet change. Order level analysis of bacteria was used initially to screen for diet effects and later for correlations with behavior. There was a significant main effect of Bacterial Order (F (9,150) = 625.8, p<.0001) and a significant interaction between Diet and Bacterial Order (F (18,150) = 74.55 p<.0001) in percent of the microbiome represented by each order after the diet change (Fig. 2 (Post-diet change); Table 2). Mice that remained on the normal diet post-diet change had a larger percentage of the microbiome as Bacterioidales than those on high fat diet, which had a significantly higher percentage than the high sucrose group (p<.0001; Fig. 2; Table 2). The high sucrose animals had a higher percentage of Lactobacillales than both the normal (p<.0001) and high fat (p<.05) diet groups (Fig. 2; Table 2). Both the high fat and high sucrose groups had

larger percentages of the microbiome represented by Clostridiales than normal (p<.0001) and the high fat group's percentage of Erysipelothrichales was higher than normal (p<.05; Fig. 2; Table 2). Tenericutes; Mollicutes; Anaeroplasmatales was only found in the mice on the normal diet (Table 2).

In order to determine whether all identified bacteria within an order were affected by diet similarly, analysis was also performed at the genus level. There was a significant main effect of Bacterial Genera ($F(56,840) = 235.8$, $p<.0001$) and a significant interaction between Diet and Bacterial Genera ($F(112,840) = 30.35$, $p<.0001$) in percent of the microbiome represented by each genera (Table 2). Three genera of Bacteroidiales showed significantly lower and two of Clostridiales showed significantly higher percentages in both the high fat and high sucrose groups, as compared to the mice remaining on normal diet (Table 2). The mice on the high sucrose diet had higher percentages of Lactobacillus and Lactococcus than those on normal diet (p<.001-.0001). Lactobacillales; Enterococcus was only found in the high sucrose diet group (Table 2).

3.3 Step-down latency and open field exploration

In order to ultimately address the hypothesis that specific changes in the microbiota can influence behavior, the effects of diet on anxiety in this study were examined with a simple step-down latency task and open field exploration. There was no significant effect of Diet ($F(2,15)=.09$, $p=.92$), but a significant effect of Session ($F(1,15)=11.4$, $p =.004$) on step-down latency (Fig. 3A). On average, mice had lower latencies after the diet change than before, when data was collapsed across diet groups (Fig. 3A). During the habituation trial for the novel object and location tasks, there was a near significant effect of Diet ($F(2,15)=3.4$, $p=.06$) and a significant effect of Session ($F(1,15)=44$, $p<.0001$) on percent time spent in the center of the open field (Fig. 3B). Averaged across diet groups, mice spent a larger percentage of time in the

center following the diet change than before (Fig. 3B). There was no effect of Diet on the path length or average speed during open field exploration post-diet change (p=.88-.89; Fig. 3-C,D).

3.4 Novel object and location recognition

Five objects were used to assess the effects of diet on recognition memory for objects and spatial alterations of objects. There was no significant effect of Diet (p=.13-.79) or pre- vs. post-diet change Session (p=.57-.99) on novel object or novel location percent of exploration time for either 1 hour or 24 hours post-familiarization (Fig. 3E-H). For novel object recognition, mice performed above chance during the 1 hour delay session after the diet change (Fig. 3E; p=.026) and pre- and post-diet change in the 24 hour delay session (Fig. 3F; p=.0007-.036) when data were collapsed across diet groups. Exploration of objects that changed location did not occur above chance (Fig. 3G,H; p=.2-.3).

3.5 Morris water maze - Memory and cognitive flexibility

Place trials, with the platform present in the same location, in the Morris water maze were used to examine the effects of diet and associated microbial changes on acquisition of long-term memory. There was no significant effect of Diet ($F(2,15)=.15$, $p=.86$), but a significant effect of blocks of four place trials ($F(7,105)=27$, $p<.0001$) on cumulative proximity for long-term memory in the water maze (Fig. 4A). There was a significant reduction in cumulative proximity between blocks 1 and 8 of place trials ($p<.0001$; Fig. 4A), when data was collapsed across diet groups.

Probe trials, with the platform absent, were used to analyze the development of a spatial bias, as another measure of long-term memory. There was no main effect of Diet ($F(2,15)=.21$, $p=.81$), but there was a significant interaction between Diet and Trial ($F(8,60)=2.2$, $p=.04$) and a significant main effect of Trial ($F(4,60)=16.6$, $p<.0001$) on average proximity in the probe trials for long-term memory (Fig. 4B). In probe trial 1, mice on the high sucrose diet had significantly

higher average proximities than those on the normal diet (p=.04), but in probe trial 4, the mice on the high sucrose diet had a near significantly lower average proximity than the normal diet group (p=.07; Fig. 4B). Lower proximity scores are indicative of a more accurate search for the platform. There was a significant reduction in average proximity between probe trials 0 and 4 (p<.0001; Fig. 4B), when data was collapsed across diet groups.

Reversal trials, with the platform moved to the opposite quadrant, were used to assess cognitive flexibility; i.e., an animal's ability to adapt to changing conditions. There was no overall effect of Diet ($F(2,15)=2.6$, $p=.11$) on cumulative proximity to the new platform location in the reversal trials, but the high sucrose group had higher cumulative proximity than the normal mice (p=.04; Fig. 4C). There was no significant difference between diet groups in average proximity to the new platform location during the reversal probe trial ($F(2,15)=.53$, $p=.60$), but there was a significant effect of Diet ($F(2,15)=6$, $p=.01$) on proximity to the old platform location during the same trial (Fig. 4D). Both the high fat (p=.006) and high sucrose (p=.015) groups spent more time closer to the old platform location than the normal diet group (Fig. 4D). There were no differences between diet groups in swim speed in either the long-term memory or reversal trials (p=.5-.82; not shown).

The water maze was also used to test delayed short-term memory by analyzing the difference between a naive and 10 minute delayed trial across multiple sessions. There was no significant main effect of Diet ($F(2,29)=.71$, $p=.50$), but a near significant effect of Trial (naive vs. 10 min delay trials; $F(2,29)=3.6$, $p=.066$) on cumulative proximity in the delayed short-term memory task (Fig. 5A-D). The mice on the normal diet, however, showed a significantly larger difference between the naive and 10 minute delay trial than the mice eating high sucrose feed (p=.04; Fig. 5E). There was no significant effect of Diet ($F(2,15)=.14$, $p=.87$) on the associative memory control task (Fig. 5F).

3.6 Behavioral correlations

Correlational analyses were used to address the hypothesis that specific diet-related changes in the microbiome were related to the behavioral changes. Analysis was limited to those orders of bacteria and behavioral measurements that showed significant differences between diet groups. There was a significant correlation between both cumulative proximity averaged across reversal trials (Fig. 6A; Table 3) and the average proximity to the old platform location in the reversal probe trial (Fig. 6B; Table 3) and the order of Firmacutes; Clostridia; Clostridiales. Higher percentages of the microbiome as Clostridiales were associated with poorer performance (higher cumulative proximities) in the reversal trials and searching closer to the old platform location (lower average proximities) in the reversal probe trial. Lower expression of Bacteroidales was also associated with a lower average proximity to the old platform location in the reversal probe trial (Fig. 6C; Table 3). Higher percentages of Lactobacillales showed a trend for poorer performance in the first probe trial (Fig. 6D; Table 3).

4. DISCUSSION

This study showed alterations in multiple orders and genera of bacteria in the feces of male mice after only 2 weeks on high-energy diets. Some similarities in alterations in the microbiome were seen when comparing the normal diet to both the high fat and high sucrose diets (e.g., Clostridiales), but the percentage decreases in Bacteroidales were greater in the high sucrose diet mice. In contrast, Lactobacillales was only significantly increased in the high sucrose diet group and Erysipelotrichales was only significantly affected by the high fat diet. The mice on the high sucrose diet had significant problems with cognitive flexibility, working memory, and development of a spatial bias early in training for long-term memory. Higher percentages of Clostridiales and lower percentages of Bacteroidales in the high fat and high sucrose diet groups were associated with poorer performance in the reversal trials for assessing cognitive flexibility.

Many changes in the microbiome from the normal diet were found in both the high sucrose and high fat groups, but the animals on the high sucrose diet showed more significant alterations. There were no differences in bacterial expression between the assigned groups prior to the diet change, suggesting that the cage rotation was successful in normalizing the microbiota across subjects. Post-diet change, the order Bacteriodales, as well as members within its family Porphyromonadaceae, were significantly reduced in representation within the fecal microbiome in the high fat diet group, as compared to mice on the normal diet. The high sucrose group had even greater declines, having significantly lower expression than both the normal and high fat diet groups. Bacteriodales includes gram-negative, anaerobic, bacteria that are commonly found in the environment, gut and skin (Eckburg et al., 2005). Bacteria within the phylum Firmacutes tended to increase in prevalence in the high fat and/or the high sucrose diet groups. Bacteria within Firmacutes are gram-positives organisms and include the gut commensal Clostridia (Ludwig et al., 2009). Similar changes in Bacteriodales and Firmacutes have been reported in animals on Western diets and in obese mice with a leptin mutation (Ley et al., 2005, Turnbaugh et al., 2008, Turnbaugh et al., 2009, Ohland et al., 2013, Patterson et al., 2014) and in obese humans (Ley et al., 2006b), but see (Vrieze et al., 2012).

There was some variation in dietary effects on the orders within the Firmacutes phylum. The order Clostridiales and bacteria within its Lachnospiraceae and Ruminococcaceae families showed increased expression in both the high sucrose and high fat diet groups. Clostridiales bacteria are gram positive, obligate anaerobes. Lactobacillales and its genera Lactobacillus and Lactococcus were significantly increased, compared to normal, only in animals on the high sucrose diet. The genus Enterococcus was only expressed in the high sucrose diet group, but the percentage was less than .2%. Lactobacillales bacteria are gram-positive and Lactobacillus is commonly found in probiotics (Reid, 1999). Erysipelotrichales bacteria were only significantly increased from normal in the high fat diet mice. A similar increase in this order has been seen with humanized mice on a diet enriched in fat (Turnbaugh et al., 2009).

Significant declines in performance in the water maze were confined to the mice on the high sucrose diet. Four weeks after the diet change, mice on the high sucrose diet showed impairment in retrieval of long-term memory in the probe trial on the first day of testing. There was, however, no significant effect of diet overall on place trial learning for long-term memory. This suggests that performance was similar across groups when the platform was present, but the high sucrose diet animals had not developed a strong enough spatial bias on the first day to retrieve the memory one hour after training (Gallagher et al., 1993). Long Evans rats that were supplemented with sucrose showed impairment in the water maze throughout long-term memory testing after 5 weeks on the supplemented diet (Jurdak et al., 2008), suggesting that continued exposure to the diet may exacerbate memory deterioration. However, Sprague-Dawley rats on a high-energy diet showed spatial long- and short-term memory problems in a radial maze task after only 72 hours, and maintained these behavioral changes up to 90 days (Kanoski and Davidson, 2010). Non-spatial long- and short-term memory deficits also emerged, but only after 30 days (Kanoski and Davidson, 2010). The relatively small effect of diet on long-term memory in the present study could be due to species and/or task differences. The mice on the high sucrose diet in this study did, however, show significant deficits in spatial short-term memory, similar to the rats (Kanoski and Davidson, 2010).

Several types of Lactobacillales, including Enterococcus, Lactococcus and Lactobacillus, increased in prevalence in the high sucrose diet group, which also had the greatest deficits in early development of a spatial bias and in spatial short-term memory. There was also a trend for animals with higher percentages of Lactobacillales to be more impaired in developing a spatial bias early in training. Interestingly, Lactobacillus is often included in probiotic treatments (Reid, 1999), which have been shown to reduce stress-induced memory problems; altering short-term memory impairments and N-methyl-D-aspartate (NMDA) receptor and BDNF changes associated with stress (Collins et al., 2012). Lactobacillus- and bifidobacterium-containing probiotics enhance place learning in the water maze in diabetic and control rats and memory

and long-term potentiation in diabetic rats (Davari et al., 2013). It is possible that the increases seen in Lactobacillus in this study were too small to counteract the negative effects of other bacterial or dietary changes, or that the high sucrose diet enhanced the prevalence of non-beneficial species of Lactobacillales. No significant relationships were found between any of the altered bacterial orders and short-term memory performance in this study.

The high sucrose group showed continued improvement in long-term memory, even showing a trend for improved performance over the normal diet mice by Day 4, indicating that the mice were able to develop a spatial bias with increased repetition. However, the mice on the high sucrose diet were then impaired in learning a new platform location, which suggests the mice had deficits in cognitive flexibility. There was not a significant difference between diet groups in proximity to the new platform location during the probe trial after one day of reversal training, but both the high fat- and high sucrose-fed mice spent more time during that probe trial near the old platform location than the chow-fed mice. Rats on high fat diets rich in dextrose show impairments in discrimination reversal learning, which is related to reductions in BDNF in both the prefrontal cortex and hippocampus (Kanoski et al., 2007). Whether the performances in the reversal training and probe trials was due to enhanced hippocampal learning following extended training or problems with perseveration, involving the prefrontal cortex (Moffat et al., 2007), remains to be determined.

Both Bacteroidales and Clostridiales showed progressive changes across diets and relationships to reduced cognitive flexibility. Increased Clostridiales in the high-energy diet groups was related to impaired performance in reversal trials with the platform present and an increased search near the old platform position in the reversal probe trial. Lower percentages of Bacteroidales were also related to searching more for the old platform position than the normal animals.

Both Clostridiales and Bacteroidales have been associated with autism. In a study looking at autism and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), the most

commonly recruited population in afflicted individuals was Clostridiales; Ruminococcaceae (De Angelis et al., 2013), which was also increased in expression in both of the high energy diet groups in the present study. Treatment with Bacteroides fragillis improves gut permeabiilty and decreases autism spectrum disorders in the maternal immune activation mouse model (Hsiao et al., 2013). Prenatal exposure to valproic acid, another mouse model of autism spectrum disorders, causes increases in Clostridiales and decreases in Bacteroidales, similar to high-energy diets. These animals have deficits in social and repetitive behaviors (de Theije et al., 2014), which involve prefrontal cortex (Mundy, 2003, Lopez et al., 2005). These repetitive behaviors are influenced by alterations in working memory and response inhibition (Lopez et al., 2005). Most of the microbiota/brain interaction studies show changes in BDNF expression or electrophysiology in the hippocampus and/or amygdala (Bercik et al., 2011, Gareau et al., 2011, Neufeld et al., 2011, Collins et al., 2012, Davari et al., 2013). However, there is evidence of changes in GABA receptors in the prefrontal cortex with Lactobacillus rhamnosus treatment (Bravo et al., 2011). These studies support a relationship between alterations in Clostridiales and Bacteroidales and perseveration in the reversal trials. However, in individuals with cirrhosis of the liver and hepatic encephalopathy, higher expression of members of the Clostridiales, including Lachnospiraceae and Ruminococcaceae families, and lower expression of Bacteroidales; Porphyromonadaceae and Rikenellaceae were associated with good cognition (Bajaj, 2014). It seems likely that Clostridiales and Bacteroidales interact with the status of other systems in the body to influence higher brain function.

Mice on the high fat diet did not significantly differ from the chow-fed mice in behavioral testing, except for remaining focused on the old platform position during the reversal probe trial. Rats on high fat diets for 90 days are impaired in reversal discrimination learning, working memory and rules learning (Greenwood and Winocur, 1990, Kanoski et al., 2007). Sprague-Dawley rats that became obese on a high fat diet showed impaired hippocampal function and breached blood brain barrier (Davidson et al., 2012). The high fat diet mice in this study showed

enhanced weight gain as compared to both other groups across all weeks, but only exhibited greater body weight than the group on the high sucrose diet on the last weighing. Long Evans rats that were supplemented with Crisco showed impairment in the water maze on day 1 of testing after 5 weeks (Jurdak et al., 2008). Mice fed 45% fat for 12 months, however, showed no significant deficits in the water maze at 5 and 10 months (Mielke et al., 2006). Interestingly, mice fed 33% fat, in the form of lean ground beef, showed better long- and short-term memory than those on 12% fat (Li et al., 2009).

There was no effect of diet on step-down latency or time in the center of the open field, indicating that the diets did not significantly affect anxiety. The lack of differences in speed of movement and distance traveled in the open arena and/or water maze suggest that the water maze results were not likely to be due to mobility differences. There were no effects of the diets on novel object or location recognition in this study, although Long-Evans rats fed sucrose for a longer duration (8 weeks) were impaired in the 1 hour retention test (Jurdak and Kanarek, 2009). It is likely that the novel location task was too difficult, since none of the groups performed above chance.

5. CONCLUSION

This study showed that both high-energy diets caused shifts in the composition of the microbiome, but more bacterial orders and genera were altered by the high sucrose diet than the high fat group. The high sucrose diets also interfered with early development of a spatial bias, cognitive flexibility and working memory to a greater degree than high fat diets. A diet-induced increase in bacteria from the order Clostridiales and a decrease in Bacteroidales were both associated with poor cognitive flexibility. These results suggest that specific bacterial orders within the microbiota may contribute to the effects of Western diets on cognitive function.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

Anisman H, Hayley S, Kelly O, Borowski T, Merali Z (2001) Psychogenic, neurogenic, and systemic stressor effects on plasma corticosterone and behavior: mouse strain-dependent outcomes. Behav Neurosci 115:443-454.

Bajaj JS (2014) The role of microbiota in hepatic encephalopathy. Gut microbes 5:397-403.

Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, Collins SM (2011) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141:599-609, 609 e591-593.

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF (2011) Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America 108:16050-16055.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature methods 7:335-336.

Collins SM, Surette M, Bercik P (2012) The interplay between the intestinal microbiota and the brain. Nature reviews Microbiology 10:735-742.

Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J (2005) Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr 81:341-354.

Das SR, Jensen R, Kelsay R, Shumaker M, Bochart R, Brim B, Zamzow D, Magnusson KR (2012a) Reducing expression of GluN1(0XX) subunit splice variants of the NMDA receptor interferes with spatial reference memory. Behavioural Brain Research 230:317-324.

Das SR, Jensen R, Kelsay R, Shumaker M, Bochart R, Brim B, Zamzow D, Magnusson KR (2012b) Reducing expression of GluN1(0XX) subunit splice variants of the NMDA receptor interferes with spatial reference memory. Behavioural brain research 230:317-324.

Das SR, Magnusson KR (2008) Relationship between mRNA expression of splice forms of the zeta1 subunit of the N-methyl-D-aspartate receptor and spatial memory in aged mice. Brain Res 1207:142-154.

Daulatzai M (2014) Obesity and Gut's Dysbiosis Promote Neuroinflammation, Cognitive Impairment, and Vulnerability to Alzheimer's disease: New Directions and Therapeutic Implications. J Mol Genet Med S1:005.

Davari S, Talaei SA, Alaei H, Salami M (2013) Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. Neuroscience 240:287-296.

Davidson TL, Monnot A, Neal AU, Martin AA, Horton JJ, Zheng W (2012) The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats. Physiol Behav 107:26-33.

De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, Francavilla R (2013) Fecal microbiota and metabolome of

children with autism and pervasive developmental disorder not otherwise specified. PLoS One 8:e76993.

de Theije CG, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, Garssen J, Kraneveld AD, Oozeer R (2014) Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav Immun 37:197-206.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308:1635-1638.

Francis HM, Stevenson RJ (2011) Higher reported saturated fat and refined sugar intake is associated with reduced hippocampal-dependent memory and sensitivity to interoceptive signals. Behav Neurosci 125:943-955.

Gallagher M, Burwell R, Burchinal M (1993) Severity of spatial learning impairment in aging: Development of a learning index for performance in the Morris water maze. Behav Neurosci 107:618-626.

Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT, Philpott DJ, Macqueen G, Sherman PM (2011) Bacterial infection causes stress-induced memory dysfunction in mice. Gut 60:307-317.

Greenwood CE, Winocur G (1990) Learning and memory impairment in rats fed a high saturated fat diet. Behav Neural Biol 53:74-87.

Hohmann CF, Walker EM, Boylan CB, Blue ME (2007) Neonatal serotonin depletion alters behavioral responses to spatial change and novelty. Brain Research 1139:163-177.

Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, Patterson PH, Mazmanian SK (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155:1451-1463.

Jurdak N, Kanarek RB (2009) Sucrose-induced obesity impairs novel object recognition learning in young rats. Physiol Behav 96:1-5.

Jurdak N, Lichtenstein AH, Kanarek RB (2008) Diet-induced obesity and spatial cognition in young male rats. Nutritional neuroscience 11:48-54.

Kalmijn S (2000) Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. J Nutr Health Aging 4:202-207.

Kanoski SE (2012) Cognitive and neuronal systems underlying obesity. Physiol Behav 106:337-344.

Kanoski SE, Davidson TL (2010) Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. Journal of experimental psychology Animal behavior processes 36:313-319.

Kanoski SE, Davidson TL (2011) Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav 103:59-68.

Kanoski SE, Meisel RL, Mullins AJ, Davidson TL (2007) The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. Behav Brain Res 182:57-66.

Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America 102:11070-11075.

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. Science 320:1647-1651.

Ley RE, Peterson DA, Gordon JI (2006a) Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124:837-848.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006b) Microbial ecology: human gut microbes associated with obesity. Nature 444:1022-1023.

Li W, Dowd SE, Scurlock B, Acosta-Martinez V, Lyte M (2009) Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav 96:557-567.

Lopez BR, Lincoln AJ, Ozonoff S, Lai Z (2005) Examining the relationship between executive functions and restricted, repetitive symptoms of Autistic Disorder. Journal of autism and developmental disorders 35:445-460.

Ludwig W, Schleifer KH, Whitman WB (2009) Revised road map to the phylum Firmicutes. In: Bergey's Manual of Systematic Bacteriology, vol. 3 (DeVos, P. et al., eds), pp 1-17 New York: Springer.

Magnusson KR, Scruggs B, Aniya J, Wright KC, Ontl T, Xing Y, Bai L (2003a) Age-related deficits in mice performing working memory tasks in a water maze. Behavioral Neuroscience 117:485-495.

Magnusson KR, Scruggs B, Aniya J, Wright KC, Ontl T, Y.Xing, Bai L (2003b) Age-related deficits in mice preforming working memory tasks in a water maze. Behav Neurosci 117:485-495.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6:610-618.

Mielke JG, Nicolitch K, Avellaneda V, Earlam K, Ahuja T, Mealing G, Messier C (2006) Longitudinal study of the effects of a high-fat diet on glucose regulation, hippocampal function, and cerebral insulin sensitivity in C57BL/6 mice. Behav Brain Res 175:374-382.

Moffat SD, Kennedy KM, Rodrigue KM, Raz N (2007) Extrahippocampal contributions to age differences in human spatial navigation. Cereb Cortex 17:1274-1282.

Mundy P (2003) Annotation: the neural basis of social impairments in autism: the role of the dorsal medial-frontal cortex and anterior cingulate system. Journal of child psychology and psychiatry, and allied disciplines 44:793-809.

Neufeld KM, Kang N, Bienenstock J, Foster JA (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 23:255-264, e119.

Ohland CL, Kish L, Bell H, Thiesen A, Hotte N, Pankiv E, Madsen KL (2013) Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. Psychoneuroendocrinology 38:1738-1747.

Pasinetti GM (2002) From epidemiology to therapeutic trials with anti-inflammatory drugs in Alzheimer's disease: the role of NSAIDs and cyclooxygenase in beta-amyloidosis and clinical dementia. J Alzheimers Dis 4:435-445.

Patterson E, RM OD, Murphy EF, Wall R, O OS, Nilaweera K, Fitzgerald GF, Cotter PD, Ross RP, Stanton C (2014) Impact of dietary fatty acids on metabolic activity and host intestinal microbiota composition in C57BL/6J mice. The British journal of nutrition 1-13.

Reid G (1999) The scientific basis for probiotic strains of Lactobacillus. Applied and environmental microbiology 65:3763-3766.

Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, Guyonnet D, Legrain-Raspaud S, Trotin B, Naliboff B, Mayer EA (2013) Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology 144:1394-1401, 1401 e1391-1394.

Turnbaugh PJ, Backhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell host & microbe 3:213-223.

Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI (2009) The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine 1:6ra14.

Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143:913-916 e917.

FIGURE LEGENDS

Fig. 1. Effects of diet on body weight (A), change in body weight (B) and average consumption per day (C). Mice on the high fat diet had a significantly higher body weight in week 5 than the high sucrose-fed animals (#; A) and greater increases in body weight across the weeks, from the weight before the diet change, than both the normal and high sucrose diet groups (#; B). C) The mice in the normal diet group consumed more per day than either the high fat- or high sucrose-fed animals across all weighings (*). Means ± SEM. N = 6. $p < .05$ for difference from high fat diet (#) or normal diet (*) groups (ANOVA & Tukey's post-hoc test).

Fig. 2. Effects of diet on the percentages of bacterial classes in the fecal microbiome pre- and post-diet change in individual animals. Numbers on the x-axis indicate cage numbers of siblings housed together prior to the diet change. The A animals remained on normal diet. The B & C animals were changed from normal to high fat or high sucrose diets, respectively. N = 6. Means and SEM detailed in Table 2.

Fig. 3. There were no effects of diet on step-down latency (A), time spent in the center of an open field (B), distance traveled (C) and speed of exploration (D) in an open field; and percent exploration of novel objects (E,F) and objects in novel locations (G,H) after a one hour (E,G) and 24 hour (F,H) delay. On average across diet groups, animals stepped down more quickly (A) and spent more time in the center of an open field (B) post-diet change, as compared to pre-diet change (§), and animals explored the novel object above chance after a 1 hour delay post-diet change (*; E) and after a 24 hour delay both pre- and post-diet change (*; F). Means ± SEM. N = 6. § indicates $p < .05$ for difference between testing pre- versus post-diet change (ANOVA & Fisher's PLSD post-hoc test). * indicates $p < .05$ for difference from a chance level of 20% exploration time within a session (one-sample t-test).

Fig. 4. Effects of diet on water maze performance for long-term memory (A,B) and cognitive flexibility (C,D). The mice on a high sucrose diet had higher proximity scores (searched more away from the platform) during probe trial 1 for long-term memory (B) and in reversal trials (C). Both the high sucrose and high fat groups searched closer to the old platform location in the reversal probe trial than the mice on normal diet (D). Means ± SEM. N = 6. * indicates $p < .05$ for difference from the normal diet group (ANOVA & Fisher's PLSD post-hoc test).

Fig. 5. Effects of diet on delayed short-term memory (A-E) and a control task of associative memory (F). A-D) Naive and 10 minute delay trials for animals on normal (A), high fat (B), and high sucrose (C) diets for each session and averaged across sessions (D). E) The high sucrose-fed mice had significantly less difference between the naive and delayed short-term memory trials than the normal diet group. F) There was no effect of diet on the associative memory task. Means ± SEM. N = 6. * indicates $p < .05$ for difference from the normal diet group (ANOVA & Fisher's PLSD post-hoc test).

Fig. 6. Correlations between bacterial orders and cognitive functions among individuals across diets. A,B) Higher percentages of Clostridiales in the microbiome were associated with poorer performance (higher proximity scores) for learning the new platform location in reversal trials (A) and with searching closer to the old platform location (lower proximity scores) in the reversal probe trial (B). C) Lower expressions of Bacteriodales were also associated with lower proximity scores for the old platform location. D) Higher percentages of Lactobacillales showed a trend for poorer performance in the first probe trial. Low proximity scores indicate a search pattern near the platform location. High proximity scores indicate that more of the search was spent away from the platform location. Significance level set to $p < .01$. N = 6.

Table 1. Comparison of diets

	Normal (chow) PicoLab Rodent Diet 20 [a]	High fat TD.88137 [b]	High sucrose TD.98090 [b]
Kcal / Kg diet	4,070	4,500	4,000
Percent of Kcal provided by:			
Protein	24.7	17.3	17.7
Carbohydrate	62.1	42.7	70.4
Fat	13.2	42.0	11.8
Sucrose, gm / Kg diet	31.8	341.46	645.6

[a] purchased from LabDiet (St. Louis, MO)

[b] purchased from Harlan Laboratories (Madison, WI)

Figure 1

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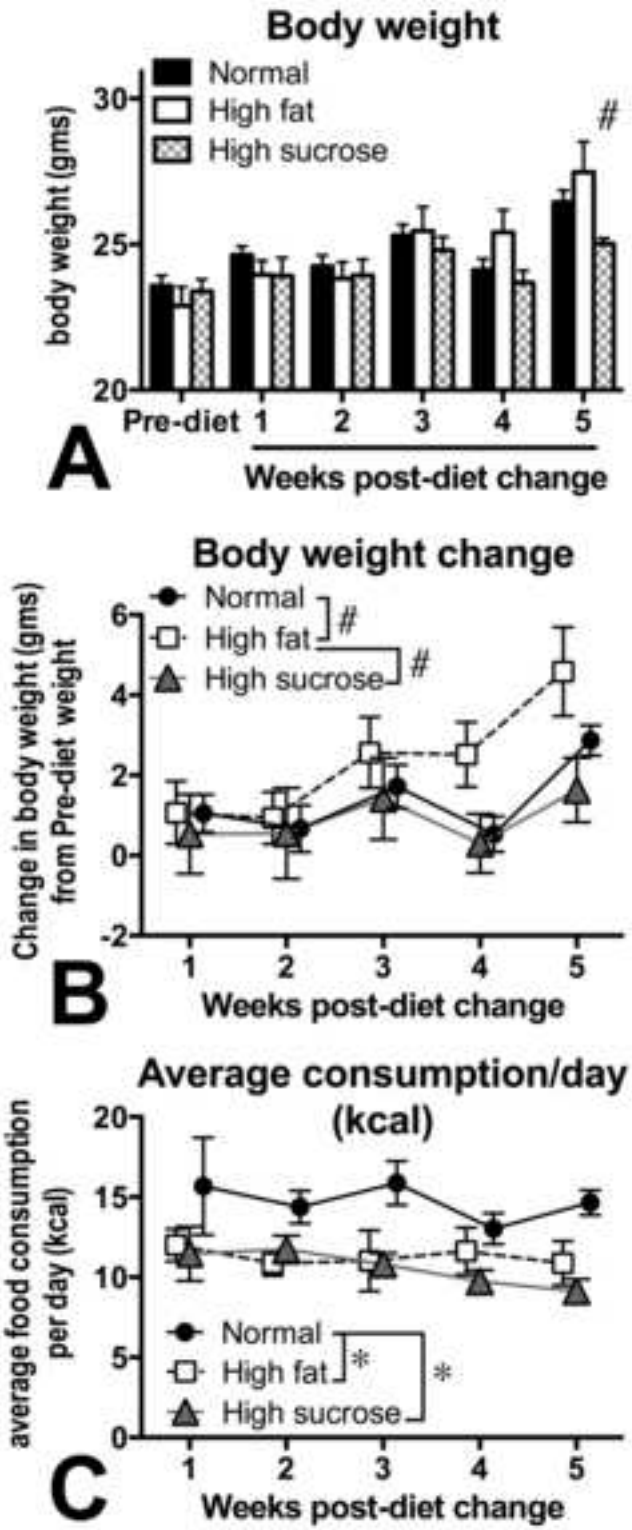

Figure 1

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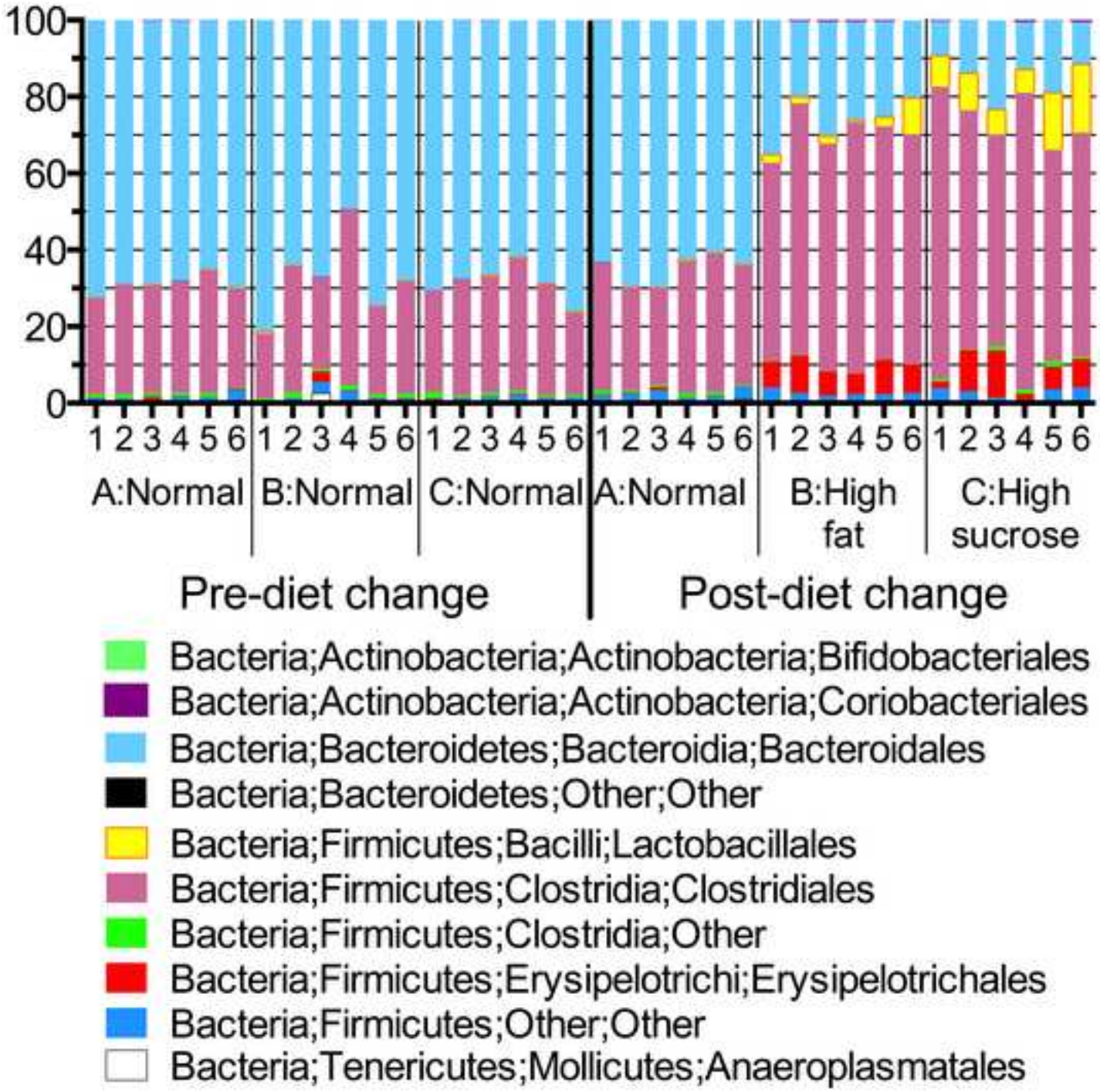

Figure 2

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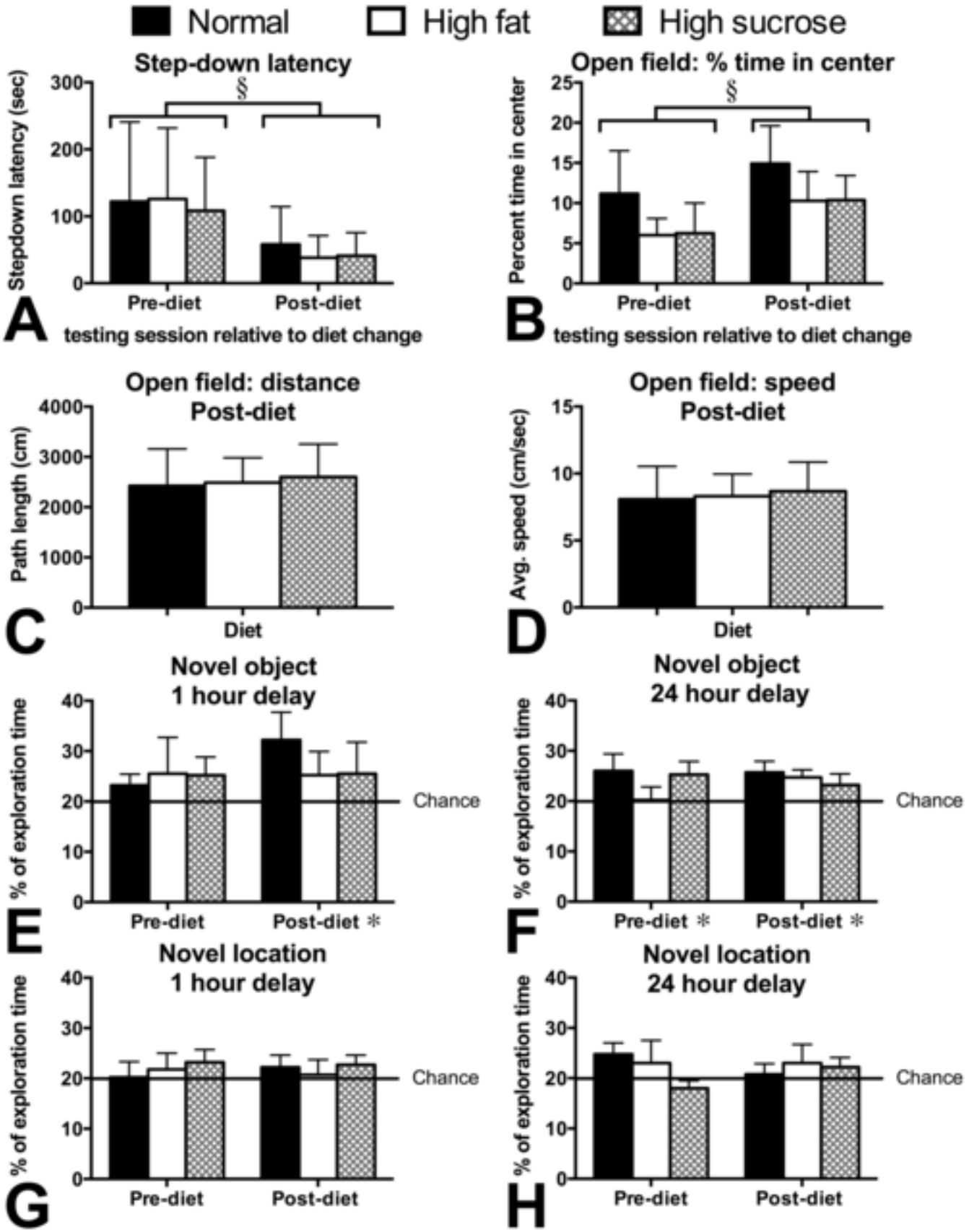

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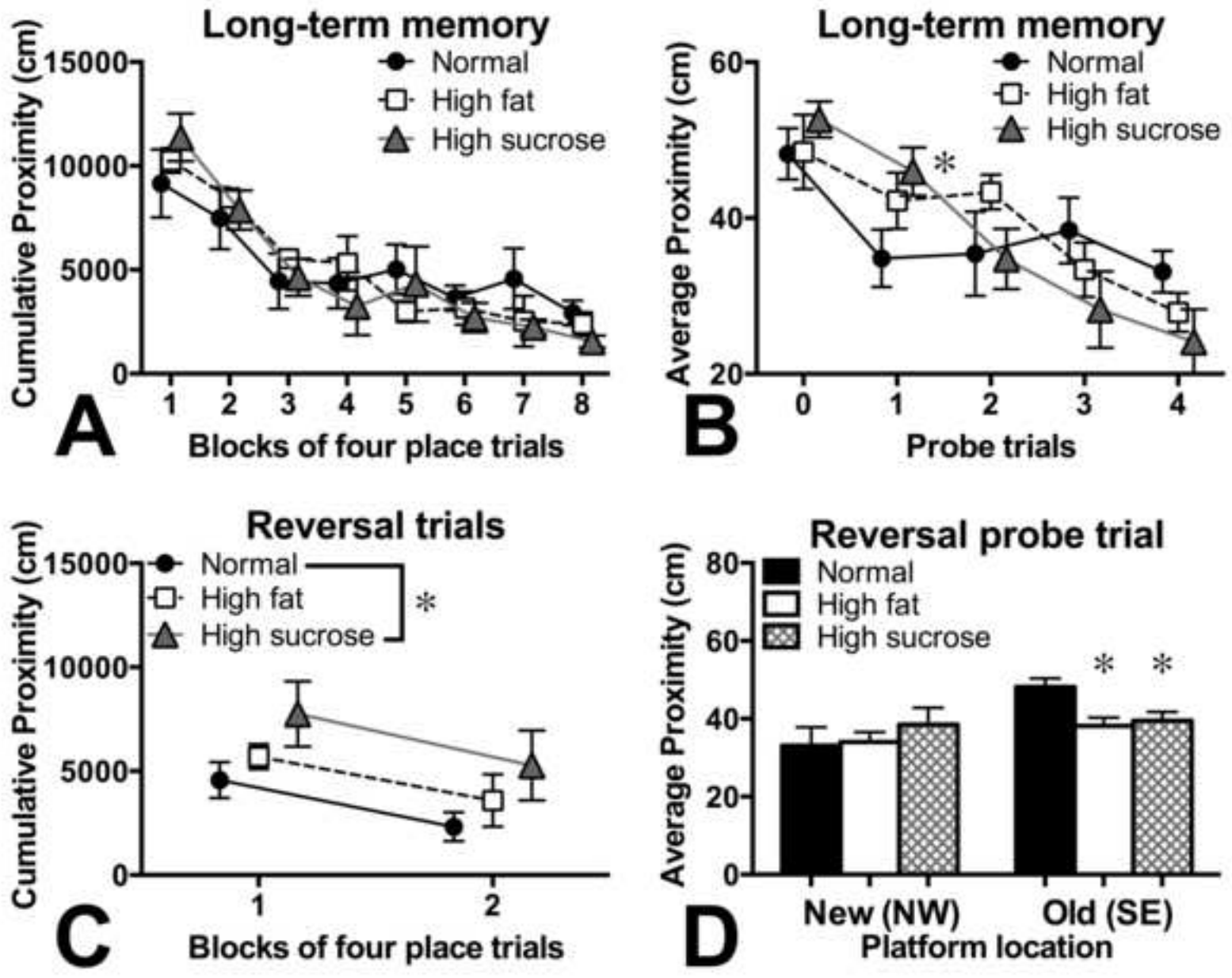

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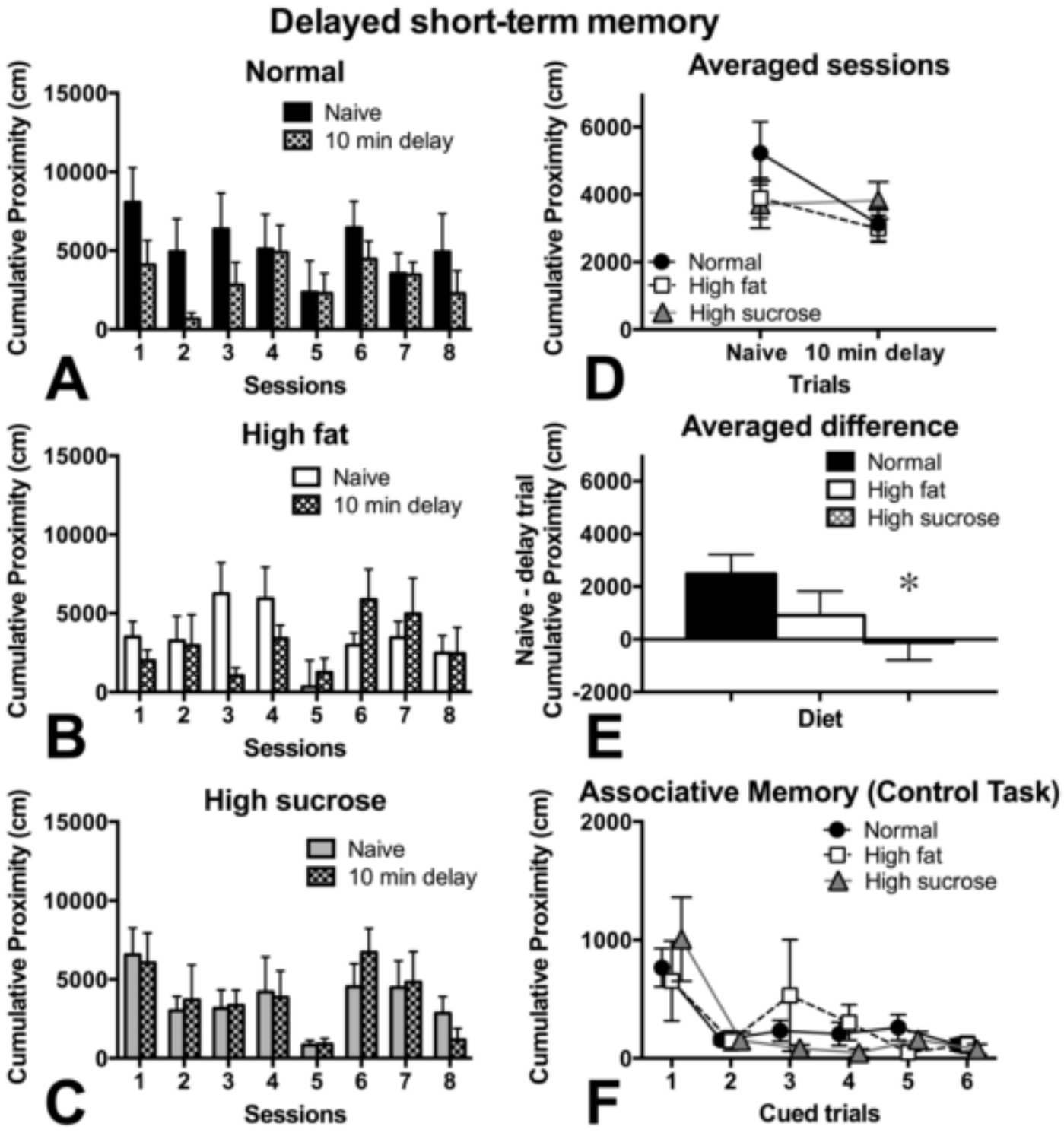

Figure 5

Figure 6

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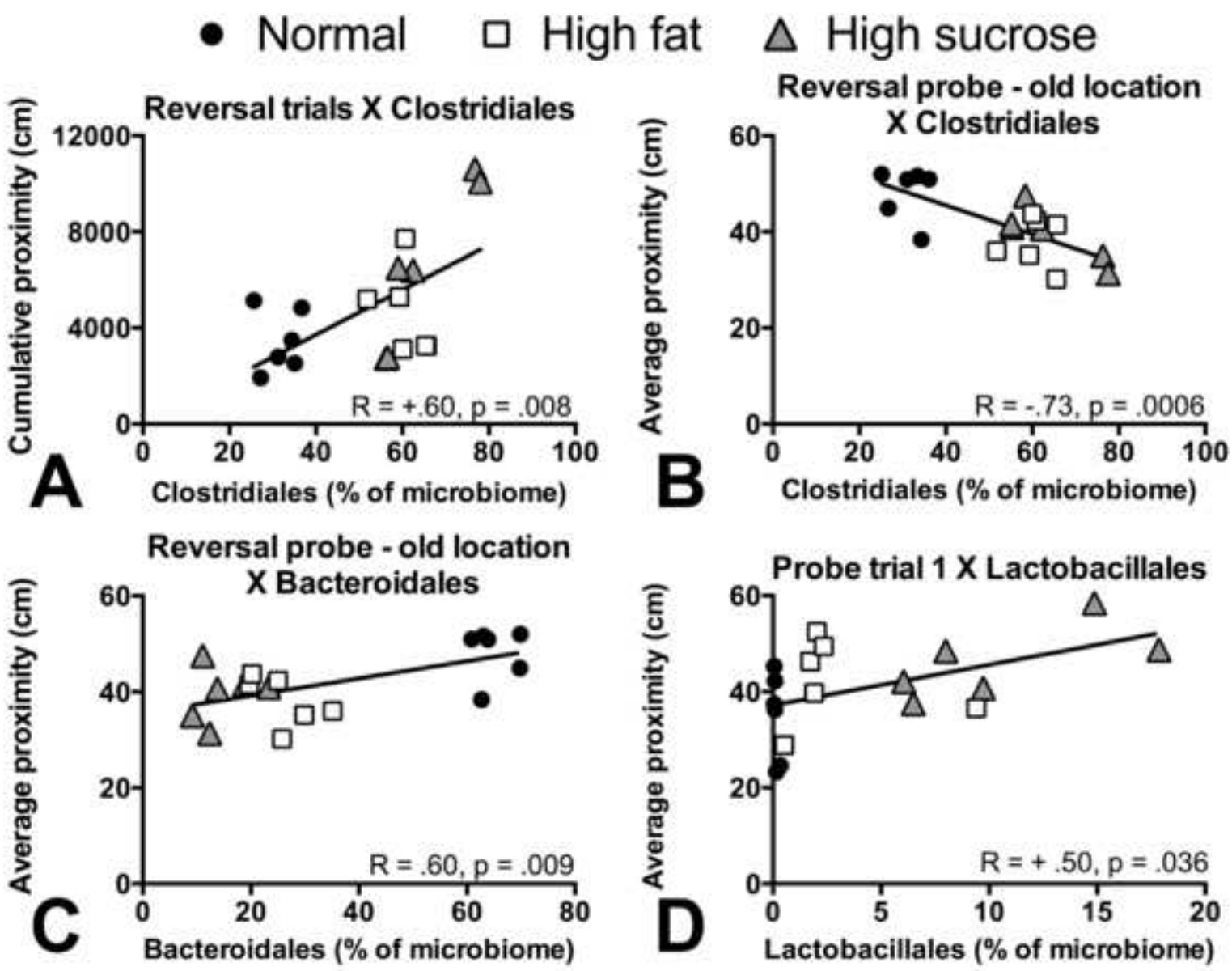

Figure 6

Table 2. Average percent of microbiome for each bacterial order and genera (detected at >.01%) two weeks after the change in diet.

	Normal diet		High fat diet			High sucrose diet		
	Mean	SEM	Mean	SEM		Mean	SEM	
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales								
Bifidobacteriaceae;Bifidobacterium	**0.000**	**0.000**	**0.026**	**0.017**		**0.004**	**0.004**	
Bacteria;Actinobacteria;Actinobacteria;Coriobacteriales	**0.010**	**0.010**	**0.221**	**0.069**		**0.205**	**0.085**	
Coriobacteriaceae;Enterorhabdus	0.005	0.005	0.190	0.068		0.161	0.077	
Coriobacteriaceae;Other	0.005	0.005	0.030	0.016		0.043	0.016	
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales	**65.03**	**1.574**	**25.946**	**2.396**	********	**14.772**	**2.171**	****** ^^^^**
Other;Other	0.207	0.037	0.113	0.043		0.118	0.018	
Porphyromonadaceae;Barnesiella	8.192	0.952	3.619	0.922	*****	3.211	0.337	******
Porphyromonadaceae;Other	48.126	2.022	20.226	1.911	********	10.522	1.850	****** ^^^^**
Porphyromonadaceae;Tannerella	0.025	0.009	0.000	0.000		0.003	0.003	
Rikenellaceae;Alistipes	8.478	0.838	1.988	0.447	********	0.918	0.212	********
Bacteria;Bacteroidetes;Other;Other	**0.039**	**0.013**	**0.038**	**0.011**		**0.013**	**0.009**	
Bacteria;Firmicutes;Bacilli;Lactobacillales	**0.135**	**0.044**	**2.989**	**1.309**		**10.501**	**1.967**	****** ^**
Enterococcaceae;Enterococcus	0.000	0.000	0.000	0.000		0.156	0.024	
Lactobacillaceae;Lactobacillus	0.130	0.039	1.953	1.347		5.813	2.143	*******
Other;Other	0.005	0.005	0.009	0.009		0.031	0.011	
Streptococcaceae;Lactococcus	0.000	0.000	1.027	0.180		4.501	0.673	*****
Bacteria;Firmicutes;Clostridia;Clostridiales	**31.123**	**1.778**	**60.526**	**2.082**	********	**64.160**	**4.171**	********
Incertae Sedis XIV;Blautia	0.109	0.035	0.048	0.014		0.007	0.007	
Lachnospiraceae;Coprococcus	0.444	0.087	2.269	0.741		1.102	0.228	
Lachnospiraceae;Dorea	0.034	0.018	0.505	0.099		0.474	0.090	
Lachnospiraceae;Johnsonella	0.277	0.120	2.094	0.617		1.978	1.078	
Lachnospiraceae;Other	14.492	0.825	35.174	3.548	********	35.343	3.802	********
Lachnospiraceae;Syntrophococcus	0.803	0.133	0.016	0.016		0.000	0.000	
Other;Other	9.616	0.900	7.804	0.974		9.6627	0.567	
Peptostreptococcaceae;Other	0.000	0.000	0.945	0.257		1.773	0.416	
Peptostreptococcaceae;Sporacetigeni	0.000	0.000	0.013	0.009		0.024	0.019	
Ruminococcaceae;Acetivibrio	0.077	0.036	0.000	0.000		0.000	0.000	
Ruminococcaceae;Anaerotruncus	0.024	0.009	0.383	0.076		0.114	0.025	
Ruminococcaceae;Butyricicoccus	0.046	0.014	0.004	0.004		0.021	0.008	
Ruminococcaceae;Oscillibacter	2.203	0.292	1.907	0.277		3.177	0.133	
Ruminococcaceae;Other	2.896	0.339	9.294	0.737	********	10.449	0.595	********

Ruminococcaceae;Papillibacter	0.020	0.013	0.039	0.017		0.023	0.011
Ruminococcaceae;Ruminococcus	0.072	0.029	0.004	0.004		0.000	0.000
Veillonellaceae;Other	0.010	0.010	0.028	0.019		0.049	0.017
Bacteria;Firmicutes;Clostridia;Other	**0.796**	**0.110**	**0.123**	**0.028**		**0.876**	**0.174**
Bacteria;Firmicutes;Erysipelotrichia;Erysipelotrichales	**0.280**	**0.117**	**7.405**	**0.684**	*****	**6.612**	**1.818**
Erysipelotrichaceae;Coprobacillus	0.065	0.014	5.245	0.883	******	3.486	1.188
Erysipelotrichaceae;Holdemania	0.050	0.017	0.424	0.110		0.368	0.057
Erysipelotrichaceae;Other	0.111	0.059	0.707	0.561		2.740	0.836
Turicbactor	0.054	0.054	1.029	0.560		0.017	0.017
Bacteria;Firmicutes;Other;Other	**2.316**	**0.331**	**2.726**	**0.311**		**2.857**	**0.553**
Bacteria;Tenericutes;Mollicutes;Anaeroplasmatales							
Anaeroplasmataceae;Anaeroplasma	**0.270**	**0.159**	**0.000**	**0.000**		**0.000**	**0.000**

*** indicates p<.001 and **** indicates p < .0001 for difference from normal diet group.
^ indicates p<.05 and ^^^^ indicates p<.0001 for difference from high fat diet group.
Two-way ANOVA, followed by Tukey's post-hoc analysis with correction for multiple comparisons with one family for all comparisons.
Bolded Orders are also shown in Fig. 5 and bold numbers represent sums of the unbolded subtaxa (averaged for each diet group) listed below on the table.
Only orders and genera expressed at > .01% in at least one diet group were included in the analysis. The remaining taxa were summed and expressed as a percentage of the total remaining taxa.

Table 3

Table 3. Pearson's correlation coefficients for relationships between bacterial orders and behavior in the water maze that exhibited significant differences between diets.

Bacterial orders	Long-term memory - probe trial 1 proximity	Reversal learning - averaged trials	Reversal probe - old location proximity	Short-term memory - trial differences
Bacteriodales	- .47	- .51	+ .60 **	+ .39
Lactobacillales	+ .50	+ .26	- .09	- .18
Clostridiales	+ .37	+ .60 **	- .73 **	- .44
Erysipelotrichales	+ .34	+ .002	- .28	- .17

** indicates $p < .01$ for a significant correlation.