

Coprophagy prevention alters microbiome structure and function and impairs memory in a small mammal

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Abstract

Background In the long process of adaptive evolution, many small mammals engage in coprophagy, or the behavior of consuming feces, as a means to meet nutritional requirements when feeding on low-quality foods. In addition to nutritional benefits, coprophagy may also help herbivores retain necessary gut microbial diversity and function, which may have downstream physiological effects, such as maintaining energy balance and cognitive function. Here, we used collars to prevent Brandt's vole (*Lasiopodomys brandtii*) from engaging in coprophagy and monitored changes in microbial community structure, energy metabolism, and memory formation.

Results In this research, we found that coprophagy prevention decreased alpha diversity of the gut microbiota, and altered abundances of microbial taxa such as Bacteroidetes, Firmicutes and Oscillospira. The coprophagy prevention decreased body mass while increased food intake and ghrelin level in serum. Importantly, coprophagy prevention decreased vole's memory and neurotransmitters in brain. The administration of acetate compensated for the energy loss and decline of spatial memory caused by coprophagy prevention and partially rescued the composition and structure of gut microbiota.

Conclusions These findings identify relationship between coprophagy behavior and interactions between the gut microbiota, energy metabolism and neurological function. Our results suggest that coprophagy may have a positive effect on animal's nutritional requirements and ecological adaptations by improving memory function.

Background

The mammalian gut hosts a diverse community of microbes that have profound effects on host nutrition, physiology, and behavior[1]. The gut microbiota has been implicated in regulation of many physiological functions including aiding digestion, energy metabolism and regulating the neuroendocrine system[2,3,4]. Diverse gut microbiota communities are especially important for herbivorous hosts, where they ferment indigestible dietary fibers into compounds that are more amenable to absorption and utilization by the host, such as short-chain fatty acids[5]. Therefore, the gut microbiota of herbivores is more complex than that of omnivores or carnivores [6].

To capitalize on the benefits provided by gut microbial communities, many hindgut fermenting herbivores engage in coprophagy, or the behavior of consuming feces. This behavior is common in rodents, lagomorphs, and even primates[7,8,9]. Coprophagy is considered an adaptation to the metabolic disadvantages of small body size, low-quality foods, and microbial fermentation occurring in the hindgut (distal to the major site of nutrient absorption: of the small intestine) as opposed to the foregut[10,11]. Therefore, many hindgut fermenting herbivores consume feces such that nutrients that were liberated in the hindgut can be absorbed. The practice of coprophagy aides in the uptake of essential amino acids,

vitamin B and vitamin K, short-chain fatty acids, and trace elements that are not fully absorbed, thus avoiding the loss of such nutrients.

Animal nutrition and the gut microbiota can have downstream impacts on cognitive function of animals[12,13]. Recent work demonstrates that gut microbiota play a central role in aspects of animal behavior and memory[3,14,15]. Gut microbes can elicit signals via the vagal nerve to the brain and vice versa[16,17]. Large numbers of studies have also demonstrated that the gut microbiota impacts nutrition and energy homeostasis[18,19]. So, the gut microbiota may affect the memory of animals directly through the gut-brain axis, or indirectly by affecting energy balance. Therefore, we predict that coprophagy and the nutritional benefits provided by this behavior could have influences on animal learning and memory.

We tested this notion in Brandt's vole (*Lasiopodomys brandtii*), a small mammalian herbivore distributed in the Inner Mongolia of China, Mongolia and in the Beigaer region of Russia. Brandt's voles need to consume large amounts of fibers to meet their energy needs, because of the shortage of high-nutrition food in the field[20]. Coprophagy can help Brandt's vole to obtain maximum energy yields from limited resources. Liu and Wang[10] reported that Brandt's vole engages in coprophagy behavior and has a rhythmic fecal intake (Brandt's voles ingested 68.8 ± 7.4 fecal pellets per day, averaging $17 \pm 2\%$ of total faeces produced). We studied the relationship among coprophagy, the gut microbiota, energy metabolism and memory ability, and specifically addressed three questions: (i) does the prevention of coprophagy behavior influence the community structure of the vole gut microbiota? (ii) does prevention of coprophagy affect vole's memory? (iii) if so, can provisioning of microbial products (specifically short-chained fatty acids) rescue the phenotype?

Methods

Animals

Brandt's voles for experiments are laboratory colonies in the Chinese Academy of Sciences in Beijing. Voles were housed in groups (3-4 individuals per cage) in plastic cages ($30 \times 15 \times 20 \text{ cm}^3$) with sawdust bedding, and were maintained at the room temperature of $23 \pm 1^\circ\text{C}$, under a photoperiod of 16Light: 8Dark. Our specific studies were carried out using adult male Brandt's voles(4-5 months) with free access to water and food the commercial rabbit pellets (containing 18% protein, 3% fat, 12% fiber, and 47% carbohydrate, Beijing KeAo Bioscience Co.).

Experimental device

We experimentally prevented coprophagy behavior using hard plastic collars that are described in the supplement[21]. The collars were cut along a radial line and placed on the necks of the voles and are roughly 3%-5% of the vole's body mass. We used two types of collars: for the "coprophagy prevention"

treatment (CP), the width of the collar was 1.5-2cm and prevented animals from consuming their feces, and animals were placed in cages with wire-bottoms, where animals did not have access to feces that fell through the screen. To control the potentially stressful effects of collars, we added a stress control treatment (SCP) where we used sham-ring collars with a width of 0.5cm, and there was padding underneath the wire-screen, so that animals could access their feces and engage in coprophagy.

Experiment 1

Before the experiment, eight male voles were moved into individual cages and kept for at least 2 weeks. These voles were then placed in collars and wire-bottomed cages for 2 weeks (CP treatment), and then animals were moved to solid bottom cages and collars were removed for the following 2 weeks. This process of coprophagy prevention and recovery was repeated again. We collected feces at 0 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks to inventory the gut microbiota (Fig. S1a). All the animals remained at $23\pm 1^{\circ}\text{C}$ and 16Light: 8Dark. Microbial inventories are described in more detail below.

Experiment 2

Eighteen male voles were moved into individual cages and kept for at least 2 weeks and then were randomly assigned into 3 groups (6 animals in each group). The treatment group was coprophagy prevention maintained (CP), the control group was without any device (Con). Another group was a sham-ring group (SCP), to control for potentially stressful effects of the collars. All the groups remained at $23\pm 1^{\circ}\text{C}$ and 16Light: 8Dark for 4 weeks (Fig. S1b). We measured several metabolic indicators including body mass, food intake, fecal production, resting metabolic rates (RMR), non-shivering thermogenesis (NST) and concentrations of thyroid hormones in the serum. Indicators of digestive physiology included small intestine morphology and concentrations of short chain fatty acids in the cecum. We also measured their memory using a Y-maze, and assayed neurological parameters. These procedures are described in more detail below.

Experiment 3

Twenty-one male voles were moved into individual cages and kept for at least 2 weeks and then divided into three groups: coprophagy prevention group gavaged with acetate (CP-Ace 4.5mmol/d), coprophagy prevention group gavaged with PBS (CP-PBS), control group gavaged with PBS (CON). Groups were gavaged with about 0.2 ml each day. These 3 groups remained at $23\pm 1^{\circ}\text{C}$ and 16Light: 8Dark for 6 weeks (Fig. S1c). After acclimation, we collected feces to inventory the gut microbiota. We measured their metabolic metrics, and digestive physiology measurements, and neurological parameters similar to

Experiment 2. We also measured their memory behavior using the Y-maze, novel object test, and conspecific recognition test (described below).

Measurement of body mass, food intake and fecal production

Preliminary experiments demonstrated that collars do not affect the animals' ability to eat or drink. In the described experiments we measured body mass and food intake at 9 am every day. Body weight and food weight were measured by using an electronic balance (Sartorius Model BL 1500, $\pm 0.1\text{g}$). Voles were placed in a cage with a known amount of food. Twenty-four hours later, the amount of remaining food was measured with the difference viewed as food intake per 24 h. We measured fecal production by collecting all feces produced during a 24-hour period. Feces were dried (used oven at $60\text{ }^{\circ}\text{C}$ for one week) and weighed.

Measurement of resting metabolic rate and non-shivering thermogenesis

Resting metabolic rates (RMR) was measured as oxygen consumption using an open-circuit respirometry system (TSE labmaster, Germany). RMR were measured at 30°C , and inside 5.8 L transparent metabolic chambers (Type I for rats) we placed double-layered meshes for the separation of voles during measurements. The air flow rate was 0.8 L/minute. Oxygen consumption measurements were taken every 6 minutes for 3 hours. We took the average of the 3 lowest consecutive readings as the RMR.

The cost of nonshivering thermogenesis (NST) was measured at 25°C using a previously established method. We injected animals subcutaneously with norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) to elicit an elevated metabolic rate. The dosage of NE was calculated by the formula $\text{NE (mg/kg)} = 2.53\text{BM}^{0.4}$ for Brandt's voles, and the cost of NST is estimated as the 3 highest consecutive readings of oxygen consumption during 1 hour of measurement.

Behavioral tests

We conducted a suite of behavioral assays for voles in Experiments 2 and 3. We assayed working memory using a Y-maze[22], which requires voles to use memory to find the food arm and represents a hippocampal dependent-spatial working memory. The apparatus consisted of a white plastic maze with three arms (40 cm long, 30 cm high and 8 cm wide) that intersect at 120° . The three arms were marked with different colored shapes at the end of the arms. The voles began to test after a 12h fasting. First, we only opened two arms and put food in one them, called the food arm. Voles were placed at one end of the "beginning" arm for 5 minutes to adapt and memorize the location of the food and association with a colored shape marker. Voles were then removed from the Y-maze for one hour. Next, voles were again

placed in the “beginning” arm, but this time both arms were open (the “food” arm and a “novel” arm) with no food in either. Animals were allowed to move freely for 10 minutes, and we calculated the distance travelled and amount of time spend in the “food” arm and the “novel” arm.

We assayed novelty-seeking and recognition behaviors by exposing animals to new and familiar objects. Tests occurred in a plastic cage (42 × 27 × 18 cm) with a hyaline lid, and consisted of a 10-minute training phase and a 5-minute testing phase at 1-hour intervals. Two plastic toys with different shapes (one is a cube, the other is a pyramid) were used as the familiar object and novel object, respectively. During the 10-minute training period, voles were kept with the pyramid toy to become familiar with it (defined as the familiar object). One hour later, they were exposed to the pyramid and cube toys (the latter was defined as novel subject) together in the cage for 5-minute test. Novelty and familiar preferences (i.e., the duration and frequency of investigating familiar or novel object) during 5-minute test were recorded. The toys and cage were wiped with 75% ethanol after each training and test.

We investigated social interactions and conspecific recognition by repeatedly exposing voles to an unrelated individual, and then exposing them to a novel unrelated individual[23]. Each subject was placed into the experimental cage (25×45×20 cm³) and allowed to habituate for 10 min. Thereafter, an unrelated young male (approximately 30–40 days of age) was introduced into the experimental cage for 5 min (trial 1, Tr 1) and then removed. Thirty minutes later, the same stimulus young was reintroduced for another 5 min (trial 2, Tr 2). This process was repeated once more to total three times (Tr 1, Tr 2, Tr 3). During the fourth trial (New), a new, unrelated young was introduced into the arena. All 5-minute interaction periods were video recorded; the frequency and duration of the subject’s behaviors including olfactory investigation (i.e., sniffing of the conspecific’s anogenital or head region), close pursuit (i.e., closely following the conspecific), and escape behavior (i.e., moving away from the conspecific) were quantified.

Last, we used the open field test as a general method for measuring animal’s activity[22,23]. Voles were placed in the unfamiliar arena (60cm diameter with 45 cm high walls, lined with grey card, divided into two regions: peripheral and central zone) for 5 min to adapt the environment. Later, voles were placed in the field again and their behavior was recorded for 10 minutes using computerised tracking. We calculate moving distance travelled in the field in cm.

The apparatus for behavior tests was cleaned thoroughly with 75% ethanol between uses to remove odor cues from previous individuals. All the behavioral data were analyzed with EthoVision Image Tracking System 20.0 (Noldus, Holland).

Sample collection

Animals sacrificed between 9 a.m. and 11 a.m. The serum was collected and centrifuged at 4°C (30min, 1500r). We get the extracted the entire gastrointestinal tract (stomach, small intestine, cecum and colon).

Each section was laid flat to measure length. Contents of each section were removed, tissues were rinsed with saline 5 times, and then we measured wet mass of each empty gut region. A small section from the middle of the small intestine was rinsed 3 times with 4% paraformaldehyde and then quickly put into a 4% paraformaldehyde and fixed for more than 24 hours. After routine paraffin embedding, a paraffin microtome was used to cut 5 µm sections continuously, and 6-8 sections were placed horizontally on cation-adhering slides. H&E staining of the gut was done as previously described, neutral gum seals, number and preservation. Then we measured the length and width of small intestinal villi used the ImageJ[24].

Gut Microbiota Profiling

Fresh feces and gut contents collected from the caecum were immediately frozen, and were stored in -80°C. DNA was extracted by 2×Cetyltrimethyl Ammonium Bromide (2×CTAB) and Phenol chloroform mixture (phenol: chloroform: isoamyl alcohol = 25:24:1), and was then isolated by a spin column using SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, 273 B518131-0100). Universal primers were used for PCR amplification of the V3-V4 hypervariable regions of 16S rRNA genes and contained Illumina 3' adaptor sequences as well as a 12-bp barcode. Sequencing was done on an Illumina HiSeq 2500. Raw sequencing reads were denoised, filtered according to barcode and primer sequences (Forward primer-341F, CCTACGGGNGGCWGCAG; Reverse primer-805R, GACTACHVGGGTATCTAATCC), and classified with the Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) software suite. The open-reference Operation Taxonomic Unit (OTU) picking strategies in QIIME software suite were used to identify OTUs, using a 97% sequence similarity. Resulting sequences were then searched against the Greengenes reference database of 16S rRNA gene sequences, clustered at 97% by uclust. Alpha diversity was investigated by measuring Faith's phylogenetic diversity after rarefying OTU tables to sequences per sample. Beta diversity of the gut microbiome was also compared. Nonmetric multidimensional scaling (NMDS) based on unweighted UniFrac distance was used to visualize the structure of microbial community using R vegan package. Statistical testing among variations in bacterial community compositions (Bray-Curtis distance metric) was carried out using analysis of similarity (ANOSIM, permutations=999). PICRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) is a bioinformatics software package based on marker genes to predict functional components of the microbiome (include citation for PICRUST. We used PICRUST to predict the function of microbiota, and used STAMP (*t*-test) to do statistical analysis[25].

Measurement of short chain fatty acids

We measured six short chain fatty acids in the cecal contents collected at dissection: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid. Cecum contents were centrifuged, supernatant removed, and then treated with phosphoric acid for 3 hours to make them

stable. Short chain fatty acids were measured by high performance gas chromatography (GC) (Agilent 7890A; Agilent Technologies, Germany) with a GC autosampler and a FID system. Separations were performed in a 30 m×0.25 mm×0.25 µm DB-WAX column (Agilent Technologies). 99.998% hydrogen was used as carrier gas at a flow-rate of 1.0 ml/min. The system was operated at 250°C. Injections were performed in the splitless mode at 230 °C, and 0.5 µl for each injection. The oven temperature was programmed from 60 °C (1 min) at 5 °C/min to 200 °C, then from 200 °C at 10 °C/min to 230 °C. The total running time of each sample is 32min.

Measurements of serum hormones

We measured concentrations of hormones as an indicator of energy metabolism. Concentrations of triiodothyronine (T3) and thyroxine (T4) in serum were determined by radioimmunoassay using ¹²⁵I RIA kits (Beijing North Institute of Biological Technology, Beijing, China). Intra- and inter-assay coefficients of variation were 2.4% and 8.8% for T3, and 4.3% and 7.6% for T4.

Serum ghrelin (a des-acylated form) concentrations were measured by Ghrelin EIA kit (Catalog No. EK-031-31, Phoenix Pharmaceuticals, USA). The minimum detected concentration by this kit was 0.13 ng/mL. The intra- and inter-assay variations for ghrelin EIA were 10% and 15%, respectively.

Measurement of protein expression by western blot

Samples of the intestinal jejunum (1.5cm segment, whole thickness of tissue), hypothalamus, and hippocampus were homogenized in RIPA buffer and cleared by centrifugation, according to standard techniques. Protein placed in loading buffer (4% SDS, 20% glycerol, 0.1% bromophenol blue, 250 mM Tris HCl pH 6.9, 0.2% 2-beta mercaptoethanol) and denatured by heating at 100 °C for 3 min. The total protein separated by SDS–PAGE using a Mini Protean apparatus (Bio-Rad Laboratories, PA, USA) then transferred to PVDF membranes. In order to reduce non-specific antibody binding, membranes were incubated for 12 h at 4°C or 2h at room temperature in 5% skimmed milk powder blocking buffer. The membranes were then exposed to primary antibodies more than 12 hours at 4°C. Then the appropriate horseradish peroxidase-conjugated secondary antibody incubated for 2 h at room temperature. Antibody concentration is depended on the instructions. The reaction products were revealed by chemiluminescence (ECL, Yesen). Protein was expressed as relative units (RU) and quantified with Lab image Software (BioRad, USA). Western blots of whole tissue lysates were probed with primary antibodies against: UCP1 (ab155117, Abcam), TH (AB152, Merck Millipore), FFAR2/GPR43 (ABC299, Merck Millipore), AVP (AB1565, Merck Millipore), AVP-R1(ab187753, Abcam), BDNF (ab203573, Abcam), TrkB(4603, Cell signaling), PCNA(13110, Cell signaling), Oxytocin(AB911, Merck Millipore), Oxytocin-receptor(ab217212, Abcam), GFAP(ab7260, Abcam), NeuN (MAB377, Merck Millipore), CRF(ab184238, Abcam), CRF-receptor(CRF1, ab95023, Abcam). β-tubulin (A01030HRP, Abbkine), GAPDH

(A01020,Abbkine). The second antibody was peroxidase-conjugated goat anti-rabbit IgG (111-035-003, Jackson) or peroxidase-conjugated goat anti-mice IgG (115-035-003, Jackson).

Measurement of neuropeptide by Real-time -qPCR

We assayed the influence of food intake on coprophagy by measuring the gene expression of agouti-related protein (AgRP), neuropeptide Y (NPY), cocaine- and amphetamine-regulated transcript (CART), and pro-opiomelanocortin (POMC) in the hypothalamus. Total RNA was extracted from the hypothalamus using TRIzol agent, then reversed transcription into cDNA according to the requirements of the specification (Code No. RR820Q/A/B, TAKARA, Dalian, China). Real-time RT-qPCR analysis was carried out as follows: The cDNA samples (2 μ L) were used as a template for the subsequent PCR reaction using gene-specific primers (Table S1). The final reaction volume of 20 μ L contained 10 μ L of 2 \times SYBR Premix EX Tag II (TAKARA), 0.4 μ L of 50 \times ROX Reference Dye, 2 μ L cDNA template, 0.8 μ L of forward prime and reverse primer (final concentration 0.4 μ M per primer) and 6 μ L dH₂O. The qPCR was performed using Piko Real Software 2.2 (Piko Real 96, Thermo scientific, America). After an initial polymerase activation step at 95 °C for 60 s, amplification was followed by 40 cycles (95 °C for 5 s, 55 °C for 30 s and 72 °C for 30 s). The reaction was finished by the built-in melt curve. All samples were quantified for relative quantity of gene expression by using actin expression as an internal standard.

Determination of monoamine neurotransmitters and Turnover ratio (TR)

We investigated changes in memory-related neurotransmitters by measuring concentrations of 5 neurotransmitters: 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NE, norepinephrine; DA, dopamine and DOPAC, 3,4-dihydroxyphenylacetic acid. We used high performance liquid chromatography with electrochemical detection (HPLC-ECD). The internal standard was 3,4-dihydroxybelzamine (DHBA). Average weights of tissue sampled were as follows: Hippocampus~0.01g, Hypothalamus~0.015g. Samples were homogenized in 0.1 M cold perchloric acid, ultrasonic oscillation for 10 seconds and centrifuged at 13,000 RPM at 4°C for 30 min. Supernatant was filtered using 0.2 mm nylon filter. Aliquots of 30 μ l were manually injected using Hamilton syringe. The mobile phase (pH=4.0) consisted of 90 mM NaH₂PO₄·2H₂O, 50 mM citric acid monohydrate, 1.7 mM alkylsulfonate (SAS), 50 μ M EDTA, 2 mM NaCl, and 8% Acetonitrile. The detector (Thermo Scientific Ultimate 3000 RS Electrochemical Detector with 6041RS Ultra Amperometric Cell (6070.3000), highly efficient glassy carbon electrode (6070.3200), 6041RS sensor, and Pd reference electrode) was set at 150 mV, and the column (Acclaim™ 120, C18 3 m 120Å, 2.1 \times 150 mm) was placed at 8°C. Compounds were identified by comparing their retention times with those of authentic reference compounds. The level of each monoamine was calculated by the internal standard method using Chromeleon 7 software (ThermoFisher Scientific, MA, USA).

Quantitative calculation:

(1) The samples were quantified according to the correction factor + internal standard method and were converted into ng/mg of wet tissue.

Correction factor (f) = (peak area of internal standard substance / concentration of internal standard substance) / (peak area of standard / concentration of standard)

(2) Content (sample) = f × {peak area of sample / (peak area of internal standard substance / concentration of internal standard substance)}

(3) The amount of material was measured by $W \text{ (ng)} = \text{Content (sample)} \times V \text{ (volume of liquid to be measured)}$. Monoamine and metabolite levels are expressed as ug/g of wet tissue. Ratio of metabolite(s)/amine was calculated as an index of rate of metabolism; DOPAC/DA indicated dopamine turnover rate and 5-HIAA/5-HT indicated 5-HT turnover rate.

Many studies have indicated that levels of the dopamine metabolites DOPAC in tissue are useful indexes of neurotransmitter function. By calculating the tissue DOPAC/DA and 5-HIAA/5-HT ratios, we were able to obtain an indication of dopamine and 5-HT utilization in tissue regions of the brain. Based on previous studies the TR for 5-HT was calculated from the equation $\text{TR} = 5\text{-HIAA content} / 5\text{-HT content}$. The TR for dopamine was calculated from the equation $\text{TR} = \text{DOPAC content} / \text{DA content}$.

Statistical analysis

Statistical analysis was carried out using the SPSS 20.0 software package. Differences in body mass, food intake, and fecal production were compared between treatment groups using a repeated-measures ANOVA. Measurements on the final days of trials were compared using ANOVAs. Microbial measurements (abundances of taxa or functions, and alpha diversity metrics) were compared using independent t-tests or ANOVAs (depending on the number of groups). Beta diversity was compared using the ANOSIM test as described above. One-way ANOVAs were used to compare measurements of RMR, NST, measurements of digestive anatomy, serum concentrations of hormones, SCFA concentrations measurements from behavioral trials, and expression of neurotransmitters. For all of these tests, post-hoc differences between groups were conducted using the Student-Newman-Keuls method. Results were deemed statistically significant if $p < 0.05$.

Results

Coprophagy prevention changes the gut microbiota composition

We collected feces continuously during experiment 1 to detect changes in the gut microbiota. Alpha diversity was lower in 2 weeks and 6 weeks group compared to the 0, 4, 8 weeks groups ($F_{(4,35)} = 3.409$, $p = 0.019$, Table 1, Fig. 1a). For beta diversity, analysis based on unweighted UniFrac distance showed significant differences among 5 sample types (across time and treatments; ANOSIM, unweighted: $r =$

0.141, $p = 0.005$, Fig. 1b, weighted: $r = 0.194$, $p = 0.001$), and we can clearly see that the samples collected at 2 weeks and 6 weeks (when animals were during the coprophagy prevention period) cluster together, while the other 3 time points (when animals were during the coprophagy period) also cluster together (Table S3). We use repeated measures to analyze phylum-level differences: Bacteroidetes, Firmicutes, Verrucomicrobia, Cyanobacteria, Deferribacteres, Proteobacteria, Spirochaetes, TM7 and Tenericutes (Table 2, Fig. S2a). Coprophagy prevention significantly decreased the relative abundances of Firmicutes ($F_{(4,35)} = 9.676$, $p < 0.01$) and increased Bacteroidetes ($F_{(4,35)} = 9.368$, $p < 0.01$, Fig. 1c). Specifically, compared between 0 week and 2 weeks, relative abundance of the phylum Firmicutes decreased from $60.1\% \pm 2.5\%$ at week 0 to $46.3\% \pm 2.1\%$ after 2 weeks of coprophagy prevention ($t = 4.254$, $df = 14$, $p = 0.001$; Fig. 1c). Relative abundances of Bacteroidetes increased from $35.1\% \pm 2.5\%$ at week 0 to $49.1\% \pm 1.8\%$ at week 2 ($t = 4.46$, $df = 14$, $p = 0.001$; Fig. 1c).

We analyzed the influence coprophagy prevention on the microbiome at a finer scale by comparing the abundances of the top 30 most abundant OTUs between 0 week group and 2 weeks group (Fig. S2b). OTU identified as belonging to the genera, *Oscillospira*, *Ruminococcus* and *Clostridium* were significantly lower at the 2-week time point compared to week 0, while OTUs belonging to YRC22, *Allobaculum*, *Roseburia* and *Dorea* increased. Additionally, OTUs identified as *Prevotella copri* increased and *Ruminococcus flavefaciens* decreased in 2 weeks group (Table 3). The functional potential of bacterial assemblages associated to each stool sample was predicted with PICRUST using level 3 of Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs. The gut microbiome of samples collected at the 2-week time point harbored significantly different functional categories, compared to 0 week ($p < 0.05$, Fig. 1d). These different functional categories were related to carbohydrate metabolism (e.g., glycolysis/gluconeogenesis, starch and sucrose metabolism), lipid metabolism (e.g., fatty acid biosynthesis), amino acid metabolism (e.g., Ala, asp and glu acid metabolism), and cell signaling molecules (e.g. ABC transporters, Cell cycle- caulobacter).

Coprophagy affected the energy metabolism of animals

In Experiment 2, the body mass in the Con group, CP group and SCP group were not significantly different ($F_{(2,15)} = 1.418$, $p = 0.273$, Fig. S2a) although acclimation time has a significant effect on it ($F_{(14,210)} = 2.219$, $p = 0.008$, Fig. S2a). Body mass on the last day of the experiment was significantly different across the three treatment groups ($F_{(2,15)} = 3.802$, $p = 0.046$), with the CP group exhibiting a body mass 20.1% lower than the Con and SCP groups. During coprophagy prevention, food intake was significantly different across the 3 groups ($F_{(2,15)} = 16.352$, $p < 0.01$, Fig. 2a), with CP group showing the highest food intake and SCP group exhibiting the lowest. Food intake did not differ depending on acclimation time ($F_{(4,60)} = 9.112$, $p < 0.01$, Fig. 2a).

RMR was 28.2% lower in the CP group compared to the Con group ($F_{(2,15)} = 4.956$, $p = 0.022$, Fig. 2b). Groups did not exhibit significant differences in energetic costs of NST ($F_{(2,15)} = 3.176$, $p = 0.071$, Fig. 2c). Thyroid hormone T3 is an important hormone for thermogenesis; the T3 in serum of CP group was

significantly lower than Con and SCP groups ($F_{(2,15)} = 5.449$, $p = 0.017$, Fig. 2d). We also found that animal activity, measured by the open-field test, demonstrated that animals in the CP group moved significantly more than the Con and SCP groups ($F_{(2,15)} = 7.662$, $p = 0.005$, Fig. 2e).

Coprophagy prevention decreased intestinal absorptive capacity

We observed a marked increase in the mass of the cecum in the CP group compared with other groups ($F_{(2,15)} = 7.819$, $p = 0.005$, Fig. 2g). There was no difference among groups of colon length ($F_{(2,15)} = 2.733$, $P = 0.097$, Fig. S3f), and small intestine ($F_{(2,15)} = 1.953$, $p = 0.176$, Fig. S3e). To further investigate the changes in the intestinal morphology, we measured the intestinal villus length and crypt depth, and we found villus length was significantly decreased in the CP group ($F_{(2,15)} = 5.519$, $p = 0.016$, Fig. 2f).

We measured concentrations of several short-chain fatty acids (SCFAs), which are associated with gut microbiota activity using high performance gas chromatography. Specifically, cecal contents of voles in the CP treatment exhibited significantly lower concentrations of butyrate ($F_{(2,15)} = 6.396$, $p = 0.01$, Fig. 2h), acetic acid ($F_{(2,15)} = 6.273$, $p = 0.01$, Fig. 2h), and propionic acid ($F_{(2,15)} = 15.337$, $p < 0.01$, Fig. 2h) compared to Con and SCP voles, suggesting that coprophagy prevention decreases fermentation activity of the gut microbiome.

Coprophagy prevention decreased memory and dopamine (DA) and 5-HT turnover in the brain

From the locus of movement, the total moving distance of three groups showed no difference ($F_{(2,15)} = 0.244$, $p = 0.786$, Fig. 3b). Voles in the CP group spent significantly less time in the “food” arm compared to the other groups ($F_{(2,15)} = 5.146$, $p = 0.02$, Fig. 3a). The proportion of distance in food arm was not different among 3 groups ($F_{(2,15)} = 0.188$, $p = 0.831$, Fig. 3a).

We investigated potential changes in concentrations of monoamines across the 3 groups, as these neurotransmitters have been associated with memory. The levels of NE did not differ across groups in the hypothalamus ($F_{(2,15)} = 1.571$, $p = 0.24$, Fig. 3c) and hippocampus ($F_{(2,15)} = 1.566$, $p = 0.241$, Fig. 3d). The content of DA was significantly lower in the hippocampus tissue of CP voles compared to the other groups ($F_{(2,15)} = 6.11$, $p = 0.011$, Fig. 3d). The content of DOPAC in hypothalamus of CP group was lower than other treatment groups ($F_{(2,15)} = 4.067$, $p = 0.039$, Fig. 3c). Concentrations of 5-HT and its metabolite 5-HIAA in the hippocampus tissues of CP voles were lower than Con and SCP groups ($F_{(2,15)} = 4.017$, $p = 0.04$; $F_{(2,15)} = 4.167$, $p = 0.036$, Fig. 3d). The turnover ratio of 5-HT and DA did not exhibit significant differences among our three groups ($p > 0.05$, Fig. S3h,i). We also found that the TH ($F_{(2,15)} = 8.338$, $p = 0.004$) and AVP ($F_{(2,15)} = 4.59$, $p = 0.028$) in the hippocampus tissues of the CP group were lower than Con and SCP groups (Fig. 3e-h).

Supplementation of SCFAs can protect the damage of energy metabolism caused by coprophagy prevention

In Experiment 3, we supplemented CP animals with acetate to investigate whether phenotypes were rescued. The body masses of voles in the CP-Ace group did not decrease as much as those in the CP-PBS group (Time: $F = 6.353$, $p < 0.001$; Group: $F = 0.035$, $p = 0.965$; Fig. S4a). Food intake did not differ between the CP-Ace group and CON groups at 2,3,4,5 weeks, while the CP-PBS group exhibited significantly higher food intakes (Time: $F = 9.903$, $p < 0.001$; Group: $F = 5.260$, $p = 0.016$; 0 week: $F_{(2,18)} = 0.124$, $p = 0.884$; 1 weeks: $F_{(2,18)} = 5.941$, $p = 0.012$; weeks: $F_{(2,18)} = 4.003$, $p = 0.036$; 3 weeks: $F_{(2,18)} = 5.559$, $p = 0.013$; 4 weeks: $F_{(2,18)} = 5.261$, $p = 0.016$; 5 weeks: $F_{(2,18)} = 7.911$, $p = 0.003$, Fig. 4a). The length of small intestine and colon were different among three groups ($F_{(2,18)} = 3.748$, $p = 0.044$, Fig. S4d; $F_{(2,18)} = 4.387$, $p = 0.028$, Fig. 4b). Because we added acetate artificially, the acetate content in CP-Ace group was higher than CP-PBS group, but there was no difference with CON group ($F_{(2,18)} = 4.34$, $p = 0.029$, Fig. 4c). The propionic acid content decreased in CP-PBS group ($F_{(2,18)} = 5.804$, $p = 0.011$, Fig. 4c) and the content of isovaleric acid increased ($F_{(2,18)} = 7.176$, $p = 0.005$, Fig. 4d).

To further explain the changes in food intake, we measured ghrelin and neuropeptide in hypothalamus. Ghrelin content of group CP-PBS was significantly higher than that of other groups ($F_{(2,18)} = 4.828$, $p = 0.021$, Fig. 4e). The feeding related neuropeptide NPY was significantly lower in the CP-Ace group compared to the other groups ($F_{(2,15)} = 4.588$, $p = 0.028$, Fig. 4f). CART, a neuropeptide that inhibits appetite, was more highly expressed in CP-Ace group than CP-PBS group, but still lower than the control group ($F_{(2,15)} = 7.236$, $p = 0.006$, Fig. 4f).

Supplementation of SCFAs can change the gut microbiota

Feces were from three groups of animals and analyzed the changes of gut microbiota by 16S rRNA sequencing. Alpha diversities of three groups were not significantly different (Table S4). For the beta diversity, analysis based on unweighted UniFrac distance showed significant differences among the 3 groups (ANOSIM, 3 groups: unweighted: $r = 0.198$, $p = 0.004$; weighted: $r = 0.341$, $p = 0.001$) though pairwise comparisons were not statistically significant (Table S5, Fig. 4g). Abundances of the phylum Firmicutes was lower in the CP-PBS group compared to CON ($F_{(2,18)} = 6.297$, $p = 0.008$, Fig. 4h) and abundance of the phylum Bacteroidetes was higher in the CP-PBS group compared to CON ($F_{(2,18)} = 7.297$, $p = 0.005$, Fig. 4h); abundances of these phyla were intermediate in the CP-Ace group. The relative abundance of Spirochaetes was significantly lower in the CP-PBS group ($F_{(2,18)} = 5.114$, $p = 0.017$, Fig. 4h), and abundances were recovered by supplementation of acetate. acetate supplementation did not reverse the effect of coprophagy prevention on Actinobacteria ($F_{(2,18)} = 9.835$, $p = 0.001$, Fig. 4h).

To assess how coprophagy prevention and supplementation altered predicted functions of microbial communities, we applied LEfSe method with LDA score > 2 to PICRUSt results (Fig. S4h). This analysis identified one discriminative feature in the microbiota of CP-Ace voles, 16 in CP-PBS, and 8 in CON voles.

Many functional categories like carbohydrate metabolism (e.g., Carbohydrate metabolism, Pyruvate metabolism and Galactose metabolism), amino acid metabolism (e.g., Amino sugar and nucleotide sugar metabolism) were not affected by acetate supplementation, because that CP-PBS voles were no difference between CP-Ace voles, and they were lower than those in the CON group (Fig. S4i).

To evaluate the relationship between the growth promoting effect of coprophagy and the change of gut microbiota, we used MaAsLin, a multivariate statistical tool, to calculate the correlation between body mass and gut microbial community structure of voles while controlling for other variables, including treatment groups (Table S6). We found that abundances of *Paludibacter* and *Lachnospira* were positively correlated with body mass; while abundances of *Adlercreutzia* exhibited a negative correlation body mass. After removing OTUs that were present in less than 10% of the samples, we then analyzed the association between OTUs and body mass by MaAsLin. The results showed that there were 293 OTUs correlated with body mass, of which 213 were positively correlated with body mass and 80 were negatively correlated with body mass. The results of association between OTUs and coprophagy prevention (Table S7-8) showed that *Ruminococcaceae*, *RF16* and *Paraprevotellaceae* were negatively correlated with CP-PBS treatment. *Sphaerochaeta*, *YRC22* and *Prevotella* were positively correlated with CP-PBS treatment. *Lachnospira* were positively correlated with acetate supplement. *Oscillospira* were negatively correlated with both CP-PBS and CP-Ace.

Supplementation of SCFAs can increase the memory during coprophagy prevention

We used acetate with the highest proportion of short chain fatty acids as the gavage reagent. In order to fully determine the effects of coprophagy prevention on animal memory, we conducted three behavioral tests from different perspectives. The results of Y- maze test showed that there was no difference in the total moving distance across the three groups ($F_{(2,18)} = 1.579$, $p = 0.233$), and the distance in food arm was significantly lower in the CON and CP-Ace group ($F_{(2,18)} = 6.963$, $p = 0.006$, Fig. 5a). The proportion of time in food arm was lower in the CP-PBS group than other groups ($F_{(2,18)} = 1.065$, $p = 0.366$, Fig. 5b). The proportion of distance in food arm was not different among 3 groups ($F_{(2,18)} = 3.627$, $p = 0.047$, Fig. 5b). The object recognition test, showed that animals explore novel object by sniffing them and quickly avoiding them, and they were more willing to stay next to familiar objects. Comparing the three groups together, frequency of investigation of novel object showed that voles in CP-PBS group were lower than CON and CP-Ace group ($F_{(2,18)} = 7.107$, $p = 0.005$, Fig. 5c). On the contrary, the residence time of familiar object in CP-PBS group was lower than CON and CP-Ace groups ($F_{(2,18)} = 4.376$, $p = 0.028$, Fig. 5d). The time spent with the novel object was lower than familiar object in CON and CP-Ace groups ($t = 2.769$, $df = 12$, $p = 0.017$; $t = 2.249$, $df = 12$, $p = 0.044$, Fig. 5d), for CP-PBS group, the residence time of novel and familiar was not different ($t = 0.408$, $df = 12$, $p = 0.691$, Fig. 5d). Voles in the Con and CP-Ace groups spent significantly more time interacting with novel objects compared to familiar objects ($t = 2.277$, $df = 12$, $p = 0.042$; $t = 2.153$, $df = 12$, $p = 0.05$, Fig. 5c), while the CP-PBS group exhibited no preference or avoidance for novel or familiar objects ($t = 0.15$, $df = 12$, $p = 0.883$, Fig. 5c). The results of individual recognition test,

showed that with the increase of recognition times, the close pursuit time of three groups of voles to the same individuals decreased significantly (Time: $F_{(3,57)} = 7.047, p < 0.001$; Groups: $F_{(2,19)} = 1.468, p = 0.255$, Fig. 5e). When exposed to the same young vole for the third time (Tr 3), the close pursuit time of CP-PBS group was higher than that of the CON and CP-Ace groups ($F_{(2,18)} = 4.093, p = 0.034$, Fig. 5e). With the increased of recognition times, the frequency of investigation in 3 groups decreased significantly (Time: $F_{(3,57)} = 49.426, p < 0.001$; Groups; $F_{(2,19)} = 1.212, p = 0.320$, Fig. 5f). The frequency of investigation in Tr3 of CP-PBS group was higher than that of the CON and CP-Ace groups ($F_{(2,18)} = 4.526, p = 0.026$, Fig. 5f).

Supplementation of SCFAs can promote the development of hippocampal neurons

To investigate potential mechanisms underlying differences in memory across groups, we measured the gene expression of neurons in the hippocampus. The BDNF was lower in CP-PBS and CP-Ace group ($F_{(2,18)} = 4.693, p = 0.023$, Fig. 5g), while the TrkB (BDNF receptor) showed no difference among the three groups ($F_{(2,18)} = 1.781, p = 0.197$, Fig. 5g). The expression of PCNA in the CP-PBS group was decreased compared to other groups ($F_{(2,18)} = 3.586, p = 0.049$, Fig. 5g). Oxytocin in the CP-PBS group was the lowest, and was increased by the acetate treatment ($F_{(2,18)} = 3.787, p = 0.042$, Fig. 5g), while the receptor of Oxytocin showed no significant differences among the three groups ($F_{(2,18)} = 2.409, p = 0.118$, Fig. 5g). For the GFAP and NeuN, the supplement of acetate increased their expression ($F_{(2,18)} = 16.537, p < 0.001$; $F_{(2,18)} = 5.284, p = 0.016$, Fig. 5g). The expression of CRF in CP-Ace group was lower than CP-PBS group ($F_{(2,18)} = 1.134, p = 0.344$, Fig. 5g). AVP and its receptor were higher in the CP-Ace group than the CP-PBS group ($F_{(2,18)} = 6.416, p = 0.008$; $F_{(2,18)} = 14.177, p < 0.001$, Fig. 5g). In the hippocampus, there are FFAR2 receptors to receive the signal from SCFAs. The expression of FFAR2 was decreased in CP-PBS group but increased in CP-Ace group ($F_{(2,18)} = 4.493, p = 0.026$, Fig. 5g). The expression of TH in CP-PBS and CP-Ace group were decreased compared to the CON group ($F_{(2,18)} = 12.259, p < 0.001$, Fig. 5g).

Discussion

Coprophagy is a widespread behavior among small- and medium-sized mammalian herbivores. This behavior can help to improve the digestive function of herbivorous animals, because many nutrients and food fragments can be digested and absorbed again by eating faeces [26]. Here, we demonstrate that prevention of coprophagy alters the community structure of the gut microbiome and affects many physiological processes and memory in Brandt's voles. This effect may be regulated by SCFAs, because we have also demonstrated that acetate supplementation can alleviate energy imbalance and improve memory in animals during coprophagy prevention. We discuss these findings more below.

Coprophagy affects host gut microbiota

In wild animals, the gut microbiota is affected by many environmental factors, including temperature, season, food quality, etc. [27,28,29,30]. Early, culture-based studies in laboratory rodents have

demonstrated that prevention of coprophagy can alter aspects of the gut microbiome[31]. For example, coprophagy prevention in rats yielded increases in the abundances of *Escherichia coli* and *Enterococcus* and decreases in *Lactobacillus*[32]. Here, we used high-throughput sequencing to more thoroughly understand the impacts of coprophagy prevention on the gut microbiome of a herbivorous rodent.

Our experiments revealed that coprophagy prevention significantly decreased abundance of the phylum *Firmicutes*, composed of families like the *Clostridiaceae*, *Lachnospiraceae* and *Ruminococcaceae*. These families of the *Firmicutes* are obligate anaerobes which reduce the oxidative state of the gut [33]. They are common constituents of the gut microbiota of healthy voles and were significantly depleted in voles where coprophagy was prevented. Highly cellulolytic bacterial species such as *Ruminococcus flavefaciens* are regarded as essential for the microbial breakdown of cellulose in the rumen[34]. In our results, *R. flavefaciens* decreased after coprophagy prevention, which may ultimately reduce cellulose fermentation capacity. Voles experiencing coprophagy prevention also exhibited significantly lower abundances of *Oscillospira*, an enigmatic bacterium has been detected from the human microbiome in association with leanness or lower body mass index in both infants and adults[35,36,37]. *Oscillospira* are not fibre degraders but rather produce butyrate by relying on fermentation products produced by other microbes or on sugars liberated from host mucins[38]. Therefore, lower abundances of *Oscillospira* could contribute to the energy imbalance in voles that are unable to engage in coprophagy.

We also predicted microbiota function from our taxonomic inventories, and found that coprophagy prevention decreased predicted abundances of genes associated with glycolysis and amino acid metabolism. The results suggest that the genes associated with glucose metabolism decrease in abundance after CP, which may reduce the energy supply and metabolic status of intestinal epithelial cells, and also affect to the proliferation of mucosal cells and the maintenance of mucosal morphology.

Coprophagy affects host energy metabolism

Our results showed that coprophagy prevention resulted in reduced body mass and increased food intake. These results are similar to previous studies in rats[39,40,41], Meadow voles (*Microtus pennsylvanicus*) and Woodland voles (*Microtus pinetorum*)[42]. The soft feces excreted by rodents are rich in vitamins, amino acids, peptides, and a variety of nutrients that are not digested and absorbed by the digestive tract[11], and experiments show that the concentrations of these nutrients in rodent faeces increases significantly as a result of coprophagy prevention [40,41]. Therefore, the prevention of coprophagy likely limits voles' access to bacteria-produced nutrients. Our measurements of gut morphology showed that the sham-ring group exhibited no differences compared to the control group in morphology of the digestive tract, but in CP group, the shortening of small intestinal villi, which is a common physiological response to malnutrition[43]. It is interesting that the higher food intake of voles experiencing coprophagy prevention still does not fully compensate for these nutritional deficiencies, as these voles still exhibited reduced body mass.

Coprophagy affects vole's memory

We found that coprophagy prevention significantly impaired the memory of Brandt's voles. Our findings are consistent with previous studies linking coprophagy with neurodevelopment. For example, rat pups which have been prevented access to maternal faeces demonstrate deficits in a variety of neuro-behavioral development tests[44]. A recent study in human has demonstrated the associations between infant gut microbiome and cognition[45]. Thus, it appears that coprophagy by impacting gut microbiome may also be important for neural development and memory. Specific brain regions, such as the hypothalamus and hippocampus, participate in learning and memory through neurotransmitters like 5-HT and DA, and the loss of these monoaminergic substances is the most important neurochemical process leading to memory loss[46,47,48,49]. Our results showed that the CP group had lower concentrations of 5-HT and its metabolite 5-HIAA in the hippocampus, which may be caused by the shortage of microbial products. It was shown that the commensal bacteria, *Bifidobacteria infantis*, could modulate tryptophan metabolism, suggesting that the normal gut microbiota can influence the precursor pool for serotonin (5-HT)[14]. Additionally, we found changes in the levels of dopamine and the metabolite DOPAC in the hippocampus. These changes in dopamine turnover highlight a dysregulation in the synthesis and degradation of this monoamine. Although not all monoamine transmitters are reduced, all results highlight that absence or changes of the gut microbiota by coprophagy prevention deeply affect neurotransmitter systems.

As the central neurohormones, both AVP and oxytocin play important roles in the regulation of central nervous functions related to spatial learning and social cognition [50,51]. Numerous studies have shown that AVP and its analogs can improve learning and memory-related performance[52,53]. For example, injection of synthetic AVP improves the performance of rats in active and short-term olfactory memory tests[54]. Administration of AVP can facilitate the consolidation and retrieval processes of both active memory[55] and reference memory in a radial maze[56]. Another study indicates that AVP via V1 receptor enhances synaptic plasticity of hippocampal synapses and ameliorates spatial learning impairments in chronic cerebral hypoperfusion, a major modulator contributing to cognitive decline[57]. OT injected into the lateral part of the septal brain area has been reported to improve juvenile recognition abilities in adult male rats[58]and oxytocin protects hippocampal memory and synaptic plasticity in response to stress[59]. Lower endogenous oxytocin levels are associated with poorer social cognition in schizophrenia[60]. In our experiment, the CP group performed poorly in the Y-maze and for social cognition, and exhibited decreased AVP and oxytocin levels in hippocampus. Coprophagy prevention may induce nutritional imbalance which could lead to impairment in neuronal development and synaptic plasticity, resulting in impaired memory.

Coprophagy-gut-microbiota-SCFAs axis promotes memory enhancement

Many physiological effects of the gut microbiome are elicited by fermentation products, including short chain fatty acids[61,62].For example, transcriptional regulation within the central nervous system can be modified by the gut microbiota and SCFAs[63,64]. In our results, coprophagy prevention not only

decreased the total concentration of short chain fatty acids, but also changed their proportions. Turnbaugh et al.[65] showed that changes in SCFAs affect the energy metabolism of the host.

When we experimentally supplemented voles that cannot engage in coprophagy with acetate, many aspects of their health and physiology are similar to that of control animals. There may be several mechanistic pathways to explain this relationship. First, acetate serves as a nutrient, and so supplementing animals with this SCFA may increase the nutritional yield from their food. SCFAs can also increase the secretion of pancreatic enzymes, which may further assist with nutrient digestion[66]. Additionally, SCFAs, especially acetate, can expand intestinal blood vessels and increase intestinal blood flow, which may contribute to the maintenance of intestinal mucosal morphology[67], and could contribute to increased nutrient absorption. So, coprophagy behavior might increase energy absorptive capacity by increasing SCFAs concentration and their downstream physiological effects. Such nutritional benefits could help vole's maintaining energy balance. From our results, acetate can directly affect the expression of neuropeptides in hypothalamus, and reduce food intake. Similar study showed that a four-fold rise in the expression of the melanocortin precursor POMC and a potent suppression of AgRP expression 30 min after acetate administration[68]. So, compared with CP-PBS group, the acetate supplement can keep energy balance while reducing food intake.

A wide range of compounds generated by gut microbiota have direct or indirect effects on the brain and behavior[63]. A recent study demonstrated that germ free mice have increased blood brain barrier permeability when compared with specific pathogen free mice containing a healthy microbiota[69]. Many studies have examined the beneficial effects of SCFAs on memory and cognition. For example, children on a high fiber diet demonstrate better cognitive control (e.g. multitasking, working memory and maintaining focus) than children who typically ate a lower fiber diet[70]. Several studies have shown that sodium butyrate has antidepressant-like effects when administered and may be used as an epigenetic drug[71,72,73]. To date, only a handful of studies have probed the mechanistic basis surrounding the beneficial neurological effects of the acetate. Since acetate can penetrate the blood-brain barrier, it may also affect memory-related brain areas. Intracellular butyrate, propionate and acetate can inhibit the activity of histone deacetylase and promote the hyperacetylation of histones. Pre-clinical studies in rodents have also shown that inhibiting histone deacetylase can enhance cognitive function in fear, anxiety and trauma[74,75].

Memory storage in the vertebrate brain uses a complex system of protein and genetic interactions[76]. Here we measured expression of BDNF-TrkB, AVP-AVPRA1, Oxytocin-OTR, CRF-CRFR1, GFAP-NeuN to elucidate potential mechanisms by which acetate influences memory (see Suppl Table 2 for the specific functions of these proteins). Brain-derived neurotrophic factor (BDNF), a ligand for the tyrosine kinase receptor B (TrkB) receptor, distributed throughout the central nervous system, especially the hippocampus and cerebral cortex[77,78,79], was decreased during coprophagy prevention. Acquisition of learning and memory involves increases in BDNF mRNA expression in hippocampus[80]. Rats with BDNF defects showed impairments in hippocampus-dependent learning and memory[81]. Our results indicate that the

acetate may increase neuronal plasticity and the memory of voles by increasing aspects of the BDNF-TrkB signaling pathway.

In our results, supplementation of acetate decreased the CRF and CRF-receptor in hippocampus, may relieve stress and anxiety caused by coprophagy prevention. Van de Wouw et al.[82] show that acute stress-induced hyperthermia and corticosterone levels in chronically stressed mice are ameliorated by SCFA supplementation, where there was a consistent decrease in CRFR1, CRFR2 and MR gene expression across all investigated tissues. In addition, we also found supplementation of acetate increased AVP, OT and their receptors, which is related to the improvement of social cognitive ability[83]. Acetate also stimulates the release of AVP and OT by acting on the central nervous system, and alleviates the memory loss caused by coprophagy prevention. These data suggest that coprophagy-gut-microbiota-SCFAs axis promotes memory enhancement.

Conclusions

The current study suggests that coprophagy prevention causes a significant disturbance to the gut bacterial community, which can impact both energy metabolism and behaviour. To our knowledge this study is among the first to demonstrate the impact of coprophagy on both memory behaviour and gut microbiota in a wild animal. Enhanced memory is extremely important for wild species that live in complex environments and must remember locations of food material and food stores. In birds, variation in spatial memory is associated with survival[84], suggesting a large fitness benefit to maintaining memory function. Therefore, the behavior of coprophagy may ensure the balance of energy metabolism, and memory, but also contribute to an animals' fitness.

Declarations

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Authors' contributions

Conceptualization: XYZ, TBB and DHW; Experiment: TBB, JW and SJT; Writing: TBB, DHW, KDK and XYZ; Supervision and Project Management: DHW and XYZ; Funding Acquisition: DHW and XYZ.

Declaration of interests

The authors declare no competing interests.

Additional file

Document S1 Fig.s S1–S4 and Tables S1–S8.

Data availability

Raw sequence data are deposited in the NCBI Sequence Read Archive under accession PRJNA588029.

References

1. Sommer F, and Backhed F. The gut microbiota—masters of host development and physiology. *Nat. Rev. Microbiol.* 2013; 11: 227-238.
2. Farzi A, Fröhlich EE, Holzer P. Gut Microbiota and the Neuroendocrine System. *Neurotherapeutics.* 2018; 15: 5-22.
3. Heijtz RD, Wang S, Anuar F, et al. Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci.* 2011;108: 3047-3052.
4. Al-Asmakh M, Anuar F, Zadjali F. et al. Gut microbial communities modulating brain development and function. *Gut Microbes.* 2012; 3: 366-373.
5. Karasov WH, Carey, H.V. Metabolic teamwork between gut microbes and host. *Microbe.* 2009; 4: 323–328.
6. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R. Evolution of mammals and their gut microbes. *Science.* 2008; 320: 1647–1651.
7. Haga R. Observation on the ecology of the Japanese pika. *Journal of Mammalogy.* 1960; 41: 200-212.
8. Matsuzawa T, Nakata M. & Tsushima M. Feeding and excretion in the Afghan Pika (*Ochotona rufescens*), a new laboratory animal. *Laboratory Animals.* 1981; 15: 319–322.
9. Hladik CM, Charles-Dominique P, Valdebouze P, Delort-Laval J & Flanzy J. La caecotrophie chez un primate phyllophage du genre *Lepilemur* et des corrélations avec les particularités de son appareil digestif. *Comptes Rendus Hebdomadaires Des Seances de L'Academie Des Sciences, Serie D – Sciences Naturelles.* 1971; 272: 3191–3194.
10. Liu QS, & Wang DH. Effects of diet quality on phenotypic flexibility of organ size and digestive function in Mongolian gerbils (*Meriones unguiculatus*). *Journal of Comparative Physiology B.* 2007; 177: 509-518.

11. Pei YX, Wang DH, Hume ID. Selective digesta retention and coprophagy in Brandt's vole (*Lasiopodomys brandtii*). J Comp Physiol B. 2001; 171: 457–464.
12. Lupien SJ, Fiocco A, Wan N, et al. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology. 2005; 30: 225-242.
13. Fink G, Bicknell RJ. Sex Hormones, Mood, Mental State and Memory. Journal of Neuroendocrinology. 2000; 12: 475-476.
14. Desbonnet L, Clarke G, Traplin A. et al. Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour. Brain Behavior & Immunity. 2015; 48: 165-173.
15. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe. 2015; 17: 565-76.
16. Borovikova LV. et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000; 405: 458-462.
17. Wang X, et al. Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. World J Gastroenterol. 2002; 8: 540-545.
18. Tremaroli V, and Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012; 489: 242–249.
19. Marika Z, Petia KD, Markiewicz LH, Marcus S, Kozak LP, & Fredrik B. Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. Cell Metabolism. 2016; 23: 1216-1223.
20. Bo TB, Zhang XY, Wen J, Deng K, Qin XW, Wang DH. The microbiota–gut–brain interaction in regulating host metabolic adaptation to cold in male Brandt's voles (*Lasiopodomys brandtii*). The journal of ISME. 2019.
21. Zhang DJ, Hendricks DG, Mahoney AW. Effect of coprophagy on bioavailability of iron from plant foods fed to anemic rats. Plant Foods for Human Nutrition. 1992; 42: 97-108.
22. Yao W, Liu W, Deng K, Wang Z, Wang DH, & Zhang XY. GnRH expression and cell proliferation are associated with seasonal breeding and food hoarding in Mongolian gerbils (*Meriones unguiculatus*). Behav. 2019; 112: 42–53.
23. Lieberwirth C, Liu Y, Jia XX, Wang ZX. Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. Horm. Behav. 2012; 62: 357-366.
24. Bo TB, Zhang XY, Wang DH. Effects of cold acclimation on the structure of small intestinal mucosa and mucosal immunity-associated cells in *Lasiopodomys brandtii*. Acta Theriol Sin. 2018; 2: 158-165.
25. Parks DH, Beiko RG. STAMP: Statistical Analysis of Metagenomic Profiles. 2015.
26. Hörnicke H. & Björnhag G. Coprophagy and related strategies of digesta utilization. Digestive Physiology and Metabolism in Ruminants. 1980; 707–730.
27. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, and Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. 2004; 101: 15718-15723.

28. Li H, Qu JP, Li T, Wirth S, Zhang Y, Zhao X, et al. Diet simplification selects for high gut microbial diversity and strong fermenting ability in high-altitude pikas. *Appl Microbiol Biotechnol*. 2018; 102: 6739-6751.
29. Sommer F, Ståhlman M, Ilkayeva O, Arnemo JM, Kindberg J, & Josefsson J, et al. The gut microbiota modulates energy metabolism in the hibernating brown bear *ursus arctos*. *Cell Reports*. 2016; 14: 1655-1661.
30. Zhao M, Cai H, Jiang Z, Yang LI, Zhang H, & Feng F. High fat diet, gut microbiota and metabolic health. *Food Science*. 2018; 39: 336-343.
31. Klaasen HL, Koopman JP, Poelma FGJ, & Beynen AC. Intestinal, segmented, filamentous bacteria. *FEMS Microbiology Reviews*. 1992; 8: 165.
32. Fitzgerald RJ, Gustafsson BE, Mcdaniel EG. Effects of coprophagy prevention on intestinal microflora in rats. *Journal of Nutrition*. 1964; 84: 155.
33. Sagheddu V, Patrone V, Miragoli F, Puglisi E, Morelli L. Infant early gut colonization by Lachnospiraceae: high frequency of *Ruminococcus gnavus*. *Front. Pediatr*. 2016; 4: 57.
34. Kirsti E. Præsteng PB. Pope KO. Cann RI. Mackie Svein D. Mathiesen Lars P, Folkow Vincent GH. Eijsink, Monica A. Sundset. Probiotic Dosing of *Ruminococcus flavefaciens* Affects Rumen Microbiome Structure and Function in Reindeer. *Microbial Ecology*. 2013; 66: 840-849.
35. Tims S, Derom C, Jonkers DM, Vlietinck R, Saris WH, Kleerebezem M, de Vos WM, Zoetendal EG. Microbiota conservation and BMI signatures in adult monozygotic twins. *ISME J*. 2013; 7: 707–717.
36. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT. et al. Human genetics shape the gut microbiome. *Cell*. 2014; 159: 789–99.
37. Verdum FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, Buurman WA, de Vos WM, Rensen SS. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity*. 2013; 21: 607–15 .
38. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014; 505: 559–63.
39. Cree TC, Wadley DM, & Marlett JA. Effect of preventing coprophagy in the rat on neutral detergent fiber digestibility and apparent calcium absorption. *Journal of Nutrition*. 1986; 116: 1204.
40. Sukemori S, Ikeda S, Kurihara Y, & Ito S. Amino acid, mineral and vitamin levels in hydrous faeces obtained from coprophagy-prevented rats. *Journal of Animal Physiology & Animal Nutrition*. 2010; 87: 213-220.
41. Stillings BR, & Hackler LR. Effect of coprophagy on protein utilization in the rat. *Journal of Nutrition*. 1966; 90: 19-24.
42. Cranford JA. and Johnson EO. Effects of coprophagy and diet quality on two microtine rodents (*Microtus pennsylvanicus* and *Microtus pinetorum*). *Journal of Mammalogy*. 1989; 70: 494-502.

43. Dunel-Erb S, Chevalier C, Laurent P, Bach A, Decrock F, & Maho YL. Restoration of the jejunal mucosa in rats refed after prolonged fasting. *Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology*. 2001; 129: 933-947.
44. Lee TM, & Moltz H. The maternal pheromone and brain development in the preweanling rat. *Physiology & Behavior*. 1984; 33: 385–390.
45. Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, Thompson AL, Geng X, Gilmore JH, Knickmeyer RC. Infant Gut Microbiome Associated With Cognitive Development. *Biol Psychiatry*. 2018; 83: 148-159.
46. Luine V, Hearn M. Spatial memory deficits in aged rats: contributions of the cholinergic system assessed by ChAT. *Brain Research*. 1990; 523: 321-324.
47. Bowman RE, Beck KD, Luine VN. Chronic stress effects on memory: sex differences in performance and monoaminergic activity. *Hormones & Behavior*. 2003; 43: 48-59.
48. Deng XH, Yi L, & Chen ZG. Memory-based evolutionary game on small-world network with tunable heterogeneity. *Physica A Statistical Mechanics & Its Applications*. 2010; 389: 5173-5181.
49. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *Journal of Neuroscience the Official Journal of the Society for Neuroscience*. 2000; 20: 9104.
50. Liebsch G, Wotjak CT, Landgraf R, Engelmann M. Septal vasopressin modulates anxiety-related behaviour in rats. *Neuroscience Letters*. 1996; 217: 101-104.
51. Cilz NI, Cymerblit-Sabba A, Young WS. Oxytocin and vasopressin in the rodent hippocampus. *Genes Brain Behav*. 2019; 18: e12535.
52. Hamburger-Bar R, Klein A, Belmaker RH. The effect of chronic vs. acute injection of vasopressin on animal learning and memory. *Peptides*. 1985; 6: 23-25.
53. Bluthé RM, Koob GF, Dantzer R. Hypertonic saline mimics the effects of vasopressin and social recognition in rats. *Behavioural Pharmacology*. 1991; 2: 513-516.
54. Bonus B, URBAN I. et al. Opposite effects of oxytocin and vasopressin on avoidance behavior and hippocampal theta rhythm in the rat. *Neuropharmacology*. 1978; 17: 239-247.
55. Metzger D, Alescio-Lautier B, Bosler O, Devigne C, & Soumireu-Mourat B. Effect of changes in the intrahippocampal vasopressin on memory retrieval and relearning. *Behav Neural Biol*. 1993; 59: 29-48.
56. Dietrich A, Allen JD. Vasopressin and memory: II. Lesions to the hippocampus block the memory enhancing effects of AVP 4–9 in the radial maze. *Behavioural Brain Research*. 1997; 87: 201-8.
57. Yang C, Zhang X, Gao J, Wang M, Yang Z. Arginine vasopressin ameliorates spatial learning impairments in chronic cerebral hypoperfusion via V1a receptor and autophagy signaling partially. *Transl Psychiatry*. 2017; 7: 1174.
58. Popik P, Van Ree JM. Long-term facilitation of social recognition in rats by vasopressin related peptides: a structure-activity study. *Life Sciences*. 1992; 50: 567-572.

59. Lee SY, Park SH, Chung C, Kim JJ, Choi SY, Han JS. Oxytocin Protects Hippocampal Memory and Plasticity from Uncontrollable Stress. *Sci Rep.* 2015; 5: 18540.
60. Strauss GP, Chapman HC, Keller WR, Koenig JI, Gold JM, Carpenter WT, Buchanan RW. Endogenous oxytocin levels are associated with impaired social cognition and neurocognition in schizophrenia. *J Psychiatr Res.* 2019; 112: 38-43.
61. Scott KP, Gratz SW, Sheridan PO, et al. The influence of diet on the gut microbiota. *Pharmacol Res.* 2013; 69: 52–60.
62. Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. A comprehensive and up-to-date review on the metabolites of saccharolytic and proteolytic fermentation in the colon. *J AOAC Int.* 2012; 95: 50–60.
63. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain and behaviour—epigenetic regulation of the gut-brain axis. *Genes Brain Behav.* 2014; 13: 69–86.
64. Luczynski P, Neufeld MV, Oriach CS. et al. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. *International Journal of Neuropsychopharmacology.* 2016; 19: 20.
65. Turnbaugh PJ, Ley RE, Mahowald MA. et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006; 444: 1027-1031.
66. Harada E. Effect of short-chain fatty acids on the secretory response of the ovine exocrine pancreas. *Am. J. Physiol.* 1983; 244: 284-90.
67. Kvietys PR, Granger DN. Effect of volatile fatty acids on blood and oxygen uptake by the dog colon. *Gastroenterology.* 1981; 80: 962-969.
68. Frost G, Sleeth ML, Sahuriarisoylu M. et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications.* 2014; 5: 3611.
69. Braniste V, Alasmakh M, Kowal C. et al. The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine.* 2014; 6: 263.
70. Khan NA, Raine LB, Drollette ES. et al. Dietary fiber is positively associated with cognitive control among prepubertal children. *Journal of Nutrition.* 2015; 145: 143-149.
71. Gundersen BB, Blendy JA. Effects of the histone deacetylase inhibitor sodium butyrate in models of depression and anxiety. *Neuropharmacology.* 2009; 57: 67–74.
72. Valvassori SS, Varela RB, Arent CO, Dal-Pont GC, Bobsin TS, Budni J, Reus GZ, Quevedo J. Sodium butyrate functions as an antidepressant and improves cognition with enhanced neurotrophic expression in models of maternal deprivation and chronic mild stress. *Curr Neurovasc Res.* 2014; 11: 359–366.
73. Wei YB, Melas PA, Wegener G, Mathé AA, Lavebratt C. Antidepressant-like effect of sodium butyrate is associated with an increase in TET1 and in 5-hydroxy-methylation levels in the Bdnf gene. *International Journal of Neuropsychopharmacology.* 2015; 18: pyu032.

74. Whittle N, Singewald N. Brain Disorders Across the Lifespan: Translational Neuroscience from Molecule to Man: HDAC inhibitors as cognitive enhancers in fear, anxiety and trauma therapy: where do we stand? *Biochemical Society Transactions*. 2014; 42: 569-81.
75. Singewald N, Schmuckermair C, Whittle N. et al. Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol Ther*. 2015; 149: 150-190.
76. Nelson TJ, Backlund PS, Alkon DL. Hippocampal protein-protein interactions in spatial memory. *Hippocampus*. 2010; 14: 46.
77. McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. *Annu Rev Neurosci*. 1999; 22: 295–318.
78. Egan MF, Kojima M, Callicott JH. et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*. 2003; 112: 257-269.
79. Heldt SA, Stanek L, Chhatwal JP. et al. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry*. 2007; 12: 656-670.
80. Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *Journal of Neuroscience the Official Journal of the Society for Neuroscience*. 2000; 20: 7116–21.
81. Meier P. Neurotrophins as synaptic modulators. *Nat Rev Neurosci*. 2001; 2: 24–32.
82. Van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O’Sullivan O, Cryan JF. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. *The Journal of Physiology*. 2018; 596: 4923–4944.
83. Song Z, Larkin TE, Malley MO, et al. Oxytocin (OT) and arginine-vasopressin (AVP) act on OT receptors and not AVP V1a receptors to enhance social recognition in adult Syrian hamsters (*Mesocricetus auratus*). *Horm. Behav*. 2016; 81: 20-27.
84. Kowialiewski B, Majerus S. Verbal working memory and linguistic long-term memory: Exploring the lexical cohort effect. *Memory & Cognition*. 2019; 1: 1-15.

Tables

Table 1 The alpha diversity of vole gut microbiota in experiment 1.

Groups	Observed-OTUs	Faith's phylogenetic Diversity	Shannon
0 week	13797.6±135.2	579.8±4.0	10.5±0.05
2 weeks	12461.3±223.7	535.0±8.17	9.9±0.16
4 weeks	13452.4±610.7	564.01±18.6	10.3±0.18
6 weeks	12599.13±158.8	531.3±7.1	10.1±0.08
8 weeks	13211.9±300.7	559.4±11.7	10.2±0.2
F(4,35)	2.878	3.409	2.36
<i>p</i>	0.037	0.019	0.072

Table 2. Repeated measures of abundance in phylum level.

Phylum	<i>F</i>	<i>p</i>	Phylum	<i>F</i>	<i>p</i>
Actinobacteria	0.248	0.908	Firmicutes	9.446	<0.001
Bacteroidetes	9.062	<0.001	Proteobacteria	1.809	0.155
Cyanobacteria	0.213	0.929	Spirochaetes	1.331	0.283
Deferribacteres	0.641	0.638	TM7	2.416	0.072
Verrucomicrobia	2.405	0.073	Tenericutes	1.255	0.311

Table 3. Different OTU between 0 week group and 2 weeks group.

	OUT	Specise	<i>t</i>	<i>p</i>
	New.2126	g_Ruminococcus s_	13.83	0.001
	290253	g_Oscillospira s_	13.27	0.001
	New.823113	g_Helicobacter s_	12.48	0.0011
	581933	g_Oscillospira s_	12.22	0.0012
	New.926860	g_Prevotella s_copri	10.36	0.0026
	234912	g_Clostridium s_	9.17	0.0044
	724472	g_[Ruminococcus] s_gnavus	8.15	0.0069
	New.1334	g_Ruminococcus s_flavefaciens	7.39	0.0098

Figures

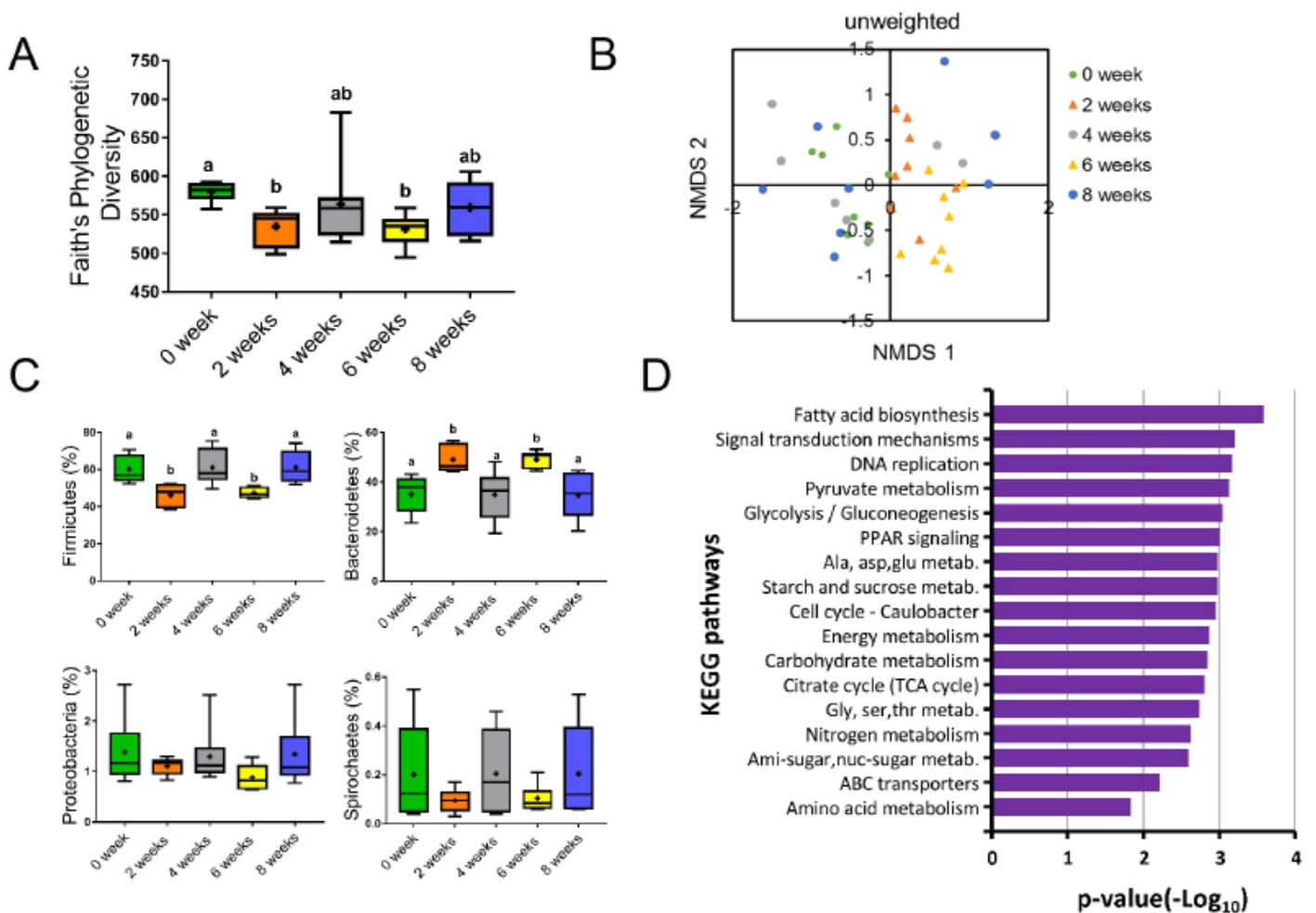


Figure 1

(a) Alpha diversity (Faith's phylogenetic diversity) of bacterial communities across groups (n = 8 per group, one-way ANOVA). (b) NMDS plot based on unweighted UniFrac distance metrics representing the differences in fecal microbial community structure in different groups (ANOSIM). (c) Abundance represented as the proportions of OTUs classified at the phylum rank. (d) The different functional categories were predicted with PICRUST using level 3 of KEGG orthologs. Data are means \pm SEM (n =8 per group). Significant differences in different letter(a,b,c) representations.

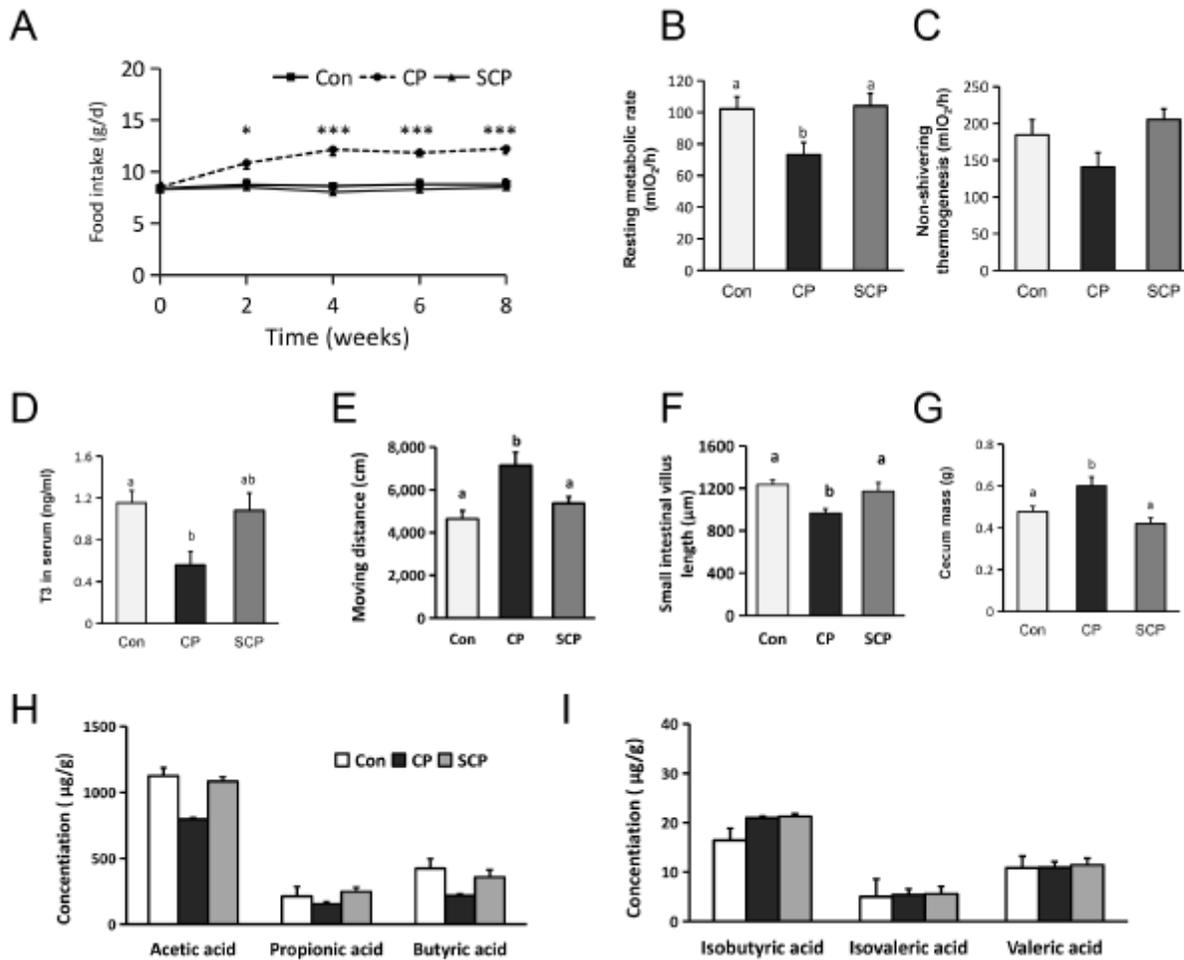


Figure 2

Coprophagy prevention induce alterations in metabolic phenotypes in voles (a)Changes of food intake with time of acclimation (repeated measures ANOVA). (b) RMR in 3 groups. (c) NST in 3 groups. (d)T3 in serum. (e) Moving distance in 3 groups. Digestive tract differences among three groups in small intestinal villus length (f)and cecum mass(g). (h,i)The concentrations of six short-chain fatty acids (SCFAs) in caecal contents. Data are means \pm SEM (n = 6 per group, one-way ANOVA with SNK post hoc tests). Significant differences in different letter(a,b,c) representations.

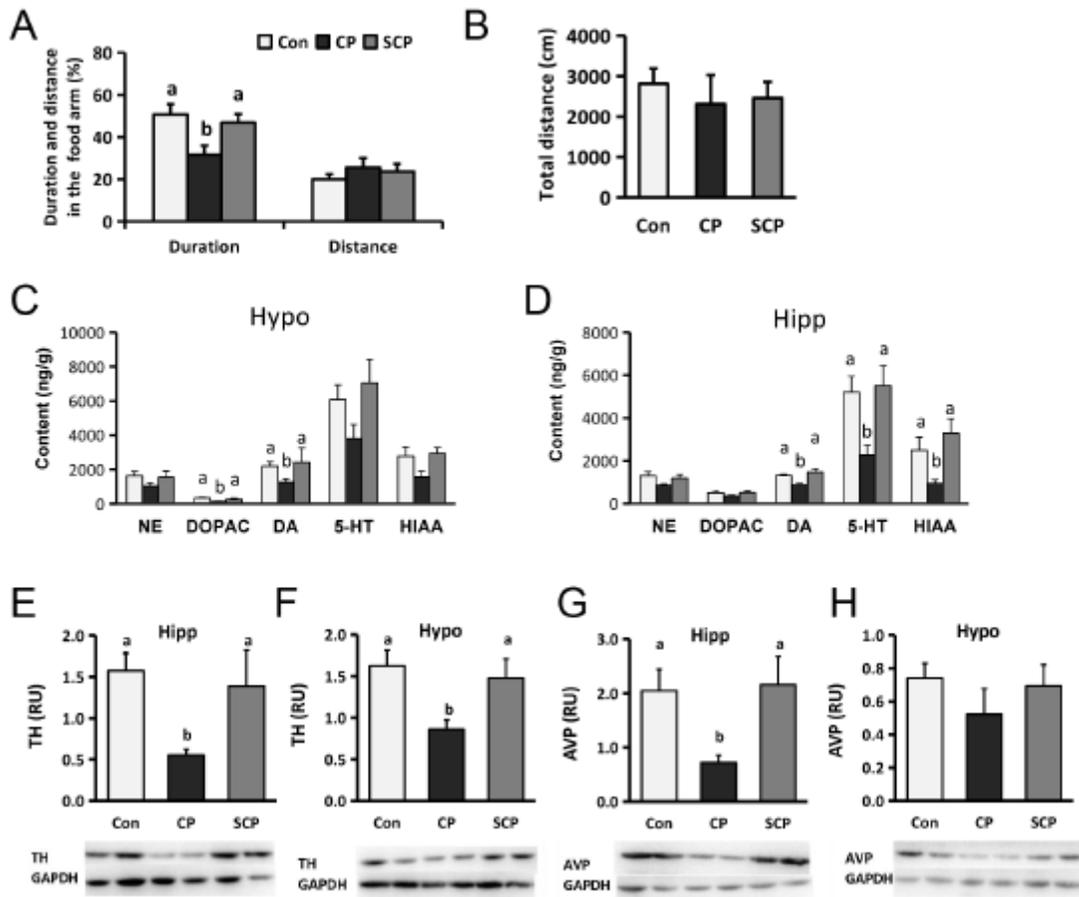


Figure 3

Coprophagy prevention induce alterations in memory and neurodevelopment in voles (a)The duration and distance in the “food arm”. (b)The total moving distance of three groups. The content of neurotransmitters in hypothalamus(c) and hippocampus(d). (e,f)Tyrosine hydrogenase (TH) expression in hypothalamus and hippocampus. (g,h)Arginine vasopressin(AVP) expression in hypothalamus and hippocampus. Data are means \pm SEM (n = 6 per group, one-way ANOVA with S-N-K post hoc tests). Significant differences in different letter(a,b,c) representations.

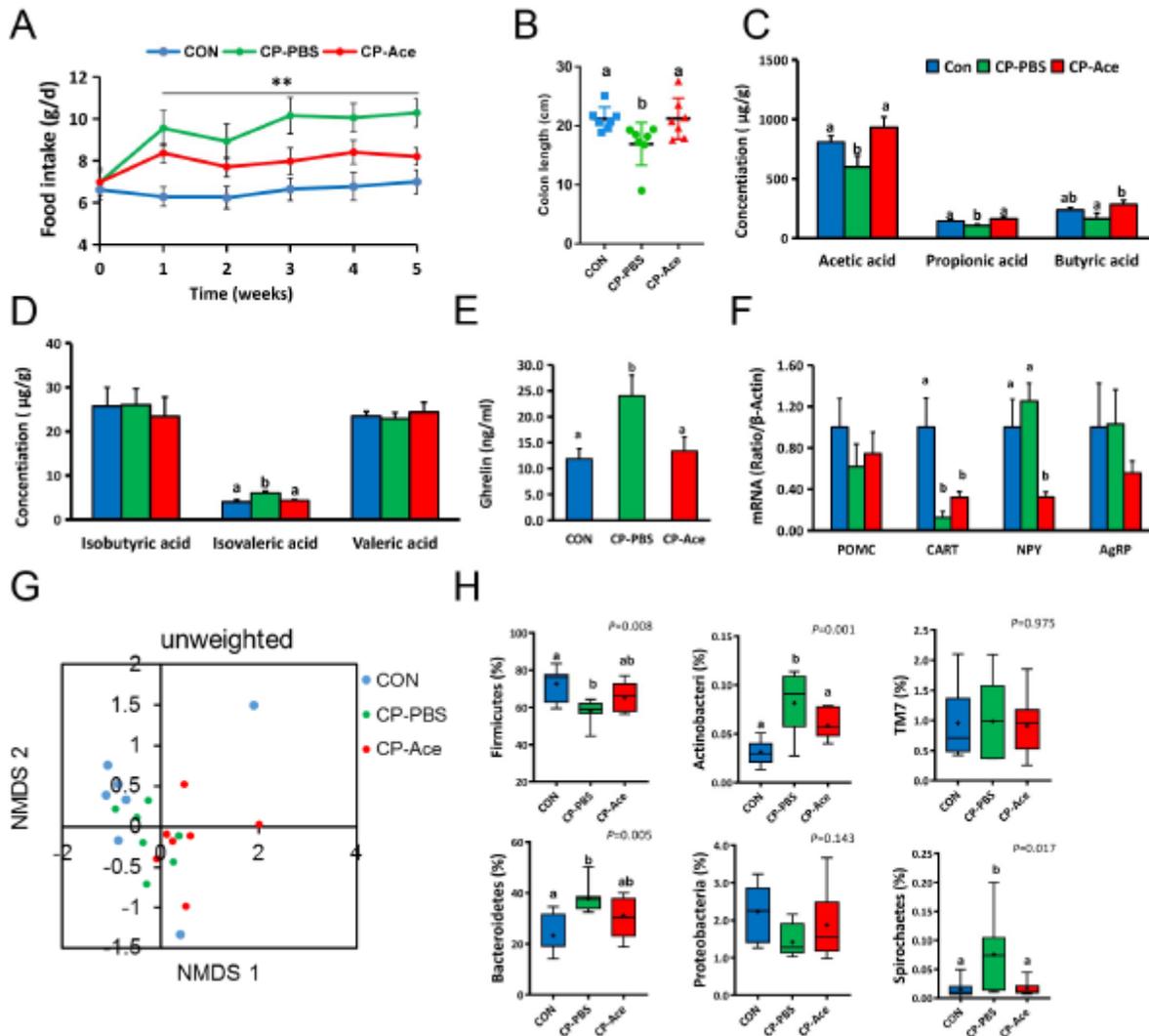


Figure 4

Effects of acetate on metabolism and gut microbiota during coprophagy prevention in voles (a) Changes of food intake with time of acclimation (repeated measures ANOVA). (b) Colon length in 3 groups. (c,d) The concentrations of six short-chain fatty acids (SCFAs) in caecal contents. (e) Ghrelin in serum. (f) Expression of neuropeptides in hypothalamus (POMC, CART, NPY, AgRP). (g) NMDS plot based on unweighted UniFrac distance metrics representing the differences in fecal microbial community structure in different groups (ANOSIM). (h) Abundance represented as the proportions of OTUs classified at the phylum rank. Data are means \pm SEM (n = 7 per group). Significant differences in different letter (a, b, c) representations.

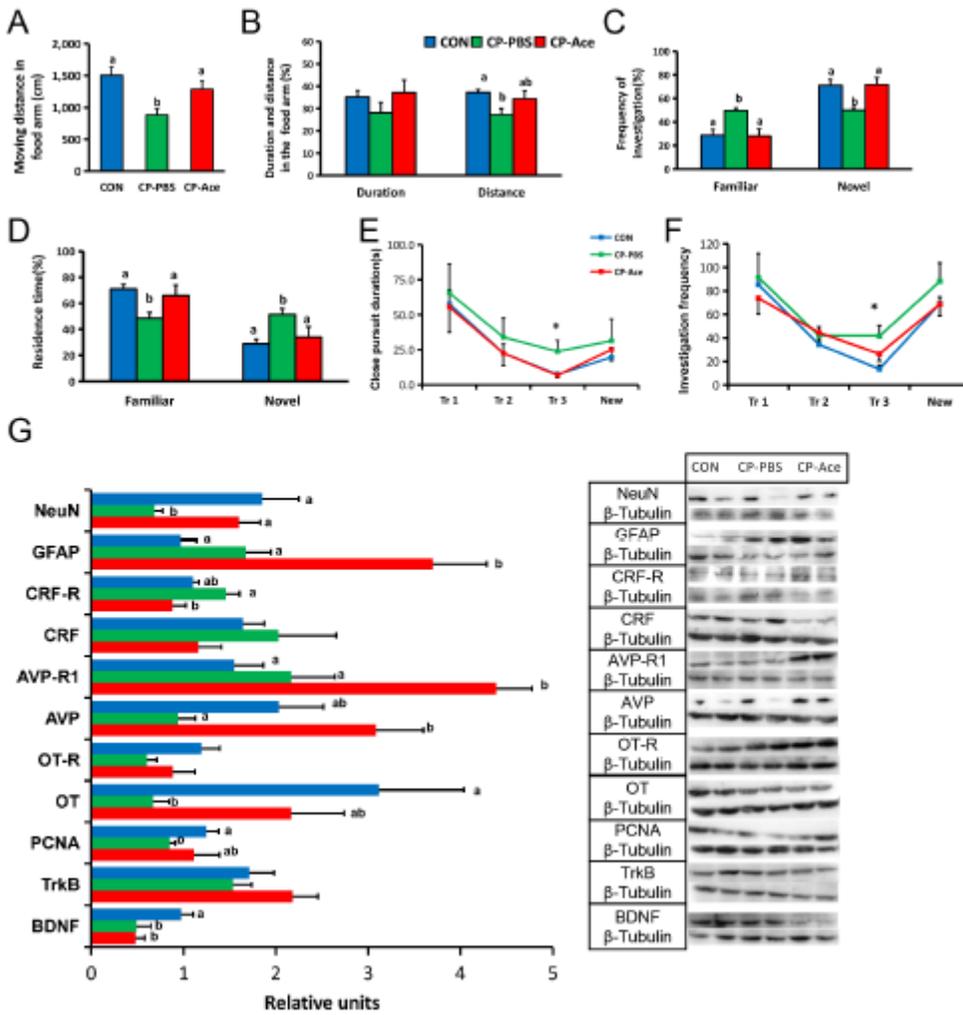


Figure 5

Supplementation of acetate can promote the memory and development of hippocampal neurons during coprophagy prevention in voles (a)The moving distance in the “food arm”. (b) The proportion of duration and distance in the “food arm”. (c) The proportion of frequency of visits to familiar and novel objects. (d)The proportion of residence time in familiar and novel objects. (e) Close pursuit duration and (f) frequency of visits in four times. Data are means \pm SEM (n =7 per group). (g) The content of neurotransmitters in hippocampus. Data are means \pm SEM (n =7 per group). Significant differences in different letter(a,b,c) representations.

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