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ORIGINAL ARTICLE

Cafeteria diet and probiotic therapy: cross talk among memory, neuroplasticity, serotonin receptors and gut microbiota in the rat

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The western diet is known to have detrimental effects on cognition and the gut microbiota, but few studies have investigated how these may be related. Here, we examined whether a probiotic could prevent diet-induced memory deficits. Rats were pre-exposed to vehicle, low or high doses of VSL#3 for 2 weeks before half were switched from chow to a cafeteria diet (Caf) for 25 days; VSL#3 treatment continued until death. High-dose VSL#3 prevented the diet-induced memory deficits on the hippocampal-dependent place task, but the probiotic caused deficits on the perirhinal-dependent object task, irrespective of diet or dose. No differences were observed in anxiety-like behaviour on the elevated plus maze. Gut microbial diversity was dramatically decreased by Caf diet and here, VSL#3 was able to increase the abundance of some taxa contained in the probiotic such as *Streptococcus* and *Lactobacillus* and also other taxa including *Butyrivibrio,* which were decreased by the Caf diet. This affected the predicted profile of microbial metabolic pathways related to antioxidant and bile biosynthesis, and fat and carbohydrate metabolism. In the hippocampus, the Caf diet increased the expression of many genes related to neuroplasticity and serotonin receptor (5HT) 1A, which was normalised in Caf-High rats. Distance-based linear modelling showed that these genes were the best predictors of place memory, and related to microbiota principal component (PC) 1. Neuroplasticity genes in the perirhinal cortex were also affected and related to PC1 but object memory performance was correlated with perirhinal 5HT2C expression and microbiota PC3. These results show that probiotics can be beneficial in situations of gut dysbiosis where memory deficits are evident but may be detrimental in healthy subjects.

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INTRODUCTION

Western style diets high in saturated fat and sugar have been consistently shown to have detrimental effects on the brain and cognitive function. The hippocampus is a crucial brain area for learning and memory and is particularly susceptible to dietary insult with memory deficits evident after less than 1 week of diet.[1,2] Prolonged exposure to these diets promotes obesity and also impairs memory functions dependent on other brain regions including the perirhinal cortex.[3] The cause of these diet-induced memory deficits remains unclear but two commonly proposed mechanisms are elevated levels of pro-inflammatory cytokines[4,5] and decreased neuroplasticity markers.[6,7]

An emerging body of literature suggests that microbiota, specifically within the gut, can also influence cognition via the gut-brain axis. Bidirectional communication occurs between the gastrointestinal tract and the central nervous system via endocrine, immune and neural pathways. This links the emotional and cognitive centres of the brain with the peripheral functioning of the digestive tract.[8–10] Germ-free and antibiotic-induced gut dysbiosis models have demonstrated causal associations between disruption of the gut microbial community and impairments of memory and reduced anxiety-like behaviour.[11–13]

Microbiota obtain energy for growth through the metabolism of dietary nutrients and therefore, diet is a crucial factor which can alter the gut microbial composition.[14–16] Studies on humans[17] and in animal models[17–20] have shown that consuming a western style diet, even acutely, can rapidly alter the composition and metabolic activity of the microbiota. Moreover, we have recently shown that consuming a diet high in saturated fat or sugar, but matched in energy to controls, for only 2 weeks impairs memory and these deficits were associated with macronutrient-specific changes in the microbiota composition.[21] Similar memory deficits have also been reported when healthy mice were transplanted with microbiota from high fat diet fed mice.[22]

Many factors have been suggested to mediate the relationship between gut microbial changes and the observed behavioural deficits. These include increased gut permeability and elevated inflammation,[22–25] reduced synaptic plasticity[13,26] and alterations in neurotransmitter signalling, receptors and associated metabolites.[27] One potential way to correct for diet-induced dysbiosis and in turn, potentially combat these changes is through the use of probiotics. VSL#3 is a commonly use probiotic in humans and has been shown to impact the gut microbiota composition.[28,29] It reduces gut permeability and inflammation in conditions such as colitis[30,31] and has anti-inflammatory effects in peripheral tissue including the liver.[32,33] Moreover, VSL#3 has been shown to restore age-related deficits in long-term potentiation (LTP)[34] and ameliorate sickness behaviour.[35]

In the current study therefore, we investigated whether pre-exposure to VSL#3 would prevent diet-induced memory deficits

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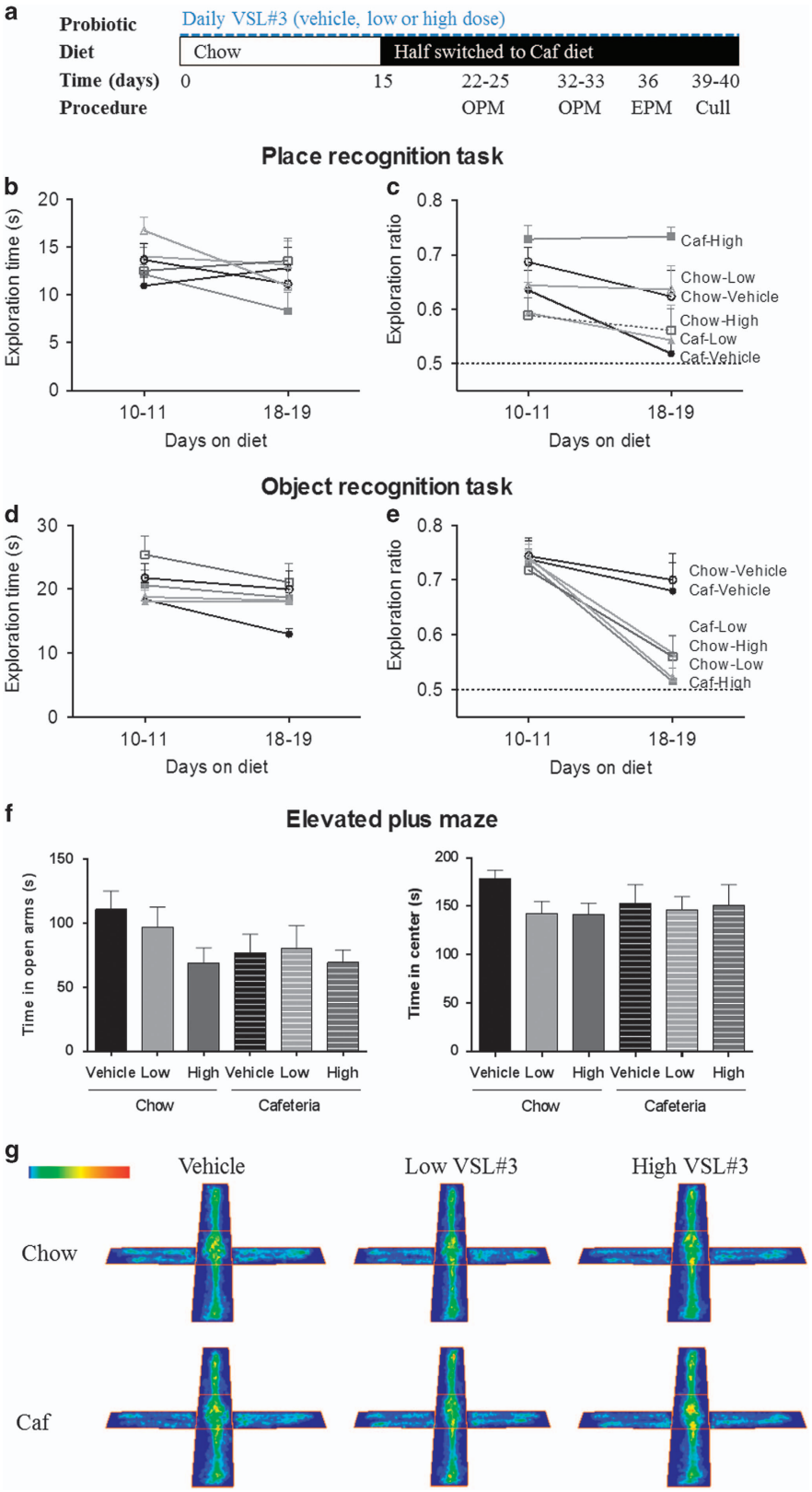
and assessed its effect on anxiety-like behaviours. In addition, we examined if the western style diet and VSL#3 would change the faecal microbiota composition, plasma endotoxin concentrations and numerous brain signalling pathways in the hippocampus and perirhinal cortex including plasticity, inflammation, dopamine, serotonin and glutamate receptors.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Animal Resource Centre, Perth, WA, Australia) were housed in a temperature (18–22 °C) and light (12 h cycle, lights on 0700h) controlled colony room. Rats weighed approximately 200g on arrival and were weight-matched across groups and cages ($n = 10$/group,



2/cage). Sample size was based on previous similar studies.[36] Rats were handled for 10 days prior to the manipulations and were maintained *ad libitum* on standard rat chow (Gordon's Premium Rat and Mouse Breeder diet, NSW, Australia) and tap water. All experimental procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales (Approval #14/45B).

Experimental design

Rats received either a vehicle, low (2.5×10^9 bacteria) or high (2.5×10^{10} bacteria) dose of VSL#3 daily in maple syrup for 2 weeks before half commenced the cafeteria (Caf) diet. VSL#3 treatment was continued during the diet intervention until death. These biologically relevant doses were calculated based on VSL#3 recommended lower and upper intakes (225 to 3 600 billion bacteria) for the dietary management of ulcerative colitis and irritable bowel syndrome. Doses within this range have been shown to colonise the gut and have beneficial effects on memory in aged rodents.[34,37] VSL#3 is a composite probiotic mixture containing multiple strains of 3 viable lyophilised bacteria species: 3 strains of bifidobacteria (*B longum* DSM 24736*, B infantis* DSM 24737, *B breve* DSM 24732), 4 strains of lactobacilli (*L acidophilus* DSM 24735*, L paracasei* DSM 24733*, L bulgaricus* DSM 24734, *L plantarum* DSM 24730), and 1 strain of *Streptococcus salivarius* subspecies *thermophilus* DSM 24731. Six groups were studied according to the diet/probiotic dose; Chow-Vehicle, Chow-Low, Chow-High, Caf-Vehicle, Caf-Low, Caf-High. Caf diet rats had access to 1 water bottle and 1 bottle of 10% sucrose solution, and standard rat chow, which was supplemented with a selection of cakes (for example, chocolate mud cake, jam roll, lamingtons), biscuits (e.g., chocolate chip, monte carlo, scotch fingers) and a protein source (for example, party pie, dim sims, dog roll). Memory was assessed on the perirhinal-dependent object, and hippocampal-dependent place, recognition tasks[38,39] after 22 and 32 days of VSL#3 and anxiety-like behaviour was quantified on elevated plus maze (EPM) on day 36 (Figure 1a; Supplementary Materials and Methods).

Sample collection and analysis

Rats were anaesthetised (xylazine/ketamine 15/100 mg kg^{-1} intraperitoneally) 3–4 days later (39–40 days VSL#3) to minimise carry-over effect of behavioural testing on the inflammation markers.[40] Trunk blood was collected to measure blood glucose (Accu-Chek, Roche Diagnostics, Sydney, NSW, Australia). The remainder of the blood was centrifuged (Microspin 12) and the plasma was separated and stored at − 30 °C for subsequent determination of plasma endotoxin concentration using a commercially available kit (Genscript ToxinSensor Chromogenic LAL Endotoxin Assay Kit; sensitivity range 0.005–1 endotoxin unit (EU) per ml). The white adipose tissue and liver was dissected and weighed and 1 faecal bolus was collected from within the distal colon of each rat. Brains were removed and coronal incisions made at the level of the optic chiasm and the rostral border of the hypothalamus (Bregma − 1.30 to − 4.52 mm). The hippocampus and perirhinal cortex were dissected, snap frozen in liquid nitrogen and stored at − 80 °C for reverse transcription–PCR.

Reverse transcription–PCR

RNA was extracted using Tri-reagent (Sigma-Aldrich, Castle Hill, NSW, Australia), quantity and purity determined (BioSpec-nano, Shimadzu Biotech, Rydalmere, NSW, Australia) and RNA integrity assessed by gel electrophoresis. After DNase I treatment (Invitrogen, Carlsbad, CA, USA), 1 μg of RNA was reverse transcribed to complementary DNA (Omniscript Reverse Transcription kit, Qiagen, Chadstone, VIC, Australia). Gene expression was quantified in the hippocampus using Taqman Array Micro Fluidic Cards (Life Technologies, Carlsbad, CA, USA) and in the hippocampus and perirhinal cortex using Taqman inventoried gene expression assays (Applied Biosystems, Foster City, CA, USA). Genes of interest were normalised against the geometric mean of the most stable housekeeping genes selected by Normfinder software. Analysis of the relative expression was performed using the $\Delta\Delta C_T$ method and was expressed relative to a calibrator sample[41] (Supplementary Materials and Methods).

Statistical analyses

Results are expressed as mean ± s.e.m. Data were excluded if they were two s.d. above or below the group mean. Normality was evaluated using Kolmogorov-Smirnov and if necessary, data were log transformed. Results were analysed using repeat measures analysis of variance (ANOVA; body weight, energy intake, memory) or two-way ANOVA (EPM, death, reverse transcription–PCR) with *post hoc* Tukey and equality of error variance was assessed. Liver scores were analysed using the Kruskal–Wallis test. Data were analysed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) and IBM SPSS Statistics 20 (St Leonards, NSW, Australia).

DNA extraction and microbial community sequencing

DNA extraction was performed using the PowerFecal DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). The composition of the microbial communities was assessed by Illumina amplicon sequencing (2×300 bp MiSeq chemistry, 27F-519R primer pair) of the 16S ribosomal RNA gene. The sequence data were then analysed using MOTHUR,[42] which included alignment with the SILVA database, removal of singletons and doubletons, chimera checking with UCHIME, and classification against the latest RDP training set. Diversity analyses and statistical analyses such as principal component analysis (PCA), non-metric multidimensional scaling (nMDS) plots, CLUSTER with SIMPROF testing, and permutational MANOVA (PERMANOVA) were performed on the reads ($n = 52{,}617 \pm 1617$ total clean reads/sample) using Primer-E[43] and GraphPad Prism 6. Linear Discriminant Analysis Effect Size (LEfSe) analysis and PICRUSt analysis[44] were performed using the Galaxy web application.[45]

RESULTS

Cafeteria fed rats ate more energy, were heavier and had larger livers and fat pads than Chow rats but had comparable blood glucose and plasma LPS concentrations

During the VSL#3 pre-exposure period rats' energy intake and body weight were comparable. When switched to the Caf diet, the Caf rats consumed approximately 2–3 times more energy ($F_{(1,24)} = 377.815$, $P < 0.001$) and were significantly heavier than the Chow rats ($F_{(1,53)} = 17.995$, $P < 0.001$). VSL#3 had no effect on energy intake or body weight (Supplementary Figure 1). At death, the Caf rats were heavier, longer, had wider girths, heavier livers, higher liver scores and more retroperitoneal, gonadal and total fat mass than the Chow rats. There was no effect of VSL#3 on any of these measures. All groups had comparable blood glucose and plasma LPS concentrations (Supplementary Table 1).

VSL#3 prevented diet-induced place deficits but impaired object memory

Exploration times on the place and object task were not affected by diet or VSL#3 treatment. We found a significant (diet*probiotic) interaction for place exploration ratios ($F_{(2,36)} = 8.386$, $P = 0.001$). After 18–19 days of diet, the Caf-High rats performed significantly

Figure 1. Experimental timeline and behavioural assays. (**a**) Rats were pre-exposed to VSL#3 (vehicle, low or high dose) for 2 weeks before half commenced the Caf diet. Rats were tested on the object and place recognition tasks (OPM) after 22 and 32 days of VSL#3, the elevated plus maze (EPM) after 36 days and were killed after 39–40 days. (**b**) All groups had similar exploration times on the place task. (**c**) VSL#3 prevented the diet-induced memory deficits in the Caf-High, but not Caf-Low, rats on the place task. (**d**) All groups had similar exploration times on the object task. (**e**) VSL#3 caused memory deficits on the object task (18–19 days), irrespective of diet or dose. Memory data were analysed by repeated measures ANOVA followed by *post hoc* Tukey ($n = 6$–10 rats/group); Chow-Vehicle = open circle, Chow-Low = open triangle, Chow-High = open square, Caf-Vehicle = closed circle, Caf-Low = closed triangle, Caf-High = closed square. (**f**) All rats spent similar amounts of time on the open arms and in the centre of the maze ($n = 9$–10 per group). (**g**) ANYmaze heat map of average group occupancy plot of the rats' centre position; the blue end of this range indicates less time, the red end indicates more time ($n = 9$–10 per group).

Table 1. The effects of Caf diet and VSL#3 exposure on hippocampal and perirhinal cortex gene expression

	Chow			*Caf*			P-*values*		
Gene	*Vehicle*	*Low*	*High*	*Vehicle*	*Low*	*High*	*Diet*	*Drug*	*Diet* Drug*
Hippocampus									
Neuroplasticity									
Homer1a	0.94 ± 0.08	1.10 ± 0.09	1.06 ± 0.06	1.23 ± 0.15	1.19 ± 0.10	1.48 ± 0.11	0.004	NS	NS
IGF1	0.91 ± 0.07	1.06 ± 0.09	0.94 ± 0.08	1.36 ± 0.16	1.25 ± 0.16	1.28 ± 0.16	0.004	NS	NS
Syn1	1.04 ± 0.11	1.15 ± 0.08	0.93 ± 0.05	1.21 ± 0.15	1.24 ± 0.10	1.45 ± 0.12	0.007	NS	NS
BDNF	0.91 ± 0.07	0.82 ± 0.12	0.88 ± 0.07	1.05 ± 0.14	1.04 ± 0.08	1.18 ± 0.11	0.014	NS	NS
CREB1	1.04 ± 0.11	1.56 ± 0.13	1.32 ± 0.08	1.71 ± 0.26	1.68 ± 0.12	1.37 ± 0.09	0.025	NS	0.076
mTOR	1.03 ± 0.09	1.00 ± 0.08	1.06 ± 0.07	1.07 ± 0.15	1.32 ± 0.09	1.24 ± 0.07	0.03	NS	NS
GAD67	0.75 ± 0.10	0.99 ± 0.07	1.43 ± 0.15	1.36 ± 0.21	1.43 ± 0.21	1.12 ± 0.13	0.068	NS	0.015
DCX	1.01 ± 0.06	1.35 ± 0.14	1.26 ± 0.12	1.40 ± 0.20	1.35 ± 0.12	1.61 ± 0.24	0.07	NS	NS
NGF	1.02 ± 0.08	0.90 ± 0.08	1.14 ± 0.11^	1.09 ± 0.12	1.06 ± 0.10	1.35 ± 0.14^	NS	0.048	NS
FGF2	0.91 ± 0.12	0.53 ± 0.04	0.86 ± 0.07	0.69 ± 0.07	0.76 ± 0.09	0.70 ± 0.04	NS	0.093	0.009
TrkB	1.05 ± 0.12	0.86 ± 0.08	0.80 ± 0.03	0.95 ± 0.12	0.85 ± 0.12	1.15 ± 0.07	NS	NS	0.062
Pvalb	1.02 ± 0.07	1.27 ± 0.17	1.06 ± 0.04	1.23 ± 0.12	1.31 ± 0.15	1.22 ± 0.11	NS	NS	NS
Reln	1.03 ± 0.09	1.23 ± 0.11	1.12 ± 0.05	1.05 ± 0.09	1.15 ± 0.09	1.08 ± 0.06	NS	NS	NS
Inflammation									
IKBKB#	1.03 ± 0.04	1.11 ± 0.03	1.04 ± 0.06	1.01 ± 0.03	1.00 ± 0.03	0.96 ± 0.04	0.027	NS	NS
GFAP	1.04 ± 0.11	1.24 ± 0.09	1.07 ± 0.04	1.38 ± 0.18	1.31 ± 0.13	1.24 ± 0.06	0.049	NS	NS
Ptgs2	1.01 ± 0.05	1.06 ± 0.17	1.23 ± 0.14	1.31 ± 0.19	1.29 ± 0.11	1.39 ± 0.13	0.052	NS	NS
IL-1β#	1.14 ± 0.15	1.20 ± 0.14	0.85 ± 0.13	0.79 ± 0.10	1.02 ± 0.12	0.84 ± 0.09	0.074	NS	NS
Ager	0.48 ± 0.06	0.60 ± 0.09	0.59 ± 0.09	0.49 ± 0.04	1.23 ± 0.35	0.78 ± 0.16	NS	NS	NS
Cd68	1.09 ± 0.15	1.27 ± 0.18	0.89 ± 0.07	0.85 ± 0.13	0.90 ± 0.10	1.02 ± 0.20	NS	NS	NS
Myd-88	1.03 ± 0.08	1.20 ± 0.10	0.99 ± 0.06	1.20 ± 0.09	1.12 ± 0.06	1.11 ± 0.07	NS	NS	NS
SOCS-3#	1.01 ± 0.07	1.14 ± 0.08	0.99 ± 0.06	0.98 ± 0.10	0.98 ± 0.08	0.93 ± 0.06	NS	NS	NS
IL-15#	0.94 ± 0.06	1.20 ± 0.14	0.98 ± 0.05	1.01 ± 0.09	1.04 ± 0.06	1.08 ± 0.08	NS	NS	NS
TNF-α#	1.04 ± 0.09	1.00 ± 0.09	0.90 ± 0.04	0.97 ± 0.07	0.96 ± 0.03	0.97 ± 0.05	NS	NS	NS
Dopamine receptors									
Drd2	1.02 ± 0.11^	5.97 ± 1.51	1.34 ± 0.22^	1.48 ± 0.21^	2.09 ± 0.37	2.54 ± 0.54^	NS	0.001	0.003
Drd1	0.84 ± 0.05^	2.60 ± 0.60	1.10 ± 0.15^	1.07 ± 0.12^	1.30 ± 0.19	1.22 ± 0.21^	NS	0.004	0.024
Serotonin receptors									
5HT2c	1.97 ± 0.66	1.75 ± 0.46	1.23 ± 0.34	1.02 ± 0.25	1.05 ± 0.28	0.74 ± 0.24	0.037	NS	NS
5HT1A	1.01 ± 0.06	0.99 ± 0.09	1.05 ± 0.09	1.25 ± 0.17	1.31 ± 0.13	1.07 ± 0.08	0.037	NS	NS
Glutamate receptors									
mGLUR5	1.03 ± 0.09	1.21 ± 0.05	1.06 ± 0.06	1.34 ± 0.20	1.52 ± 0.13	1.43 ± 0.11	0.002	NS	NS
Grin2b	1.06 ± 0.11	1.14 ± 0.11	1.29 ± 0.07	1.29 ± 0.20	1.48 ± 0.15	1.30 ± 0.13	0.089	NS	NS
Grin2a	1.04 ± 0.10	1.16 ± 0.08	1.10 ± 0.05	1.10 ± 0.15	1.24 ± 0.11	1.27 ± 0.08	NS	NS	NS
mGLUR1a	0.76 ± 0.09	1.38 ± 0.21	1.78 ± 0.46	1.20 ± 0.26	1.82 ± 0.22	1.35 ± 0.28	NS	NS	NS
Oxidative stress									
NOS2	1.18 ± 0.22	1.85 ± 0.44	0.75 ± 0.17	0.67 ± 0.23	1.19 ± 0.30	0.88 ± 0.22	NS	0.035	NS
SOD1	1.02 ± 0.08	1.24 ± 0.07	1.16 ± 0.04	1.18 ± 0.10	1.09 ± 0.09	1.19 ± 0.09	NS	NS	NS
MAP kinases									
MAPK8	1.01 ± 0.06	1.10 ± 0.05	1.12 ± 0.06	1.31 ± 0.12	1.40 ± 0.07	1.21 ± 0.09	0.001	NS	NS
MAPK10	1.02 ± 0.08	1.14 ± 0.07	1.15 ± 0.11	1.32 ± 0.15	1.28 ± 0.07	1.28 ± 0.09	0.025	NS	NS
Signal transducer and activator of transcription									
STAT1	1.05 ± 0.11	1.44 ± 0.10	1.09 ± 0.07	1.21 ± 0.11	1.17 ± 0.08	1.04 ± 0.06	NS	0.044	0.079
STAT3	1.03 ± 0.09	1.30 ± 0.04	1.17 ± 0.07	1.32 ± 0.12	1.30 ± 0.11	1.16 ± 0.07	NS	NS	NS
Glucocorticoid receptor									
Nr3c1	1.01 ± 0.06	1.17 ± 0.05	1.01 ± 0.04	1.18 ± 0.12	1.31 ± 0.07	1.30 ± 0.12	0.007	NS	NS
Insulin and glucose regulation									
GLUT3	1.03 ± 0.09	1.02 ± 0.07	0.95 ± 0.06	1.36 ± 0.16	1.29 ± 0.08	1.29 ± 0.08	< 0.001	NS	NS
INSR	1.03 ± 0.08^	1.40 ± 0.10	1.10 ± 0.06	1.25 ± 0.14^	1.42 ± 0.09	1.27 ± 0.13	NS	0.027	NS
Neurod1	0.90 ± 0.06	1.14 ± 0.17	0.78 ± 0.05	1.07 ± 0.12	0.93 ± 0.10	1.00 ± 0.07	NS	NS	NS
AKT kinases									
Akt1	1.04 ± 0.10	1.16 ± 0.05	1.10 ± 0.04	1.33 ± 0.13	1.17 ± 0.08	1.13 ± 0.07	NS	NS	NS
Akt3	1.04 ± 0.10	1.13 ± 0.10	1.06 ± 0.03	1.10 ± 0.15	1.08 ± 0.10	1.07 ± 0.04	NS	NS	NS
Others									
C-Jun	1.01 ± 0.06	1.14 ± 0.07	0.96 ± 0.04	1.33 ± 0.09	1.24 ± 0.12	1.40 ± 0.12	< 0.001	NS	NS

Table 1. (Continued)

	Chow			*Caf*			P-*values*		
Gene	*Vehicle*	*Low*	*High*	*Vehicle*	*Low*	*High*	*Diet*	*Drug*	*Diet* Drug*
JAK2	1.03 ± 0.09	1.18 ± 0.12	1.11 ± 0.07	1.23 ± 0.15	1.37 ± 0.04	1.49 ± 0.12	0.006	NS	NS
NOD2	1.06 ± 0.14	1.29 ± 0.16	1.13 ± 0.14	1.42 ± 0.19	1.64 ± 0.19	1.43 ± 0.19	0.022	NS	NS
NCAM1	1.03 ± 0.08	1.31 ± 0.11	1.25 ± 0.09	1.39 ± 0.16	1.34 ± 0.12	1.35 ± 0.12	0.095	NS	NS
MMP-9	0.85 ± 0.25	1.01 ± 0.29	1.02 ± 0.17	1.27 ± 0.35	1.18 ± 0.33	0.82 ± 0.22	NS	NS	NS
Perirhinal cortex									
Neuroplasticity									
BDNF#	0.88 ± 0.03	0.98 ± 0.04	0.88 ± 0.04	1.03 ± 0.05	1.07 ± 0.04	1.04 ± 0.04	< 0.001	NS	NS
CREB1#	0.85 ± 0.06	0.88 ± 0.03	0.92 ± 0.04	0.87 ± 0.03	0.95 ± 0.04	0.94 ± 0.03	NS	NS	NS
Syn1#	1.01 ± 0.07	1.05 ± 0.05	1.03 ± 0.04	1.00 ± 0.04	1.07 ± 0.02	1.08 ± 0.03	NS	NS	NS
GAD67#	0.89 ± 0.07	0.90 ± 0.03	0.94 ± 0.07	0.96 ± 0.06	0.92 ± 0.04	0.86 ± 0.05	NS	NS	NS
Inflammation									
MCP-1#	1.25 ± 0.16	1.06 ± 0.12	1.09 ± 0.10	0.97 ± 0.14	1.48 ± 0.19	0.97 ± 0.08	0.042	NS	NS
TNF-α#	1.03 ± 0.13	1.10 ± 0.14	0.73 ± 0.06	0.88 ± 0.08	1.24 ± 0.14	1.09 ± 0.10	NS	NS	NS
IL-1β#	1.13 ± 0.13	1.08 ± 0.14	1.01 ± 0.10	0.81 ± 0.09	0.99 ± 0.11	0.92 ± 0.09	NS	NS	NS
Dopamine receptors									
DRD1#	0.75 ± 0.10	0.91 ± 0.14	1.16 ± 0.16	0.98 ± 0.11	0.75 ± 0.08	0.76 ± 0.07	NS	NS	0.02
DRD2#	0.82 ± 0.14	0.91 ± 0.16	1.02 ± 0.20	0.95 ± 0.15	0.93 ± 0.14	0.85 ± 0.10	NS	NS	NS
Serotonin receptors									
5HT1A#	1.00 ± 0.06	1.12 ± 0.08	0.98 ± 0.06	0.90 ± 0.06	1.03 ± 0.04	0.98 ± 0.04	NS	NS	NS
5HT2C#	0.90 ± 0.07	0.99 ± 0.07	0.90 ± 0.11	0.95 ± 0.09	0.89 ± 0.02	0.82 ± 0.05	NS	NS	NS
Glutamate receptors									
Grin2b#	0.88 ± 0.06	0.95 ± 0.05	0.91 ± 0.04	0.87 ± 0.03	1.03 ± 0.03	0.98 ± 0.05	NS	NS	< 0.001
mGLUR5#	0.90 ± 0.06	0.87 ± 0.04	0.93 ± 0.05	0.90 ± 0.04	0.95 ± 0.03	0.91 ± 0.04	NS	NS	NS

Abbreviation: NS, not significant. In the hippocampus, mRNA expression was measured using the microfluidic card platform (Life Technologies) and some inflammatory genes were quantified using Taqman gene assays (Applied Biosystems); respectively, $n = 6$–8 and $n = 8$–10 rats per group. In the perirhinal cortex, all genes were quantified using Taqman gene assays (Applied Biosystems), $n = 8$–10 rats per group. Taqman gene assays are marked#. Results are expressed as mean ± s.e.m. Data were analysed by two-way ANOVA followed by *post hoc* Tukey, ^$P < 0.05$ versus low dose VSL#3. Interaction simple main effects are discussed in-text.

better than the Caf-Vehicle ($P = 0.025$) and Caf-Low ($P = 0.045$) rats. Object memory ratios were significantly affected by VSL#3 ($F_{(2,42)} = 12.872$, $P < 0.001$) such that VSL#3 treated rats had lower exploration ratios than Vehicle rats irrespective of diet or dose (Ps < 0.001; Figures 1b–e).

No effect of diet or VSL#3 on anxiety-like behaviour on the EPM

All groups performed comparably on the EPM in respect to the number of entries, total time spent, mean visit time, time immobile, time spent stretching, dipping, rearing or grooming in the centre space and open and closed arms of the EPM (Figure 1f).

Cafeteria diet altered gene expression in the hippocampus and perirhinal cortex

As detailed below, genes serving multiple functions were modified by the Caf diet (15 upregulated, 2 downregulated), VSL#3 affected 4 genes and 4 genes showed significant (diet*probiotic) interactions (Table 1). Neuroplasticity: The most consistent finding was that the Caf diet elevated genes related to neuroplasticity including CREB1, Syn1, BDNF, Homer1, mTOR, IGF1 and there was a trend for elevated DCX. VSL#3 affected NGF expression with increased expression in the high versus low dose group ($P = 0.041$). There were significant interactions for FGF2 and GAD67. Pvalb, Reln or TrkB were not affected.

Inflammation. Some inflammation genes were increased in the Caf rats (GFAP, trend for Ptgs2) and others were decreased (IKBKB, trend for interleukin-1β). Ager, CD68, Myd-88, SOCS-3, interleukin-15 and tumour necrosis factor-α were not affected.

Dopamine receptors. There were significant interactions for DRD1 and DRD2 with both elevated in the Chow-Low group compared to Caf-Low group ($P < 0.01$).

Serotonin receptors. Caf diet decreased 5HT2C and increased 5HT1A expression.

Glutamate receptors. Caf diet upregulated mGLUR5 and there was a trend for increased Grin2b. There were no differences in mGLUR1a or Grin2a. Oxidative stress: VSL#3 affected NOS2 expression with higher levels in the low than high-dose groups ($P = 0.034$). There were no differences in SOD1.

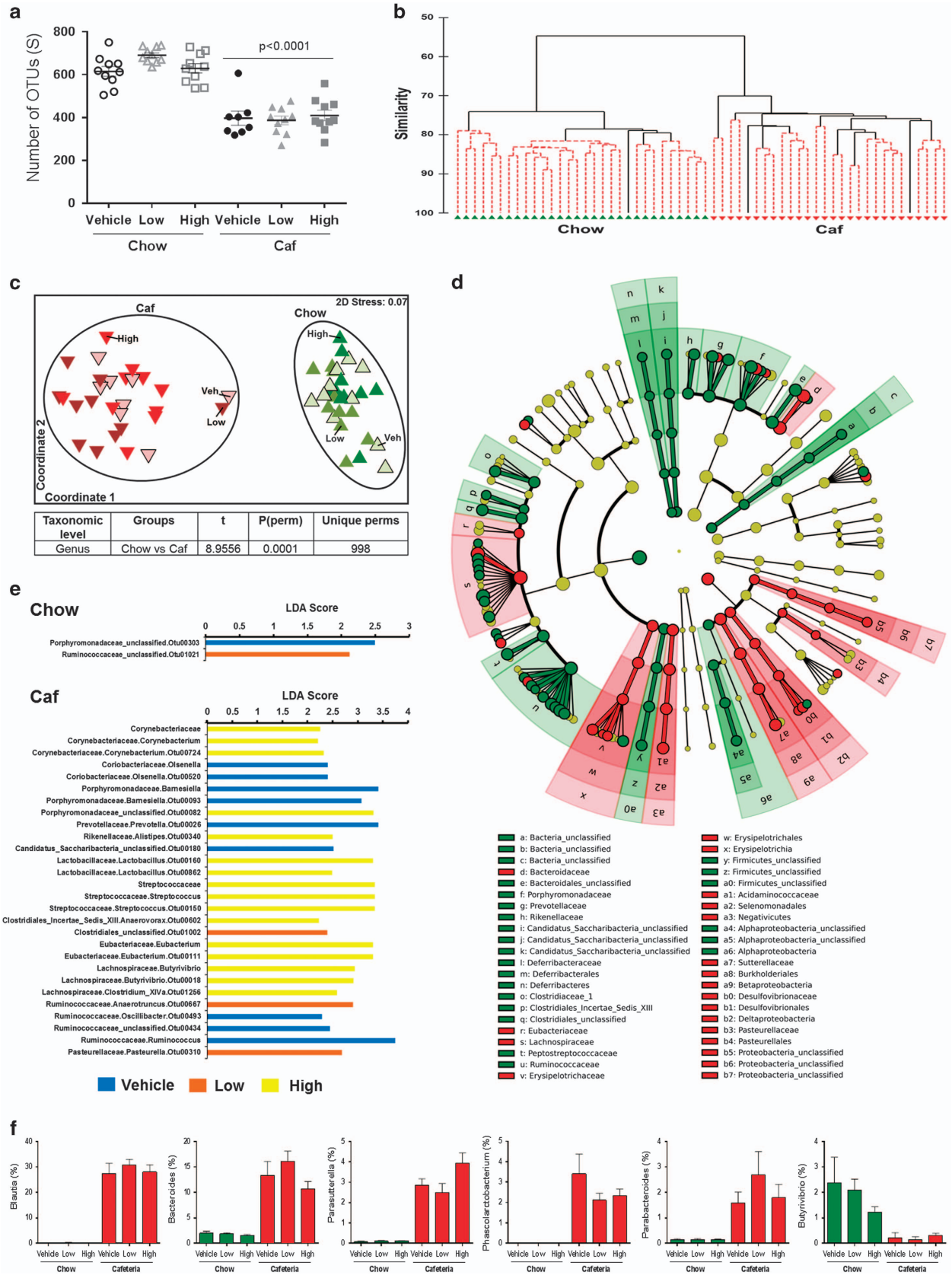
MAP kinases. Caf diet significantly upregulated MAPK8 and MAPK10.

Signal transducer and activator of transcription. VSL#3 affected STAT1 expression with higher levels in the low than high-dose groups ($P = 0.044$). There was no effect on STAT3.

Glucocorticoid receptor. The glucocorticoid receptor Nr3c1 was significantly upregulated by the Caf diet.

Insulin and glucose regulation. Caf diet significantly increased the expression of GLUT3. VSL#3 affected INSR expression with increased levels in the low dose versus vehicle groups ($P = 0.031$). Neurod1 was not affected.

AKT kinase. Akt1 and 3 were comparable between groups. Others: Caf diet increased the expression of C-Jun, JAK2 and NOD2. NCAM1 and MMP-9 were comparable between groups.



Taxonomic level	Groups	t	P(perm)	Unique perms
Genus	Chow vs Caf	8.9556	0.0001	998

In the perirhinal cortex, we measured neuroplasticity and inflammation related genes and dopamine, serotonin and glutamate receptors (Table 1).

Neuroplasticity. BDNF expression was significantly increased by the Caf diet, with no effect on CREB1, Syn1 and GAD67.

Inflammation. There was a significant interaction for MCP-1 with expression higher in the Caf-Low than Chow-Low group ($P = 0.042$). Tumour necrosis factor-α and interleukin-1β were not affected.

Dopamine receptors. DRD1 had a significant interaction with expression higher in the Chow-High than the Caf-High group ($P = 0.013$). DRD2 was not affected.

Serotonin receptors. There was no effect of diet or VSL#3 on 5HT1A or 5HT2C.

Glutamate receptors. Grin2b had a significant interaction with expression higher in the Chow-High than the Caf-High rats ($P < 0.001$). mGLUR5 expression was not affected.

Hippocampal and perirhinal gene expression correlated with memory

We examined whether there were any associations between hippocampal gene expression and hippocampal-dependent place memory. We found a negative correlation between place recognition memory and hippocampal 5HT1A ($r = -0.35$, $P = 0.029$) and GAD67 ($r = -0.34$, $P = 0.043$) mRNA expression. The DistLM analysis showed that hippocampal Pvalb best predicted place recognition memory (Pseudo-$F = 6.36$, $P = 0.018$) and the best predictive cluster of hippocampal genes were Pvalb, DCX, TrkB and Igf1 (AIC = -117.24, $R^2 = 0.51$). Of note, 5HT1A expression positively correlated with GAD67 ($r = 0.49$, $P = 0.0006$), Pvalb ($r = 0.43$, $P = 0.003$), DCX ($r = 0.52$, $P = 0.0002$) and TrkB ($r = 0.32$, $P = 0.02$). We also examined the relationship between perirhinal gene expression and perirhinal-dependent object memory and found that 5HT2C was positively correlated with object memory ($r = 0.35$, $P = 0.018$). The DistLM analysis showed that 5HT2C was the only gene to show a relationship with object memory (Pseudo-$F = 4.72$, $P = 0.03$).

Cafeteria diet led to dramatic changes in the gut microbiota

The Caf diet significantly reduced microbial diversity compared to Chow, as evidenced by a decrease in the number of OTUs by up to 40% ($F_{(1,52)} = 180.315$, $P < 0.0001$) and decreased Shannon's diversity ($F_{(1,52)} = 227.565$, $P < 0.0001$). VSL#3 had no effect on these (Figure 2a; Supplementary File 1, Sheet: 1). A similar significant drop in number of OTUs and other alpha-diversity measures were observed following rarefaction of all samples down to 24917 reads. (Supplementary File, Sheet: 2). Rats consuming Caf and Chow diets had significantly different microbiota across all taxonomic levels (Phylum: $t = 6.17$, $P = 0.001$, Class: $t = 6.52$, $P = 0.001$, Order: $t = 6.41$, $P = 0.001$, Family: $t = 7.58$, $P = 0.001$, Genus: $t = 8.96$, $P = 0.001$, OTU: $t = 6.93$, $P = 0.001$) and this was confirmed by pairwise PERMANOVAs. The differentiation was further established using PCA, nMDS and CLUSTER analyses which revealed distinct clustering of the diet groups (Figures 2b and c; Supplementary File 1, Sheets: 3–4).

In order to identify microbial taxa significantly contributing to differences between the groups, we determined their effect size using a linear discriminant analysis (LDA score > 2, $P < 0.05$). Diet significantly affected 531 taxa across all taxonomic levels. In the Caf rats 137 taxa increased in abundance and 394 taxa decreased (Figure 2d; Supplementary File 1, Sheet: 5). The main bacterial taxa that increased were *Blautia, Bacteroides, Phascolarctobacterium, Parasutterella* and *Erysipelotrichaceae* while *Butyrivibrio* decreased (Figure 2f).

Overall, the probiotic treatment increased the abundance of 2 taxa in the high-dose groups. When we examined Chow and Caf separately we respectively found that 2 and 28 taxa were affected by VSL#3 and the majority of changes were evident in the Caf-High group (16/28 taxa) (Figure 2e; Supplementary File 1, Sheets: 6–7). The Caf-High group had increased abundance of taxa present in the probiotic such as *Streptococcaceae* and *Lactobacillus*, as well as other taxa including *Butyrivibrio* which were reduced by the Caf diet. Further effects of the probiotics on the microbiota can be seen in the two-way LEfSe analysis (Supplementary file 1, Sheets 8–11) between each probiotic dose and vehicle rats across both diet types.

The gut microbial profile significantly correlated with object memory

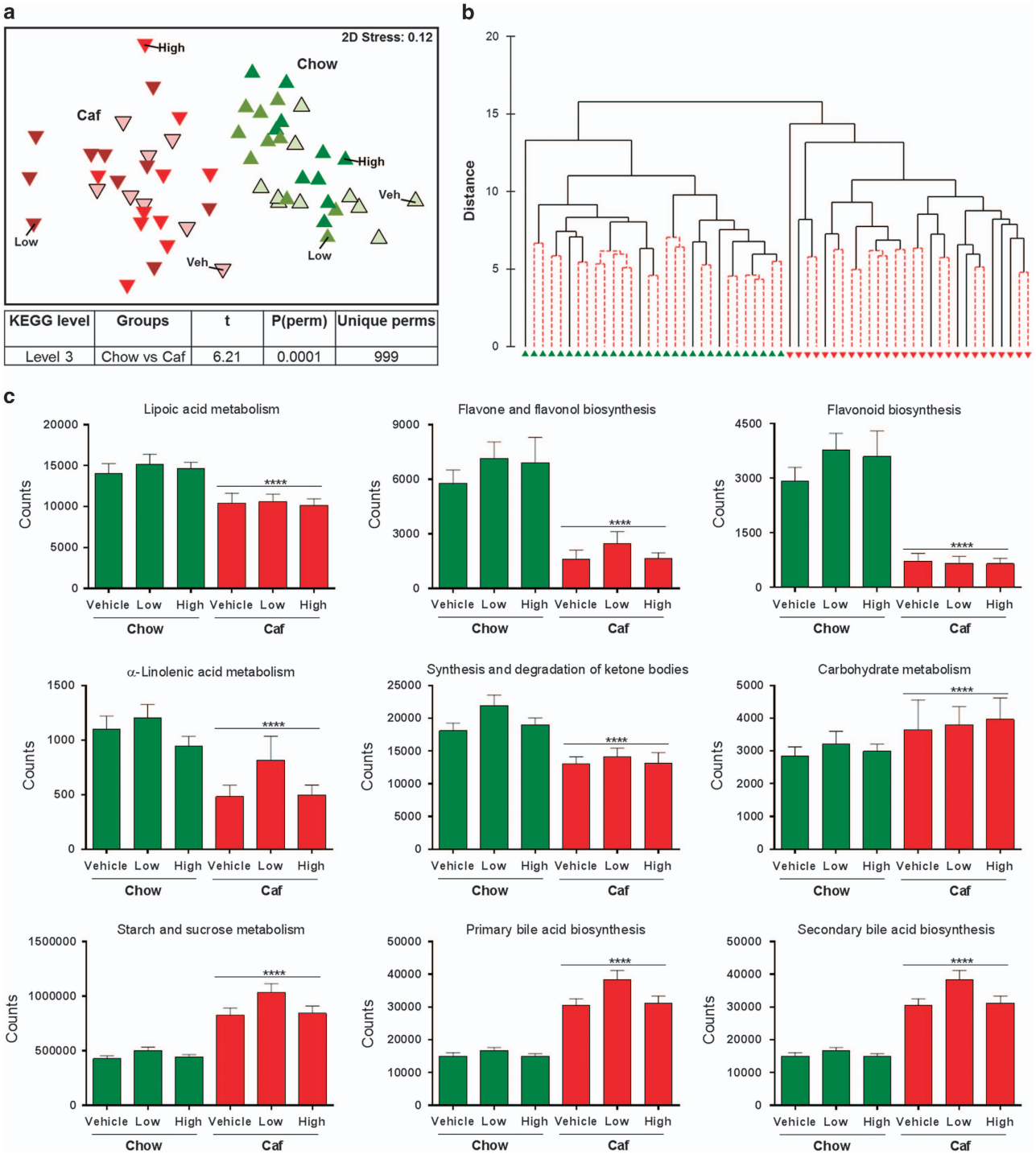
We performed a DistLM analysis between the PC scores and memory exploration ratios to determine whether the microbial profiles were related to memory performance. PC3 was significantly related to object memory (Genus $P = 0.001$, Family $P = 0.001$, trend at Order $P = 0.108$ and Class $P = 0.113$) and there were no relationships between the PC scores and place memory (Supplementary File 1, Sheet 12).

We then explored the relationship between microbiota taxa at the genus level and memory using DistLM (Supplementary File 1, Sheets: 13-14). This revealed a number of taxa related to object recognition memory including *Clostridium XI* (Pseudo-$F = 6.81$, $P = 0.013$), *Clostridium sensu stricto* (Pseudo-$F = 5.18$, $P = 0.029$) and *Turicibacter* (Pseudo-$F = 8.52$, $P = 0.003$) and these significantly contributed to PC3. Place recognition memory was significantly related to *Bacteroidetes unclassified* (Pseudo-$F = 5.62$, $P = 0.017$) and showed a trend with *Streptococcus* (Pseudo-$F = 3.71$, $P = 0.068$) and *Parabacteroides* (Pseudo-$F = 2.87$, $P = 0.096$).

The gut microbial profile strongly correlates with expression of neuroplasticity genes

We examined the relationship between the microbiota PC scores and categories of hippocampal and perirhinal genes outlined in Table 1. The DistLM analysis highlighted that PC1 was significantly related to the neuroplasticity genes in the perirhinal cortex and

Figure 2. Caf diet strongly alters the gut microbiota of rats. (**a**) The number of OTUs significantly decreased in Caf fed rats as compared to those fed chow. Similar results were observed for Shannon's diversity and phylogenetic diversity. (**b**) Cluster analysis with SIMPROF testing following square-root transformation and Bray-Curtis resemblance demonstrated that rats are distributed into two clusters based on the diet they consumed. Black lines indicate significant differentiation while red dotted lines indicate samples cannot be significantly differentiated. (**c**) Non-metric multidimensional scaling plot and PERMANOVA following square-root transformation and Bray-Curtis resemblance confirms that rats are differentiated into two clusters based on diet. (**d**) Circular representation of microbial taxa identified to be significantly different in abundance across diets using LEfSe analysis. The plot shows microbial taxa from phylum to genus level, with taxa significantly enriched (LDA score > 2.0, $P < 0.05$) in Chow rats shown in green and those in Caf rats shown in red. Abbreviations of microbial names are listed below the graph. (**e**) Microbial taxa identified to be significantly different in abundance (LDA score > 2.0, $P < 0.05$) across probiotic groups using LEfSe analysis. The analysis was performed for Chow and Caf rats separately. (**f**) Relative abundance (%) of six bacterial genera identified to be important in the LEfSe analysis.



KEGG level	Groups	t	P(perm)	Unique perms
Level 3	Chow vs Caf	6.21	0.0001	999

Figure 3. Caf diet strongly affected predicted bacterial metabolic contributions. (**a**) Non-metric multidimensional scaling plot and PERMANOVA following log transformation and Euclidean distance resemblance of PICRUSt predictions (KEGG Level 3) demonstrated that the total predicted metabolic contributions are distributed into two clusters based on the diet the rats consumed. (**b**) Cluster analysis with SIMPROF testing following log transformation and Euclidean distance resemblance of PICRUSt predictions (KEGG Level 3) confirmed the presence of two clusters related to diet. (**c**) Predicted counts of metabolic pathways of interest in the six diet-probiotic groups.

hippocampus and the serotonin receptors in the hippocampus at numerous taxonomic levels (Supplementary File 1, Sheets 15–17). When we investigated the contribution of specific microbial genera to this relationship, we found a large overlap between microbial taxa that were related to the hippocampus serotonin receptors and to the hippocampus and perirhinal cortex neuroplasticity genes (Supplementary Table 2).

Cafeteria diet significantly affected predicted microbial metabolic profiles

Given the microbial changes following exposure to the Caf diet and VSL#3, we employed PICRUSt to predict the metagenomes and determine potential changes in microbial metabolic pathways resulting from the diet or probiotic. PERMANOVA and pairwise comparisons revealed that diet significantly affected microbial

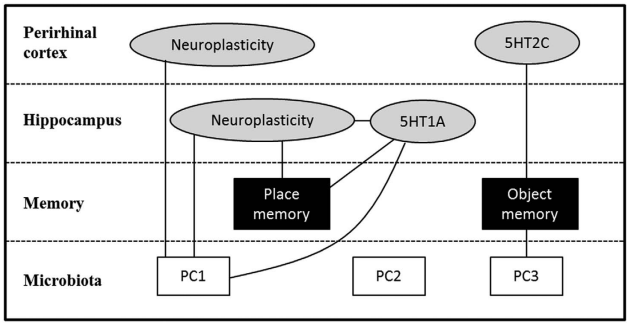


Figure 4. The key relationships between gut microbiota PC, memory and brain gene expression from the Distance-based linear models in Supplementary File 1. Microbiota PC1 is related to neuroplasticity gene clusters in the hippocampus and perirhinal cortex and hippocampal serotonin receptors. Place memory is related to hippocampal neuroplasticity and 5HT1A gene expression. Microbiota PC3 is related to object memory, and object memory is related to perirhinal 5HT2C gene expression. PC, principal components; 5HT1A, serotonin receptor 1A; 5HT2C, serotonin receptor 2C.

metabolic profiles ($t = 6.21$, $P = 0.001$). This was confirmed using nMDS plots and CLUSTER analysis. To determine the effect of VSL#3, we then analysed the Chow and Caf groups separately and found that Chow-Vehicle and Chow-Low groups were significantly different ($t = 1.64$, $P = 0.034$). Antioxidant related pathways (for example, lipoic acid metabolism, flavone and flavonol biosynthesis and flavonoid biosynthesis) and fat metabolism pathways (for example, α-linolenic acid metabolism, synthesis and degradation of ketone bodies) were depleted in the Caf rats while pathways related to primary and secondary bile acid biosynthesis and carbohydrate, starch and sugar metabolism were enriched (Figure 3; Supplementary File 1, Sheet 18).

DISCUSSION

To the best of our knowledge, we show here for the first time, that pre-exposure to a probiotic can prevent diet-induced hippocampal-dependent cognitive deficits on the place task, but cause memory deficits on the perirhinal-dependent object task. The Caf diet decreased gut microbial diversity in 25 days and in these rats, VSL#3 was able to increase specific taxa contained in the probiotic such as *Streptococcus* and *Lactobacillus* and also other taxa including *Butyrivibrio,* which were decreased by the Caf diet. Caf diet exposure increased the expression of many hippocampal genes, most notably, those related to neuroplasticity and also 5HT1A, which was normalised in the Caf-High group who had intact place memory. DistLM showed that these genes clusters were the best predictors of place memory and that they were highly correlated with each other and microbiota PC1. Perirhinal neuroplasticity genes were also highly related to microbiota PC1 but object memory was related to microbiota PC3 and perirhinal 5HT2C. VSL#3 however, had no effect on metabolic outcomes including body weight or adiposity. These results highlight the potential causative role of the gut microbiota composition in diet-induced memory deficits and the importance of considering the relationships between the gut microbiota, behaviour and the brain.

Many previous studies have shown that high-energy diets rapidly cause hippocampal-dependent memory deficits[1,2,4,6] but very few have associated these deficits with diet-induced microbiota changes.[21,46,47] In our present work we show that a probiotic could prevent these diet-induced spatial memory deficits however, irrespective of diet, VSL#3 caused memory deficits on the object task. Importantly, these changes were not due to differences in activity level or stress as comparable exploration times were observed on the memory and anxiety-like behaviour tasks. Probiotics have previously been shown to restore or prevent hippocampal-dependent memory deficits in aged[37] and diabetic[48] rodents, but this is the first time this has been shown in the context of diet-induced memory deficits. Interestingly, antibiotics have previously been shown to impair object, but not spatial, memory in mice after 10 days exposure.[49] Moreover, one study in healthy humans found that extended exposure to a probiotic impaired semantic memory after 20, but not 10 days exposure.[50] In the current study, deficits on the object task also took longer to develop and were only evident at the later memory test.

We then investigated brain pathways known to be affected by high-energy diets and/or probiotics to determine which genes may be responsible for the behavioural outcomes. The Caf diet increased expression of a range of neuroplasticity related genes in the hippocampus and BDNF mRNA in the perirhinal cortex. DistLM showed that a cluster of these hippocampal neuroplasticity genes were the best overall predictors of place memory. These results are in contrast to a number of studies which link decreased hippocampal neuroplasticity to the diet-induced memory deficits[6,7,51] but this may be related to the length of the diet exposure. Elevated neuroplasticity markers were however reported in the hippocampus following a Caf diet,[52] and in regions such as the amygdala and nucleus accumbens.[53,54]

We found that microbiota PC1 was highly related to neuroplasticity gene expression in both the hippocampus and perirhinal cortex. Several commensal species are able to modulate levels of BDNF in the gut with consequent changes in brain levels.[26,27] In addition, other species produce neurotoxins that target proteins which enable synaptic vesicles to dock and fuse with presynaptic plasma membranes.[13,55] Recent data indicate that neurotoxin signals affect neuronal tissue via pattern recognition receptors expressed on the microglia surface[56] and this could be an avenue of future investigation. The Caf diet did not appear to affect plasma endotoxin concentration which indicates that the short diet intervention did not influence gut permeability. Studies which have investigated whether high-energy diets affect gut permeability are generally chronic in nature[57,58] and therefore, to further elucidate whether gut permeability may be affected at this earlier point, future studies could assess gut tight junction protein expression. Our results however suggest that after 24 days of diet, alternative pathways of microbiota-brain communication should be examined.

Mechanistic studies have outlined a regulatory relationship between the serotonergic system and neurotrophic signalling which influences the survival, differentiation and plasticity of neurons.[59] In the current study we found a strong relationship between hippocampal 5HT1A mRNA and the neuroplasticity genes. Microbiota PC1 was strongly related to the serotonin receptor genes and many of the specific contributing taxa overlapped with those related to the neuroplasticity genes. In the hippocampus, the Caf diet decreased the expression of 5HT2C and increased 5HT1A, which was normalised by high-dose VSL#3 (along with place memory) and 5HT1A also negatively correlated with place memory. In terms of the impact of the probiotic on the gut microbiome, VSL#3 had a small effect in the Chow rats but was able to influence 28 bacterial taxa in the Caf rats and 16 of these were affected by the high dose. For example, *Streptococcus*, which has the capacity to produce serotonin,[60] was exclusively increased in the Caf-High group. In the perirhinal cortex, 5HT2C was the best gene predictor of object memory and positively correlated with the exploration ratios. Serotonergic projections from the midbrain raphe nuclei have been shown to modulate LTP and memory and 5HT1A antagonists or 5HT2C agonists can prevent memory impairments.[61,62]

Figure 4 summarises the relationships between the gut microbiota, behavioural measures and brain changes. Briefly, microbiota PC1 was related to expression of neuroplasticity genes in the hippocampus and perirhinal cortex and also to hippocampal serotonin receptor genes. There was a large overlap of specific taxa that were related to each of these categories (Supplementary Table 2). Hippocampal neuroplasticity genes and 5HT1A were associated with each other and with place recognition memory. Finally, microbiota PC3 was related to object memory, which was correlated with perirhinal 5HT2C mRNA expression.

The altered microbial composition predicted changes in a range of microbial metabolic pathways. The Caf diet decreased the abundance of microbial pathways related to antioxidant production that have a neuroprotective role in the brain such as protection from neurotoxins.[63,64] *Pseudoflavonifractor*, a bacterium that is unable to cleave quercetin[65] but is highly related to the flavonoid-metabolising genus *Flavonifractor*, was strongly correlated with hippocampal serotonin receptors and neuroplasticity genes, and the perirhinal neuroplasticity genes, and thus warrants future investigation in the context of diet-induced memory deficits. The Caf diet also decreased microbial pathways related to fatty acid metabolism and increased those related to carbohydrate and sucrose metabolism and primary and secondary bile acid biosynthesis. This is in line with previous work from our laboratory showing that 16 weeks of Caf diet caused decreased antioxidant related pathways and increased bile acid biosynthesis.[66]

Given these striking results, it would be informative for future studies to determine whether these outcomes can be recapitulated in a faecal transplant model in which the microbiota from Caf rats and Caf rats receiving the high dose of VSL#3 are transferred into a germ-free rodent that is fed a chow diet. A previous study has shown that transfer of microbiota from mice fed a high fat diet to healthy mice leads to memory deficits[22] in the recipients however, this has not been investigated in the context of a probiotic intervention.

In conclusion, our data suggest that probiotics can promote better outcomes for some types of memory and associated brain mechanisms when the microbiota is grossly dysregulated. This dysregulation could be caused by diet, as in the current study, or by a range of other factors including the ageing process.[34,37] Probiotics may however be detrimental to healthy subjects as evidenced by the object memory deficits and another study in humans.[50] These results support a customised approach to develop probiotics which may be more effective at restructuring the gut microbiota towards a healthy profile.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed experiments: JEB, MJM. Performed the experiments: JEB. Behavioural data: JEB. Brain data: JEB, JM. Microbiota data: JEB, NOK. Wrote the paper and interpreted data: JEB, NOK, JM, MJM. All authors approved the manuscript.

REFERENCES

1 Beilharz JE, Maniam J, Morris MJ. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain Behav Immun* 2014; **37**: 134–141.
2 Kanoski SE, Davidson TL. Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. *J Exp Psychol Anim Behav Process* 2010; **36**: 313–319.
3 Jurdak N, Kanarek RB. Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiol Behav* 2009; **96**: 1–5.
4 Beilharz JE, Maniam J, Morris MJ. Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation. *Behav Brain Res* 2016; **306**: 1–7.
5 Hsu TM, Konanur VR, Taing L, Usui R, Kayser BD, Goran MI *et al.* Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus* 2015; **25**: 227–239.
6 Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* 2002; **112**: 803–814.
7 Reichelt AC, Maniam J, Westbrook RF, Morris MJ. Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression. *Brain Behav Immun* 2015; **43**: 68–75.
8 Simpson HL, Campbell BJ. Review article: dietary fibre-microbiota interactions. *Aliment Pharmacol Ther* 2015; **42**: 158–179.
9 Mayer EA, Knight R, Mazmanian SK, Cryan JF. Gut microbes and the brain: paradigm shift in neuroscience. *J Neurosci* 2014; **34**: 15490–15496.
10 Burokas A, Moloney RD, Dinan TG, Cryan JF. Microbiota regulation of the Mammalian gut-brain axis. *Adv Appl Microbiol* 2015; **91**: 1–62.
11 Frohlich EE, Farzi A, Mayerhofer R, Reichmann F, Jacan A, Wagner B *et al.* Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain Behav Immun* 2016; **56**: 140–155.
12 Desbonnet L, Clarke G, Traplin A, O'Sullivan O, Crispie F, Moloney RD *et al.* Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour. *Brain Behav Immun* 2015; **48**: 165–173.
13 Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A *et al.* Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA* 2011; **108**: 3047–3052.
14 Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology* 2014; **146**: 1564–1572.
15 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010; **107**: 14691–14696.
16 Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012; **486**: 222–227.
17 David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**: 559–563.
18 Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A *et al.* Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104–108.
19 Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R *et al.* Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut* 2014; **63**: 116–124.
20 Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009; **1**: 6ra14.
21 Beilharz JE, Kaakoush NO, Maniam J, Morris MJ. The effect of short-term exposure to energy-matched diets enriched in fat or sugar on memory, gut microbiota and markers of brain inflammation and plasticity. *Brain Behav Immun* 2016; **57**: 304–313.
22 Bruce-Keller AJ, Salbaum JM, Luo M, Blanchard ET, Taylor CM, Welsh DA *et al.* Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biol Psychiatry* 2015; **77**: 607–615.
23 McCusker RH, Kelley KW. Immune-neural connections: how the immune system's response to infectious agents influences behavior. *J Exp Biol* 2013; **216**: 84–98.
24 Krabbe KS, Reichenberg A, Yirmiya R, Smed A, Pedersen BK, Bruunsgaard H. Low-dose endotoxemia and human neuropsychological functions. *Brain Behav Immun* 2005; **19**: 453–460.
25 Takechi R, Pallebage-Gamarallage MM, Lam V, Giles C, Mamo JC. Nutraceutical agents with anti-inflammatory properties prevent dietary saturated-fat induced disturbances in blood-brain barrier function in wild-type mice. *J Neuroinflamm* 2013; **10**: 73.
26 Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J *et al.* The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* 2011; **141**: 599–609, 609.e591-593.

27 Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 2011; **23**: 255–264, e119.
28 Brigidi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Res Microbiol* 2001; **152**: 735–741.
29 Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P *et al.* Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999; **13**: 1103–1108.
30 Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C *et al.* Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; **121**: 580–591.
31 Kuhbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B *et al.* Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. *Gut* 2006; **55**: 833–841.
32 Ma X, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J Hepatol* 2008; **49**: 821–830.
33 Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB *et al.* Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343–350.
34 Distrutti E, O'Reilly JA, McDonald C, Cipriani S, Renga B, Lynch MA *et al.* Modulation of intestinal microbiota by the probiotic VSL#3 resets brain gene expression and ameliorates the age-related deficit in LTP. *PLoS ONE* 2014; **9**: e106503.
35 D'Mello C, Ronaghan N, Zaheer R, Dicay M, Le T, MacNaughton WK *et al.* Probiotics improve inflammation-associated sickness behavior by altering communication between the peripheral immune system and the brain. *J Neurosci* 2015; **35**: 10821–10830.
36 Beilharz JE, Kaakoush NO, Maniam J, Morris MJ. The effect of short-term exposure to energy-matched diets enriched in fat or sugar on memory, gut microbiota and markers of brain inflammation and plasticity. *Brain Behav Immun* 2016; **57**: 304–313.
37 Jeong JJ, Kim KA, Ahn YT, Sim JH, Woo JY, Huh CS *et al.* Probiotic Mixture KF Attenuates Age-Dependent Memory Deficit and Lipidemia in Fischer 344 Rats. *J Microbiol Biotechnol* 2015; **25**: 1532–1536.
38 Aggleton JP, Brown MW. Contrasting hippocampal and perirhinal cortex function using immediate early gene imaging. *Q J Exp Psychol B* 2005; **58**: 218–233.
39 Wan H, Aggleton JP, Brown MW. Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J Neurosci* 1999; **19**: 1142–1148.
40 Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Laye S *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* 2014; **40**: 9–17.
41 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402–408.
42 Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009; **75**: 7537–7541.
43 Clarke KR. Non-parametric multivariate analyses of changes in community structure. *Austr J Ecol* 1993; **18**: 117–143.
44 Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA *et al.* Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 2013; **31**: 814–821.
45 Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* 2011; **12**: R60.
46 Magnusson KR, Hauck L, Jeffrey BM, Elias V, Humphrey A, Nath R *et al.* Relationships between diet-related changes in the gut microbiome and cognitive flexibility. *Neuroscience* 2015; **300**: 128–140.
47 Pyndt Jorgensen B, Hansen JT, Krych L, Larsen C, Klein AB, Nielsen DS *et al.* A possible link between food and mood: dietary impact on gut microbiota and behavior in BALB/c mice. *PLoS ONE* 2014; **9**: e103398.
48 Davari S, Talaei SA, Alaei H, Salami M. Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience* 2013; **240**: 287–296.
49 Frohlich EE, Farzi A, Mayerhofer R, Reichmann F, Jacan A, Wagner B *et al.* Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain Behav Immun* 2016; **56**: 140–155.
50 Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. *Eur J Clin Nutr* 2007; **61**: 355–361.
51 Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM *et al.* Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* 2008; **18**: 1085–1088.
52 Leffa DD, Valvassori SS, Varela RB, Lopes-Borges J, Daumann F, Longaretti LM *et al.* Effects of palatable cafeteria diet on cognitive and noncognitive behaviors and brain neurotrophins' levels in mice. *Metab Brain Dis* 2015; **30**: 1073–1082.
53 Sharma S, Fernandes MF, Fulton S. Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int J Obes (Lond)* 2013; **37**: 1183–1191.
54 Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int J Obes (Lond)* 2013; **37**: 382–389.
55 Popoff MR, Poulain B. Bacterial toxins and the nervous system: neurotoxins and multipotential toxins interacting with neuronal cells. *Toxins* 2010; **2**: 683–737.
56 Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; **8**: 57–69.
57 de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. 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* 2010; **299**: G440–G448.
58 Duryee MJ, Willis MS, Schaffert CS, Reidelberger RD, Dusad A, Anderson DR *et al.* Precision-cut liver slices from diet-induced obese rats exposed to ethanol are susceptible to oxidative stress and increased fatty acid synthesis. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G208–G217.
59 Galter D, Unsicker K. Sequential activation of the 5-HT1(A) serotonin receptor and TrkB induces the serotonergic neuronal phenotype. *Mol Cell Neurosci* 2000; **15**: 446–455.
60 Roshchina VV. Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. In: Lyte M, Freestone PEP (eds). *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*. Springer New York: New York, NY, USA, 2010 pp 17–52.
61 Ogren SO, Eriksson TM, Elvander-Tottie E, D'Addario C, Ekstrom JC, Svenningsson P *et al.* The role of 5-HT(1A) receptors in learning and memory. *Behav Brain Res* 2008; **195**: 54–77.
62 Jenkins TA, Nguyen JCD, Polglaze KE, Bertrand PP. Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients* 2016; **8**: 1.
63 Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP. The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* 2008; **3**: 115–126.
64 Moura FA, de Andrade KQ, dos Santos JC, Goulart MO. Lipoic acid: its antioxidant and anti-inflammatory role and clinical applications. *Curr Top Med Chem* 2015; **15**: 458–483.
65 Carlier JP, Bedora-Faure M, K'Ouas G, Alauzet C, Mory F. Proposal to unify *Clostridium orbiscindens* Winter *et al.* 1991 and *Eubacterium plautii* (Seguin 1928) Hofstad and Aasjord 1982, with description of Flavonifractor plautii gen. nov., comb. nov., and reassignment of *Bacteroides capillosus* to *Pseudoflavonifractor capillosus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2010; **60**: 585–590.
66 Kaakoush NO, Martire SI, Raipuria M, Mitchell HM, Nielsen S, Westbrook RF *et al.* Alternating or continuous exposure to cafeteria diet leads to similar shifts in gut microbiota compared to chow diet. *Mol Nutr Food Res* 2016; **61**: 1.

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