Gut microbial taxa elevated by dietary sugar disrupt memory function

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Abstract:

Emerging evidence highlights a critical relationship between gut microbiota and neurocognitive development. Excessive consumption of sugar and other unhealthy dietary factors during early life developmental periods yields changes in the gut microbiome as well as neurocognitive impairments. However, it is unclear whether these two outcomes are functionally connected. Here we explore whether excessive early life consumption of added sugars negatively impacts memory function via the gut microbiome. Rats were given free access to a sugar-sweetened beverage (SSB) during the adolescent stage of development. Memory function and anxiety-like behavior were assessed during adulthood and gut bacterial and brain transcriptome analyses were conducted. Taxa-specific microbial enrichment experiments examined the functional relationship between sugar-induced microbiome changes and neurocognitive and brain transcriptome outcomes. Chronic early life sugar consumption impaired adult hippocampal-dependent memory function without affecting body weight or anxiety-like behavior. Adolescent SSB consumption during adolescence also altered the gut microbiome, including elevated abundance of two species in the genus *Parabacteroides* (*P. distasonis* and *P. johnsonii*) that were negatively correlated with hippocampal function. Transferred enrichment of these specific bacterial taxa in adolescent rats impaired hippocampal-dependent memory during adulthood. Hippocampus transcriptome analyses revealed that early life sugar consumption altered gene expression in intracellular kinase and synaptic neurotransmitter signaling pathways, whereas *Parabacteroides* microbial enrichment altered gene expression in pathways associated with metabolic function, neurodegenerative disease, and dopaminergic signaling. Collectively these results identify a role for microbiota "dysbiosis" in

mediating the detrimental effects of early life unhealthy dietary factors on hippocampal-dependent memory function.

53 **Introduction:**

54

55 The gut microbiome has recently been implicated in modulating neurocognitive
56 development and consequent functioning [1-4]. Early life developmental periods represent
57 critical windows for the impact of indigenous gut microbes on the brain, as evidenced by
58 the reversal of behavioral and neurochemical abnormalities in germ free rodents when
59 inoculated with conventional microbiota during early life, but not during adulthood [5-7].
60 Dietary factors are a critical determinant of gut microbiota diversity and can alter gut
61 bacterial communities, as evident from the microbial plasticity observed in response to
62 pre- and probiotic treatment, as well as the "dysbiosis" resulting from consuming
63 unhealthy, yet palatable foods that are associated with obesity and metabolic disorders
64 (e.g., Western diet; foods high in saturated fatty acids and added sugar) [8]. In addition to
65 altering the gut microbiota, consumption of Western dietary factors yields long-lasting
66 memory impairments, and these effects are more pronounced when consumed during
67 early life developmental periods vs. during adulthood [9-11]. Whether diet-induced changes
68 in specific bacterial populations are functionally related to altered early life
69 neurocognitive outcomes, however, is poorly understood.

70

71 The hippocampus, which is well known for its role in spatial and episodic memory and
72 more recently for regulating learned and social aspects of food intake control [12-17], is
73 particularly vulnerable to the deleterious effects of Western dietary factors [18-20]. During
74 the juvenile and adolescent stages of development, a time when the brain is rapidly
75 developing, consumption of diets high in saturated fat and sugar [21-23] or sugar alone [24-27]
76 impairs hippocampal function while in some cases preserving memory processes that do

77 not rely on the hippocampus. While several putative underlying mechanisms have been
78 investigated, the precise biological pathways linking dietary factors to neurocognitive
79 dysfunction remain largely undetermined [11]. Here we aimed to determine whether
80 sugar-induced alterations in gut microbiota during early life are causally related to
81 hippocampal-dependent memory impairments observed during adulthood.
82
83 **Methods and Materials:**
84
85 **Experimental Subjects**
86 Juvenile male Sprague Dawley rats (Envigo; arrival post natal day (PN) 26-28; 50-70g)
87 were housed individually in standard conditions with a 12:12 light/dark cycle. All rats
88 had ad libitum access to water and Lab Diet 5001 (PMI Nutrition International,
89 Brentwood, MO; 29.8 % kcal from protein, 13.4% kcal from fat, 56.7% kcal from
90 carbohydrate), with modifications where noted. All experiments were performed in
91 accordance with the approval of the Animal Care and Use Committee at the University
92 of Southern California.
93
94 **Experiment 1**
95 Twenty one juvenile male rats (PN 26-28) were divided into two groups with equal body
96 weight and given ad libitum access to: 1) 11% weight-by-volume (w/v) solution
97 containing monosaccharide ratio of 65% fructose and 35% glucose in reverse osmosis-
98 filtered water (SUG; n=11) or 2) or an extra bottle of reverse osmosis-filtered water
99 (CTL; n=10). This solution was chosen to model commonly consumed sugar-sweetened
100 beverages in humans in terms of both caloric content and monosaccharide ratio[28].

101 Additionally, all rats were given ad libitum access to water and standard rat chow. Food
102 intake, solution intake and body weights were monitored thrice weekly except where
103 prohibited due to behavioral testing. At PN 60, rats underwent Novel Object in Context
104 (NOIC) testing, to measure hippocampal-dependent episodic contextual memory. At PN
105 67 rats underwent anxiety-like behavior testing in the Zero Maze, followed by body
106 composition testing at PN 70 and an intraperitoneal glucose tolerance test (IP GTT) at
107 PN 84. All behavioral procedures were run at the same time each day (4-6 hours into the
108 light cycle). Fecal and cecal samples were collected prior to sacrifice at PN 104.
109
110 In a separate cohort of juvenile male rats (n=6/group) animals were treated as above,
111 but on PN day 60 rats were tested in the Novel Object Recognition (NOR) and Open
112 Field (OF) tasks, with two days in between tasks. Animals were sacrificed and tissue
113 punches were collected from the dorsal hippocampus on PN day 65. Tissue punches
114 were flash frozen in a beaker filled with isopentane and surrounded dry ice and then
115 stored at -80°C until further analyses.
116
117 **Experiment 2**
118 Twenty-three juvenile male rats (PN 26-28) were divided into two groups and received a
119 gavage twice daily (12 hours apart) for 7 days (only one treatment was given on day 7) of
120 either (1) saline (SAL; n=8), or (2) a cocktail of antibiotics consisting of Vancomycin (50
121 mg/kg), Neomycin (100 mg/kg), and Metronidazole (100 mg/kg) along with
122 supplementation with 1 mg/mL of ampicillin in their drinking water (ABX; n=15), which
123 is a protocol modified from [29]. Animals were housed in fresh, sterile cages on Day 3 of
124 the antibiotic or saline treatment, and again switched to fresh sterile cages on Day 7

125 after the final gavage. All animals were maintained on sterile, autoclaved water and
126 chow for the remainder of the experiment. Rats in the ABX group were given water
127 instead of ampicillin solution on Day 7. Animals in the ABX group were further
128 subdivided to receive either gavage of a 1:1 ratio of *Parabacteroides distasonis* and
129 *Parabacteroides johnsonii* (PARA; n=8) or saline (SAL; n=7) thirty six hours after the
130 last ABX treatment. To minimize potential contamination, rats were handled minimally
131 for 14 days. Cage changes occurred once weekly at which time animals and food were
132 weighed. Experimenters wore fresh, sterile PPE and weigh boxes were cleaned with
133 sterilizing solution in between each cage change. On PN 50 rats were tested in NOIC, on
134 PN 60 rats were tested in NOR, on PN 62 rats were tested in the Zero Maze, followed by
135 Open Field on PN 64. On PN 73 rats were given an IP GTT, and on PN 76 body
136 composition was tested. Rats were sacrificed at PN 83 and dorsal hippocampus tissue
137 punches and cecal samples were collected. Tissue punches were flash frozen in a beaker
138 filled with isopentane and surrounded dry ice and cecal samples were placed in
139 microcentrifuge tubes embedded in dry ice. Samples were subsequently stored at -80°C
140 until further analyses.
141

142 **IP glucose tolerance test (IP GTT)**

143 Animals were food restricted 24 hours prior to IP GTT. Immediately prior to the test,
144 baseline blood glucose readings were obtained from tail tip and recorded by a blood
145 glucose meter (One touch Ultra2, LifeScan Inc., Milpitas, CA). Each animal was then
146 intraperitoneally (IP) injected with dextrose solution (0.923g/ml by body weight) and
147 tail tip blood glucose readings were obtained at 30, 60, 90, and 120 min after IP
148 injections, as previously described [24].

149

150 **Zero Maze**

151 The Zero Maze is an elevated circular track (63.5 cm fall height, 116.8cm outside
152 diameter), divided into four equal length sections. Two sections were open with 3 cm
153 high curbs, whereas the 2 other closed sections contained 17.5 cm high walls. Animals
154 are placed in the maze facing the open section of the track in a room with ambient
155 lighting for 5 min while the experimenter watches the animal from a monitor outside of
156 the room. The experimenter records the total time spent in the open sections (defined as
157 the head and front two paws in open arms), and the number of crosses into the open
158 sections from the closed sections.

159

160 **Novel object in context task (NOIC)**

161 NOIC measures episodic contextual memory based on the capacity for an animal to
162 identify which of two familiar objects it has never seen before in a specific context.
163 Procedures were adapted from prior reports [30,31]. Briefly, rats are habituated to two
164 distinct contexts on subsequent days (with the habituation order counterbalanced by
165 group) for 5-min sessions: Context 1 is a semi-transparent box (15in W x 24in L x 12in
166 H) with orange stripes and Context 2 is a grey opaque box (17in W x 17in L x 16in H)
167 (Context identify assignments counterbalanced by group), each context is in a separate
168 dimly lit room, which is obtained using two desk lamps pointed toward the floor. Day 1
169 of NOIC begins with each animal being placed in Context 1 containing two distinct
170 similarly sized objects placed in opposite corners: a 500ml jar filled with blue water
171 (Object A) and a square glass container (Object B) (Object assignments and placement
172 counterbalanced by group). On day 2 of NOIC, animals are placed in Context 2 with

duplicates of one of the objects. On NOIC day 3, rats are placed in Context 2 with Objects A and Object B. One of these objects is not novel to the rat, but its placement in Context 2 is novel. All sessions are 5 minutes long and are video recorded. Each time the rat is placed in one of the contexts, it is placed with its head facing away from both objects. The time spent investigating each object is recorded from the video recordings by an experimenter who is blinded to the treatment groups. Exploration is defined as sniffing or touching the object with the nose or forepaws. The task is scored by calculating the time spent exploring the Novel Object to the context divided by the time spent exploring both Objects A and B combined, which is the novelty or "discrimination index". Rats with an intact hippocampus will preferentially investigate the object that is novel to Context 2, given that this object is a familiar object yet is now presented in a novel context, whereas hippocampal inactivation impairs the preferential investigation of the object novel to Context 2 [30].

Novel Object Recognition

The apparatus used for NOR is a grey opaque box (17in W x 17in L x 16in H) placed in a dimly lit room, which is obtained using two desk lamps pointed toward the floor. Procedures are adapted from [32]. Rats are habituated to the empty arena and conditions for 10 minutes on the day prior to testing. The novel object and the side on which the novel object is placed is counterbalanced by group. The test begins with a 5-minute familiarization phase, where rats are placed in the center of the arena, facing away from the objects, with two identical copies of the same object to explore. The objects were either two identical cans or two identical bottles, counterbalanced by treatment group. The objects were chosen based on preliminary studies which determined that they are

197 equally preferred by Sprague Dawley rats. Animals are then removed from the arena and
198 placed in the home cage for 5 minutes. The arena and objects are cleaned with 10%
199 ethanol solution, and one of the objects in the arena is replaced with a different one
200 (either the can or bottle, whichever the animal has not previously seen, i.e., the "novel
201 object"). Animals are again placed in the center of the arena and allowed to explore for 3
202 minutes. Time spent exploring the objects is recorded via video recording and analyzed
203 using Any-maze activity tracking software (Stoelting Co., Wood Dale, IL).
204

205 **Open Field**

206 Open field measures general activity level and also anxiety-like behavior in the rat. A
207 large gray bin, 60 cm (L) X 56 CM (W) is placed under diffuse even lighting (30 lux). A
208 center zone is identified and marked in the bin (19 cm L X 17.5 cm W). A video camera is
209 placed directly overhead and animals are tracked using AnyMaze Software (Stoelting
210 Co., Wood Dale, IL). Animals are placed in the center of the box facing the back wall and
211 allowed to explore the arena for 10 min while the experimenter watches from a monitor
212 in an adjacent room. The apparatus is cleaned with 10% ethanol after each rat is tested.
213

214 **Body Composition**

215 Body composition (body fat, lean mass) was measured using LF90 time domain nuclear
216 magnetic resonance (Bruker NMR minispec LF 90II, Bruker Daltonics, Inc.).
217

218 **Bacterial transfer**

219 *Parabacteroides distasonis* (ATCC 8503) was cultured under anaerobic conditions at
220 37C in Reinforced Clostridial Medium (RCM, BD Biosciences). *Parabacteroides*

221 *johnsonii* (DSM 18315) was grown in anaerobic conditions in PYG medium (modified,
222 DSM medium 104). Cultures were authenticated by full-length 16S rRNA gene
223 sequencing. For bacterial enrichment, 10^9 colony-forming units of both *P. distasonis*
224 and *P. johnsonii* were suspended in 500 µL pre-reduced PBS and orally gavaged into
225 antibiotic-treated rats. When co-administered, a ratio of 1:1 was used for *P. distasonis*
226 and *P. johnsonii*.
227

228 **Gut microbiota DNA extraction and 16s rRNA gene sequencing in sugar-fed**
229 **and control rats**

230 All samples were extracted and sequenced according to the guidelines and procedures
231 established by the Earth Microbiome Project [33]. DNA was extracted from fecal and cecal
232 samples using the MO BIO PowerSoil DNA extraction kit. PCR targeting the V4 region
233 of the 16S rRNA bacterial gene was performed with the 515F/806R primers, utilizing the
234 protocol described in Caporaso et al.[34]. Amplicons were barcoded and pooled in equal
235 concentrations for sequencing. The amplicon pool was purified with the MO BIO
236 UltraClean PCR Clean-up kit and sequenced by the 2 x 150bp MiSeq platform at the
237 Institute for Genomic Medicine at UCSD. All sequences were deposited in Qiita Study
238 11255 as raw FASTQ files. Sequences were demultiplexed using Qiime-1 based "split
239 libraries" with the forward reads only dropping. Demultiplexed sequences were then
240 trimmed evenly to 100 bp and 150 bp to enable comparison to other studies for meta-
241 analyses. Trimmed sequences were matched to known OTUs at 97% identity.
242

243 **Gut microbiota DNA extraction and 16S rRNA gene sequencing for**
244 ***Parabacteroides*-enriched and control rats**

245 Total bacterial genomic DNA was extracted from rat fecal samples (0.25 g) using the
246 Qiagen DNeasy PowerSoil Kit. The library was prepared following methods from
247 (Caporaso et al., 2011). The V4 region (515F-806R) of the 16S rDNA gene was PCR
248 amplified using individually barcoded universal primers and 30 ng of the extracted
249 genomic DNA. The conditions for PCR were as follows: 94°C for 3 min to denature the
250 DNA, with 35 cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s, with a final
251 extension of 10 min at 72°C. The PCR reaction was set up in triplicate, and the PCR
252 products were purified using the Qiaquick PCR purification kit (QIAGEN). The purified
253 PCR product was pooled in equal molar concentrations quantified by nanodrop and
254 sequenced by Laragen, Inc. using the Illumina MiSeq platform and 2 x 250bp reagent
255 kit for paired-end sequencing. Amplicon sequence variants (ASVs) were chosen after
256 denoising with the Deblur pipeline. Taxonomy assignment and rarefaction were
257 performed using QIIME2-2019.10.
258
259 **Hippocampal RNA extraction and sequencing**
260 Hippocampi from rats treated with or without sugar or *Parabacteroides* were subject to
261 RNA-seq analysis. Total RNA was extracted according to manufacturer's instructions
262 using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). Total RNA was checked
263 for degradation in a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). Quality was very
264 high for all samples, and libraries were prepared from 1 ug of total RNA using a NuGen
265 Universal Plus mRNA-seq Library Prep Kit (Tecan Genomics Inc. Redwood City, CA).
266 Final library products were quantified using the Qubit 2.0 Fluorometer (Thermo Fisher
267 Scientific Inc., Waltham, MA, USA), and the fragment size distribution was determined
268 with the Bioanalyzer 2100. The libraries were then pooled equimolarly, and the final

269 pool was quantified via qPCR using the Kapa Biosystems Library Quantification Kit,
270 according to manufacturer's instructions. The pool was sequenced in an Illumina
271 NextSeq 550 platform (Illumina, San Diego, CA, USA), in Single-Read 75 cycles format,
272 obtaining about 25 million reads per sample. The preparation of the libraries and the
273 sequencing was performed at the USC Genome Core (http://uscgenomecore.usc.edu/)
274

275 **RNA-seq quality control**

276 Data quality checks were performed using the FastQC tool
277 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and low quality reads
278 were trimmed with Trim_Galore
279 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). RNA-seq reads
280 passing quality control were mapped to *Rattus novegicus* transcriptome (Rnor6) and
281 quantified with Salmon [35]. Salmon directly mapped RNA-seq reads to Rat
282 transcriptome and quantified transcript counts. Txiimport [36] were used to convert
283 transcript counts into gene counts. Potential sample outliers were detected by principle
284 component analysis (PCA) and one control and one treatment sample from the
285 *Parabacteroides* experiment were deemed outliers (Figure S7A, B) and removed.
286

287 **Identification of differentially expressed genes (DEGs)**

288 DESeq2[37] were used to conduct differential gene expression analysis between sugar
289 treatment and the corresponding controls, or between *Parabacteroides* treatment and
290 the corresponding controls. Low-abundance genes were filtered out and only those
291 having a mean raw count > 1 in more than 50% of the samples were included.
292 Differentially expressed genes were detected by DESeq2 with default settings.

293 Significant DEGs were defined as Benjamini-Hochberg (BH) adjusted false discovery
294 rate (FDR) < 0.05. For heatmap visualization, genes were normalized with variance
295 stabilization transformation implemented in DESeq2, followed by calculating a z-score
296 for each gene.
297

298 **Pathway analyses of DEGs**

299 For the pathway analyses, DEGs at an unadjusted p-value < 0.01 were used. Pathway
300 enrichment analysis were conducted using enrichr[38] by intersecting each signature with
301 pathways or gene sets from KEGG[39], gene ontology biological pathways (GOBP),
302 Cellular Component (GOCP), Molecular Function (GOMF)[40] and Wikipathways[41].
303 Pathways at FDR < 0.05 were considered significant. Unless otherwise specified, R 3.5.2
304 was used for the analysis mentioned in the RNA sequencing section.
305

306 **Additional statistical methods**

307 Data are presented as means ± SEM. For analytic comparisons of body weight, total food
308 intake, and chow intake, groups were compared using repeated measures ANOVA in
309 Prism software (GraphPad Inc., version 8.0). Taxonomic comparisons from 16S rRNA
310 sequencing analysis were analyzed by analysis of composition of microbiomes
311 (ANCOM). When significant differences were detected, Sidak post-hoc test for multiple
312 comparisons was used. Area under the curve (AUC) for the IP GTT testing was also
313 calculated using Prism. All other statistical analyses were performed using Student's
314 two-tailed unpaired t tests in excel software (Microsoft Inc., version 15.26). Normality
315 was confirmed prior to the utilization of parametric testing. For all analyses, statistical
316 significance was set at $P<0.05$.

317

318 **Results:**

319

320 **Early-life sugar consumption impairs hippocampal-dependent memory**
321 **function**

322 Results from the Novel Object in Context (NOIC) task, which measures hippocampal-
323 dependent episodic contextual memory function [31], reveal that while there were no
324 differences in total exploration time of the combined objects on days 1 or 3 of the task
325 (Figure 1A, B), animals fed sugar solutions in early life beginning at PN 28 had a
326 reduced capacity to discriminate an object that was novel to a specific context when
327 animals were tested during adulthood (PN 60), indicating impaired hippocampal
328 function (Figure 1C). Conversely, animals fed sugar solutions in early life performed
329 similarly to those in the control group when tested in the novel object recognition task
330 (NOR) (Figure 1D), which tests object recognition memory independent of context.
331 Notably, when performed using the current methods with a short duration between the
332 familiarization phase and the test phase, NOR not hippocampal-dependent but instead
333 is primarily dependent on the perirhinal cortex [31,42-44]. These data suggest that early life
334 dietary sugar consumption impairs performance in hippocampal-dependent contextual-
335 based recognition memory without affecting performance in perirhinal cortex-
336 dependent recognition memory independent of context [24].

337

338 Elevated anxiety-like behavior and altered general activity levels may influence novelty
339 exploration independent of memory effects and may therefore confound the
340 interpretation of behavioral results. Thus, we next tested whether early life sugar

341 consumption affects anxiety-like behavior using two different tasks designed to measure
342 anxiety-like behavior in the rat: the elevated zero maze and the open field task, that
343 latter of which also assesses levels of general activity [45]. Early life sugar consumption
344 had no effect on time spent in the open area or in the number of open area entries in the
345 zero maze (Figure 1E, F). Similarly, early life sugar had no effect on distance travelled or
346 time spent in the center zone in the open field task (Figure 1G, H). Together these data
347 suggest that habitual early life sugar consumption did not increase anxiety-like behavior
348 or general activity levels in the rats.
349

350 **Early life sugar consumption impairs glucose tolerance without affecting**
351 **total caloric intake, body weight, or adiposity**

352 Given that excessive sugar consumption is associated with weight gain and metabolic
353 deficits [46], we tested whether access to a sugar solution during the adolescent phase of
354 development would affect food intake, body weight gain, adiposity, and glucose
355 tolerance in the rat. Early life sugar consumption had no effect on body composition
356 during adulthood (Figure 1I, Figure S1 A, B). Early life sugar consumption also had no
357 effect on body weight or total kcal intake (Figure 1J, K), which is in agreement with
358 previous findings [24,27,47]. Animals steadily increased their intake of the 11% sugar
359 solution throughout the study (Figure 1L) but compensated for the calories consumed in
360 the sugar solutions by reducing their intake of dietary chow (Figure S1 C). However,
361 animals that were fed sugar solutions during adolescence showed impaired peripheral
362 glucose metabolism in an intraperitoneal glucose tolerance test (IP GTT) (Figure S1D).
363

364 **Gut microbiota are impacted by early life sugar consumption**

365 Principal component analyses of 16s rRNA gene sequencing data of fecal samples
366 revealed a separation between the fecal microbiota of rats fed early life sugar and
367 controls (Figure 2A). Results from LEfSe analysis identified differentially abundant
368 bacterial taxa in fecal samples that were elevated by sugar consumption. These include
369 the family *Clostridiaceae* and the genus *02d06* within *Clostridiaceae*, the family
370 *Mogibacteriaceae*, the family *Enterobacteriaceae*, the order *Enterobacteriales*, the
371 class of *Gammaproteobacteria*, and the genus *Parabacteroides* within the family
372 *Porphyromonadaceae* (Figure 2B,C). In addition to an elevated % relative abundance of
373 the genus *Parabacteroides* in animals fed early life sugar (Figure 2D), log transformed
374 counts of the *Parabacteroides* negatively correlated with performance scores in the
375 NOIC memory task (Figure 2E). Of the additional bacterial populations significantly
376 affected by sugar treatment, regression analyses did not identify any other genera as
377 being significantly correlated to NOIC memory performance. Within *Parabacteroides*,
378 levels of two operational taxonomic units (OTUs) that were elevated by sugar negatively
379 correlated with performance in the NOIC task, identified as taxonomically related to *P.*
380 *johnsonii* and *P. distasonis* (Figure 2F, G). The significant negative correlation between
381 NOIC performance and *Parabacteroides* was also present within each of the diet groups
382 alone, but when separated out by diet group only *P. distasonis* showed a significant
383 negative correlation for each diet group ($P<.05$), whereas *P. johnsonii* showed a non-
384 significant trend in both the control and sugar groups ($P=.06$, and $P=.08$, respectively;
385 Figure S2A-C). Abundance of other bacterial populations that were affected by sugar
386 consumption were not significantly related to memory task performance.
387 There was a similar separation between groups in bacteria analyzed from cecal
388 samples (Figure S3A). LEfSe results from cecal samples show elevated *Bacilli*,

389 *Actinobacteria*, *Erysipelotrichia*, and *Gammaproteobacteria* in rats fed early life sugar,
390 and elevated *Clostridia* in the controls (Figure S3B-C). Abundances at the different
391 taxonomic levels in fecal and cecal samples are shown in (Figure S4, S5). Regression
392 analyses did not identify these altered cecal bacterial populations as being significantly
393 correlated to NOIC memory performance.
394

395 **Early life *Parabacteroides* enrichment impairs memory function**

396 To determine whether neurocognitive outcomes due to early life sugar consumption
397 could be attributable to elevated levels of *Parabacteroides* in the gut, we experimentally
398 enriched the gut microbiota of naïve juvenile rats with two *Parabacteroides* species that
399 exhibited high 16S rRNA sequencing alignment with OTUs that were increased by sugar
400 consumption and were negatively correlated with behavioral outcomes in rats fed early
401 life sugar. *P. johnsonii* and *P. distasoni* species were cultured individually under
402 anaerobic conditions and transferred to a group of antibiotic-treated young rats in a 1:1
403 ratio via oral gavage using the experimental design described in Methods and outlined
404 in Figure 3A, and from [29]. To confirm *Parabacteroides* enrichment, 16SrRNA
405 sequencing was performed on rat fecal samples for SAL-SAL, ABX-SAL, and ABX-PARA
406 groups. Alpha diversity was analyzed using observed operational taxonomic units
407 (OTUs) (Figure 3B), where both ABX-SAL and ABX-PARA fecal samples have
408 significantly reduced alpha diversity when compared with SAL-SAL fecal samples,
409 suggesting that antibiotic treatment reduces microbiome alpha diversity. Further, either
410 treatment with antibiotics alone or antibiotics followed by *Parabacteroides* significantly
411 alters microbiota composition relative to the SAL-SAL group (Figure 3C). Taxonomic
412 comparisons from 16S rRNA sequencing analysis were analyzed by analysis of

413 composition of microbiomes (ANCOM). Differential abundance on relative abundance
414 at the species level (Figure 3D) was tested across samples hypothesis-free. Significant
415 taxa at the species level were corrected for using false-discovery rate (FDR)-corrected *P*-
416 values to calculate W in ANCOM. Comparing all groups resulted in the highest W value
417 of 144 for the *Parabacteroides* genus, which was enriched in ABX-PARA fecal samples
418 after bacterial gavage with an average relative abundance of 55.65% (Figure 3E). This
419 confirms successful *Parabacteroides* enrichment for ABX-PARA rats post-gavage when
420 compared to either ABX-SAL (average relative abundance of 5.47%) or ABX-SAL rats
421 (average relative abundance of 0.26%).

422 All rats treated with antibiotics showed a reduction in food intake and body
423 weight during the initial stages of antibiotic treatment, however, there were no
424 differences in body weight between the two groups of antibiotic treated animals by
425 PN50, at the time of behavioral testing (Figure S6A-C). Similar to a recent report [48],
426 *Parabacteroides* enrichment in the present study impacted body weight at later time
427 points. Animals who received *P. johnsonii* and *P. distasonis* treatment showed reduced
428 body weight 40 days after the transfer, with significantly lower lean mass (Figure S6D-
429 F). There were no differences in percent body fat between groups, nor were there
430 significant group differences in glucose metabolism in the IPGTT (Figure S6 G).
431 Importantly, the body weights in the ABX-PARA group did not significantly differ from
432 the ABX-SAL control group at the time of behavioral testing.

433 Results from the hippocampal-dependent NOIC memory task showed that while
434 there were no differences in total exploration time of the combined objects on days 1 or
435 3 of the task, indicating similar exploratory behavior, animals enriched with
436 *Parabacteroides* showed a significantly reduced discrimination index in the NOIC task

437 compared with either control group (Figure 4A-C), indicating impaired performance in
438 hippocampal-dependent memory function. When tested in the perirhinal cortex-
439 dependent NOR task [31], animals enriched with *Parabacteroides* showed impaired object
440 recognition memory compared with the antibiotic treated control group as indicated by
441 a reduced novel object exploration index (Figure 4D). These findings show that unlike
442 sugar-fed animals, *Parabacteroides* enrichment impaired perirhinal cortex-dependent
443 memory processes in addition to hippocampal-dependent memory.

444 Results from the zero maze showed no differences in time spent in the open arms
445 nor in the number of open arm entries for the *Parabacteroides*-enriched rats relative to
446 controls (Figure 4E, F), indicating that the enrichment did not affect anxiety-like
447 behavior. Similarly, there were no differences in distance travelled or time spent in the
448 center arena in the open field test, which is a measure of both anxiety-like behavior and
449 general activity in rodents (Figure 4G, H). Together these data suggest that
450 *Parabacteroides* treatment negatively impacted both hippocampal-dependent
451 perirhinal cortex-dependent memory function without significantly affecting general
452 activity or anxiety-like behavior.

453

454 **Early life sugar consumption and *Parabacteroides* enrichment alter**
455 **hippocampal gene expression profiles**

456 To further investigate how sugar and *Parabacteroides* affect cognitive behaviors, we
457 conducted transcriptome analysis of the hippocampus samples. Figure S7 (A, C) shows
458 the results of principal component analysis revealing moderate separation based on
459 RNA sequencing data from the dorsal hippocampus of rats fed sugar in early life
460 compared with controls. Gene pathway enrichment analyses from RNA sequencing data

461 revealed multiple pathways significantly affected by early life sugar consumption,
462 including four pathways involved in neurotransmitter synaptic signaling: dopaminergic,
463 glutamatergic, cholinergic, and serotonergic signaling pathways. Additionally, several
464 gene pathways that also varied by sugar were those involved in kinase-mediated
465 intracellular signalling: cGMP-PKG, RAS, cAMP, and MAPK signaling pathways (Figure
466 5A, Table S1).

467 Analyses of individual genes across the entire transcriptome using a stringent
468 false-discovery rate criterion further identified 21 genes that were differentially
469 expressed in rats fed early life sugar compared with controls, with 11 genes elevated and
470 10 genes decreased in rats fed sugar compared to controls (Figure 5B). Among the genes
471 impacted, several genes that regulate cell survival, migration, differentiation, and DNA
472 repair were elevated by early life sugar access, including *Faap100*, which encodes an FA
473 core complex member of the DNA damage response pathway [49], and *Eepd1*, which
474 transcribes an endonuclease involved in repairing stalled DNA replication forks,
475 stressed from DNA damage [50]. Other genes associated with endoplasmic reticulum
476 stress and synaptogenesis were also significantly increased by sugar consumption,
477 including *Klf9, Dgkh, Neurod2, Ppl*, and *Kirrel1* [51,52,53,54].

478 Several genes were reduced by dietary sugar, including *Tns2*, which encodes
479 tensin 2, important for cell migration [55], *RelA*, which encodes a NF/kB complex protein
480 that regulates activity dependent neuronal function and synaptic plasticity [56], and
481 *Grm8*, the gene for the metabotropic glutamate receptor 8 (mGluR8). Notably, reduced
482 expression of mGluR8 receptor may contribute to the impaired neurocognitive
483 functioning in animals fed sugar, as mGluR8 knockout mice show impaired
484 hippocampal-dependent learning and memory [57].

485 Figure S7 (A-B, D) shows the results of principal component analysis of dorsal
486 hippocampus RNA sequencing data indicating moderate separation between rats
487 enriched with *Parabacteroides* and controls. Gene pathway analyses revealed that early
488 life *Parabacteroides* treatment, similar to effects associated with sugar consumption,
489 significantly altered the genetic signature of dopaminergic synaptic signaling pathways,
490 though differentially expressed genes were commonly affected in opposite directions
491 between the two experimental conditions (Figure S8). *Parabacteroides* treatment also
492 impacted gene pathways associated with metabolic signaling. Specifically, pathways
493 regulating fatty acid oxidation, rRNA metabolic processes, mitochondrial inner
494 membrane, and valine, leucine, and isoleucine degradation were significantly affected by
495 *Parabacteroides* enrichment. Other pathways that were influenced were those involved
496 in neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease,
497 though most of the genes affected in these pathways were mitochondrial genes (Figure
498 5D, Table S2).
499 At the level of individual genes, dorsal hippocampal RNA sequencing data
500 revealed that 15 genes were differentially expressed in rats enriched with
501 *Parabacteroides* compared with controls, with 13 genes elevated and two genes
502 decreased in the *Parabacteroides* group compared with controls (Figure 6C). Consistent
503 with results from gene pathway analyses, several individual genes involved in metabolic
504 processes were elevated by *Parabacteroides* enrichment, such as *Hmgcs2*, which is a
505 mitochondrial regulator of ketogenesis and provides energy to the brain under
506 metabolically taxing conditions or when glucose availability is low [58], and *Cox6b1*, a
507 mitochondrial regulator of energy metabolism that improves hippocampal cellular
508 viability following ischemia/reperfusion injury [59]. *Parabacteroides* enrichment was also

associated with incased expression of *Slc27A1* and *Mfrp*, which are each critical for the transport of fatty acids into the brain across capillary endothelial cells [60,61].

Discussion:

Dietary factors are a key source of gut microbiome diversity [29,47,62-64] and emerging evidence indicates that diet-induced alterations in the gut microbiota may be linked with altered neurocognitive development [29,64-66]. Our results identify species within the genus *Parabacteroides* that are elevated by habitual early life consumption of dietary sugar and are negatively associated with hippocampal-dependent memory performance. Further, targeted microbiota enrichment of *Parabacteroides* perturbed both hippocampal- and perirhinal cortex-dependent memory performance. These findings are consistent with previous literature in showing that early life consumption of Western dietary factors impair neurocognitive outcomes [10,11], and further suggest that altered gut bacteria due to excessive early life sugar consumption may functionally link dietary patterns with cognitive impairment.

Our previous data show that rats are not susceptible to habitual sugar consumption-induced learning and memory impairments when 11% sugar solutions are consumed ad libitum during adulthood, in contrast to effects observed in the present and previous study in which the sugar is consumed during early life development [24]. It is possible that habitual sugar consumption differentially affects the gut microbiome when consumed during adolescence vs. adulthood. However, a recent report showed that adult consumption of a high fructose diet (35% kcal from fructose) promotes gut microbial “dysbiosis” and neuroinflammation and cell death in the hippocampus, yet without impacting cognitive function [67], suggesting that perhaps neurocognitive

533 function is more susceptible to gut microbiota influences during early life than during
534 adulthood. Indeed, several reports have identified early life critical periods for
535 microbiota influences on behavioral and neurochemical endpoints in germ free mice [5,7
536 6]. However, the age-specific profile of sugar-associated microbiome dysbiosis and
537 neurocognitive impairments remains to be determined.

538 Given that the adolescent rats consuming SSBs compensated for these calories by
539 consuming less chow, it is possible that reduced nutrient (e.g., dietary protein)
540 consumption may have contributed to the deficits in hippocampal function. However,
541 we think this is unlikely, as adolescent SSB access did not produce any substantial
542 nutrient deficiency that would restrict growth, as evidenced by the similarities in body
543 weight between the experimental and control group. Furthermore, prior studies that
544 directly examined the effects of adolescent caloric (and thereby nutrient) restriction on
545 learning and memory in rats found that there were no differences in hippocampal-
546 dependent memory function when rats were restricted by ~40% from PN 25-PN 67 [68],
547 Importantly, the parameters in this study closely match those in the present study, as
548 our adolescent SSB access was given over a similar developmental period prior to
549 behavioral testing, and produced a ~40% reduction in total chow kcal consumption.
550 Thus, it is likely that excessive sugar consumption and not nutrient deficiency led to the
551 memory deficits, although future work is needed to more carefully examine these
552 variables independently.

553 While our study reveals a strong negative correlation between levels of fecal
554 *Parabacteroides* and performance in the hippocampal-dependent contextual episodic
555 memory NOIC task, as well as impaired NOIC performance in rats given access to a
556 sugar solution during adolescence, sugar intake did not produce impairments in the

557 perirhinal cortex-dependent NOR memory task. This is consistent with our previous
558 report in which rats given access to an 11% sugar solution during adolescence were
559 impaired in hippocampal-dependent spatial memory (Barne's maze procedure), yet
560 were not impaired in a nonspatial task of comparable difficulty that was not
561 hippocampal-dependent [24]. Present results revealing that early life sugar consumption
562 negatively impacts hippocampal-dependent contextual-based object recognition
563 memory (NOIC) without influencing NOR memory performance is also consistent with
564 previous reports using a cafeteria diet high in both fat content and sugar [69,70]. On the
565 other hand, enrichment of *P. johnsonii* and *P. distasonis* in the present study impaired
566 memory performance in both tasks, suggesting a broader impact on neurocognitive
567 functioning with this targeted bacterial enrichment approach.

568 Gene pathway analyses from dorsal hippocampus RNA sequencing identified
569 multiple neurobiological pathways that may functionally connect gut dysbiosis with
570 memory impairment. Early life sugar consumption was associated with alterations in
571 several neurotransmitter synaptic signaling pathways (e.g., glutamatergic, cholinergic)
572 and intracellular signaling targets (e.g., cAMP, MAPK). A different profile was observed
573 in *Parabacteroides*-enriched animals, where gene pathways involved with metabolic
574 function (e.g., fatty acid oxidation, branched chain amino acid degradation) and
575 neurodegenerative disease (e.g., Alzheimer's disease) were altered relative to controls.
576 Given that sugar has effects on bacterial populations in addition to *Parabacteroides*,
577 and that sugar consumption and *Parabacteroides* treatment differentially influenced
578 peripheral glucose metabolism and body weight, these transcriptome differences in the
579 hippocampus are not surprising. However, gene clusters involved with dopaminergic
580 synaptic signaling were significantly influenced by both early life sugar consumption

581 and *Parabacteroides* treatment, thus identifying a common pathway through which
582 both diet-induced and gut bacterial infusion-based elevations in *Parabacteroides* may
583 influence neurocognitive development. Though differentially expressed genes were
584 commonly affected in opposite directions in *Parabacteroides* enriched animals
585 compared with early life sugar treated animals, it is possible that perturbations to the
586 dopamine system play a role in the observed cognitive dysfunction. For example, while
587 dopamine signaling in the hippocampus has not traditionally been investigated for
588 mediating memory processes, several recent reports have identified a role for dopamine
589 inputs from the locus coeruleus in regulating hippocampal-dependent memory and
590 neuronal activity [71,72]. Interestingly, endogenous dopamine signaling in the
591 hippocampus has recently been linked with regulating food intake and food-associated
592 contextual learning [73], suggesting that dietary effects on gut microbiota may also impact
593 feeding behavior and energy balance-relevant cognitive processes.

594 It is important to note that comparisons between the gene expressional analyses
595 in the *Parabacteroides* enrichment and sugar consumption experiments should be
596 made cautiously given that there were slight differences in timing of the hippocampus
597 tissue harvest between the two experiments (PN 65 for sugar consumption vs PN 83 for
598 the *Parabacteroides* enrichment). Further, future work is needed to determine whether
599 differences in gene expression observed in each experiment translates to differential
600 expression at the protein level. It is also worth emphasizing that the levels of
601 *Parabacteroides* conferred by our enrichment study were substantially higher than in
602 the dietary sugar study, and thus it is not surprising that *Parabacteroides* enrichment
603 would confer a different impact on host physiology, hippocampal gene expression, and
604 neurocognition compared to *Parabacteroides* elevations associated with SSB

605 consumption. Regardless of these caveats in comparing the two models, our data extend
606 the field by highlighting a specific bacterial population that 1) is capable of negatively
607 impacting neurocognitive development when experimentally enriched, and 2) is
608 elevated by early-life consumption of dietary sugar with levels correlating negatively
609 with hippocampal-dependent memory performance.

610 Many of the genes that were differentially upregulated in the hippocampus by
611 *Parabacteroides* enrichment were involved in fat metabolism and transport. Thus, it is
612 possible that *Parabacteroides* conferred an adaptation in the brain, shifting fuel
613 preference away from carbohydrate toward lipid-derived ketones. Consistent with this
614 framework, *Parabacteroides* was previously shown to be upregulated by a ketogenic
615 diet in which carbohydrate consumption is drastically depleted and fat is used as a
616 primary fuel source due. Furthermore, enrichment of *Parabacteroides merdae* together
617 with *Akkermansia muciniphila* was protective against seizures in mice [29]. It is possible
618 that *P. distasonis* reduces glucose uptake from the gut, enhances glucose clearing from
619 the blood, and/or alters nutrient utilization in general, an idea further supported by
620 recent finding that *P. distasonis* is associated with reduced diet- and genetic-induced
621 obesity and hyperglycemia in mice [48].

622 The present findings produce several opportunities for further mechanistic
623 investigation. For example, how do diet-induced alterations in gut bacteria impact the
624 brain? Several possible mechanisms have been investigated and proposed, such as
625 impaired gut barrier function and endotoxemia [64,74], perhaps related to altered short
626 chain fatty acid production [67,75]. Moreover it is well known that the liver is negatively
627 impacted by excessive fructose consumption [76], and emerging evidence highlights a gut
628 microbiome-liver axis with crosstalk via bile acids and cytokines [77]. It is possible that

629 dietary sugar induced microbiota changes alter the hepatic-gut axis, thus contributing to
630 altered cognitive function. Indeed, an altered bile acid profile due to gut microbiota
631 produced bile acid secondary metabolites is associated with cognitive dysfunction in
632 Alzheimer's Disease in humans [78].

633 Taken together, our collective results provide insight into the neurobiological
634 mechanisms that link early life unhealthy dietary patters with altered gut microbiota
635 changes and neurocognitive impairments. Currently probiotics, live microorganisms
636 intended to confer health benefits, are not regulated with the same rigor as
637 pharmaceuticals but instead are sold as dietary supplements. Our findings suggest that
638 gut enrichment with certain species of *Parabacteroides* is potentially harmful for
639 neurocognitive mnemonic development. These results highlight the importance of
640 conducting rigorous basic science analyses on the relationship between diet,
641 microorganisms, brain, and behavior prior to widespread recommendations of bacterial
642 microbiome interventions for humans.

643

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652

Conflicts of interest:

The authors declare no competing interests.

References:

1 Vuong, H. E., Yano, J. M., Fung, T. C. & Hsiao, E. Y. The Microbiome and Host Behavior. *Annu Rev Neurosci* **40**, 21-49, doi:10.1146/annurev-neuro-072116-031347 (2017).

2 Noble, E. E., Hsu, T. M. & Kanoski, S. E. Gut to Brain Dysbiosis: Mechanisms Linking Western Diet Consumption, the Microbiome, and Cognitive Impairment. *Front Behav Neurosci* **11**, 9, doi:10.3389/fnbeh.2017.00009 (2017).

3 Lach, G. *et al.* Enduring neurobehavioral effects induced by microbiota depletion during the adolescent period. *Transl Psychiatry* **10**, 382, doi:10.1038/s41398-020-01073-0 (2020).

4 Morais, L. H. *et al.* Enduring Behavioral Effects Induced by Birth by Caesarean Section in the Mouse. *Curr Biol* **30**, 3761-3774 e3766, doi:10.1016/j.cub.2020.07.044 (2020).

5 Neufeld, K. A., Kang, N., Bienenstock, J. & Foster, J. A. Effects of intestinal microbiota on anxiety-like behavior. *Commun Integr Biol* **4**, 492-494, doi:10.4161/cib.4.4.15702 (2011).

6 Sudo, N. *et al.* Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* **558**, 263-275, doi:10.1113/jphysiol.2004.063388 (2004).

7 Diaz Heijtz, R. *et al.* Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* **108**, 3047-3052, doi:10.1073/pnas.1010529108 (2011).

8 Cryan, J. F. *et al.* The Microbiota-Gut-Brain Axis. *Physiological reviews* **99**, 1877-2013, doi:10.1152/physrev.00018.2018 (2019).

9 Kanoski, S. E. & Davidson, T. L. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav* **103**, 59-68, doi:10.1016/j.physbeh.2010.12.003 (2011).

10 Noble, E. E., Hsu, T. M., Liang, J. & Kanoski, S. E. Early-life sugar consumption has long-term negative effects on memory function in male rats. *Nutritional neuroscience*, 1-11, doi:10.1080/1028415X.2017.1378851 (2019).

11 Noble, E. E. & Kanoski, S. E. Early life exposure to obesogenic diets and learning and memory dysfunction. *Curr Opin Behav Sci* **9**, 7-14, doi:10.1016/j.cobeha.2015.11.014 (2016).

12 Hsu, T. M. *et al.* Hippocampus ghrelin receptor signaling promotes socially-mediated learned food preference. *Neuropharmacology* **131**, 487-496, doi:10.1016/j.neuropharm.2017.11.039 (2018).

13 Hsu, T. M. *et al.* A hippocampus to prefrontal cortex neural pathway inhibits food motivation through glucagon-like peptide-1 signaling. *Mol Psychiatry* **23**, 1555-1565, doi:10.1038/mp.2017.91 (2018).

14 Hsu, T. M. *et al.* Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. *Elife* **4**, doi:10.7554/eLife.11190 (2015).

15 Kanoski, S. E., Fortin, S. M., Ricks, K. M. & Grill, H. J. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol Psychiatry* **73**, 915-923, doi:10.1016/j.biopsych.2012.07.002 (2013).

16 Davidson, T. L. *et al.* Contributions of the hippocampus and medial prefrontal cortex to energy and body weight regulation. *Hippocampus* **19**, 235-252, doi:10.1002/hipo.20499 (2009).

17 Kanoski, S. E. & Grill, H. J. Hippocampus Contributions to Food Intake Control: Mnemonic, Neuroanatomical, and Endocrine Mechanisms. *Biol Psychiatry* **81**, 748-756, doi:10.1016/j.biopsych.2015.09.011 (2017).

18 Kanoski, S. E. & Davidson, T. L. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav* **103**, 59-68, doi:10.1016/j.physbeh.2010.12.003 (2011).

19 Davidson, T. L., Sample, C. H. & Swithers, S. E. An application of Pavlovian principles to the problems of obesity and cognitive decline. *Neurobiology of learning and memory* **108**, 172-184, doi:10.1016/j.nlm.2013.07.014 (2014).

20 Baym, C. L. *et al.* Dietary lipids are differentially associated with hippocampal-dependent relational memory in prepubescent children. *Am J Clin Nutr* **99**, 1026-1032, doi:10.3945/ajcn.113.079624 (2014).

21 Valladolid-Acebes, I. *et al.* Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? *Neurobiol Learn Mem* **106**, 18-25, doi:10.1016/j.nlm.2013.06.012 (2013).

22 Boitard, C. *et al.* Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. *Brain Behav Immun* **40**, 9-17, doi:10.1016/j.bbi.2014.03.005 (2014).

23 Boitard, C. *et al.* Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. *Hippocampus* **22**, 2095-2100, doi:10.1002/hipo.22032 (2012).

24 Hsu, T. M. *et al.* Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus* **25**, 227-239, doi:10.1002/hipo.22368 (2015).

25 Kendig, M. D., Boakes, R. A., Rooney, K. B. & Corbit, L. H. Chronic restricted access to 10% sucrose solution in adolescent and young adult rats impairs spatial memory and alters sensitivity to outcome devaluation. *Physiol Behav* **120**, 164-172, doi:10.1016/j.physbeh.2013.08.012 (2013).

26 Reichelt, A. C., Killcross, S., Hambly, L. D., Morris, M. J. & Westbrook, R. F. Impact of adolescent sucrose access on cognitive control, recognition memory, and parvalbumin immunoreactivity. *Learn Mem* **22**, 215-224, doi:10.1101/lm.038000.114 (2015).

27 Noble, E. E., Hsu, T. M., Liang, J. & Kanoski, S. E. Early-life sugar consumption has long-term negative effects on memory function in male rats. *Nutr Neurosci* **22**, 273-283, doi:10.1080/1028415X.2017.1378851 (2019).

28 Walker, R. W., Dumke, K. A. & Goran, M. I. Fructose content in popular beverages made with and without high-fructose corn syrup. *Nutrition* **30**, 928-935, doi:10.1016/j.nut.2014.04.003 (2014).

29 Olson, C. A. *et al.* The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell* **173**, 1728-1741 e1713, doi:10.1016/j.cell.2018.04.027 (2018).

30 Martinez, M. C., Villar, M. E., Ballarini, F. & Viola, H. Retroactive interference of object-in-context long-term memory: role of dorsal hippocampus and medial prefrontal cortex. *Hippocampus* **24**, 1482-1492, doi:10.1002/hipo.22328 (2014).

740 31 Balderas, I. *et al.* The consolidation of object and context recognition memory involve
741 different regions of the temporal lobe. *Learn Mem* **15**, 618-624,
742 doi:10.1101/lm.1028008 (2008).
743 32 Beilharz, J. E., Maniam, J. & Morris, M. J. Short exposure to a diet rich in both fat and
744 sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain*
745 *Behav Immun* **37**, 134-141, doi:10.1016/j.bbi.2013.11.016 (2014).
746 33 Thompson, L. R. *et al.* A communal catalogue reveals Earth's multiscale microbial
747 diversity. *Nature* **551**, 457-463, doi:10.1038/nature24621 (2017).
748 34 Caporaso, J. G. *et al.* Ultra-high-throughput microbial community analysis on the
749 Illumina HiSeq and MiSeq platforms. *ISME J* **6**, 1621-1624, doi:10.1038/ismej.2012.8
750 (2012).
751 35 Patro, R., Duggal, G., Love, M. I., Irizarry, R. A. & Kingsford, C. Salmon provides fast and
752 bias-aware quantification of transcript expression. *Nat Methods* **14**, 417-419,
753 doi:10.1038/nmeth.4197 (2017).
754 36 Soneson, C., Love, M. I. & Robinson, M. D. Differential analyses for RNA-seq: transcript-
755 level estimates improve gene-level inferences. *F1000Res* **4**, 1521,
756 doi:10.12688/f1000research.7563.2 (2015).
757 37 Anders, S. & Huber, W. Differential expression analysis for sequence count data.
758 *Genome Biol* **11**, R106, doi:10.1186/gb-2010-11-10-r106 (2010).
759 38 Kuleshov, M. V. *et al.* Enrichr: a comprehensive gene set enrichment analysis web server
760 2016 update. *Nucleic Acids Res* **44**, W90-97, doi:10.1093/nar/gkw377 (2016).
761 39 Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. KEGG: new
762 perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* **45**, D353-
763 D361, doi:10.1093/nar/gkw1092 (2017).
764 40 The Gene Ontology, C. Expansion of the Gene Ontology knowledgebase and resources.
765 *Nucleic Acids Res* **45**, D331-D338, doi:10.1093/nar/gkw1108 (2017).
766 41 Slenter, D. N. *et al.* WikiPathways: a multifaceted pathway database bridging
767 metabolomics to other omics research. *Nucleic Acids Res* **46**, D661-D667,
768 doi:10.1093/nar/gkx1064 (2018).
769 42 Aggleton, J. P. & Brown, M. W. Contrasting hippocampal and perirhinal cortex function
770 using immediate early gene imaging. *Q J Exp Psychol B* **58**, 218-233,
771 doi:10.1080/02724990444000131 (2005).
772 43 Albasser, M. M., Davies, M., Futter, J. E. & Aggleton, J. P. Magnitude of the object
773 recognition deficit associated with perirhinal cortex damage in rats: Effects of varying
774 the lesion extent and the duration of the sample period. *Behav Neurosci* **123**, 115-124,
775 doi:10.1037/a0013829 (2009).
776 44 Cohen, S. J. & Stackman, R. W., Jr. Assessing rodent hippocampal involvement in the
777 novel object recognition task. A review. *Behav Brain Res* **285**, 105-117,
778 doi:10.1016/j.bbr.2014.08.002 (2015).
779 45 Sestakova, N., Puzserova, A., Kluknavsky, M. & Bernatova, I. Determination of motor
780 activity and anxiety-related behaviour in rodents: methodological aspects and role of
781 nitric oxide. *Interdiscip Toxicol* **6**, 126-135, doi:10.2478/intox-2013-0020 (2013).
782 46 Goran, M. I. *et al.* The obesogenic effect of high fructose exposure during early
783 development. *Nat Rev Endocrinol* **9**, 494-500, doi:10.1038/nrendo.2013.108 (2013).

784 47 Noble, E. E. *et al.* Early-Life Sugar Consumption Affects the Rat Microbiome
785 Independently of Obesity. *J Nutr* **147**, 20-28, doi:10.3945/jn.116.238816 (2017).
786 48 Wang, K. *et al.* Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions
787 via Production of Succinate and Secondary Bile Acids. *Cell Rep* **26**, 222-235 e225,
788 doi:10.1016/j.celrep.2018.12.028 (2019).
789 49 Ling, C. *et al.* FAAP100 is essential for activation of the Fanconi anemia-associated DNA
790 damage response pathway. *EMBO J* **26**, 2104-2114, doi:10.1038/sj.emboj.7601666
791 (2007).
792 50 Kim, H. S. *et al.* Endonuclease EEPD1 Is a Gatekeeper for Repair of Stressed Replication
793 Forks. *J Biol Chem* **292**, 2795-2804, doi:10.1074/jbc.M116.758235 (2017).
794 51 Zucker, S. N. *et al.* Nrf2 amplifies oxidative stress via induction of Klf9. *Mol Cell* **53**, 916-
795 928, doi:10.1016/j.molcel.2014.01.033 (2014).
796 52 Yasuda, S. *et al.* Diacylglycerol kinase eta augments C-Raf activity and B-Raf/C-Raf
797 heterodimerization. *J Biol Chem* **284**, 29559-29570, doi:10.1074/jbc.M109.043604
798 (2009).
799 53 Murdoch, H. *et al.* Periplakin interferes with G protein activation by the melanin-
800 concentrating hormone receptor-1 by binding to the proximal segment of the receptor
801 C-terminal tail. *J Biol Chem* **280**, 8208-8220, doi:10.1074/jbc.M405215200 (2005).
802 54 Gerke, P. *et al.* Neuronal expression and interaction with the synaptic protein CASK
803 suggest a role for Neph1 and Neph2 in synaptogenesis. *J Comp Neurol* **498**, 466-475,
804 doi:10.1002/cne.21064 (2006).
805 55 Chen, H., Duncan, I. C., Bozorgchami, H. & Lo, S. H. Tensin1 and a previously
806 undocumented family member, tensin2, positively regulate cell migration. *Proc Natl*
807 *Acad Sci U S A* **99**, 733-738, doi:10.1073/pnas.022518699 (2002).
808 56 O'Mahony, A. *et al.* NF-kappaB/Rel regulates inhibitory and excitatory neuronal function
809 and synaptic plasticity. *Mol Cell Biol* **26**, 7283-7298, doi:10.1128/MCB.00510-06 (2006).
810 57 Gerlai, R., Adams, B., Fitch, T., Chaney, S. & Baez, M. Performance deficits of mGluR8
811 knockout mice in learning tasks: the effects of null mutation and the background
812 genotype. *Neuropharmacology* **43**, 235-249, doi:10.1016/s0028-3908(02)00078-3
813 (2002).
814 58 Shao, X. *et al.* HMG-CoA synthase 2 drives brain metabolic reprogramming in cocaine
815 exposure. *Neuropharmacology* **148**, 377-393, doi:10.1016/j.neuropharm.2017.10.001
816 (2019).
817 59 Yang, S., Wu, P., Xiao, J. & Jiang, L. Overexpression of COX6B1 protects against
818 I/Rinduced neuronal injury in rat hippocampal neurons. *Mol Med Rep* **19**, 4852-4862,
819 doi:10.3892/mmr.2019.10144 (2019).
820 60 Ochiai, Y. *et al.* The blood-brain barrier fatty acid transport protein 1 (FATP1/SLC27A1)
821 supplies docosahexaenoic acid to the brain, and insulin facilitates transport. *J*
822 *Neurochem* **141**, 400-412, doi:10.1111/jnc.13943 (2017).
823 61 Kautzmann, M. I. *et al.* Membrane-type frizzled-related protein regulates lipidome and
824 transcription for photoreceptor function. *FASEB J* **34**, 912-929,
825 doi:10.1096/fj.201902359R (2020).
826 62 David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature*
827 **505**, 559-563, doi:10.1038/nature12820 (2014).

63 de La Serre, C. B. *et al.* Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol* **299**, G440-448, doi:10.1152/ajpgi.00098.2010 (2010).
64 Bruce-Keller, A. J. *et al.* Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biol Psychiatry* **77**, 607-615, doi:10.1016/j.biopsych.2014.07.012 (2015).
65 Leigh, S. J., Kaakoush, N. O., Westbrook, R. F. & Morris, M. J. Minocycline-induced microbiome alterations predict cafeteria diet-induced spatial recognition memory impairments in rats. *Transl Psychiatry* **10**, 92, doi:10.1038/s41398-020-0774-1 (2020).
66 Leigh, S. J., Kaakoush, N. O., Bertoldo, M. J., Westbrook, R. F. & Morris, M. J. Intermittent cafeteria diet identifies fecal microbiome changes as a predictor of spatial recognition memory impairment in female rats. *Transl Psychiatry* **10**, 36, doi:10.1038/s41398-020-0734-9 (2020).
67 Li, J. M. *et al.* Dietary fructose-induced gut dysbiosis promotes mouse hippocampal neuroinflammation: a benefit of short-chain fatty acids. *Microbiome* **7**, 98, doi:10.1186/s40168-019-0713-7 (2019).
68 Alamy, M., Errami, M., Taghzouti, K., Saddiki-Traki, F. & Bengelloun, W. A. Effects of postweaning undernutrition on exploratory behavior, memory and sensory reactivity in rats: implication of the dopaminergic system. *Physiol Behav* **86**, 195-202, doi:10.1016/j.physbeh.2005.07.008 (2005).
69 Kendig, M. D., Westbrook, R. F. & Morris, M. J. Pattern of access to cafeteria-style diet determines fat mass and degree of spatial memory impairments in rats. *Sci Rep* **9**, 13516, doi:10.1038/s41598-019-50113-3 (2019).
70 Yang, Y. *et al.* Early-life high-fat diet-induced obesity programs hippocampal development and cognitive functions via regulation of gut commensal Akkermansia muciniphila. *Neuropsychopharmacology* **44**, 2054-2064, doi:10.1038/s41386-019-0437-1 (2019).
71 Takeuchi, T. *et al.* Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature* **537**, 357-362, doi:10.1038/nature19325 (2016).
72 Kempadoo, K. A., Mosharov, E. V., Choi, S. J., Sulzer, D. & Kandel, E. R. Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. *Proc Natl Acad Sci U S A* **113**, 14835-14840, doi:10.1073/pnas.1616515114 (2016).
73 Azevedo, E. P. *et al.* A Role of Drd2 Hippocampal Neurons in Context-Dependent Food Intake. *Neuron* **102**, 873-886 e875, doi:10.1016/j.neuron.2019.03.011 (2019).
74 Ou, Z. *et al.* Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. *Nutr Diabetes* **10**, 12, doi:10.1038/s41387-020-0115-8 (2020).
75 Hu, L. *et al.* High Salt Elicits Brain Inflammation and Cognitive Dysfunction, Accompanied by Alternations in the Gut Microbiota and Decreased SCFA Production. *J Alzheimers Dis* **77**, 629-640, doi:10.3233/JAD-200035 (2020).
76 Stanhope, K. L. Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit Rev Clin Lab Sci* **53**, 52-67, doi:10.3109/10408363.2015.1084990 (2016).

872 77 Cerdo, T., Dieguez, E. & Campoy, C. Early nutrition and gut microbiome:
873 interrelationship between bacterial metabolism, immune system, brain structure, and
874 neurodevelopment. *Am J Physiol Endocrinol Metab* **317**, E617-E630,
875 doi:10.1152/ajpendo.00188.2019 (2019).
876 78 MahmoudianDehkordi, S. *et al.* Altered bile acid profile associates with cognitive
877 impairment in Alzheimer's disease-An emerging role for gut microbiome. *Alzheimers*
878 *Dement* **15**, 76-92, doi:10.1016/j.jalz.2018.07.217 (2019).
879

880

881 **Figure Legends**

882

883 **Figure 1: Early-life sugar consumption negatively impacts hippocampal-**
884 **dependent memory function.** (A, B) Early life sugar consumption had no effect on
885 total exploration time on days 1 (familiarization) or day 3 (test day) of the Novel Object
886 in Context (NOIC) task. (C) The discrimination index was significantly reduced by early
887 life sugar consumption, indicating impaired hippocampal function (P<.05, n=10,11;
888 two-tailed, type 2 Student's T-test). (D) There were no differences in exploration index
889 in the Novel Object Recognition (NOR task) (n=6; two-tailed, type 2 Student's T-test).
890 (E, F) There were no differences in time spent in the open arm or the number of entries
891 into the open arm in the Zero Maze task for anxiety-like behavior (n=10; two-tailed, type
892 2 Student's t-test). (G, H) There were no differences in distance travelled or time spent
893 in the center arena in the Open Field task (n=8; two-tailed, type 2 Student's T-test). (I)
894 There were no differences in body fat % during adulthood between rats fed early life
895 sugar and controls (n=10,11; two-tailed, type 2 Student's T-test). (J-K) Body weights and
896 total energy intake did not differ between the groups (n=10,11; two- way repeated
897 measures ANOVA), despite (L) increased kcal consumption from sugar sweetened

898 beverages in the sugar group. CTL=control, SUG= sugar, PN= post-natal day; data
899 shown as mean ± SEM.
900
901
902 **Figure 2: Effect of adolescent sugar consumption on the gut microbiome in**
903 **rats.** (A) Principal component analysis showing separation between fecal microbiota of
904 rats fed early life sugar or controls (n=11, 10; dark triangles= sugar, open circles=
905 control). (B) Results from LEfSe analysis showing Linear Discriminate Analysis (LDA)
906 scores for microbiome analysis of fecal samples of rats fed early life sugar or controls.
907 (C) A cladogram representing the results from the LEfSe analysis with class as the outer
908 most taxonomic level and species at the inner most level. Taxa in red are elevated in the
909 sugar group. (D) Relative % abundance of fecal *Parabacteroides* are significantly
910 elevated in rats fed early life sugar (P<.05; n=11, 10, two-tailed, type 2 Student's T-test).
911 (E) Linear regression of log normalized fecal *Parabacteroides* counts against shift from
912 baseline performance scores in the novel object in context task (NOIC) across all groups
913 tested (n=21). (E, F) Linear regression of the most abundant fecal *Parabacteroides*
914 species against shift from baseline performance scores in NOIC across all groups tested
915 (n=21). *P<0.05; data shown as mean ± SEM.
916
917
918 **Figure 3: Intestinal *Parabacteroides* is enriched by antibiotic treatment**
919 **and oral gavage of *P. distasonis* and *P. johnsonii*.** A) Schematic showing the
920 timeline for the experimental design of the *Parabacteroides* transfer experiment. B)
921 Alpha diversity based on 16S rRNA gene profiling of fecal matter (n=7-8) represented by

922 observed operational taxonomic units (OTUs) for a given number of sample sequences.
923 C) Principal coordinates analysis of weighted UniFrac distance based on 16S rRNA gene
924 profiling of feces for SAL-SAL, ABX-SAL, and ABX-PARA enriched rats (n=7-8). D)
925 Average taxonomic distributions of bacteria from 16S rRNA gene sequencing data of
926 feces for SAL-SAL, ABX-SAL, and ABX-PARA enriched animals (n=7-8). E) Relative
927 abundances of *Parabacteroides* in fecal microbiota for SAL-SAL, ABX-SAL, and ABX-
928 PARA enriched animals (n=7-8) (ANCOM). PN= post-natal day, IP GTT=
929 intraperitoneal glucose tolerance test. Data are presented as mean ± S.E.M. * $p < 0.05$,
930 ** $p < 0.01$, *** $p < 0.001$. n.s.=not statistically significant. SAL-SAL= rats treated with
931 saline, ABX-SAL=rats treated with antibiotics followed by sterile saline gavage, ABX-
932 PARA= rats treated with antibiotics followed by a 1:1 gavage of *Parabacteroides*
933 *distasonis* and *Parabacteroides johnsonii*.
934
935
936 **Figure 4: Early-life enrichment with *Parabacteroides* negatively impacts**
937 **neurocognitive function.** (A, B) Early-life enrichment with a 1:1 ratio of *P. johnsonii*
938 and *P. distasonis* had no effect on total exploration time in the Novel Object in Context
939 (NOIC) task. (C) Discrimination index was significantly reduced by enrichment with *P.*
940 *johnsonii* and *P. distasonis*, indicating impaired hippocampal function (n=7,8; $F_{(2, 19)} =$
941 4.92; P< .05, one-way ANOVA with Tukey's multiple comparison test). (D) There was a
942 significant reduction in the exploration index in the Novel Object Recognition (NOR
943 task), indicating impaired perirhinal cortex function (n=7,8; $F_{(2, 19)} = 3.61$; P< .05, one-
944 way ANOVA with Tukey's multiple comparison test). (E, F) There were no differences in
945 time spent or number of entries into the open arm by animals with *P. johnsonii* and *P.*

946 *distasonis* enrichment in the Zero Maze task for anxiety-like behavior (n=7,8; one-way
947 ANOVA). (G, H) There were no differences in distance travelled or time spent in the
948 center arena in the Open Field task (n=7,8; one-way ANOVA). SAL-SAL=saline-saline
949 control, ABX-SAL= antibiotics-saline control, ABX-PARA= antibiotics-*P. johnsonii* and
950 *P. distasonis* enriched, PN= post-natal day; data shown as mean ± SEM; * *P*<.05.
951
952
953 **Figure 5: Effect of early life sugar or targeted *Parabacteroides* enrichment**
954 **on hippocampal gene expression.** (A) Pathway analyses for differentially expressed
955 genes (DEGs) at a p-value < 0.01 in hippocampal tissue punches from rats fed early life
956 sugar compared with controls. Upregulation by sugar is shown in red and
957 downregulation by sugar in blue. (B) A heatmap depicting DEGs that survived the
958 Benjamini-Hochberg corrected FDR of P< 0.05 in rats fed early life sugar compared
959 with controls. Warmer colors (red) signify an increase in gene expression and cool
960 colors (blue) a reduction in gene expression by treatment (CTL=control, SUG= early life
961 sugar; n=7/group). (C) A heatmap depicting DEGs that survived the Benjamini-
962 Hochberg corrected FDR of P< 0.05 in rats with early life *Parabacteroides* enrichment
963 compared with combined control groups. Warmer colors (red) signify an increase in
964 gene expression and cool colors (blue) a reduction in gene expression by treatment
965 (n=7, 14). (D) Pathway analyses for differentially expressed genes (DEGs) at a P-value <
966 0.01 in rats enriched with *Parabacteroides* compared with combined controls.
967 Upregulation by *Parabacteroides* transfer is shown in red and downregulation in blue.
968 Dotted line indicates ±0.25 log2 fold change.

Figure 1

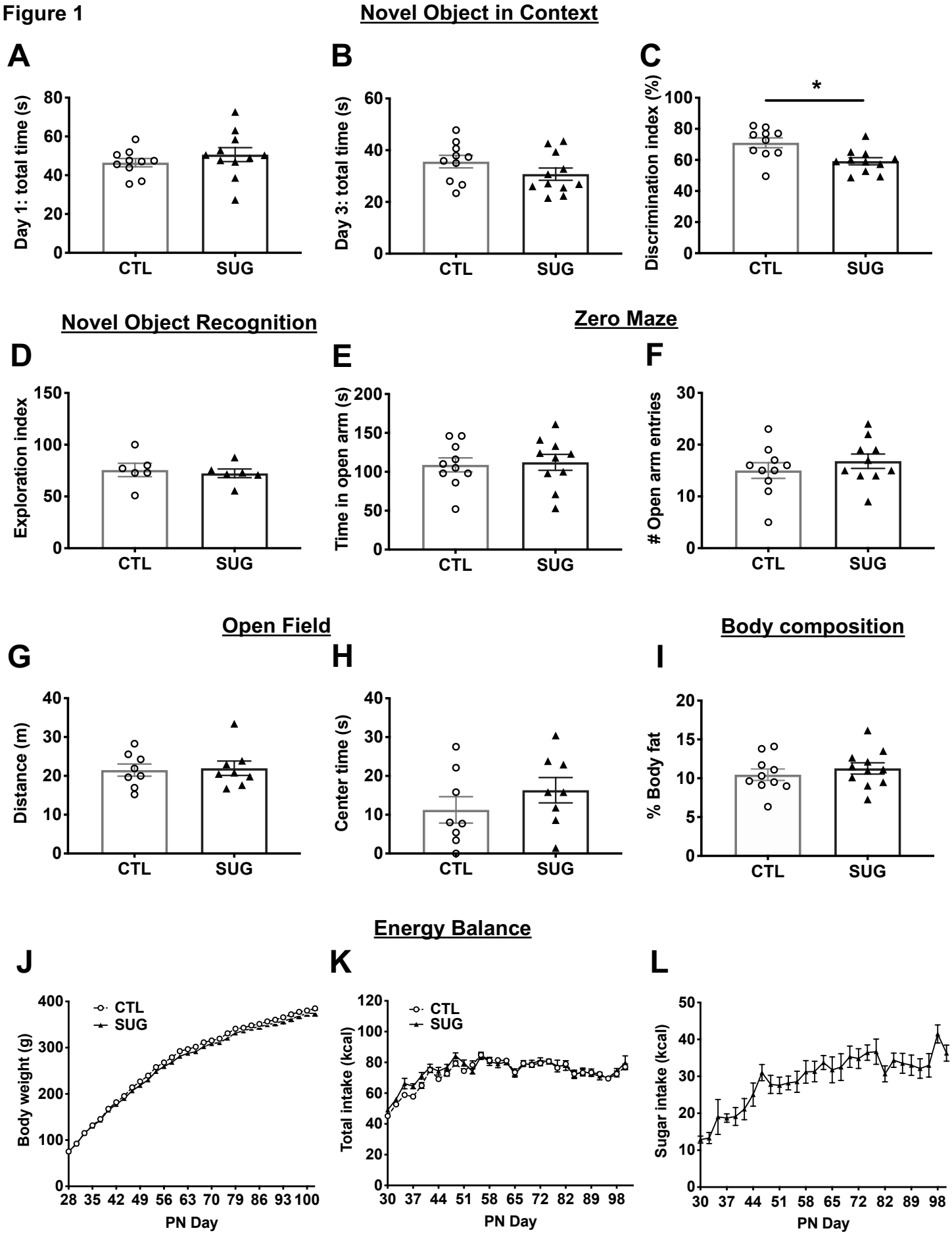

Novel Object in Context
A
Day 1: total time (s)
CTL
SUG
B
Day 3: total time (s)
C
Discrimination index (%)
*
Novel Object Recognition
D
Exploration index
Zero Maze
E
Time in open arm (s)
F
Open arm entries
Open Field
G
Distance (m)
H
Center time (s)
Body composition
I
% Body fat
Energy Balance
J
Body weight (g)
PN Day
K
Total intake (kcal)
L
Sugar intake (kcal)

Figure 2

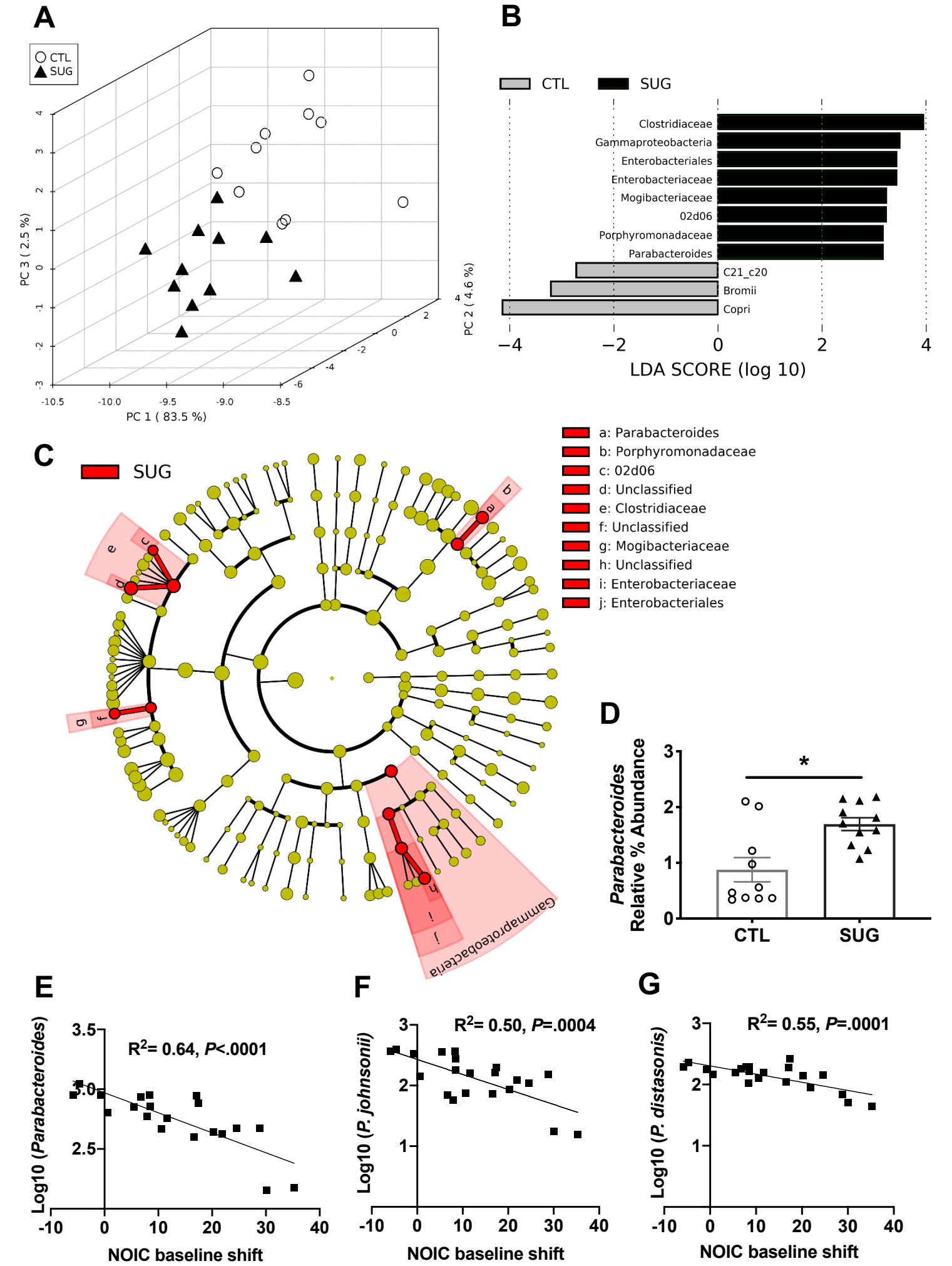
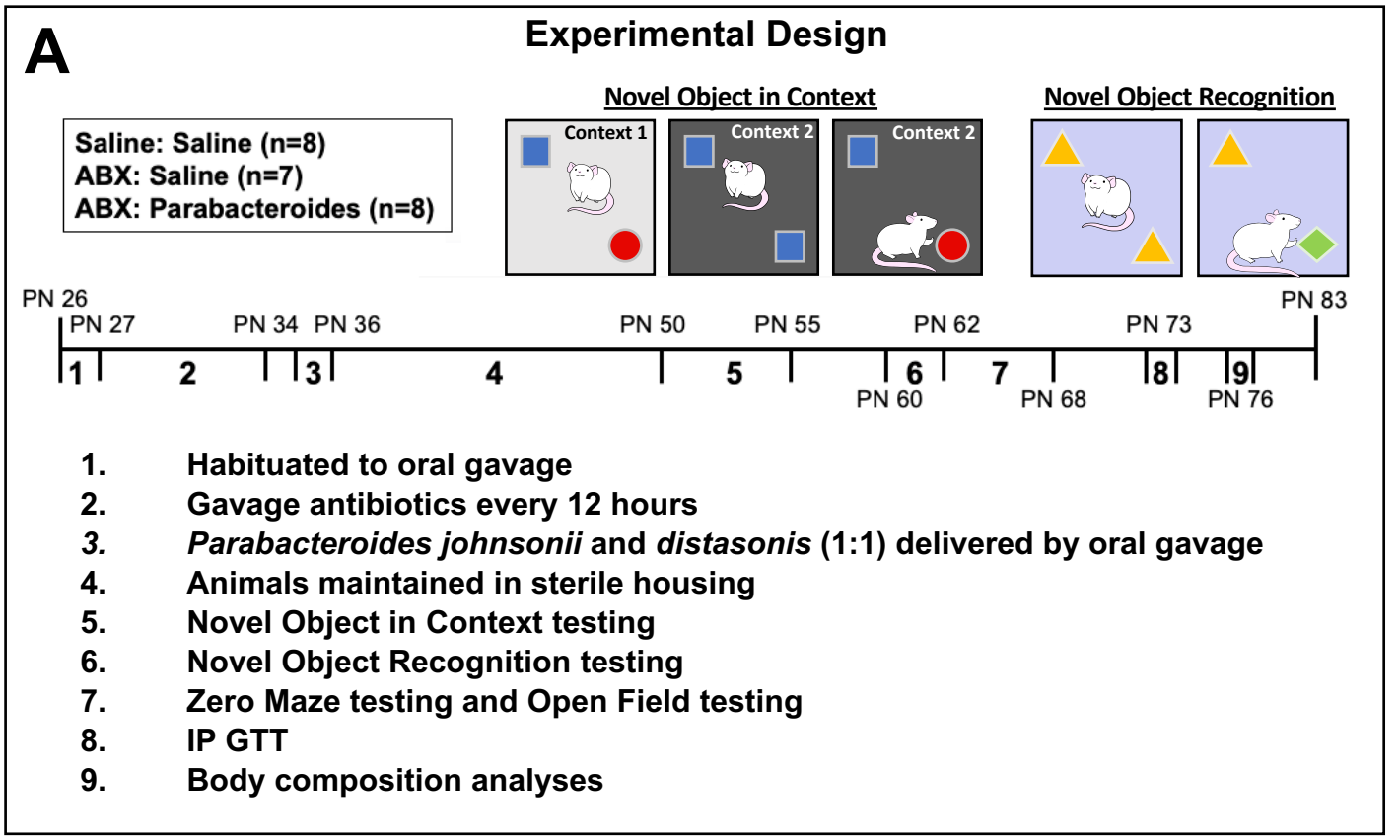
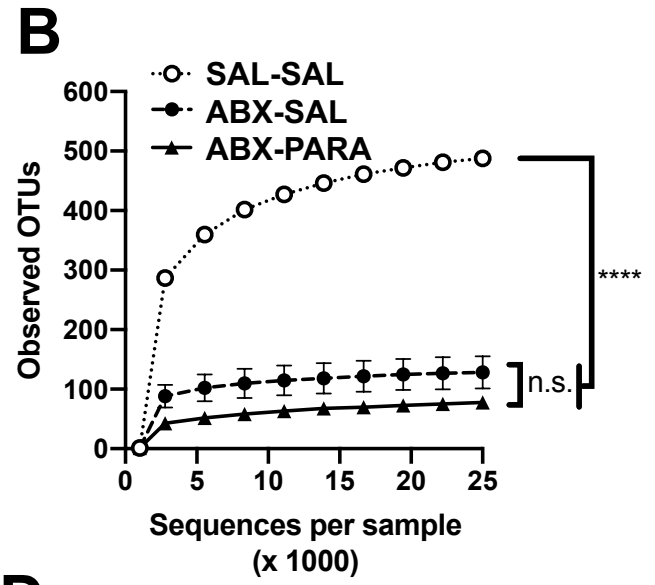
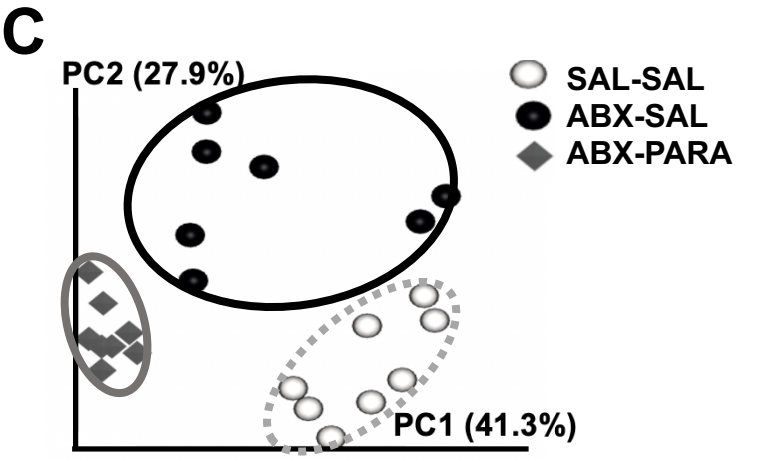
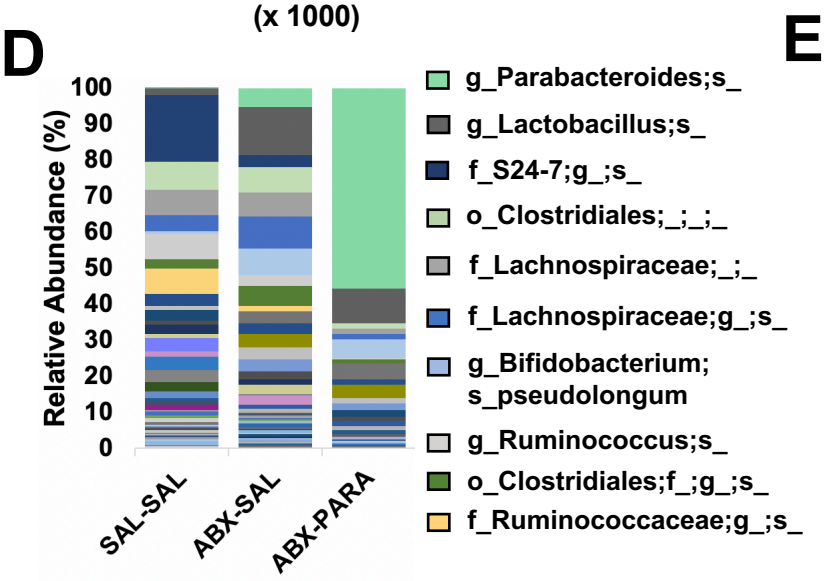

A
CTL
SUG
PC 3 (2.5 %)
PC 1 (83.5 %)
PC 2 (4.6 %)
B
CTL
SUG
Clostridiaceae
Gammaproteobacteria
Enterobacteriales
Enterobacteriaceae
Mogibacteriaceae
02d06
Porphyromonadaceae
Parabacteroides
C21_c20
Bromii
Copri
LDA SCORE (log 10)
C
SUG
a: Parabacteroides
b: Porphyromonadaceae
c: 02d06
d: Unclassified
e: Clostridiaceae
f: Unclassified
g: Mogibacteriaceae
h: Unclassified
i: Enterobacteriaceae
j: Enterobacteriales
Gammaproteobacteria
D
Parabacteroides Relative % Abundance
CTL
SUG
E
R2= 0.64, P<.0001
Log10 (Parabacteroides)
NOIC baseline shift
F
R2= 0.50, P=.0004
Log10 (P. johnsonii)
NOIC baseline shift
G
R2= 0.55, P=.0001
Log10 (P. distasonis)
NOIC baseline shift

Figure 3


A
Experimental Design
Saline: Saline (n=8)
ABX: Saline (n=7)
ABX: Parabacteroides (n=8)
Novel Object in Context
Context 1
Context 2
Context 2
Novel Object Recognition
PN 26
PN 27
PN 34
PN 36
PN 50
PN 55
PN 62
PN 73
PN 83
PN 60
PN 68
PN 76
1
2
3
4
5
6
7
8
9
1. Habituated to oral gavage
2. Gavage antibiotics every 12 hours
3. Parabacteroides johnsonii and distasonis (1:1) delivered by oral gavage
4. Animals maintained in sterile housing
5. Novel Object in Context testing
6. Novel Object Recognition testing
7. Zero Maze testing and Open Field testing
8. IP GTT
9. Body composition analyses


B
SAL-SAL
ABX-SAL
ABX-PARA
Observed OTUs
600
500
400
300
200
100
0
0
5
10
15
20
25
Sequences per sample
(x 1000)
****
n.s.


C
PC2 (27.9%)
PC1 (41.3%)
SAL-SAL
ABX-SAL
ABX-PARA


D
Relative Abundance (%)
100
90
80
70
60
50
40
30
20
10
0
SAL-SAL
ABX-SAL
ABX-PARA
g_Parabacteroides;s_
g_Lactobacillus;s_
f_S24-7;g_;s_
o_Clostridiales;_;_;_
f_Lachnospiraceae;_;_
f_Lachnospiraceae;g_;s_
g_Bifidobacterium;
s_pseudolongum
g_Ruminococcus;s_
o_Clostridiales;f_;g_;s_
f_Ruminococcaceae;g_;s_

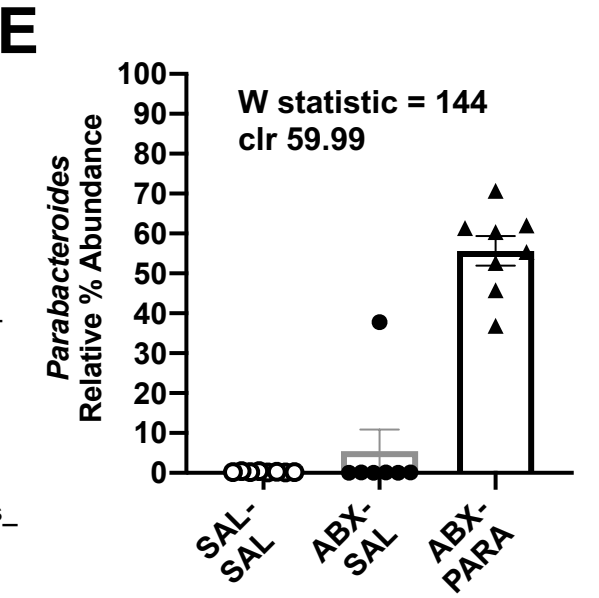
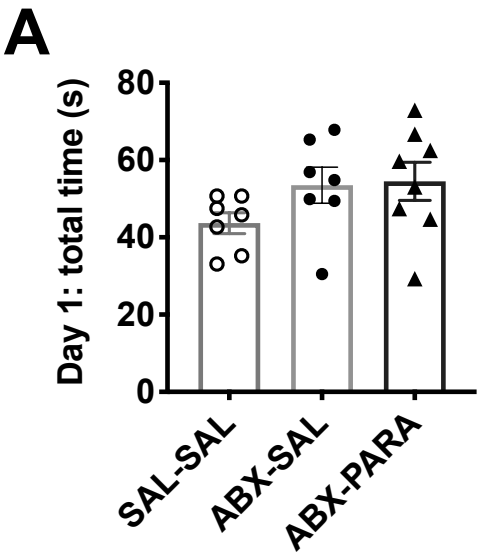
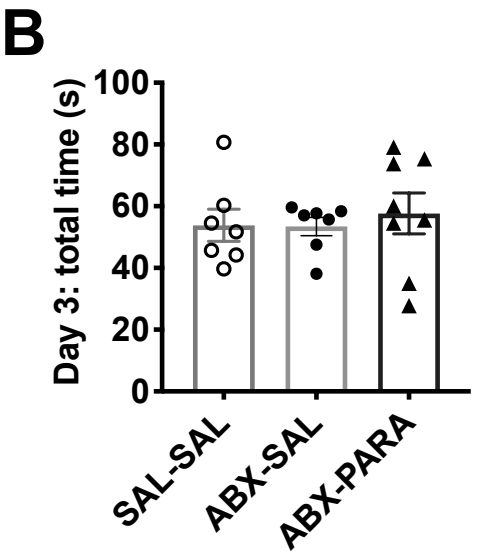
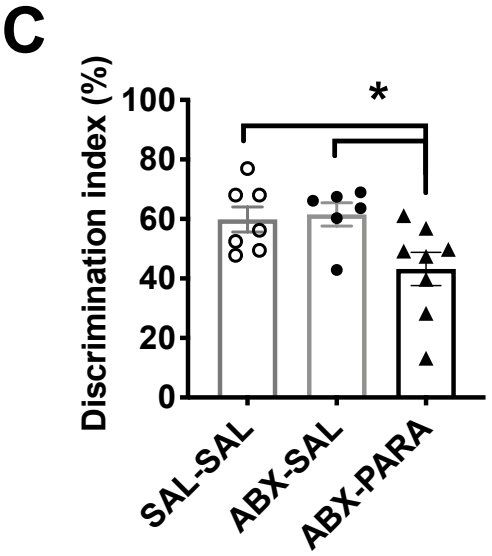

E
Parabacteroides
Relative % Abundance
W statistic = 144
clr 59.99
100
90
80
70
60
50
40
30
20
10
0
SAL-SAL
ABX-SAL
ABX-PARA

Figure 4

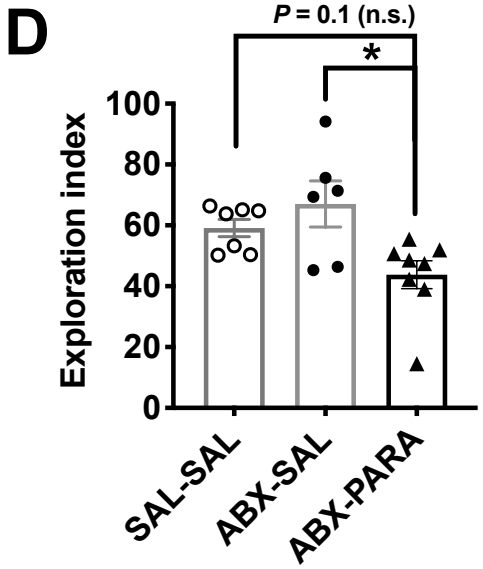
Novel Object in Context



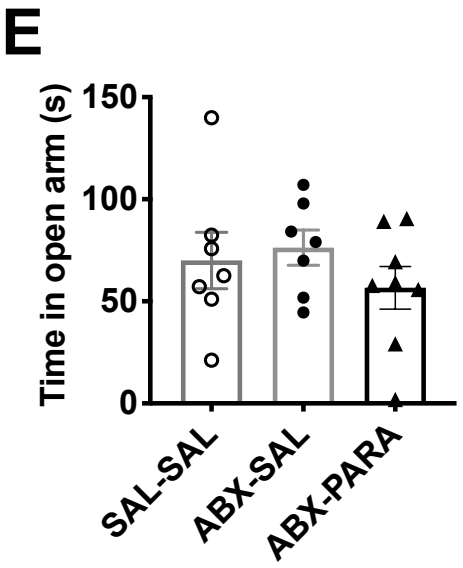


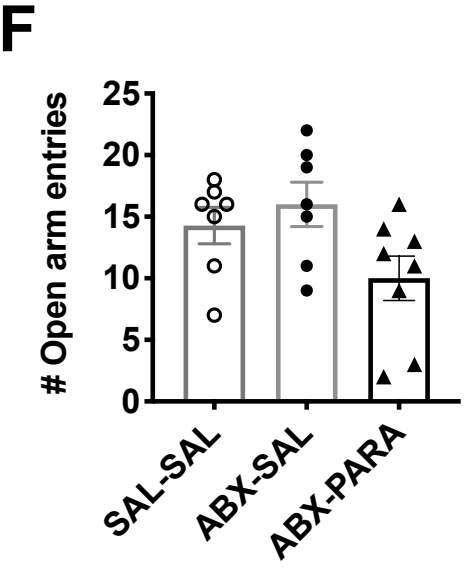


Novel Object Recognition

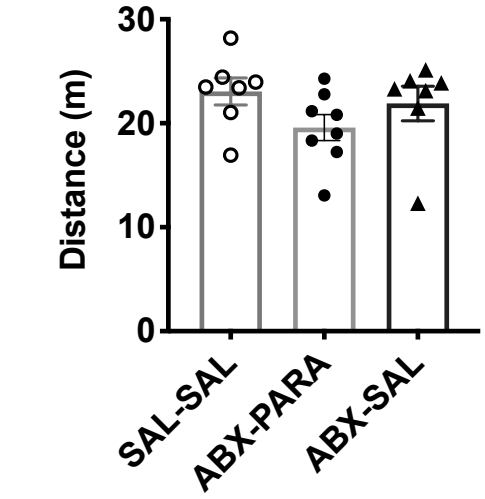


Zero Maze





Open Field



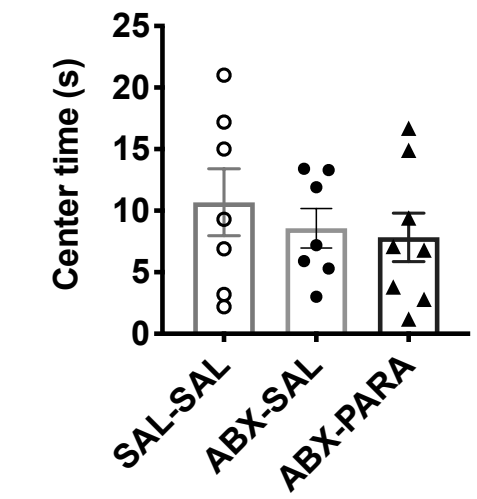
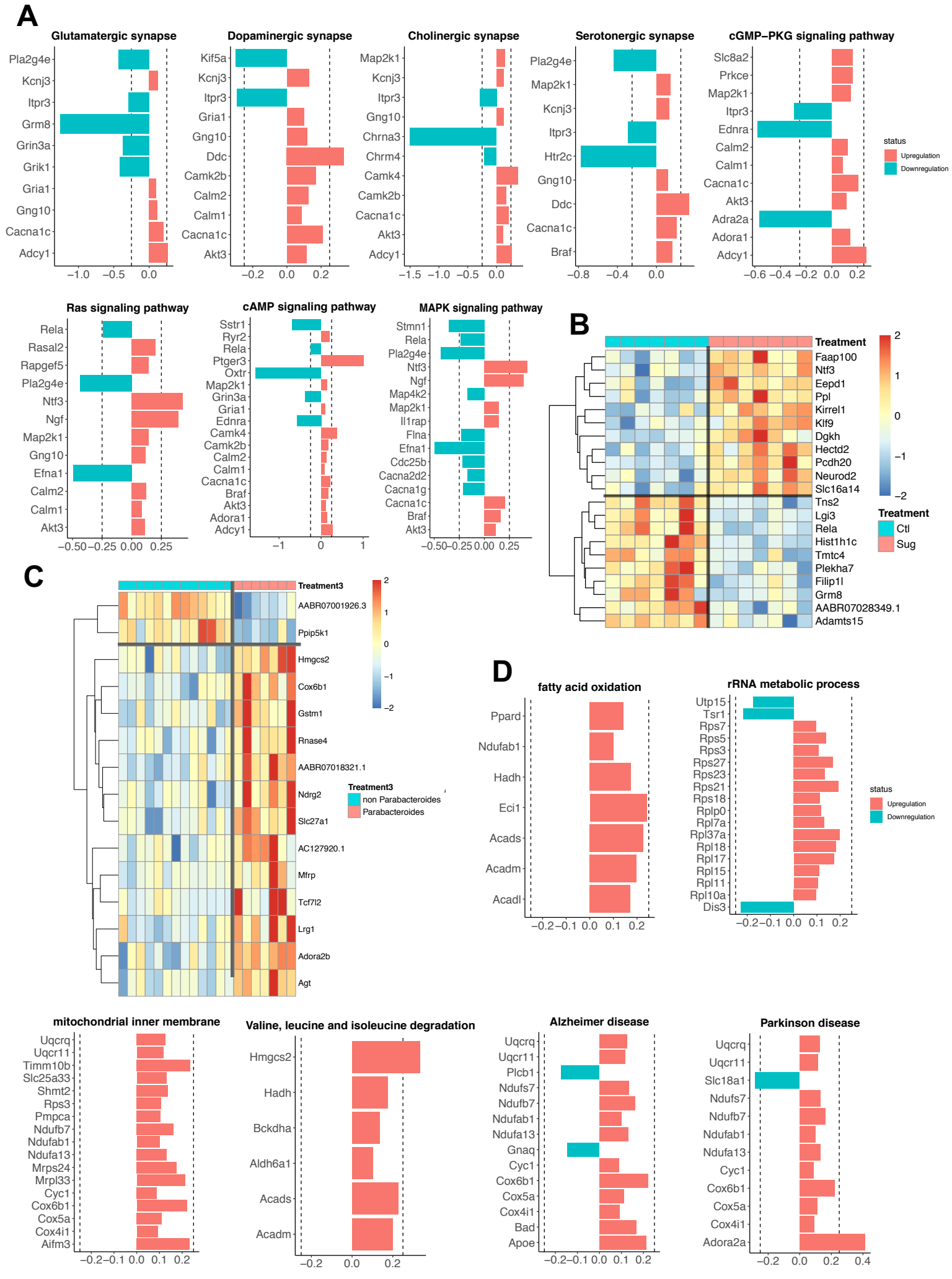


Figure 5

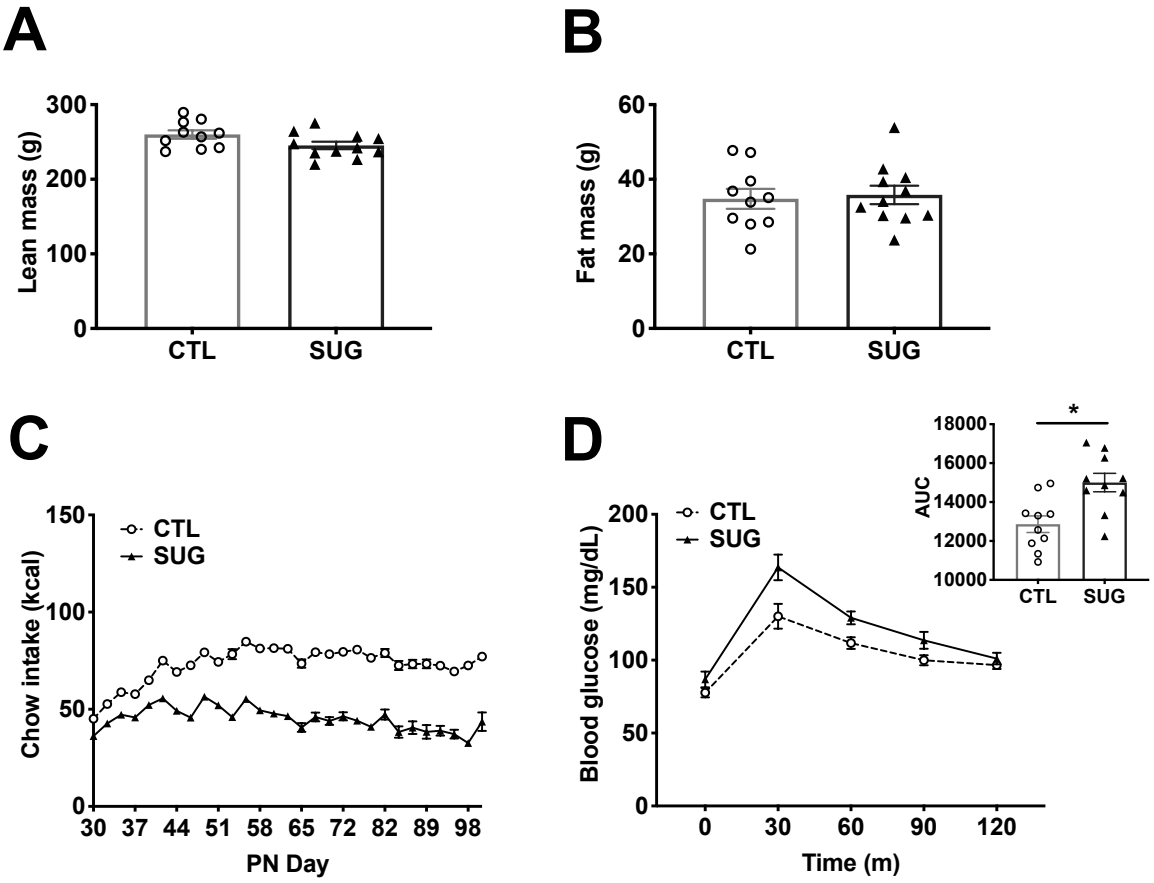

A
Glutamatergic synapse
Pla2g4e
Kcnj3
Itpr3
Grm8
Grin3a
Grik1
Gria1
Gng10
Cacna1c
Adcy1
−1.0 −0.5 0.0
Dopaminergic synapse
Kif5a
Kcnj3
Itpr3
Gria1
Gng10
Ddc
Camk2b
Calm2
Calm1
Cacna1c
Akt3
−0.2 0.0 0.2
Cholinergic synapse
Map2k1
Kcnj3
Itpr3
Gng10
Chrna3
Chrm4
Camk4
Camk2b
Cacna1c
Akt3
Adcy1
−1.5 −1.0 −0.5 0.0
Serotonergic synapse
Pla2g4e
Map2k1
Kcnj3
Itpr3
Htr2c
Gng10
Ddc
Cacna1c
Braf
−0.8 −0.4 0.0
cGMP−PKG signaling pathway
Slc8a2
Prkce
Map2k1
Itpr3
Ednra
Calm2
Calm1
Cacna1c
Akt3
Adra2a
Adora1
Adcy1
−0.6 −0.4 −0.2 0.0 0.2
status
Upregulation
Downregulation
Ras signaling pathway
Rela
Rasal2
Rapgef5
Pla2g4e
Ntf3
Ngf
Map2k1
Gng10
Efna1
Calm2
Calm1
Akt3
−0.50 −0.25 0.00 0.25
cAMP signaling pathway
Sstr1
Ryr2
Rela
Ptger3
Oxtr
Map2k1
Grin3a
Gria1
Ednra
Camk4
Camk2b
Calm2
Calm1
Cacna1c
Braf
Akt3
Adora1
Adcy1
−1 0 1
MAPK signaling pathway
Stmn1
Rela
Pla2g4e
Ntf3
Ngf
Map4k2
Map2k1
Il1rap
Flna
Efna1
Cdc25b
Cacna2d2
Cacna1g
Cacna1c
Braf
Akt3
−0.50 −0.25 0.00 0.25
B
Treatment
Faap100
Ntf3
Eepd1
Ppl
Kirrel1
Klf9
Dgkh
Hectd2
Pcdh20
Neurod2
Slc16a14
Tns2
Lgi3
Rela
Hist1h1c
Tmtc4
Plekha7
Filip1l
Grm8
AABR07028349.1
Adamts15
2
1
0
−1
−2
Treatment
Ctl
Sug
C
Treatment3
AABR07001926.3
Ppip5k1
Hmgcs2
Cox6b1
Gstm1
Rnase4
AABR07018321.1
Ndrg2
Slc27a1
AC127920.1
Mfrp
Tcf7l2
Lrg1
Adora2b
Agt
2
1
0
−1
−2
Treatment3
non Parabacteroides
Parabacteroides
D
fatty acid oxidation
Ppard
Ndufab1
Hadh
Eci1
Acads
Acadm
Acadl
−0.2 −0.1 0.0 0.1 0.2
rRNA metabolic process
Utp15
Tsr1
Rps7
Rps5
Rps3
Rps27
Rps23
Rps21
Rps18
Rplp0
Rpl7a
Rpl37a
Rpl18
Rpl17
Rpl15
Rpl11
Rpl10a
Dis3
−0.2 −0.1 0.0 0.1 0.2
status
Upregulation
Downregulation
mitochondrial inner membrane
Uqcrq
Uqcr11
Timm10b
Slc25a33
Shmt2
Rps3
Pmpca
Ndufb7
Ndufab1
Ndufa13
Mrps24
Mrpl33
Cyc1
Cox6b1
Cox5a
Cox4i1
Aifm3
−0.2 −0.1 0.0 0.1 0.2
Valine, leucine and isoleucine degradation
Hmgcs2
Hadh
Bckdha
Aldh6a1
Acads
Acadm
−0.2 0.0 0.2
Alzheimer disease
Uqcrq
Uqcr11
Plcb1
Ndufs7
Ndufb7
Ndufab1
Ndufa13
Gnaq
Cyc1
Cox6b1
Cox5a
Cox4i1
Bad
Apoe
−0.2 −0.1 0.0 0.1 0.2
Parkinson disease
Uqcrq
Uqcr11
Slc18a1
Ndufs7
Ndufb7
Ndufab1
Ndufa13
Cyc1
Cox6b1
Cox5a
Cox4i1
Adora2a
−0.2 0.0 0.2 0.4

Term	Overlap	P.value	Adjusted.P.value	Odds.Ratio	Combined.Score
cAMP signaling pathway	18/211	9.08710002926925e-07	0.000275339130886858	3.922209511	54.56279715
Long-term potentiation	10/67	1.75113958513324e-06	0.000265297647147685	6.862240522	90.96067106
Vascular smooth muscle contraction	14/140	2.31824837751272e-06	0.000234143086128785	4.597701149	59.65378698
Oxytocin signaling pathway	14/154	7.11610557675168e-06	0.000539044997438939	4.179728318	49.54294651
Circadian entrainment	11/99	1.02664310479357e-05	0.000622145721504906	5.108556833	58.68010783
Amphetamine addiction	9/68	1.58699502114579e-05	0.000801432485678624	6.085192698	67.24797055
Calcium signaling pathway	15/189	1.74937542122293e-05	0.000757229646615067	3.648969166	39.96959184
Cholinergic synapse	11/113	3.60773936379843e-05	0.00136643128403866	4.475638287	45.78508194
Axon guidance	14/180	4.14870567165781e-05	0.00139673090945813	3.575989783	36.08219845
Apelin signaling pathway	12/138	4.9577399145753e-05	0.00150219519411632	3.998001	39.62808792
Neurotrophin signaling pathway	11/121	6.78430713999647e-05	0.00188676823947175	4.179728318	40.11834187
Dopaminergic synapse	11/135	0.000181335935857366	0.00457873238039848	3.746275011	32.2747558
Glutamatergic synapse	10/114	0.000194050732212181	0.00452287475848392	4.033071184	34.47223604
Aldosterone synthesis and secretion	9/102	0.0003852671882922	0.00833828271803832	4.056795132	31.89279298
cGMP-PKG signaling pathway	12/172	0.000397324053301256	0.00802594587668537	3.207698476	25.11871164
Inflammatory mediator regulation of TRP channels	10/127	0.000464586599843255	0.00879810873453165	3.620237126	27.78301233
MAPK signaling pathway	16/294	0.000759189930175949	0.0135314440496066	2.502150285	17.97359249
GnRH signaling pathway	8/90	0.000767481845084928	0.0129192777255963	4.086845466	29.31247298
Glioma	7/75	0.0012196493427778	0.0194501974137724	4.291187739	28.79040196
Renin secretion	7/76	0.00131872717084366	0.0199787166382814	4.234724743	28.08083358
Retrograde endocannabinoid signaling	10/150	0.00167349865616307	0.0241461948960672	3.0651341	19.59490832
Neuroactive ligand-receptor interaction	17/348	0.00171069778931894	0.0235609740983472	2.246003435	14.30895981
Serotonergic synapse	9/132	0.00241588944480672	0.0318267174685407	3.134796238	18.88930332
Fc gamma R-mediated phagocytosis	7/87	0.00287625632706458	0.0363127361291903	3.699299775	21.64558595
Regulation of actin cytoskeleton	12/217	0.00294032709499641	0.0356367643913564	2.542507548	14.82087258
Dilated cardiomyopathy (DCM)	7/90	0.00347972133870684	0.0405521371395451	3.575989783	20.24297392
Apoptosis	9/141	0.00375836444668354	0.0421772010127819	2.934702861	16.38670992
Cocaine addiction	5/48	0.00377562901206963	0.040857699666325	4.789272031	26.7202504
Morphine addiction	7/92	0.00393256649864465	0.041088539623769 9	3.498250875	19.37493308
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6/72	0.00476843186679707	0.0481611618546504	3.831417625	20.48175394
Proteoglycans in cancer	11/203	0.00502723467090038	0.0491371646865424	2.491365155	13.18650979
Ras signaling pathway	12/233	0.00518599384881057	0.0491048792559251	2.367914755	12.45947913
response to calcium ion (GO:0051592)	11/80	1.2352727119088e-06	0.00630359664887059	6.32183908	86.00368202
axon guidance (GO:0007411)	15/159	2.11788224048725e-06	0.00540377653660322	4.337453915	56.66924273
nervous system development (GO:0007399)	25/456	2.46003232910435e-05	0.0418451499180649	2.52066949	26.75123758
semaphorin-plexin signaling pathway (GO:0071526)	6/30	3.8991177233796e-05	0.0497429943560153	9.195402299	93.35333482
regulation of cAMP biosynthetic process (GO:0030817)	5/19	4.29910702140572e-05	0.0438766862604667	12.09921355	121.651762
integral component of plasma membrane (GO:0005887)	61/1464	7.0402625056926e-07	0.00031399570775389	1.915708812	27.13879347
dendrite (GO:0030425)	16/216	2.18212117029006e-05	0.00486613020974683	3.405704555	36.55216023
Hypothetical Network for Drug Addiction WP1246	6/31	4.74677094198248e-05	0.00835431685788917	8.898776418	88.59142051

Term	Overlap	P.value	Adjusted.P.value	Odds.Ratio	Combined.Score
Ribosome	25/170	1.8140743921693e-10	5.49664540827297e-08	4.617231508	103.5657751
Valine, leucine and isoleucine degradation	11/56	1.26913532735964e-06	0.00019227400209498	6.167302086	83.73453805
Alzheimer disease	19/175	3.33806655284383e-06	0.00033714472183722	3.408836062	42.98582769
Huntington disease	19/192	1.28962346143454e-05	0.00097688977203666	3.107012036	34.98052859
Parkinson disease	16/144	1.46258452357674e-05	0.00088632622128750	3.488574917	38.83732905
Oxidative phosphorylation	15/134	2.46175398478414e-05	0.00124318576231599	3.514609058	37.29721186
Renal cell carcinoma	10/68	5.50407863682206e-05	0.00238247975279583	4.617231508	45.28320287
Hepatocellular carcinoma	16/171	0.00011932397329175	0.00451939548842524	2.937747299	26.53863464
Fatty acid degradation	8/50	0.00016689013768195	0.00561863463529261	5.023547881	43.69569768
Cardiac muscle contraction	10/78	0.00017913885562032	0.00542790732529572	4.025278751	34.7274859
Pathways in cancer	33/535	0.00024656992139553	0.00679188056207689	1.936648132	16.08941113
Non-alcoholic fatty liver disease (NAFLD)	14/151	0.00034292783199551	0.00865892775788684	2.910996288	23.22390084
Propanoate metabolism	6/31	0.00038022110318012	0.00886207648181358	6.076872436	47.85389755
Thermogenesis	18/231	0.00045634785343122	0.00987667139926156	2.446533059	18.81935665
Colorectal cancer	10/88	0.00048364517887962	0.00976963261336839	3.567860711	27.23761603
Lysine degradation	8/59	0.00053628083889564	0.0101558183865863	4.257243967	32.06067671
Chronic myeloid leukemia	9/76	0.00067780917368503	0.0120809517427391	3.718086425	27.12955585
Acute myeloid leukemia	8/69	0.0015316936421722	0.0257835096432332	3.640252087	23.59386144
Endometrial cancer	7/58	0.00236407898597343	0.0377008385657867	3.789314134	22.91537237
Glyoxylate and dicarboxylate metabolism	5/31	0.00275840767079955	0.0417898762126132	5.064060364	29.84302272
Gastric cancer	12/150	0.00312702923091649	0.0451185646175094	2.51177394	14.48708786
Retrograde endocannabinoid signaling	12/150	0.00312702923091649	0.0430677207771259	2.51177394	14.48708786
beta-Alanine metabolism	5/32	0.00318487576286933	0.0419572763543221	4.905808477	28.20517071
Prostate cancer	9/97	0.00377722652917845	0.0476874849308779	2.913139879	16.25172356
cotranslational protein targeting to membrane (GO:0006613)	23/94	1.49568324253231e-14	7.63247158664239e-11	7.682287318	244.5549244
viral transcription (GO:0019083)	25/114	1.65246783633483e-14	4.21627168440831e-11	6.885345231	218.4990049
protein targeting to ER (GO:0045047)	23/98	3.94454541709055e-14	6.70967175447103e-11	7.36872457	227.4272657
SRP-dependent cotranslational protein targeting to membrane (GO:0006614)	22/90	5.73311848802441e-14	7.31402591109714e-11	7.674864818	234.0061039
viral gene expression (GO:0019080)	24/111	7.76259023219601e-14	7.92249959097925e-11	6.788578217	204.9259636
nuclear-transcribed mRNA catabolic process, nonsense-mediated decay (GO:000018	24/113	1.18161750327455e-13	1.00496568653501e-10	6.66842639	198.4971942
nuclear-transcribed mRNA catabolic process (GO:0000956)	27/175	1.07890372161453e-11	7.86520813056991e-09	4.844135456	122.3264849
ncRNA processing (GO:0034470)	27/228	4.67386230344937e-09	2.98133991681276e-06	3.718086425	71.31765701
rRNA metabolic process (GO:0016072)	25/201	6.38357070676274e-09	3.61948459073447e-06	3.905121176	73.6878333
rRNA processing (GO:0006364)	25/203	7.82676247376835e-09	3.99399689036399e-06	3.866647076	72.17373963
peptide biosynthetic process (GO:0043043)	23/175	8.94006953384337e-09	4.14737953010934e-06	4.126485759	76.47501535
viral process (GO:0016032)	26/221	1.03523973593864e-08	4.40235697707908e-06	3.693785206	67.91411105
mitochondrial ATP synthesis coupled electron transport (GO:0042775)	16/86	1.13438922557321e-08	4.45291401392316e-06	5.841334745	106.864803
ribosome biogenesis (GO:0042254)	26/227	1.81118251672489e-08	6.60176027346223e-06	3.596152117	64.10752778
cellular protein metabolic process (GO:0044267)	40/485	4.37588247153129e-08	1.48867521681495e-05	2.58945767	43.87725333
respiratory electron transport chain (GO:0022904)	16/95	4.96518726560543e-08	1.58358441352403e-05	5.287945138	88.93387612
translation (GO:0006412)	25/233	1.23203616731831e-07	3.69828268342666e-05	3.368795521	53.59560786
gene expression (GO:0010467)	34/412	4.49194120183821e-07	0.00012734653307211	2.591028943	37.86998856
cytoplasmic translation (GO:0002181)	11/55	1.04973492845598e-06	0.00028193670247389	6.279434851	86.44880925
cellular macromolecule biosynthetic process (GO:0034645)	28/368	2.09983328625941e-05	0.00535772462989088	2.388915432	25.7311694
glutamate receptor signaling pathway (GO:0007215)	7/37	0.00014404472041709	0.0350028670613536	5.94000594	52.54164983
cytosolic ribosome (GO:0022626)	22/125	6.4024267746916e-11	2.85548234151245e-08	5.525902669	129.7026553
cytosolic part (GO:0044445)	22/160	8.08431374352751e-09	1.80280196480664e-06	4.31711146	80.44220663
polysomal ribosome (GO:0042788)	9/29	1.80391663386915e-07	2.68182272901881e-05	9.743950631	151.3053851
cytosolic large ribosomal subunit (GO:0022625)	13/70	2.74848263799486e-07	3.06455814136427e-05	5.83090379	88.08773562
large ribosomal subunit (GO:0015934)	13/73	4.57645374086965e-07	4.08219673685572e-05	5.591277607	81.61683671
ribosome (GO:0005840)	13/77	8.67324705016003e-07	6.44711364061896e-05	5.300821627	73.98808595
polysome (GO:0005844)	11/64	5.03105411073612e-06	0.00032055001905547	5.396389325	65.83530776
mitochondrial matrix (GO:0005759)	26/309	7.14751780482095e-06	0.00039847411761876	2.641833432	31.30241179
cytosolic small ribosomal subunit (GO:0022627)	9/50	2.48344308134448e-05	0.00123068401586626	5.651491366	59.9243427
small ribosomal subunit (GO:0015935)	9/54	4.70643775513193e-05	0.00209907123878884	5.232862376	52.14021014
focal adhesion (GO:0005925)	26/357	8.48584001310998e-05	0.00344062245315 5	2.286628937	21.43606373
mitochondrial inner membrane (GO:0005743)	24/342	0.00027325451243327	0.0101559593787699	2.203310474	18.07839801
mitochondrion (GO:0005739)	53/1027	0.00038185672014572	0.013100622860384	1.620302079	12.75253096
mitochondrial respiratory chain complex I (GO:0005747)	7/52	0.00123936844220466	0.0394827375159486	4.226542688	28.28889835
RNA binding (GO:0003723)	72/1388	2.85108813407164e-05	0.0328160244231646	1.628671863	17.04441708
Cytoplasmic Ribosomal Proteins WP163	23/92	9.02670968051791e-15	1.58870090377115e-12	7.849293564	253.8350743
Fatty Acid Beta Oxidation WP1269	8/34	8.83809769884804e-06	0.00077775259749862 6	7.387570413	85.9650117
Mitochondrial LC-Fatty Acid Beta-Oxidation WP401	5/16	0.00010523398308386 3	0.00617372700758661	9.811616954	89.86778136
Electron Transport Chain WP295	11/103	0.00044042239073246 6	0.0193785851922285	3.35309628	25.911978

1 **Supplemental Figures:**

Figure S1



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4 **Figure S1: Effect of early life sugar consumption on food intake and**

5 **metabolic measures** (A, B) There were no differences in lean mass or in fat mass

6 between animals fed sugar solutions or control animals (n=10,11; two-tailed, type 2

7 Student's T-test). (C) Kcals from chow intake were lower throughout the feeding period

8 in animals fed early life sugar (n=10,11). (D) Results from the intraperitoneal glucose

9 tolerance test show an elevated area under the curve (AUC) in rodents fed sugar

10 solutions during early life (n=10,11; two-tailed, type 2 Student's T-test; P<.05).

11 CTL=control, SUG= sugar, PN= post-natal day; data shown as mean ± SEM.

Figure S2

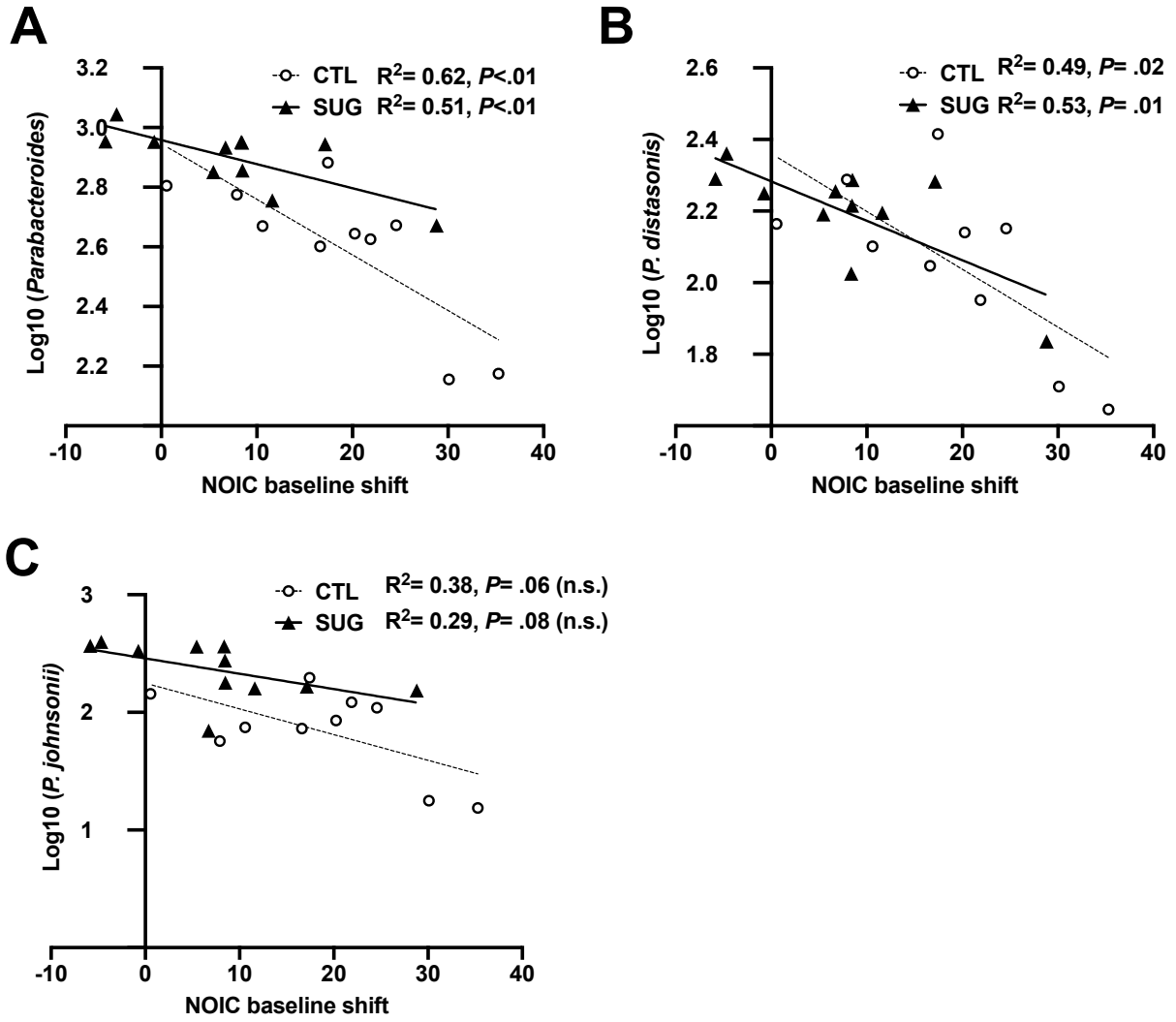
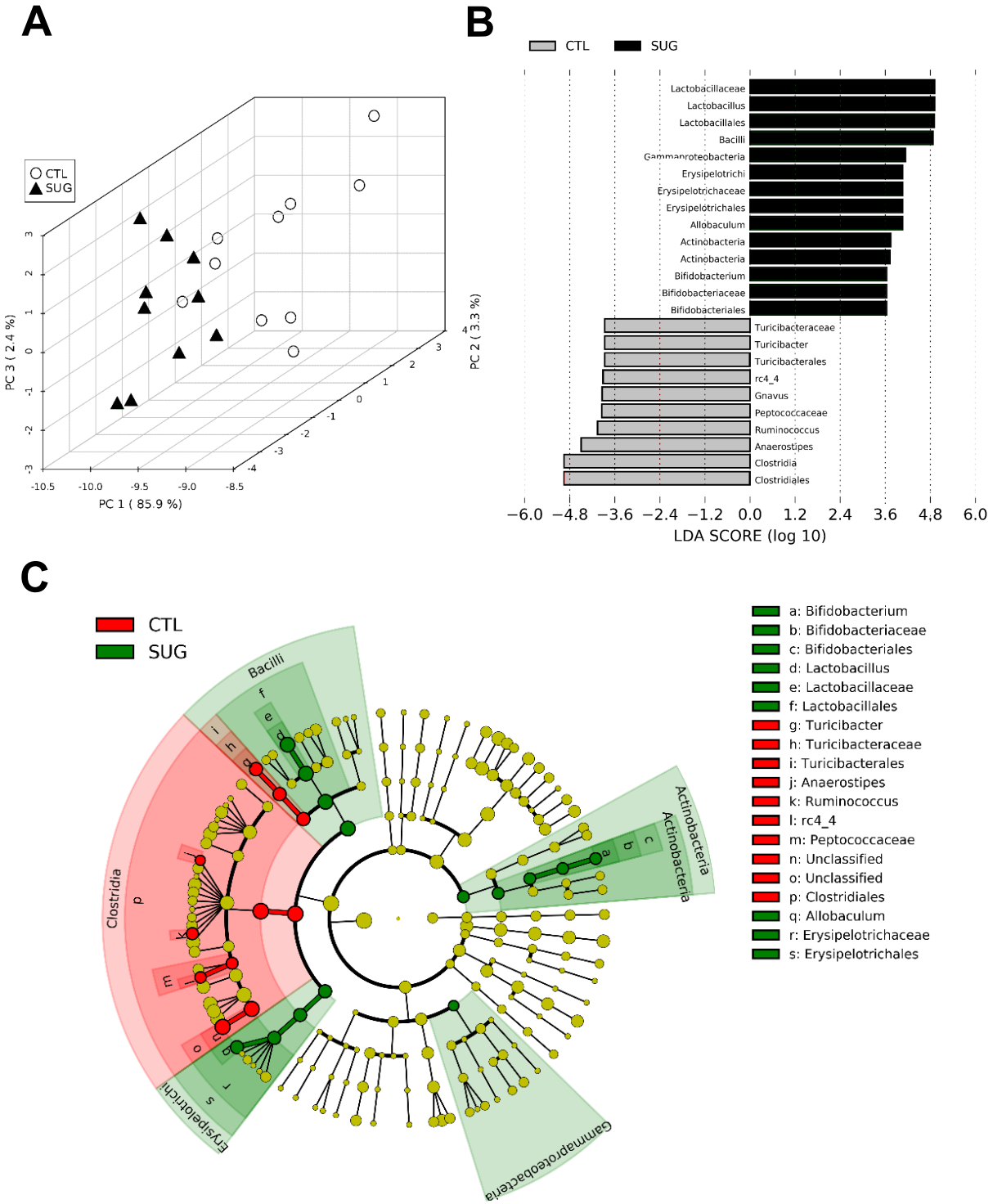


Figure S2: Relationship between *Parabacteroides* and behavioral outcomes in the Novel Object in Context task (NOIC) A) Linear regression of log normalized fecal *Parabacteroides* counts against shift from baseline performance scores in the NOIC task in sugar (SUG) and control (CTL) groups (n=10, 11). (B, C) Linear regression of the most abundant fecal *Parabacteroides* species against shift from baseline performance scores in NOIC across all groups tested (n=10, 11). *P<0.05; data shown as mean ± SEM.

Figure S3



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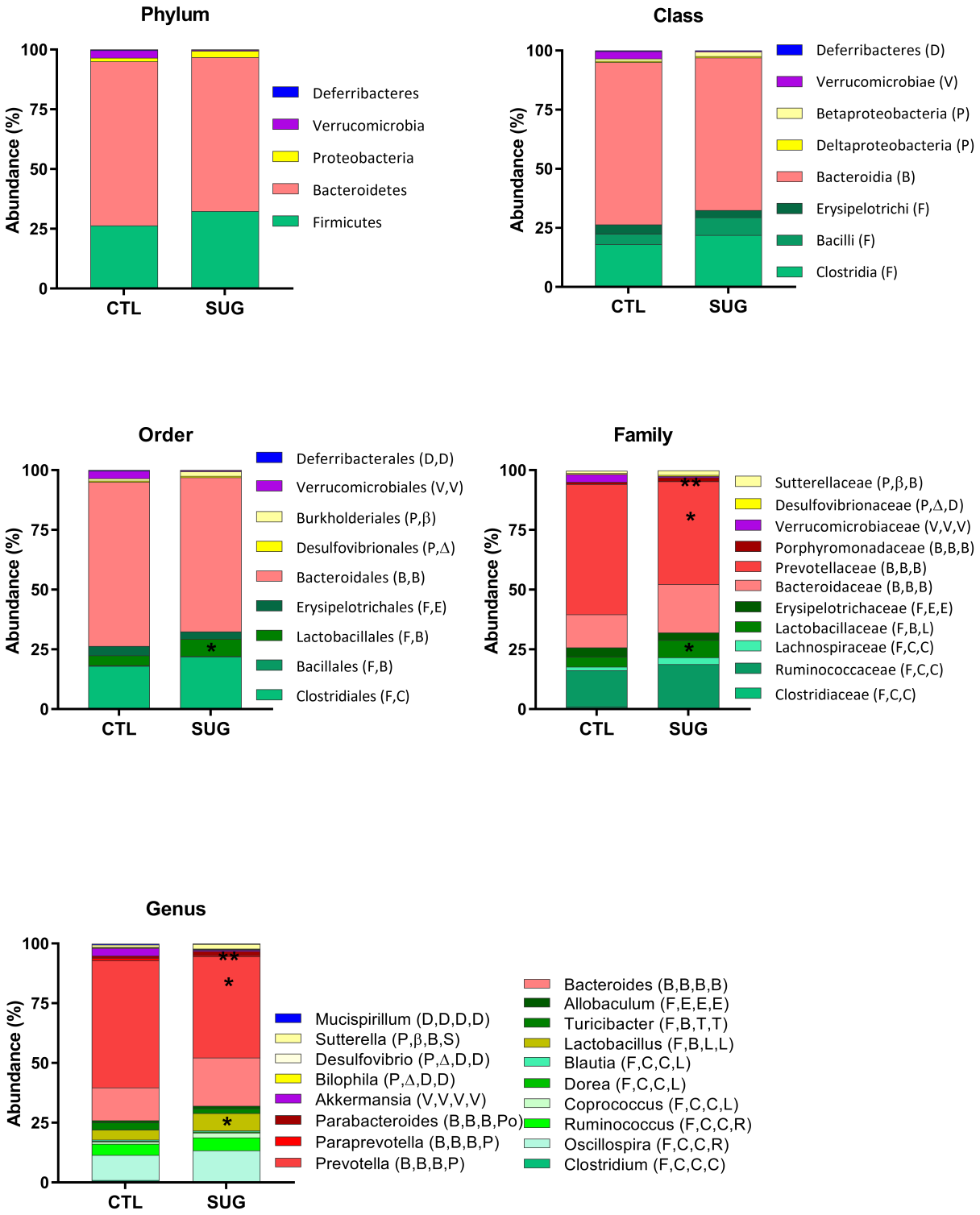
23 **Figure S3: Effect of early life sugar consumption on the rat cecal microbiota**

24 (A) Principal component analysis (PCA) was run using all phylogenic levels (112
25 normalized taxa abundances) and shows different clustering patterns based on overall
26 cecal microbial profiles. (B) Linear discriminant analysis (LDA) Effect Size (LEfSe), run
27 using the GALAXY platform, identified characteristic features of the cecal microbiota of
28 rats fed a control diet or early life sugar. Relative differences among groups were used to
29 rank the features with the LDA score set at 2. (C) Identified taxa are displayed by scores
30 and on a phylogenic cladogram. CTL=control, SUG= sugar.

Figure S4



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32

33 **Figure S4: Effect of early life sugar consumption on the rat fecal microbiota**.

34 Filtered bacterial abundances by taxonomic levels phylum, class, order, family, genus in

35 fecal samples from rats fed a control diets or early life sugar. Differences in abundances

36 were assessed by Mann-Whitney non-parametric test. * $p<0.05$, *** $p<0.001$.

37 CTL=control, SUG= sugar.

Figure S5

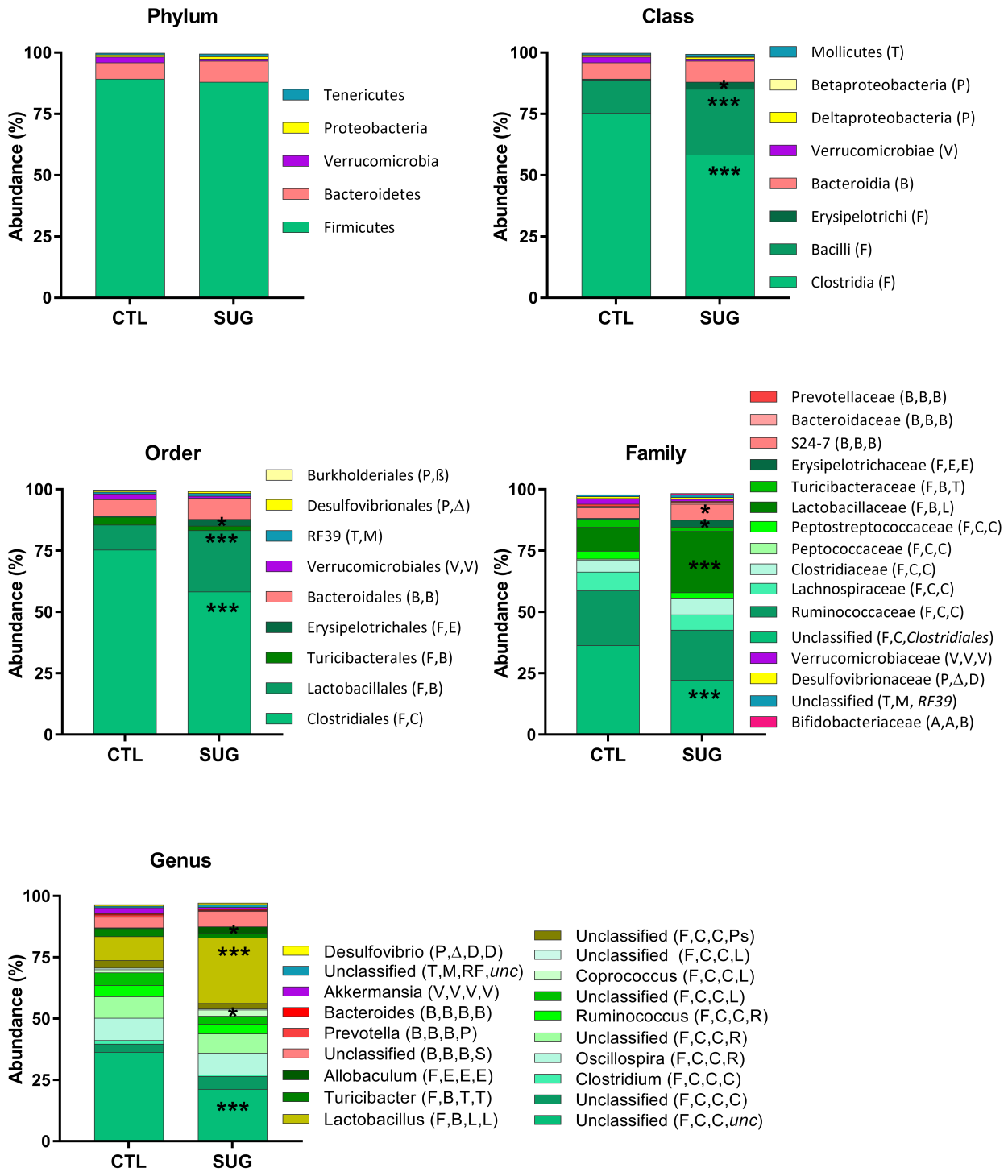

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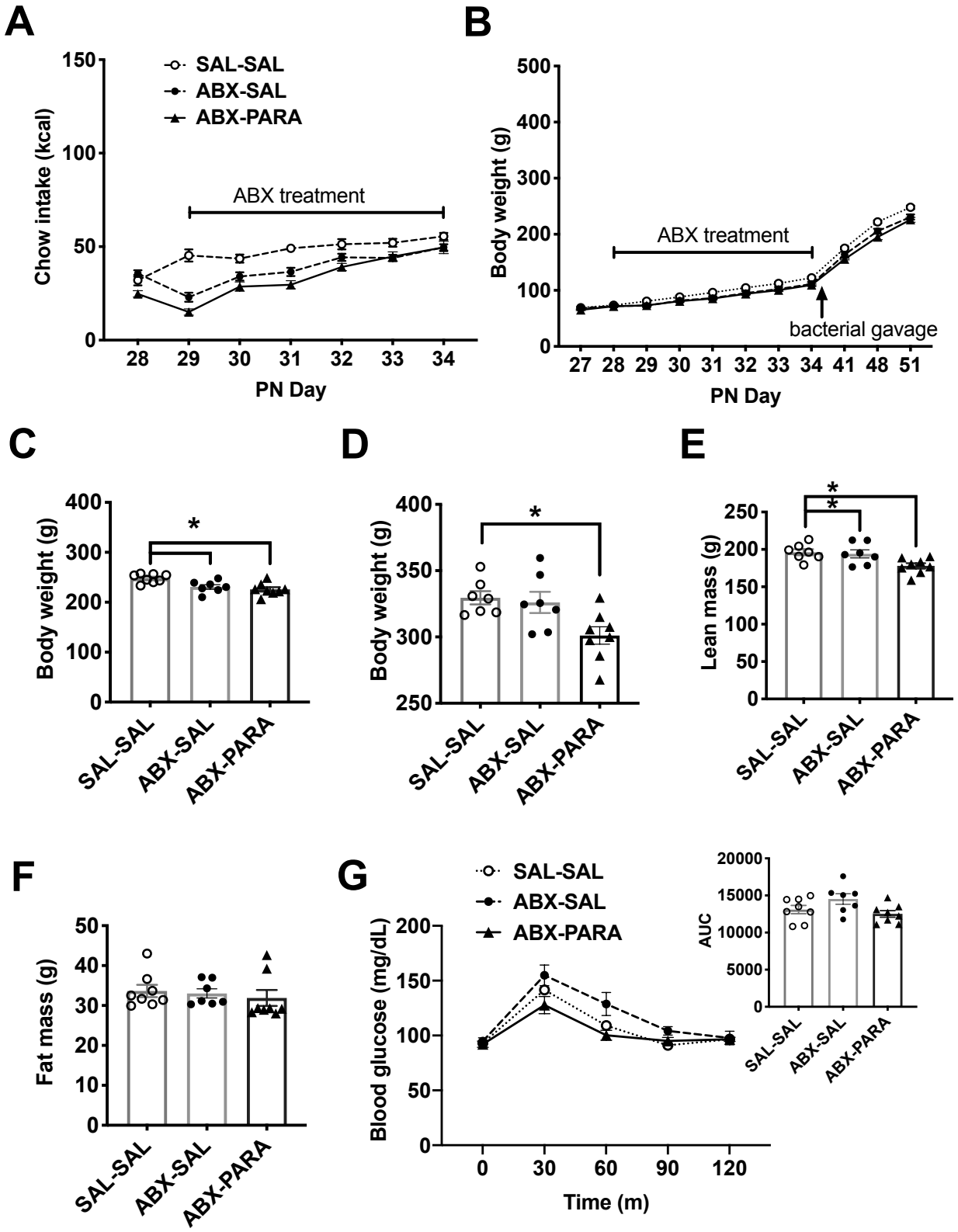
Figure S5: Effect of early life sugar consumption on the rat cecal microbiota: Filtered bacterial abundances by taxonomic levels phylum, class, order, family, genus in cecal samples from rats fed a control diets or early life sugar. Differences in abundances were assessed by Mann-Whitney non-parametric test. * $p<0.05$, *** $p<0.001$. CTL=control, SUG= sugar.

Phylogenic taxonomy legend for Figure S4, S5:

Phylum		Class		Order		Family		Genus
F	*Firmicutes*	C	*Clostridia*	C	*Clostridiales*	C	*Clostridiaceae*	*Clostridium*
						L	*Lachnospiraceae*	*Coprococcus*
								Blautia
								Dorea
						P	*Peptococcaceae*	
						Ps	*Peptostreptococcaceae*	
						R	*Ruminococcaceae*	*Oscillospira*
								Ruminococcus
		B	*Bacilli*	L	*Lactobacillales*	L	*Lactobacillaceae*	*Lactobacillus*
				T	*Turicibacterales*	T	*Turicibacteraceae*	*Turicibacter*
				B	*Bacillales*			
		E	*Erysipelotrichi*	E	*Erysipelotrichales*	E	*Erysipelotrichaceae*	*Allobaculum*
B	*Bacteroidetes*	B	*Bacteroidia*	B	*Bacteroidales*	S	*S24-7*	
						B	*Bacteroidaceae*	*Bacteroides*
						P	*Prevotellaceae*	*Prevotella*
								Paraprevotella
						Po	*Porphyromonadaceae*	*Parabacteroides*
V	*Verrucomicrobia*	V	*Verrucomicrobiae*	V	*Verrucomicrobiales*	V	*Verrucomicrobiaceae*	*Akkermansia*
P	*Proteobacteria*	β	*Betaproteobacteria*	B	*Burkholderiales*	S	*Sutterellaceae*	*Sutterella*
		Δ	*Deltaproteobacteria*	D	*Desulfovibrionales*	D	*Desulfovibrionaceae*	*Desulfovibrio*
								Bilophila
T	*Tenericutes*	M	*Mollicutes*	RF	*RF39*			
D	*Deferribacteres*	D	*Deferribacteres*	D	*Deferribacterales*	D	*Deferribacteraceae*	*Mucispirillum*
A	*Actinobacteria*	A	*Actinobacteria*	B	*Bifidobacter*	B	*Bifidobacteriaceae*	

Figure S6


A
SAL-SAL
ABX-SAL
ABX-PARA
Chow intake (kcal)
ABX treatment
PN Day
B
Body weight (g)
ABX treatment
bacterial gavage
PN Day
C
Body weight (g)
D
Body weight (g)
E
Lean mass (g)
F
Fat mass (g)
G
Blood glucose (mg/dL)
Time (m)
AUC

52

53 **Figure S6: Experimental design, food intake, and metabolic measures for**
54 **gut *Parabacteroides* enrichment** (A) Effect of antibiotic treatment on food intake
55 (B) and body weight (n=7,8). (C) Effect of gut *Parabacteroides* enrichment on body
56 weight at PN 51 prior to the start of behavioral testing (n=7,8; one way ANOVA with
57 Tukey's post hoc test, $F_{(2,20)}$= 8.79; *P<.05) (D) Effect of gut *Parabacteroides*
58 enrichment on body weight (n=7,8; one way ANOVA with Tukey's post hoc test, $F_{(2,19)}$=
59 5.7; *P<.05) (E) lean mass (n=7,8; one way ANOVA with Tukey's post hoc test, $F_{(2,19)}$=
60 5.33; *P<.05) (F) and body fat (one way ANOVA, n.s.) at PN 76. (G) Blood glucose levels
61 during an interaperitoneal glucose tolerance test (IP GTT) (n=7,8 one way ANOVA for
62 AUC; n.s.) SAL-SAL=saline-saline control, ABX-SAL= antibiotics-saline control, ABX-
63 PARA= antibiotics-*P. johnsonii* and *P. distasonis* enriched, PN= post-natal day; data
64 shown as mean ± SEM.

Figure S7

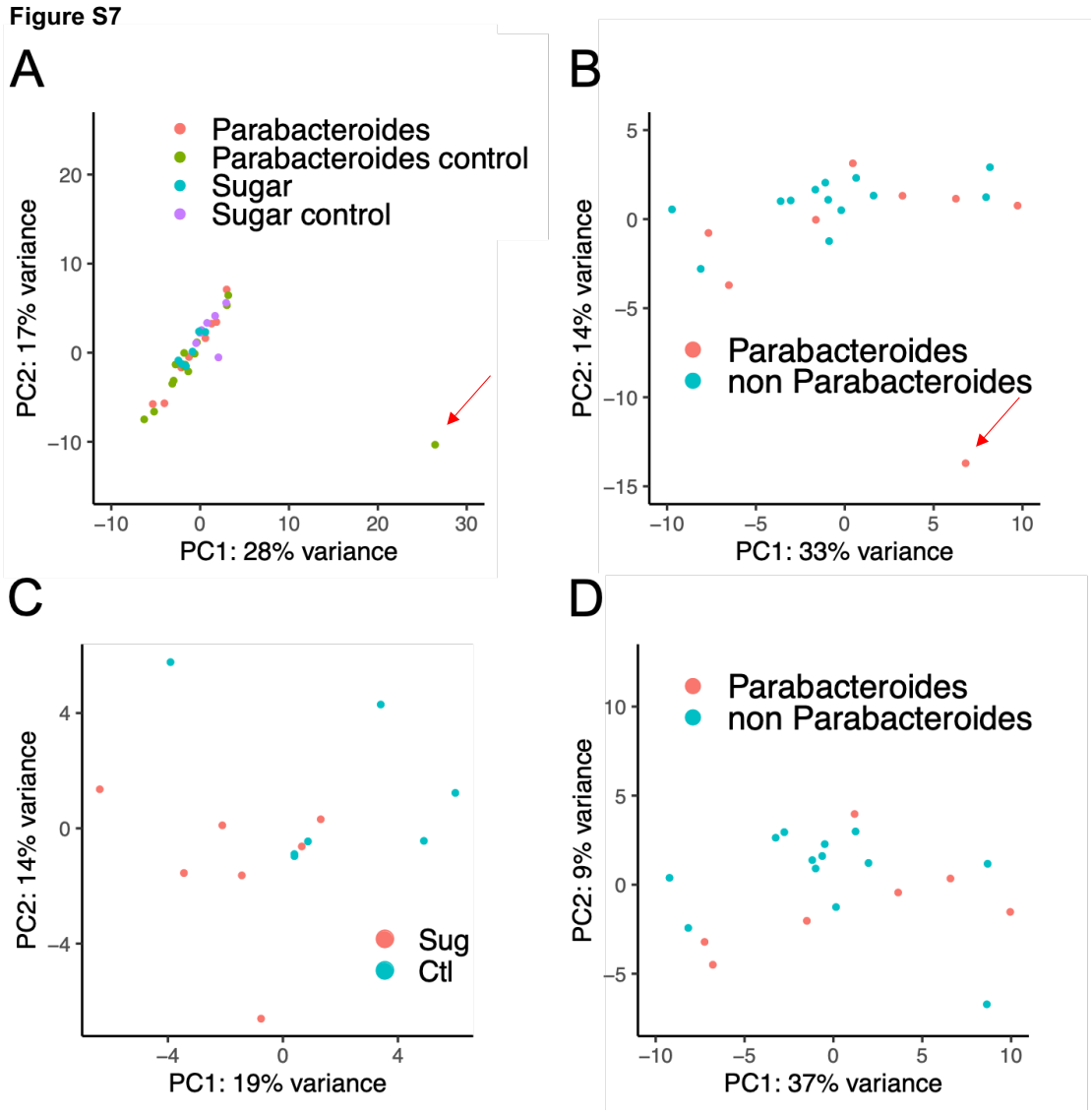


Figure S7: Principal component analyses (PCA) of hippocampal gene expression data to identify outliers (A) PCA identified one control sample (red arrow) as an outlier when all samples from both sugar and *Parabacteroides* enrichment experiments were considered. (B) PCA identified one treatment sample (red arrow) from the *Parabacteroides* experiment as an outlier. After removing the outliers, PCA for the remaining samples from the sugar treatment experiment (C) and those from the *Parabacteroides* enrichment experiments (D).

Figure S8

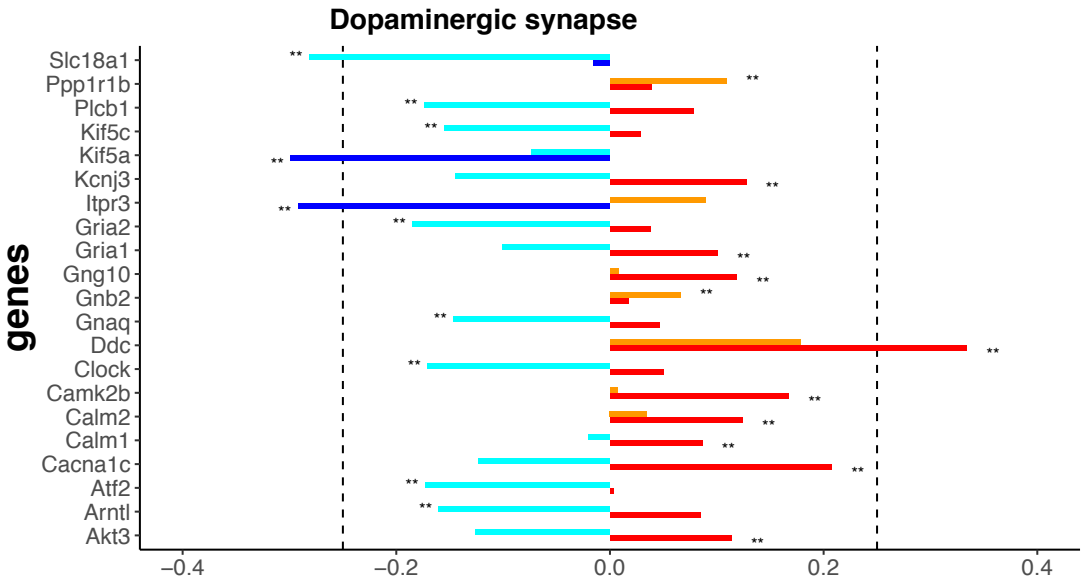


Figure S8: Comparison of hippocampal gene expression pathways altered by sugar and *Parabacteriodes*. The dopaminergic synapse pathway overlaps in the sugar and *Parabacteroides* transfer experiments. Red= upregulated by sugar, dark blue= downregulated by sugar, orange= upregulated by *Parabacteroides*, light blue= downregulated by *Parabacteroides*. * $P < 0.05$ and ** $P < 0.01$. Dotted line indicated ± 0.25 log2 fold change.