

An intermittent hypercaloric diet alters gut microbiota, prefrontal cortical gene expression and social behaviours in rats

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26 1. Introduction

27

28 The global rate of obesity is rapidly growing, and it is of great concern that the incidence of
29 overweight and obesity is increasing amongst young people and children [1], who most
30 frequently consume hypercaloric high fat and high sucrose (HFHS) ‘junk’ foods [2]. Studies
31 in rodents have indicated that chronic exposure to hypercaloric diets causes multiple changes
32 to behavioural processes and reward systems, including decreased dopamine turnover in the
33 mesolimbic system [3]. The effects of chronic HFHS diet consumption may be particularly
34 pronounced during critical windows of neurodevelopment. This is supported by emerging data
35 indicating that adolescence may be a sensitive period for susceptibility to diet-induced
36 behavioural changes in mood [4], reward seeking [5, 6] and cognition [7].

37

38 Beyond a role in cognition, recent studies have suggested that hypercaloric diet-induced
39 obesity may evoke changes to social behaviour in rodents [8-10]. High fat diet consumption
40 increased social interaction in adult male mice, but impaired recognition memory for a novel
41 *versus* familiar mouse [11], and social recognition is reduced in juvenile rats following short
42 term exposure to high fat diets [10]. Social play, a characteristic adolescent social behaviour in
43 rats that decreases into adulthood [12], was shown to be reduced following neonatal
44 overfeeding, suggesting that early-life nutrition may impact the expression of this behaviour in
45 rats [8]. However, the litter size manipulation utilised in neonatal rodent overfeeding protocols
46 may have also contributed to the altered social repertoires observed.

47

48 Previous research has demonstrated overlapping neuronal substrates supporting social
49 behaviour and those that are altered by HFHS diet. Maturation of the prefrontal cortex (PFC)
50 throughout adolescence [13] represents a critical period of vulnerability to diet-evoked

51 cognitive deficits [14]. The PFC has a critical role in social processing [15, 16], and the
52 appropriate maturation of this region is fundamental for the development of social cognition
53 [17]. Further experimental evidence highlights that the rodent homologue of the medial PFC
54 and the hippocampus are important for social behaviour, including social memory and
55 sociability [18-20]. As aspects of social interaction are rewarding, it is proposed that the
56 increased dopamine efflux and ongoing refinement of reward-associated neural connections
57 within the PFC across adolescence accentuate this behaviour in young rats [12]

58

59 Previous studies have highlighted that dysfunction in the PFC is induced through
60 intermittent access to a HFHS diet [21], or a continuously-available high fat diet [7] during
61 adolescence, supporting evidence that PFC neuropathology underpins social deficits [22]. In
62 particular, intermittent access to palatable foods has been shown to impact on reward
63 neurocircuitry [23, 24], and furthermore allows examination of behaviour both immediately
64 following palatable food consumption, and when animals have not had recent access to the
65 same palatable food source.

66

67 Moreover, dietary manipulations also influence gut microbial composition [25], and
68 alterations to gut flora has been linked to changes in cognition, mood and behaviour [26, 27].
69 Studies utilising germ-free (GF) mice demonstrated that the presence, composition, and
70 functionality of the gut microbiota is crucial for normal social behaviours, which are reduced
71 in GF mice [26]. GF mice and antibiotic-induced gut dysbiosis rodent models have
72 demonstrated associations between the disruption of the gut microbial community and
73 cognitive, social and emotional alterations [26, 27].

74

75 Building on the hypothesis that intermittent exposure to a HFHS diet during the juvenile
76 developmental phase alters cognitive control and neurotransmitter systems within the brain,
77 we sought to examine the effects of intermittent HFHS food consumption on social interaction
78 and social memory in young rats. Spontaneous novel object recognition and odour recognition
79 memory were examined to assess potential HFHS diet effects on long-term memory and
80 olfaction. To highlight putative molecular pathways impacted by intermittent HFHS food
81 consumption, we examined the expression of specific genes associated with neuroplasticity,
82 monoamine signalling, and neuroinflammation in the PFC and hippocampus. Furthermore, we
83 examined faecal microbiota composition to explore diet-induced alterations. Exploratory
84 statistical analyses through linear modelling were performed to determine associations between
85 faecal microbiota composition, behaviour and cortical gene expression.

86

87 **2. Methods**

88

89 ***2.1. Animals***

90 Male ($n = 32$) albino Sprague Dawley rats (Animal Resources Centre, Western
91 Australia) arrived at postnatal day (P)21 (mean body weight = ~50 g) and were housed in
92 groups of four in a temperature ($21\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$; humidity $55 \pm 5\%$) and light (12 h cycle lights
93 on at 0700) controlled colony room. Standard laboratory rat chow (Meat Free Rat and Mouse
94 Diet, Specialty Feeds, Western Australia; energy composition of 14 kJ/g; 23% protein, 12%
95 fat, 65% carbohydrates) and water was available *ad libitum* throughout the experiment.
96 Behavioural tests were performed between 0800 and 1400 and procedures were approved by
97 the institution's Animal Care and Ethics Committee.

98

2.2. Diet administration

100 Rats were allocated to diet conditions: Control (normal rodent chow-fed, $n = 8$) or
101 HFHS condition ($n = 8$). An additional age/weight matched cohort ($n = 16$) were allocated as
102 sample animals for social memory and social interaction. Body weights were standardised in
103 all treatment groups prior to the commencement of the diet (control: 75.5 ± 2.0 g; HFHS: 76.4
104 ± 2.0 g), and rats were habituated to handling by the experimenters for seven days prior to
105 commencing diet manipulations. Group-housing was used to negate confounding effects of
106 social isolation stress [28]. Rats in the HFHS diet condition were provided with 2 h daily
107 homecage access (between 0900-1100) to semi-pure HFHS pellets (Specialty Feeds, Western
108 Australia, SP04-025; 18.4kJ/g digestible energy; composed of 20% fat (lard), 39.6% sucrose,
109 19.4% protein, providing 36% energy from lipids and 55% from sucrose), in addition to *ad*
110 *libitum* standard chow and water access. Consumption of HFHS diet was calculated in the 2 h
111 diet access period. Body weight was recorded at baseline before the diet began, and thereafter
112 twice per week. Total 24 h energy intake per cage of four rats was calculated by measuring
113 chow consumption and HFHS diet consumption as mass difference twice a week [29].

114

115 2.3. Behavioural analysis

116 A timeline of the general experimental procedures is presented in Figure 1A. Diet
117 administration began on P28, coinciding the commencement of adolescence in male rats [13].
118 Behavioural tests were conducted in a room illuminated at 30 Lux, and sessions were recorded
119 with a ceiling-mounted video camera. Social interaction, social memory, social odour
120 preference, novel object recognition and odour recognition memory was assessed. Behaviours
121 were scored by an observer who was blind to the group allocations using ODLog (v2.7,
122 Macropod Software, Australia).

123

124 2.3.1. *Social interaction*

125 Social interaction tests were conducted in a square test arena (dimensions: 50 cm [length] x 50
126 cm [width] x 60 cm [height]) constructed from black Perspex. All rats were habituated to the
127 arena 24 h prior to testing by being placed individually into the arena for 10 minutes.

128

129 Rats were held in individual cages for 15 minutes prior to social interaction testing. In
130 the social interaction test, one rat from either the control or HFHS diet condition rat was placed
131 in the arena with an unfamiliar partner matched for body weight (+/- 10g). To differentiate
132 between animals, one rat was marked on its back with a black odourless fabric pen marker 24
133 h prior to testing. Test session duration was 10 min. The two rats were placed into the test arena
134 simultaneously facing each other in opposing corners. Rats in the HFHS diet condition were
135 tested 1 h after access to the HFHS pellets (post), and 23 h after HFHS pellet access (pre),
136 counterbalanced across days and animals. The arena was cleaned with 70% ethanol between
137 testing sessions to eliminate residual odour cues.

138

139 As social behaviour in rats has been shown to depend on the playfulness of its partner,
140 both animals in a sample pair were considered as one experimental unit [12]. Videos were
141 scored to measure i) the total time (s) spent in social interaction; ii) frequency of social
142 investigation behaviour (sniffing, licking, grooming); iii) frequency of social play behaviour
143 (pinning, pouncing); and iv) frequency of aggressive-like behaviour (biting, boxing, overt
144 physical harm).

145

146 2.3.2. *Social memory*

147 Social memory testing was performed immediately after HFHS consumption to reduce
148 confounding effects of reduced social contact in the HFHS diet rats. Social memory tests were

149 conducted in a circular arena (dimensions: 100 cm diameter, 50 cm height) constructed from
150 grey Perspex. The arena contained two wire chambers with plastic bases (dimensions: 18 cm
151 [length] x 20 cm [width] x 22 cm [height]). The wires were interspaced 1 cm apart to allow the
152 test rat to interact with the sample rats without physical contact. Sample, control and HFHS
153 diet rats were habituated to the testing apparatus 24 h prior to testing by being placed
154 individually into the arena with the empty chambers for 10 minutes.

155

156 Social memory was tested in two phases (see Supplementary Figure 1A). In Phase 1,
157 rats were placed in the arena for 5 min with one sample rat in a chamber and the other chamber
158 left empty. Time exploring the chamber containing the sample rat *versus* the empty chamber
159 was used as a measure of sociability [30]. The experimental rat was then removed and placed
160 into individual holding cages for a 5 min inter-trial interval (ITI) period. In Phase 2, the arena
161 contained the original sample rat (familiar) in a chamber and the previously empty chamber
162 contained a novel rat. The experimental rat was returned to the arena to explore for a 3 min
163 period. Between test phases the arena was cleaned with 70% ethanol to eliminate odour cues.

164

165 Videos were scored to measure the duration of time the rat spent exploring the chambers
166 during each phase. Sociability was quantified as the time spent exploring the chamber
167 containing the sample rat as opposed to the empty chamber, and social recognition memory
168 was measured as the time spent in proximity to the chamber containing the novel rat versus the
169 familiar sample rat.

170

171 2.3.3. *Social odour preference*

172 The wire chambers used for social recognition were either filled with soiled bedding from a
173 cage of young male rats (~5 weeks of age) housed in an adjacent holding room, or clean corn

174 cob bedding. Rats were allowed to freely explore the arena for 5 min and the amount of time
175 spent exploring empty chambers containing either soiled or clean bedding was videoed and
176 then scored by an experimenter.

177

178 *2.3.4. Odour memory*

179 Odour memory was conducted in the square test arena (as described in 2.3.1). Identical
180 cylindrical stainless-steel containers (10 cm [height] x 6 cm [width]) with perforated stainless-
181 steel lids were filled with corn cob bedding and then scented with 3 mL of peppermint or
182 almond extract (Queen, Australia) to serve as odour stimuli (see Supplementary Figure 1B).
183 The odour memory test consisted of 2 phases: a 5 min sample and 3 min test. During the sample
184 phase two of the same scented containers were placed in opposite corners of the arena, and the
185 rat was allowed to explore. The rat was then removed from the arena and placed in a holding
186 cage for a 5 min retention period. The arena was thoroughly cleaned with 70% ethanol and one
187 of the scented containers was replaced with an identical container filled with a novel odour for
188 the test phase. Videoed behaviour was assessed for the duration of time the rat spent exploring
189 each of the odour containers during each phase.

190

191 *2.3.5. Object recognition memory*

192 Object recognition (Supplementary Figure 1C) was conducted in the square test arena (as
193 described in 2.3.1). Commercial objects (*e.g.* plastic bottles and tin cans) were used with
194 differing heights (16-24 cm) and widths (7-14 cm). Rats explored two identical sample objects
195 in the arena (sample phase; 5 min). The following day, 24 h after the sample phase, rats were
196 tested for recognition of a familiar *versus* a novel object (test phase; 3 mins). The time the rat
197 spent exploring each object during each phase was measured.

198

199 **2.4. Sample collection**

200 **Following 28 days of diet access, rats were sacrificed prior to receiving the HFHS diet.**

201 Rats were anaesthetised with sodium pentobarbital (100 mg/kg i.p.), brains removed and the
202 PFC and hippocampus (composed of dorsal and ventral poles) dissected and snap frozen in
203 liquid nitrogen and stored at -80°C for subsequent analysis by RT-PCR. Retroperitoneal and
204 gonadal white adipose tissues (rpWAT; gnWAT) were dissected and weighed. Livers were
205 weighed and visually scored for markers of hepatic steatosis based on previous criteria [31].
206 One faecal bolus was collected from the terminal caecum, snap frozen and stored at -80°C for
207 later microbiota analysis.

208

209 **2.5. Quantitative RT-PCR**

210 RNA was extracted using Tri-Reagent (Sigma-Aldrich) and RNeasy Mini kit (Qiagen), and
211 quantity and purity of RNA was determined by UV/Vis spectroscopy (Nanodrop; Thermo-
212 Fisher Scientific). RNA was converted to cDNA using a RT² First Strand Kit (Qiagen). Gene
213 expression was quantified by Custom RT² Profiler PCR Arrays (Qiagen) with RT² SYBR
214 Green Mastermix (Qiagen, Australia), and RT-PCR was then performed using a
215 QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems). Target genes were
216 NLR family, pyrin domain containing 3 (*Nlrp3*), glutamate decarboxylase 1 (*Gad1*), brain-
217 derived neurotrophic factor (*Bdnf*), dopamine receptor D1 (*Drd1*), dopamine receptor D2
218 (*Drd2*), monoamine oxidase A (*Maoa*), catechol-O-methyltransferase (*Comt*), 5-
219 hydroxytryptamine (serotonin) receptor 4, G-coupled (*Htr4*), tumour necrosis factor alpha
220 (*Tnf-α*), interleukin 6 (*Il6*), and integrin, alpha M (*Itgam*) (all reagents from Qiagen; see
221 Supplementary Table 1 for reference sequences). Analysis of relative gene expression was
222 normalised to the housekeeping gene beta actin (*Actb*) using the $\Delta\Delta C_T$ method [32].

223

224 **2.6. 16S rRNA gene amplicon sequencing and bioinformatics**

225 Total DNA was isolated using the Bioline ISOLATE Faecal DNA Kit (Bioline). PCR was
226 performed using Q5 DNA polymerase (New England Biolabs) with a primer set selected to
227 amplify V3-V4 region of 16S rRNA gene (forward: ACTCCTACGGGAGGCAGCAG and
228 reverse: GGACTACHVGGGTWTCTAAT). Sequencing was performed on an Illumina MiSeq
229 instrument (2 × 300bp paired-end sequencing), following the method detailed by Fadrosch, Ma
230 [33]. Sequences were joined in Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1
231 (<http://qiime.org>) using the fastq-join method. Maximum-allowed percent differences within
232 the overlapping region was zero. Sequences were de-multiplexed using the QIIME split library
233 protocol, keeping only sequences with Phred quality score higher than 20. The dataset was
234 inspected for chimeric sequences using Pintail [34]. Operational taxonomic units (OTUs) were
235 clustered at 97% sequence identity using UCLUST [35] (**min = 1443, max = 7082, median =**
236 **4466**). Taxonomic assignments were performed against the GreenGenes database [36]. OTUs
237 with a relative abundance of less than 0.01% were excluded.

238

239 **2.7. Statistical analyses**

240 **2.7.1. Behaviour, physiological parameters and brain mRNA expression**

241 Results were analysed using repeat measures analysis of variance (ANOVA; body
242 weight and energy intake), mixed design ANOVAs (social recognition memory, social
243 interaction, sociability, novel odour recognition and novel object recognition), and one-way
244 ANOVA (rpWAT, gnWAT, liver weight, RT-PCR values) with *post-hoc* Tukey and equality
245 of error variance assessed, or multivariate linear models following significant correlations with
246 *post-hoc* testing. $\Delta\Delta C_T$ values that exceeded ± 2 standard deviations from the mean were
247 excluded from analysis, resulting in group sizes of 6-8 per gene.

248

249 Social recognition memory and novel object recognition performance were converted
250 to Exploration Ratios (Time[novel-familiar]/Time[novel+familiar]) to permit exploratory
251 bivariate analysis using correlates (Pearson's *R*, one-tailed). This allowed the exploratory
252 examination of associations between cortical mRNA expression across HFHS and control diet
253 rats, and the performance of behaviours found to significantly differ between diet groups.
254 Liver scores (mass and evidence of steatosis) were analysed using the Kruskal-Wallis test. Data
255 were analysed with IBM SPSS Statistics 24, GraphPad Prism 7 and R.

256

257 2.7.2. Microbiota

258 Visualisation, alpha diversity and distance measures of microbiota were performed
259 using the R packages *phyloseq*, *vegan* and *MixOmics*. Data were total-sum scaled (*i.e.* relative
260 abundance of OTUs) and centre-log ratio transformed where appropriate [37]. Permutational
261 ANOVA (PERMANOVA) of Bray-Curtis dissimilarity index was conducted with 999
262 permutations. The *DESeq2* package was used to undertake differential abundance testing [38],
263 and multivariate analysis of variance (MANOVA) was used to test associations between
264 *Firmicutes* to *Bacteroidetes* (FB) ratio, behaviour, and gene expression.

265

266 Significance for differential abundance analyses was assessed on the basis of a
267 threshold *q*-value of 0.05 (*i.e.* *p*-value adjusted using the False Discovery Rate approach
268 Benjamini, Drai [39]). Bivariate correlations were calculated using a two-tailed Pearson's *R*
269 test.

270

271 3. Results

272

273 3.1. Body Weight, energy consumption and physiological measurements

274 Consistent with physical maturation during adolescence, all rats gained weight across the
275 experiment, however HFHS diet rats showed a significantly greater increase in body mass than
276 controls (time \times diet group $F_{(8,112)} = 5.07, p < 0.001$; Figure 1B). Overall, rats consumed
277 increasing amounts of energy across the four-week experimental period ($F_{(3,18)} = 81.4, p <$
278 0.001), and HFHS diet rats consumed more energy than control rats (diet group \times time $F_{(1,6)} =$
279 $10.8, p < 0.001$; Figure 1C). At the experimental end point, HFHS diet rats had a greater body
280 mass ($F_{(1,14)} = 4.516, p < 0.05$), rpWAT ($F_{(1,14)} = 5.54, p < 0.05$), gnWAT ($F_{(1,14)} = 4.71, p <$
281 0.05), and evidence of hepatopathology ($U = 5, p < 0.01$; Supplementary Table 2).

282 --- Figure 1 here ---

283

284 **3.2. Effect of HFHS diet on social interaction before and after HFHS feeding**

285 To assess the effect of HFHS diet consumption on social behaviour, we examined the total
286 social exploration time one-hour prior to (*pre*) or following (*post*) HFHS food access. Social
287 interaction duration during each test session did not differ in the standard chow fed control
288 animals. However, HFHS diet rats spent less time engaged in social interaction pre-HFHS food
289 access, compared to post-HFHS food access (diet access \times diet group $F_{(1,14)} = 5.66, p < 0.05$;
290 effect of diet group *pre* $F_{(1,14)} = 9.271, p < 0.01$, but not *post* $F < 1$; Figure 2A). Social
291 investigation frequency was increased in the HFHS rats post-consumption (diet access \times diet
292 group $F_{(1,14)} = 8.6, p < 0.05$; HFHS $F_{(1,14)} = 21.59, p < 0.001$, control $F < 1$; Figure 2B). No
293 significant differences were observed in the frequency of social play behaviours (Figure 2C),
294 and no aggressive behaviours were observed. Together, this data suggests that social
295 motivation is decreased in rats that consume intermittent HFHS diet when they have not had
296 access to the palatable HFHS food for a 23-hour period.

297

298 **3.3. Effect of HFHS diets on social recognition memory**

299 Social behaviour has been typically examined in mice using the ‘three-chamber’ social
300 approach test. We adapted this protocol for use in rats to examine whether changes in social
301 recognition memory was altered by HFHS diet consumption. During the social approach phase
302 of the sociability test (Figure 2D) both control and HFHS rats preferentially explored the novel
303 ‘sample’ rat compared to the empty cage ($F_{(1,14)} = 275.5, p < 0.001$) with no significant
304 differences between groups or interaction effects ($F_s < 1$). However, social recognition was
305 impaired in HFHS rats, which explored the familiar and novel rat equally, contrasting to the
306 strong preference of control rats to explore the novel rat (chamber \times diet group $F_{(1,14)} = 39.15,$
307 $p < 0.001$; control $F_{(1,14)} = 109.3, p < 0.001$; HFHS $F_{(1,14)} = 2.6, p = 0.13$; Figure 2E).
308 Exploration ratios calculated from the test data (control = 0.80 ± 0.03 ; HFHS = 0.56 ± 0.03 ; as
309 mean \pm SEM) differed significantly between groups ($F_{(1,14)} = 33.2, p < 0.001$).

310

311 ***3.4. No effect of diet on social odour preference or odour recognition memory***

312 To confirm that the lack of social recognition memory in the HFHS rats was not due to a lack
313 of olfactory sensitivity, we tested their ability to discriminate between clean and soiled bedding
314 and between two non-social odours. Control and HFHS diet rats preferentially explored the
315 chamber containing a social odour ($F_{(1,14)} = 217.8, p < 0.001$; Figure 2F).

316 **During odour recognition testing, control and HFHS diet rats showed no group or odour**
317 **preference during the sample phase (no main effect of odour $F < 1$, diet group $F_{(1,3)} = 3.7, p =$**
318 **0.15 , odour \times diet group $F_{(1,3)} = 3.4, p = 0.16$; Figure 2G). At the time of testing, both control**
319 **and HFHS rats preferentially explored the novel odour container, demonstrating odour**
320 **recognition memory (odour \times diet group $F_{(1,14)} = 3.0, p = 0.11$; Figure 2H). Together, these**
321 **results indicated that HFHS rats were unimpaired in odour discrimination, implying that the**
322 **social recognition impairment (described in section 3.3) was not due to a lack of sensitivity to**
323 **olfactory cues.**

324

325 **3.5. Effects of HFHS diet on novel object recognition**

326 HFHS diet rats were tested on their ability to explore novel compared to previously explored
327 objects. Control rats showed intact object recognition memory by preferentially exploring the
328 novel object; though HFHS rats explored the familiar and novel objects equally, indicating
329 impaired object recognition (object \times diet group, $F_{(1,14)} = 50.7, p < 0.001$; control $F_{(1,14)} = 120.5,$
330 $p < 0.001$; HFHS $F < 1$; Figure 2I). Exploration ratios calculated from the test data (control =
331 0.73 ± 0.01 ; HFHS = 0.52 ± 0.02) differed significantly between groups ($F_{(1,14)} = 60.8, p <$
332 0.001).

333

334 --- Figure 2 here ---

335

336 **3.6. Diet effects on PFC and hippocampal mRNA expression**

337 To determine whether short, intermittent periods of exposure to HFHS diet changed gene
338 expression within the hippocampus and mPFC, we quantified mRNA expression of genes
339 related to neuroplasticity, dopamine and monoamine signalling and neuroinflammation (Table
340 1). We found the majority of transcript changes occurred in the PFC. Compared to controls,
341 the HFHS diet fed rats had reduced *Maoa* expression in the PFC ($F_{(1,13)} = 8.50, p < 0.05$) and
342 hippocampus ($F_{(1,14)} = 6.89, p < 0.05$); *Comt* expression was significantly reduced in the PFC
343 ($F_{(1,14)} = 19.0, p < 0.001$), as was PFC *Bdnf* was in HFHS consuming rats ($F_{(1,13)} = 4.99, p <$
344 0.05).

345

---- Table 1 here ----

346

347 **3.7. Microbiota composition and analysis**

348 The relative abundance of a number of specific taxa differed significantly between the two diet
349 groups as shown by DESeq2 analysis (Figure 3A, Supplementary Table 3). HFHS diet
350 increased levels of bacteria from *Firmicutes* phylum *Clostridiales* family, including
351 *Lachnospiraceae* (genus *Blautia*, $q < 0.04$; unspecified genus $q < 0.03$), *Ruminococcoceae* (genus
352 unspecified $q < 0.01$) and *Veillonellaceae* (genus *Phascolarctobacterium* $q < 0.02$). HFHS diet
353 increased bacteria from *Actinobacteria* phylum, family *Bifidobacteriaceae* (genus
354 *Bifidobacterium*, $q < 0.04$), *Bacteroidetes* phylum, order *Bacteroidales* (unspecified genus $q <$
355 0.05) and *Tenericutes* phylum, order *Erysipelotrichaceae* (genus *Allobaculum* $q < 0.05$).

356

357 Alpha diversity did not differ between the HFHS and control groups measured by
358 observed species, Chao 1, Shannon or Simpson indices (see Figure 3B; $F_s < 1$). Although there
359 was visual overlap apparent on multidimensional scaling of the Bray-Curtis dissimilarity index
360 (Figure 3C), PERMANOVA revealed significant dissimilarity on the basis of diet group ($R^2 =$
361 0.18 , $p < 0.01$). PERMDISP2 revealed no significant heterogeneity of variances between the
362 two groups ($p = 0.39$). Partial least squares discriminant analysis (PLS-DA), a linear
363 classification model, identified the two components that discriminate maximally between the
364 HFHS and control diet groups, showing a large proportion of variance accounted for by the
365 first component (21%) and a lesser degree by the second (8%; Figure 3D).

366

---- Figure 3 here ----

367

368 **3.8. Associations between diet effects, behavioural performance and gene expression**

369 Correlations were performed between behaviours that differed between diet groups (social
370 interaction pre-consumption of diet, social recognition and novel object recognition) and

371 biological measurements (WAT, bodyweight; and cortical gene expression). A number of
372 significant associations were observed, in particular positive correlations between PFC
373 expression of *Maoa* and social interaction pre-HFHS diet and object memory.

374 ----- Figure 4 here -----

375 A number of bivariate correlations between physiological parameters (WAT and
376 bodyweight) and gene expression were significant (Figure 4A and B). In particular, PFC and
377 hippocampal *Itgam* expression was positively correlated with WAT (PFC: $R^2=0.52$, $p < 0.05$,
378 HPC: $R^2 = 0.66$, $p < 0.01$) and bodyweight (HPC: $R^2 = 0.67$, $p < 0.01$), and hippocampal *Maoa*
379 expression was negatively correlated with WAT ($R^2 = -0.45$, $p < 0.05$). Correlations between
380 physiological parameters (WAT and bodyweight) and behavioural performance were observed
381 (Figure 4C), in particular significant negative correlations between WAT and social
382 recognition memory ($R^2 = -0.56$, $p < 0.05$), social interaction pre-HFHS diet ($R^2 = -0.58$, $p <$
383 0.01) and novel object recognition performance ($R^2 = -0.65$, $p < 0.01$).

384
385 Total WAT was significantly associated with PFC gene expression ($F_{(1,12)} = 5.4$, $p <$
386 0.05); specifically *Tnf-a* (adjusted $R^2 = 0.41$, $p < 0.01$), *Comt* (adjusted $R^2 = 0.23$, $p < 0.05$),
387 *Maoa* (adjusted $R^2 = 0.29$, $p < 0.05$), and *Bdnf* (adjusted $R^2 = 0.74$, $p < 0.001$). A number of
388 bivariate correlations between bodyweight and gene expression were significant (Figure 4A
389 and A) however these associations did not persist in multivariate linear modelling (overall
390 model $F_{(1,12)} = 2.1$, $p = 0.17$). There were no significant associations between hippocampal gene
391 expression and body weight ($F < 1$). WAT weight predicted *Il6* expression in the hippocampus
392 ($F_{(1,13)} = 4.86$, $p < 0.05$).

393

394 Associations between hippocampal and PFC genes differentially expressed in control
395 and HFHS groups (see Table 1, Figure 4) and behavioural performance were examined. No
396 predictive relationships were observed between PFC *Bdnf*, *Comt* or *Maoa* expression and social
397 interaction pre-diet consumption, social memory or novel object recognition ($p = 0.17$; $p =$
398 0.09 ; $p = 0.16$ for overall model of each gene respectively). There was no evidence for a
399 predictive relationship between hippocampal *Maoa* expression and behaviours ($p = 0.35$).

400

401 **3.9. Associations between gut microbiota composition and social behaviour**

402 Scores on pre-diet social behaviour, social recognition memory and novel object recognition
403 tasks respectively were all significantly associated with the relative abundance of a number of
404 bacterial taxa (all associations where $q < 0.05$ presented in Table 2). Social memory
405 performance was associated with a large number of taxa. Higher social memory scores were
406 associated with a greater abundance of bacteria from the *Bifidobacteriales* and *Bacteroidales*
407 order, *Lachnospiraceae* family (*Blautia* and multiple unspecified genera), *Ruminococcaceae*
408 family and genus *Allobaculum*. Novel object recognition was negatively associated with
409 abundance of *Bacteroidales* and a number of taxa from the *Lachnospiraceae* family. Only three
410 taxa were significantly associated with social behaviour pre HFHS diet: a relative reduction of
411 *Bifidobacteriales* order and two unspecified genera from the *Lachnospiraceae* family.

412

413 ----- Table 2 here -----

414

415 **3.10. Firmicutes to Bacteroidetes ratio**

416 There were no significant differences between the diet groups on *Firmicutes* to *Bacteroidetes*
417 ratio (FB ratio; $t_{(9.63)} = -1.03$, $p = 0.33$). Samples were pooled across diet groups for subsequent
418 FB ratio analyses, with diet group included to control for potential interaction effects.

419 Multivariate linear modelling demonstrated a significant relationship between FB ratio and the
420 three behavioural dependent variables: social memory, novel object recognition and pre-diet
421 social interaction ($F_{(3,11)} = 5.26, p < 0.05$). *Post-hoc* tests demonstrated strong evidence that
422 FB ratio negatively predicted pre-diet social behaviour ($F_{(2,13)} = 11.46, p < 0.001$), but not
423 object or social recognition memory.

424

425 **3.11. Associations between gut microbiota and hippocampal and PFC gene** 426 **expression**

427 The hippocampal and PFC genes found to differ in expression between the control and HFHS
428 diet groups (PFC: *Bdnf*, *Maoa*, *Comt*; hippocampus: *Maoa*, $p_s < 0.05$) were tested for their
429 associations with differential abundance of bacterial taxa. Of these, significantly differentially
430 abundant taxa ($q < 0.05$) were apparent only for *Maoa* (Table 3). PFC *Maoa* expression was
431 positively associated with one genus of the *Lachnospiraceae* family, whilst a number of
432 bacteria across the four primary phyla were differentially abundant on the basis of hippocampal
433 *Maoa* expression in both positive and negative directions.

434 ----- Table 3 here -----

435 **4. Discussion**

436

437 The data presented in this study shows that daily intermittent consumption of a HFHS diet
438 during adolescence leads to deficits in social interaction and social memory, and impaired
439 object recognition memory in rats. This study also demonstrated associations between diet-
440 induced alterations to social behaviour with microbiota and changes in gene expression
441 associated with reward pathways and neuroplasticity.

442

443 We observed that the effects of HFHS diet on social interaction were limited to
444 immediately prior to ingestion when rats had not consumed HFHS pellets for 23 h, though not
445 after access to HFHS foodstuffs. Based on decreased expression of *Maoa* and *Comt* genes that
446 regulate catecholamine metabolism, we postulate that a junk food mimetic diet can lead to
447 altered monoamine neurotransmission and a resultant increase in anxiety-like behaviour. Thus,
448 intermittent access to a HFHS diet may influence social interaction, as comparable interaction
449 durations were observed following access to a diet rich in fats and sugars. Moreover, social
450 interaction frequency was significantly increased after rats had access to the HFHS food,
451 suggesting that the rewarding aspects of social interaction may have been amplified following
452 ingestion of a diet modelled on obesity-associated nutritional intake, and that recent HFHS diet
453 consumption may also reduce anxiety.

454

455 Social play is important for neurobehavioural development and is also intrinsically
456 linked to proliferation of neurotransmitter pathways, with the dopaminergic mesolimbic system
457 playing a major role in normal social interactions [40]. We observed no differences in
458 frequencies of social play behaviours between diet groups, though these data should be
459 interpreted with some caution. The group housing conditions and brief period of isolation used
460 prior to behavioural testing may have obscured subtle variations between groups as social
461 isolation amplifies subsequent social play behaviour [8]. Another possible explanation is that
462 social play activities tend to decline as adolescence progresses, and that the lack of measurable
463 differences could be attributed to the age of test animals representing mid-to-late adolescence
464 [12]. Extended studies focusing on both dietary habits in early adolescence and potential
465 delayed or enduring long-term effects into adulthood are needed to assess whether poor
466 nutrition reflected by a HFHS diet are associated with potential critical windows of
467 susceptibility representing social behavioural changes.

468

469 Social recognition performance differed between control and HFHS rats, with rats
470 exposed to the dietary intervention demonstrating no preference for the test chamber containing
471 the novel rat during the test phase. This is supported by a recent study showing that acute
472 exposure to a high fat diet in juvenile rats impaired social memory [10]. As rats showed
473 differences in their duration of time engaged in social interaction prior to consuming the HFHS
474 food, the social memory testing was conducted following HFHS access to ensure that any
475 memory deficits observed were not due to reduced social contact in the treated animals. Initial
476 sociability during the sample phase did not differ between HFHS and control diet rats,
477 indicating that social memory was impacted specifically by the diet constituents. Social
478 memory has been shown to depend upon both PFC and hippocampal function [18, 41], and our
479 measured alterations to markers of monoamine neurotransmission and neuroplasticity may
480 underlie the observed social changes. This is also complemented by impaired long term novel
481 object recognition, which is also associated with hippocampal dysfunction [42]. Moreover,
482 both HFHS and control diet rats showed preference for a social odour and showed intact odour
483 recognition memory. Thus, intermittent HFHS diet did not impact olfactory discrimination,
484 and indicates that social memory deficits are not associated with impaired olfactory function.

485

486 With respect to the observed variations in mRNA expression of enzymes *Maoa* and
487 *Comt* in the PFC, it is highly plausible that a HFHS diet adversely affects neurotransmitter
488 activity, specifically dopamine, that is integral to social behaviour and cognition. Dopamine
489 has a primary role in the corticolimbic circuitry involved in the regulation of food reward [43].
490 By mediating deamination of dopamine, monoamine oxidase activity has a key role in
491 controlling the availability of cortical dopamine and also functions in conjunction with
492 catecholamine-*O*-methyltransferase in dopamine breakdown and excretion as inactive

493 homovanillic acid. Changes to monoamine signalling may underpin the altered social
494 behaviour and social memory observed in HFHS diet rats, supported by reports of diet-induced
495 alterations to dopamine receptor expression in the striatum [44]. With no measurable change
496 in dopamine receptor (*Drd1a/Drd2*) expression in the hippocampus or PFC, it is suggestive
497 that impaired catecholamine metabolism, rather than reuptake is the major driver of
498 behavioural changes related to dopamine following obesogenic diet consumption [6, 45].
499 Further studies should examine whether other reward associated genes, such as serotonin and
500 μ -opioid receptors are altered by this diet protocol, and also the involvement of oxytocin
501 signalling mechanisms [10].

502

503 Reduced PFC *Bdnf* expression was observed in HFHS consuming rats, which also
504 correlated positively with novel object recognition performance. This diet-induced change may
505 reinforce the changes to social behaviours and cognition as BDNF signalling has a critical role
506 in memory encoding [46]. Decreased levels of BDNF observed in the hypothalamus, PFC,
507 and/or serum have been shown to correlate with mood disorder-like behaviours in animals and
508 humans [47] and high fat diet consumption reduces hippocampal BDNF levels [48, 49] linking
509 BDNF to emotional processes. Gut microbiota composition may influence cortical BDNF, as
510 demonstrated by previous studies indicating reduced cortical and hippocampal *Bdnf* gene
511 expression in GF mice [50], and antibiotic-induced microbiota dysbiosis altered protein levels
512 of BDNF in the amygdala and hippocampus as well as reduced anxiety-like behaviours in the
513 light-dark box [51]. Thus, microbiome influences on BDNF may be a critical factor in
514 cognition and emotional regulation.

515

516 Excessive consumption of saturated fats has been shown to induce secretion of pro-
517 inflammatory cytokines by adipocytes and macrophages, and affect the integrity of the blood-

518 brain barrier [52], allowing pro-inflammatory cytokines and immune-response cells to reach
519 the brain [53]. Interestingly, no significant changes between groups were observed in
520 inflammatory marker mRNA expression (*Il6*, *TNF- α* , *Nlrp3*, *Itgam*), and trends indicated that
521 PFC expression of *Il6* and *Nlrp3* were lower in HFHS diet rats. This may be due to the age of
522 the rats, as emergent evidence suggests that the modulatory effects of obesogenic diets on
523 inflammatory markers occur in an age-dependent manner, with younger rats showing resistance
524 to neuroinflammation [9]. However, *Itgam* (also called cluster of differentiation molecule 11b,
525 or CD11b) expression in the PFC and hippocampus positively correlated with WAT, indicative
526 that increased adiposity was associated with aspects of neuroinflammation [54]. Moreover,
527 evidence indicates that obesity-induced neuroinflammation is dependent on the type of diet in
528 terms of fat and sugar content, the duration of the diet, and regional differences in brain
529 structures [55]. Future studies utilising immunohistochemistry to examine microglia
530 morphology and astrogliosis are needed to validate the region-specific impact of obesogenic
531 diets on neuroinflammatory effects.

532

533 The effects of obesogenic high fat, high sugar and Western diets on the gut microbiome
534 have been extensively studied in rodents, with typical observations including the altered
535 abundance of the *Bacteroidetes* and *Firmicutes* phyla [56-59]. Notably, *Bacteroidetes* (order
536 *Bacteroidales*) phyla were increased in HFHS diet-fed rats. This contrasts other studies and
537 indicates that not all the members of the *Bacteroidetes* family are decreased with adiposity.
538 Whilst we did not observe an overall shift in the FB ratio, the data presented here suggests that
539 intermittent HFHS diet protocol significantly altered the gut microbiota signature, and supports
540 the concept that a phylum-wide binary distinction does not sufficiently reflect the complexity
541 of diet-induced changes to the gut microbiome as suggested in previous reports [60, 61]. FB

542 ratio changes may therefore become more prominent with more chronic hypercaloric feeding
543 schedules, and the development of pronounced obesity.

544

545 Moreover, our detected increase in the abundance of the *Firmicutes* family
546 *Ruminococcaceae* is consistent with previous studies that found these taxa to be increased in
547 mice [62] and rats [63] consuming a high fat diet. Taxa from *Lachnospiraceae* and
548 *Ruminococceae* families of the *Clostridiales* order were the most common bacterial predictors
549 of social behaviour and recognition memory, converging with clinical studies that show
550 alterations in microbiome populations in neuropsychiatric disorders including major depressive
551 disorder and autism [64, 65]. Social avoidance behaviour in non-obese diabetic mice has been
552 associated with increased abundance of *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiales*,
553 and the transfer of intestinal microbiota from these mice to microbiota-depleted recipients
554 evoked similar behavioural phenotypes [66]. As such, our observations converge with evidence
555 indicating the influence of diet on social behaviours *via* the gut-brain-microbiota axis [67, 68].

556

557 Further studies using faecal transplants from HFHS diet animals are necessary to
558 elucidate the mechanisms underpinning the neural effects of gut microbiome. Our study was
559 limited by faecal samples being taken only at the experiment endpoint, and behaviour and
560 microbiome analyses may have been more powerful if taken from the same time points.
561 Moreover, a direct comparison between HFHS diet effects and behaviour could be made if an
562 additional group that received ad libitum access to the HFHS was included in the study. In
563 addition, these animals could further serve as an additional control as behaviour around the
564 HFHS diet access period to be likely changed due to conditioning. Furthermore, as locomotor
565 activity can be influenced by motivation, anxiety and body weight, and can by itself influence

566 the results of behavioural readouts, additional examination of locomotor behaviour in this study
567 is warranted to further define diet induced alterations to social behaviour.

568

569 The results presented here support the need for further studies including generating
570 metagenomic predictions from the bacterial communities shed light onto the metabolic
571 pathways impacted by intermittent HFHS diet consumption. Modulation of the gut-brain axis
572 dynamics has clinical implications for neuropsychiatric conditions, and emerging
573 ‘psychobiotic’ treatment strategies that have been indicated to ameliorate depressive [69] and
574 anxiety-like behaviours [51] in mice, such as increased hippocampal *Bdnf* expression resulting
575 from prebiotic administration [70]. As such, harnessing the microbiome may provide a route
576 for the attenuation of diet and obesity evoked cognitive and emotional alterations.

577

578 **References**

- 579 [1] Ogden, C. L., Carroll, M. D., Kit, B. K., Flegal, K. M. Prevalence of childhood and adult
580 obesity in the United States, 2011-2012. *JAMA*. 2014,311:806-14.
- 581 [2] Braithwaite, I., Stewart, A. W., Hancox, R. J., Beasley, R., Murphy, R., Mitchell, E. A., et
582 al. Fast-food consumption and body mass index in children and adolescents: an international
583 cross-sectional study. *BMJ open*. 2014,4:e005813.
- 584 [3] Davis, J. F., Tracy, A. L., Schurdak, J. D., Tschop, M. H., Lipton, J. W., Clegg, D. J., et
585 al. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and
586 mesolimbic dopamine turnover in the rat. *Behavioral neuroscience*. 2008,122:1257-63.
- 587 [4] Baker, K. D., Loughman, A., Spencer, S. J., Reichelt, A. C. The impact of obesity and
588 hypercaloric diet consumption on anxiety and emotional behavior across the lifespan.
589 *Neurosci Biobehav Rev*. 2017,83:173-82.
- 590 [5] Reichelt, A. C. Adolescent Maturational Transitions in the Prefrontal Cortex and
591 Dopamine Signaling as a Risk Factor for the Development of Obesity and High Fat/High
592 Sugar Diet Induced Cognitive Deficits. *Front Behav Neurosci*. 2016,10:189.
- 593 [6] Naneix, F., Tantot, F., Glangetas, C., Kaufling, J., Janthakhin, Y., Boitard, C., et al.
594 Impact of Early Consumption of High-Fat Diet on the Mesolimbic Dopaminergic System.
595 *eNeuro*. 2017,4.
- 596 [7] Labouesse, M. A., Lassalle, O., Richetto, J., Iafrati, J., Weber-Stadlbauer, U., Notter, T.,
597 et al. Hypervulnerability of the adolescent prefrontal cortex to nutritional stress via reelin
598 deficiency. *Mol Psychiatry*. 2017,22:961-71.
- 599 [8] Carvalho, A. L. O., Ferri, B. G., de Sousa, F. A. L., Vilela, F. C., Giusti-Paiva, A. Early
600 life overnutrition induced by litter size manipulation decreases social play behavior in
601 adolescent male rats. *International journal of developmental neuroscience : the official
602 journal of the International Society for Developmental Neuroscience*. 2016,53:75-82.

603 [9] Teixeira, D., Ceconello, A. L., Partata, W. A., de Fraga, L. S., Ribeiro, M. F. M.,
604 Guedes, R. P. The metabolic and neuroinflammatory changes induced by consuming a
605 cafeteria diet are age-dependent. *Nutr Neurosci.* 2017;1-11.

606 [10] Yaseen, A., Shrivastava, K., Zuri, Z., Hatoum, O. A., Maroun, M. Prefrontal Oxytocin is
607 Involved in Impairments in Prefrontal Plasticity and Social Memory Following Acute
608 Exposure to High Fat Diet in Juvenile Animals. *Cereb Cortex.* 2018.

609 [11] Takase, K., Tsuneoka, Y., Oda, S., Kuroda, M., Funato, H. High-fat diet feeding alters
610 olfactory-, social-, and reward-related behaviors of mice independent of obesity. *Obesity.*
611 2016;24:886-94.

612 [12] Trezza, V., Baarendse, P. J., Vanderschuren, L. J. The pleasures of play:
613 pharmacological insights into social reward mechanisms. *Trends in pharmacological*
614 *sciences.* 2010;31:463-9.

615 [13] Spear, L. P. The adolescent brain and age-related behavioral manifestations. *Neurosci*
616 *Biobehav Rev.* 2000;24:417-63.

617 [14] Reichelt, A. C., Rank, M. M. The impact of junk foods on the adolescent brain. *Birth*
618 *Defects Res.* 2017;109:1649-58.

619 [15] Bicks, L. K., Koike, H., Akbarian, S., Morishita, H. Prefrontal Cortex and Social
620 Cognition in Mouse and Man. *Front Psychol.* 2015;6:1805.

621 [16] Kolb, B. Social behavior of rats with chronic prefrontal lesions. *J Comp Physiol*
622 *Psychol.* 1974;87:466-74.

623 [17] Kim, Y., Venkataraju, K. U., Pradhan, K., Mende, C., Taranda, J., Turaga, S. C., et al.
624 Mapping social behavior-induced brain activation at cellular resolution in the mouse. *Cell*
625 *reports.* 2015;10:292-305.

626 [18] Kogan, J. H., Frankland, P. W., Silva, A. J. Long-term memory underlying
627 hippocampus-dependent social recognition in mice. *Hippocampus.* 2000;10:47-56.

628 [19] Okuyama, T., Kitamura, T., Roy, D. S., Itohara, S., Tonegawa, S. Ventral CA1 neurons
629 store social memory. *Science.* 2016;353:1536-41.

630 [20] Rudebeck, P. H., Walton, M. E., Millette, B. H., Shirley, E., Rushworth, M. F.,
631 Bannerman, D. M. Distinct contributions of frontal areas to emotion and social behaviour in
632 the rat. *Eur J Neurosci.* 2007;26:2315-26.

633 [21] Baker, K. D., Reichelt, A. C. Impaired fear extinction retention and increased anxiety-
634 like behaviours induced by limited daily access to a high-fat/high-sugar diet in male rats:
635 Implications for diet-induced prefrontal cortex dysregulation. *Neurobiol Learn Mem.*
636 2016;136:127-38.

637 [22] Selimbeyoglu, A., Kim, C. K., Inoue, M., Lee, S. Y., Hong, A. S. O., Kauvar, I., et al.
638 Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in
639 CNTNAP2-deficient mice. *Sci Transl Med.* 2017;9.

640 [23] Furlong, T. M., Jayaweera, H. K., Balleine, B. W., Corbit, L. H. Binge-like consumption
641 of a palatable food accelerates habitual control of behavior and is dependent on activation of
642 the dorsolateral striatum. *The Journal of neuroscience : the official journal of the Society for*
643 *Neuroscience.* 2014;34:5012-22.

644 [24] Bocarsly, M. E., Hoebel, B. G., Paredes, D., von Loga, I., Murray, S. M., Wang, M., et
645 al. GS 455534 selectively suppresses binge eating of palatable food and attenuates dopamine
646 release in the accumbens of sugar-bingeing rats. *Behavioural pharmacology.* 2014;25:147-57.

647 [25] Albenberg, L. G., Wu, G. D. Diet and the intestinal microbiome: associations, functions,
648 and implications for health and disease. *Gastroenterology.* 2014;146:1564-72.

649 [26] Desbonnet, L., Clarke, G., Traplin, A., O'Sullivan, O., Crispie, F., Moloney, R. D., et al.
650 Gut microbiota depletion from early adolescence in mice: Implications for brain and
651 behaviour. *Brain, behavior, and immunity.* 2015;48:165-73.

652 [27] Frohlich, E. E., Farzi, A., Mayerhofer, R., Reichmann, F., Jacan, A., Wagner, B., et al.
653 Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain
654 communication. *Brain, behavior, and immunity*. 2016,56:140-55.

655 [28] Skelly, M. J., Chappell, A. E., Carter, E., Weiner, J. L. Adolescent social isolation
656 increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood:
657 Possible role of disrupted noradrenergic signaling. *Neuropharmacology*. 2015,97:149-59.

658 [29] Del Rio, D., Morales, L., Ruiz-Gayo, M., Del Olmo, N. Effect of high-fat diets on mood
659 and learning performance in adolescent mice. *Behavioural brain research*. 2016,311:167-72.

660 [30] Crawley, J. N., Chen, T., Puri, A., Washburn, R., Sullivan, T. L., Hill, J. M., et al. Social
661 approach behaviors in oxytocin knockout mice: comparison of two independent lines tested
662 in different laboratory environments. *Neuropeptides*. 2007,41:145-63.

663 [31] Velkoska, E., Warner, F. J., Cole, T. J., Smith, I., Morris, M. J. Metabolic effects of low
664 dose angiotensin converting enzyme inhibitor in dietary obesity in the rat. *Nutrition,
665 metabolism, and cardiovascular diseases : NMCD*. 2010,20:49-55.

666 [32] Livak, K. J., Schmittgen, T. D. Analysis of relative gene expression data using real-time
667 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001,25:402-8.

668 [33] Fadrosch, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., et al. An
669 improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the
670 Illumina MiSeq platform. *Microbiome*. 2014,2:6.

671 [34] Ashelford, K. E., Chuzhanova, N. A., Fry, J. C., Jones, A. J., Weightman, A. J. At least 1
672 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain
673 substantial anomalies. *Applied and environmental microbiology*. 2005,71:7724-36.

674 [35] Edgar, R. C. Search and clustering orders of magnitude faster than BLAST.
675 *Bioinformatics*. 2010,26:2460-1.

676 [36] DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., et al.
677 Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with
678 ARB. *Applied and environmental microbiology*. 2006,72:5069-72.

679 [37] McMurdie, P. J., Holmes, S. Waste not, want not: why rarefying microbiome data is
680 inadmissible. *PLoS Comput Biol*. 2014,10:e1003531.

681 [38] Love, M. I., Huber, W., Anders, S. Moderated estimation of fold change and dispersion
682 for RNA-seq data with DESeq2. *Genome biology*. 2014,15:550.

683 [39] Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., Golani, I. Controlling the false
684 discovery rate in behavior genetics research. *Behavioural brain research*. 2001,125:279-84.

685 [40] Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L. J.,
686 Trezza, V. Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social
687 Play Behavior in Rats. *Neuropsychopharmacology*. 2016,41:2215-23.

688 [41] Tanimizu, T., Kenney, J. W., Okano, E., Kadoma, K., Frankland, P. W., Kida, S.
689 Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social
690 Recognition Memory. *The Journal of neuroscience : the official journal of the Society for
691 Neuroscience*. 2017,37:4103-16.

692 [42] Warburton, E. C., Brown, M. W. Findings from animals concerning when interactions
693 between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for
694 recognition memory. *Neuropsychologia*. 2010,48:2262-72.

695 [43] Volkow, N. D., Wang, G. J., Baler, R. D. Reward, dopamine and the control of food
696 intake: implications for obesity. *Trends Cogn Sci*. 2011,15:37-46.

697 [44] Johnson, P. M., Kenny, P. J. Dopamine D2 receptors in addiction-like reward
698 dysfunction and compulsive eating in obese rats. *Nature neuroscience*. 2010,13:635-41.

699 [45] Naneix, F., Darlot, F., De Smedt-Peyrusse, V., Pape, J. R., Coutureau, E., Cador, M.
700 Protracted motivational dopamine-related deficits following adolescence sugar
701 overconsumption. *Neuropharmacology*. 2018,129:16-25.

702 [46] Choi, D. C., Maguschak, K. A., Ye, K., Jang, S. W., Myers, K. M., Ressler, K. J.
703 Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate
704 fear. *Proc Natl Acad Sci U S A.* 2010,107:2675-80.

705 [47] Bocchio-Chiavetto, L., Bagnardi, V., Zanardini, R., Molteni, R., Nielsen, M. G.,
706 Placentino, A., et al. Serum and plasma BDNF levels in major depression: a replication study
707 and meta-analyses. *World J Biol Psychiatry.* 2010,11:763-73.

708 [48] Molteni, R., Wu, A., Vaynman, S., Ying, Z., Barnard, R. J., Gomez-Pinilla, F. Exercise
709 reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral
710 plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience.*
711 2004,123:429-40.

712 [49] Pistell, P. J., Morrison, C. D., Gupta, S., Knight, A. G., Keller, J. N., Ingram, D. K., et al.
713 Cognitive impairment following high fat diet consumption is associated with brain
714 inflammation. *J Neuroimmunol.* 2010,219:25-32.

715 [50] Sudo, N., Aiba, Y., Oyama, N., Yu, X. N., Matsunaga, M., Koga, Y., et al. Dietary
716 nucleic acid and intestinal microbiota synergistically promote a shift in the Th1/Th2 balance
717 toward Th1-skewed immunity. *International archives of allergy and immunology.*
718 2004,135:132-5.

719 [51] Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., et al. The intestinal
720 microbiota affect central levels of brain-derived neurotropic factor and behavior in mice.
721 *Gastroenterology.* 2011,141:599-609, e1-3.

722 [52] Kanoski, S. E., Zhang, Y., Zheng, W., Davidson, T. L. The effects of a high-energy diet
723 on hippocampal function and blood-brain barrier integrity in the rat. *J Alzheimers Dis.*
724 2010,21:207-19.

725 [53] Thaler, J. P., Yi, C. X., Schur, E. A., Guyenet, S. J., Hwang, B. H., Dietrich, M. O., et al.
726 Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest.*
727 2012,122:153-62.

728 [54] Jeong, H. K., Ji, K., Min, K., Joe, E. H. Brain inflammation and microglia: facts and
729 misconceptions. *Exp Neurobiol.* 2013,22:59-67.

730 [55] Guillemot-Legris, O., Muccioli, G. G. Obesity-Induced Neuroinflammation: Beyond the
731 Hypothalamus. *Trends Neurosci.* 2017,40:237-53.

732 [56] Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., Gordon, J. I.
733 Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A.* 2005,102:11070-5.

734 [57] Duncan, S. H., Lobeley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., et al.
735 Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond).*
736 2008,32:1720-4.

737 [58] Mujico, J. R., Baccan, G. C., Gheorghe, A., Diaz, L. E., Marcos, A. Changes in gut
738 microbiota due to supplemented fatty acids in diet-induced obese mice. *Br J Nutr.*
739 2013,110:711-20.

740 [59] Serino, M., Luche, E., Gres, S., Baylac, A., Berge, M., Cenac, C., et al. Metabolic
741 adaptation to a high-fat diet is associated with a change in the gut microbiota. *Gut.*
742 2012,61:543-53.

743 [60] Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E. t., Taylor, C. M., Welsh, D.
744 A., et al. Obese-type gut microbiota induce neurobehavioral changes in the absence of
745 obesity. *Biological psychiatry.* 2015,77:607-15.

746 [61] Murphy, E. F., Cotter, P. D., Hogan, A., O'Sullivan, O., Joyce, A., Fouhy, F., et al.
747 Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in
748 diet-induced obesity. *Gut.* 2013,62:220-6.

749 [62] Kim, K. A., Gu, W., Lee, I. A., Joh, E. H., Kim, D. H. High fat diet-induced gut
750 microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway.
751 *PLoS one.* 2012,7:e47713.

752 [63] Lecomte, V., Kaakoush, N. O., Maloney, C. A., Raipuria, M., Huinao, K. D., Mitchell,
753 H. M., et al. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-
754 associated metabolic parameters. *PloS one*. 2015,10:e0126931.

755 [64] Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linlokken, A., Wilson, R., et
756 al. Correlation between the human fecal microbiota and depression. *Neurogastroenterology*
757 *and motility : the official journal of the European Gastrointestinal Motility Society*.
758 2014,26:1155-62.

759 [65] De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., De Giacomo, A., Serrazanetti,
760 D. I., et al. Fecal microbiota and metabolome of children with autism and pervasive
761 developmental disorder not otherwise specified. *PloS one*. 2013,8:e76993.

762 [66] Gacias, M., Gaspari, S., Santos, P. M., Tamburini, S., Andrade, M., Zhang, F., et al.
763 Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in
764 social behavior. *Elife*. 2016,5.

765 [67] Christian, L. M., Galley, J. D., Hade, E. M., Schoppe-Sullivan, S., Kamp Dush, C.,
766 Bailey, M. T. Gut microbiome composition is associated with temperament during early
767 childhood. *Brain, behavior, and immunity*. 2015,45:118-27.

768 [68] Parashar, A., Udayabanu, M. Gut microbiota regulates key modulators of social
769 behavior. *Eur Neuropsychopharmacol*. 2016,26:78-91.

770 [69] Bravo, J. A., Dinan, T. G., Cryan, J. F. Alterations in the central CRF system of two
771 different rat models of comorbid depression and functional gastrointestinal disorders. *The*
772 *international journal of neuropsychopharmacology*. 2011,14:666-83.

773 [70] Burokas, A., Arboleya, S., Moloney, R. D., Peterson, V. L., Murphy, K., Clarke, G., et
774 al. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-
775 like Effects and Reverse the Impact of Chronic Stress in Mice. *Biological psychiatry*.
776 2017,82:472-87.

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780 **Tables**

781 **Table 1.** The effects of intermittent high fat and high sucrose (HFHS) diet exposure on
782 prefrontal cortex and hippocampal gene expression, mean (\pm SEM), * = $P < 0.05$, ** = $P < 0.01$, #
783 = $P < 0.10$

784

785 **Table 2.** Associations between relative abundance of taxa in faecal microbiota and behavioural
786 outcomes ($q < 0.05$).

787

788 **Table 3.** Associations between relative abundance of taxa in faecal microbiota and gene
789 expression ($q < 0.05$).

790 **Table 1.** The effects of intermittent high fat and high sucrose (HFHS) diet exposure on
791 prefrontal cortex and hippocampal gene expression. Table shows Mean (SEM), * = $P < 0.05$,
792 ** = $P < 0.01$, # = $P < 0.10$

Gene	Prefrontal cortex			Hippocampus		
	Control	HFHS	<i>p</i> -value	Control	HFHS	<i>p</i> -value
Neuroplasticity						
Gad1	0.84 (0.04)	0.94 (0.07)	<i>n.s.</i>	1.00 (0.08)	0.94 (0.06)	<i>n.s.</i>
Bdnf	1.00 (0.10)	0.72 (0.03)*	0.045	1.00 (0.08)	0.96 (0.17)	<i>n.s.</i>
Dopamine receptors						
Drd1a	1.00 (0.23)	0.65 (0.13)	<i>n.s.</i>	1.00 (0.11)	0.95 (0.09)	<i>n.s.</i>
Drd2	1.00 (0.32)	0.56 (0.15)	<i>n.s.</i>	0.93 (0.03)	0.85 (0.07)	<i>n.s.</i>
Monoamine synthesis						
Maoa	1.00 (0.04)	0.86 (0.02)*	0.012	1.00 (0.04)	0.83 (0.05)*	0.02
Comt	1.00 (0.02)	0.83 (0.03)**	0.001	1.00 (0.07)	1.08 (0.09)	<i>n.s.</i>
Serotonin receptor						
Htr4	0.85 (0.07)	0.79 (0.08)	<i>n.s.</i>	1.00 (0.05)	0.96 (0.04)	<i>n.s.</i>
Inflammation						
Tnf- α	1.00 (0.16)	0.79 (0.11)	<i>n.s.</i>	1.00 (0.21)	0.65 (0.06)	<i>n.s.</i>
Il6	1.00 (0.15)	0.62 (0.08)#	0.060	0.81 (0.14)	0.59 (0.11)	<i>n.s.</i>
Nlrp3	1.00 (0.06)	0.85 (0.03)#	0.056	1.00 (0.18)	1.03 (0.14)	<i>n.s.</i>
Itgam	1.00 (0.10)	1.09 (0.09)	<i>n.s.</i>	1.00 (0.11)	1.23 (0.19)	<i>n.s.</i>

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795

Table 2. Associations between relative abundance of taxa in faecal microbiota and behavioural outcomes ($q < 0.05$).

Phylum	Class	Order	Family	Genus	Social recognition		Object recognition		Pre-diet social	
					log2FoldChange	memory	log2FoldChange	memory	log2FoldChange	interaction
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	5.13	<0.01	-7.27	0.03	-0.04	0.01
					8.51	<0.01				
					4.59	0.04				
					4.20	<0.01				
					-4.87	0.04				
					4.28	0.04				
					4.24	0.05				
					4.66	0.04				
					4.30	0.02				
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	4.72	0.01	-8.32	0.02	-0.04	0.01
					4.48	0.04	-7.89	0.03	-0.04	0.01
					4.51	0.01				
					4.91	0.01				
					5.42	0.01				
					6.06	<0.01				
					4.45	0.02				
					4.13	0.04				
					2.90	0.04				
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum						

Table 3. Associations between relative abundance of taxa in faecal microbiota and gene expression ($q < 0.05$).

Phylum	Class	Order	Family	Genus	Maoa					
					Hippocampus	Prefrontal cortex				
					log2Fold Change	log2Fold Change	q			
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	-7.19	-7.19	0.05			
					-7.87	-7.87	0.01			
					-8.18	-8.18	0.01			
					-7.18	Bifidobacteriaceae	Bifidobacterium	-7.18	-7.18	0.03
					-7.07			-7.07	-7.07	0.03
Bacteroidetes	Bacteroidia	Bacteroidales	Unspecified	Unspecified	-7.88	-7.88	0.03			
					10.40	Rikenellaceae	Alistipes	10.40	10.40	<0.01
					7.17			7.17	7.17	0.04
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	8.43	8.43	0.01			
					-7.53	Ruminococcaceae	Unspecified	-7.53	-7.53	0.03
					-8.32			-8.32	-8.32	0.02
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	-8.96	-8.96	0.01			
					-8.37			-8.37	-8.37	0.01
					9.91			9.91	9.91	0.01

799 **Figure Legends**

800 **Figure 1. Experimental design and physiological effects of HFHS diet consumption.** A)

801 Timeline of experimental procedures showing the ages of the rats at each behavioural test
802 (conducted at the same age in all animals) and at sacrifice. Social interaction testing was
803 conducted both before and after access to palatable HFHS food in the HFHS rats. As HFHS
804 rats showed differences in social interaction pre HFHS food, social memory testing, and other
805 behavioural tests were conducted after HFHS access, to ensure that any memory or behavioural
806 deficits observed were not due to reduced social contact in the HFHS diet rats. B) Mean body
807 weights of control and HFHS rats across the 4-week diet exposure period. C) Mean energy
808 consumption (kJ) per cage of rats across the 4-week diet exposure period. Error bars represent
809 +SEM. * indicates $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$.

810

811 **Figure 2. Impact of HFHS diet consumption on behaviour.** Social behaviours between

812 control / HFHS diet exposed rats and a novel weight / age matched conspecific. HFHS diet rats
813 were tested either 23h after HFHS pellet access “pre” or 1h after access to the HFHS pellets
814 “post”, A) Total duration of social contact between rats, B) frequency of social interactions,
815 and C) frequency of social play. Performance of HFHS diet and control rats in social
816 recognition memory - D) exploration times of the chamber containing the sample rat “sample”
817 and empty chamber “empty” during the sample phase of social memory testing, E) exploration
818 times of the chamber containing the familiar sample rat and chamber containing a novel sample
819 rat. F) Exploration time of chambers containing soiled bedding “social odour” or clean
820 bedding. G) Exploration of the odours during the sample phase. H) Novel odour recognition
821 performance in control and HFHS diet rats during the test phase following a 5 min delay. I)
822 Novel object recognition performance during the test phase following a 24h delay. Error bars

823 represent +SEM. ** $P < 0.01$. Error bars represent +SEM. * indicates $P \leq 0.05$, ** $P < 0.01$, ***
824 $P < 0.001$ between groups comparisons.

825

826

827 **Figure 3. Impact of HFHS diet on faecal microbiota.** A) Graphical depiction of DESeq2
828 analysis. Each coloured circle represents one bacterial genus that was more abundant in the
829 HFHS than control group ($q < 0.05$). Log2 fold change refers to the difference abundance of
830 the log2 values between diet groups for each bacterial genus. B) No significant differences in
831 alpha diversity of faecal microbiota between HFHS and control diet groups. Each panel
832 represents one alpha diversity measure as follows: Observed = total number of OTU's
833 observed; Chao1 = richness estimator (estimate of the total number of OTU's present in a
834 community); Shannon and Simpson = microbial indexes of diversity. Boxes span the first to
835 third quartiles; the horizontal line inside the boxes represents the median. Whiskers extending
836 vertically from the boxes indicate variability outside the upper and lower quartiles, and the
837 single black circles indicate outliers (all $P > 0.05$). C) Permutational ANOVA (PERMANOVA)
838 of Bray-Curtis dissimilarity index revealed significant dissimilarity on the basis of diet group.
839 D) Partial least-squares discriminant analysis (PLS-DA) Figure showing a large proportion of
840 variance accounted for by the first component (21%) and a lesser degree by the second (8%).
841 Each point represents a sample.

842

843 **Figure 4. Correlations between behaviour, cortical gene expression and physiological**
844 **effects of the HFHS diet.** Heatmap of bivariate correlations (Pearson's R^2) between the
845 behavioural assays social recognition memory, social interaction and novel object recognition
846 performance, and A) prefrontal cortex gene expression, B) hippocampal gene expression and
847 C) physiological parameters. *= $P < 0.05$, **= $P < 0.01$

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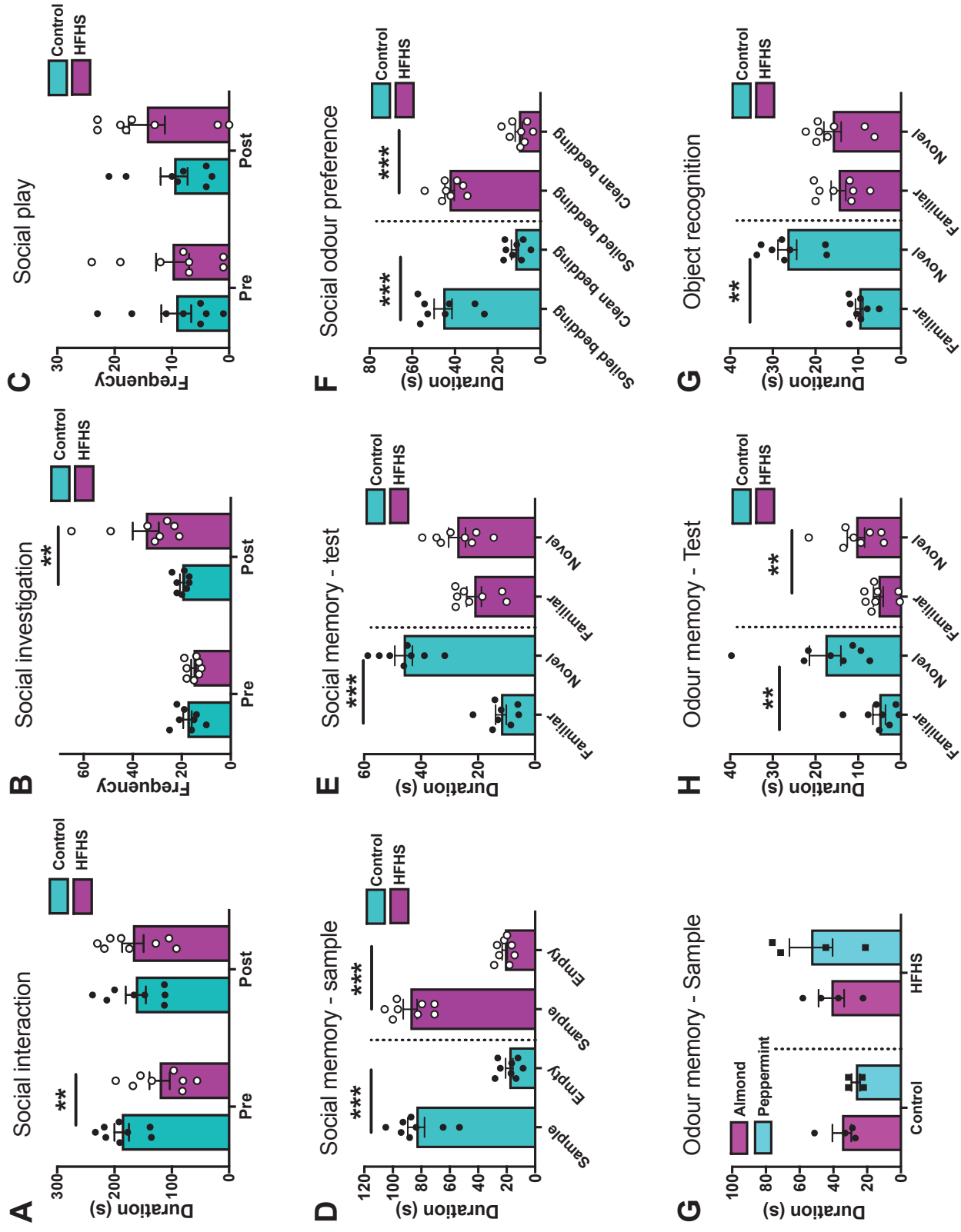


Figure 2

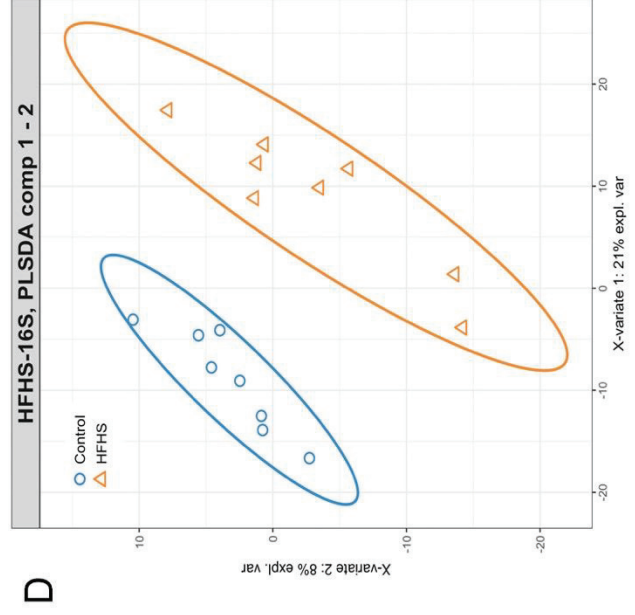
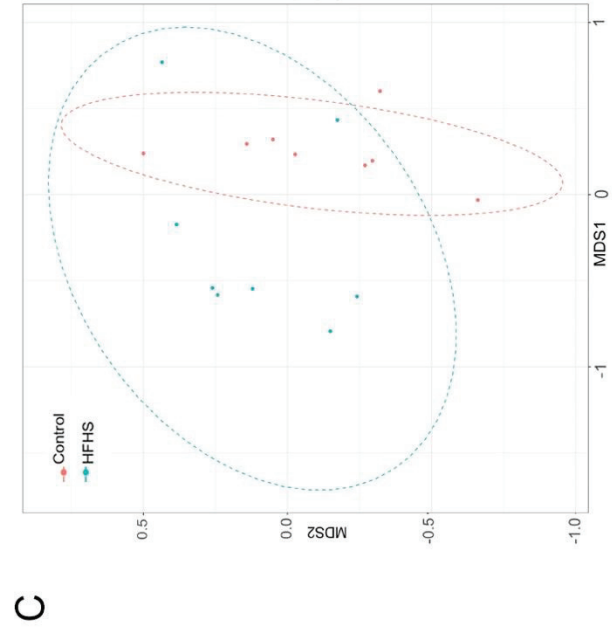
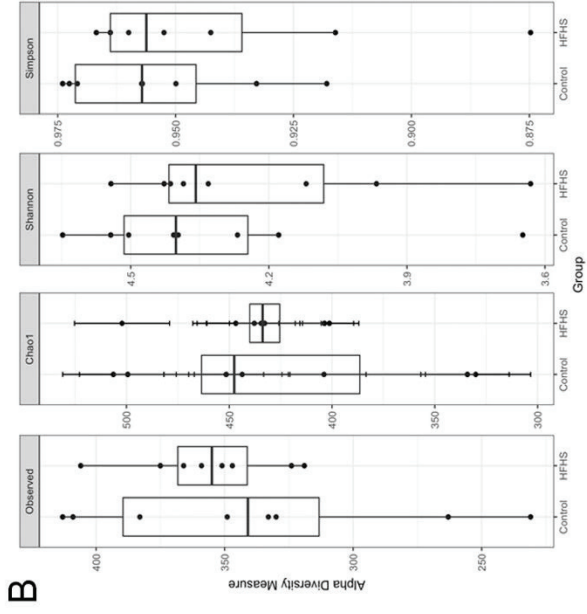
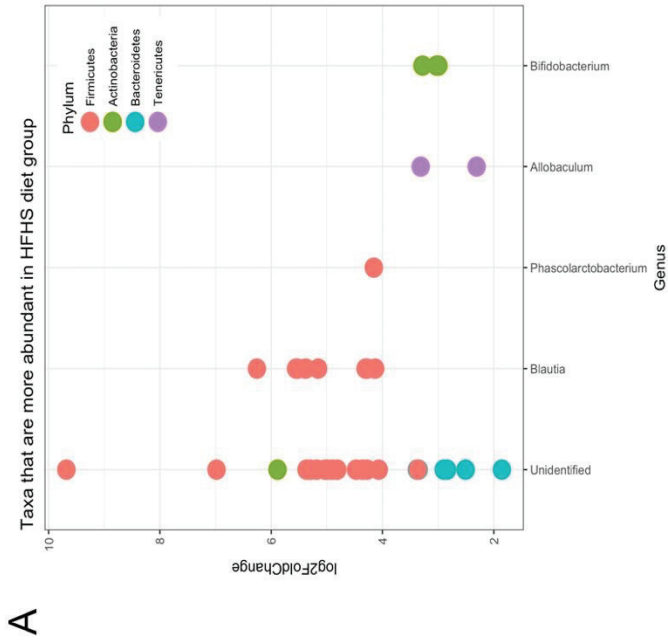


Figure 3

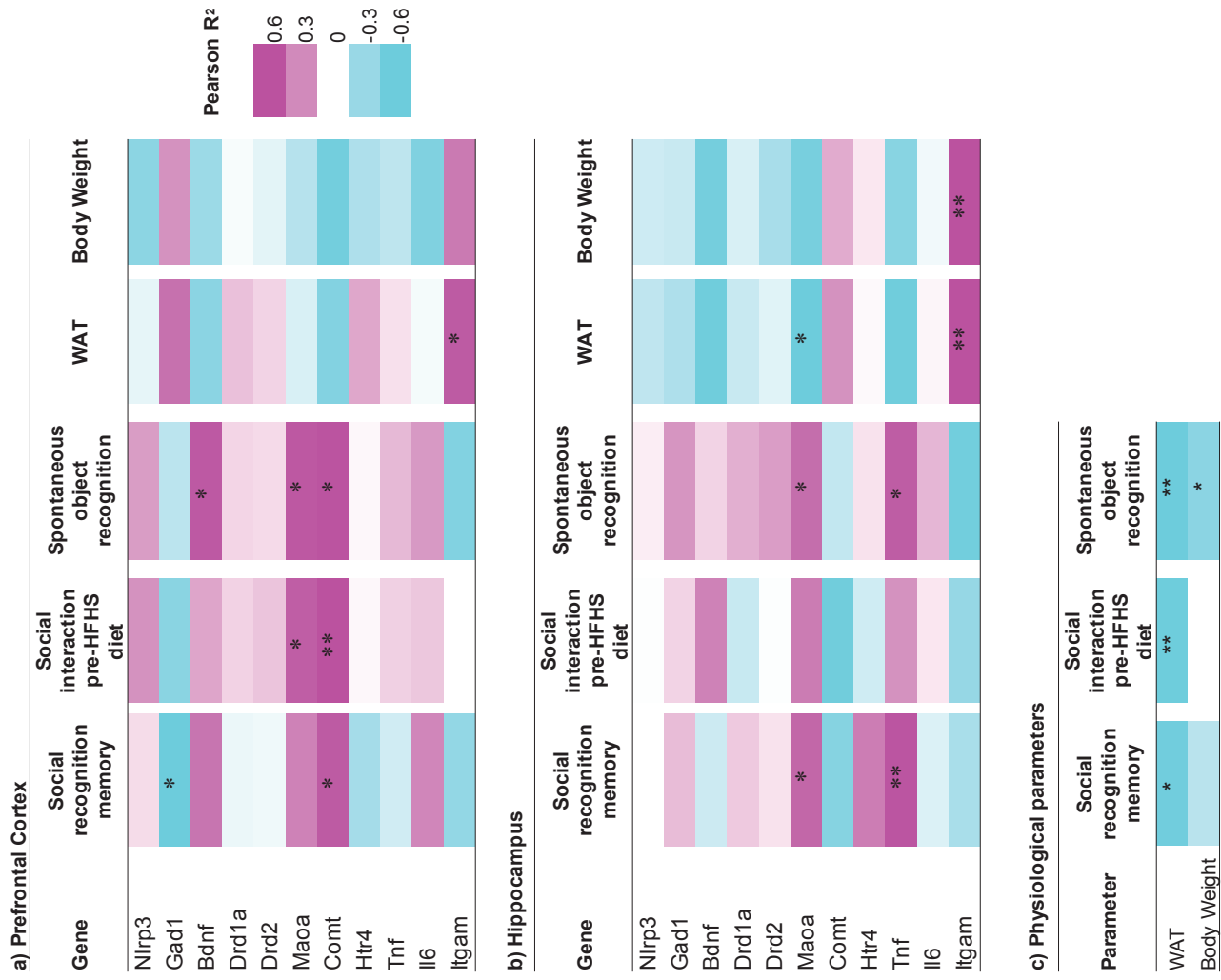


Figure 4

Supplementary Information**Supplementary Table 1.** Reference sequences of genes of interest

Gene	Gene symbol	Accession number
NLR family, pyrin domain containing 3	Nlrp3	NM_001191642
Glutamate decarboxylase 1	Gad1	NM_017007
Brain-derived neurotrophic factor	Bdnf	NM_012513
Dopamine receptor D1	Drd1a	NM_012546
Dopamine receptor D2	Drd2	NM_012547
Monoamine oxidase A	Maoa	XM_001058993, XM_343764
Catechol-O-methyltransferase	Comt	NM_012531
5-hydroxytryptamine receptor 4	Htr4	NM_012853
Tumour necrosis factor-alpha	Tnf	NM_012675
Interleukin 6	Il6	NM_012589
Integrin, alpha M	Itgam	NM_012711

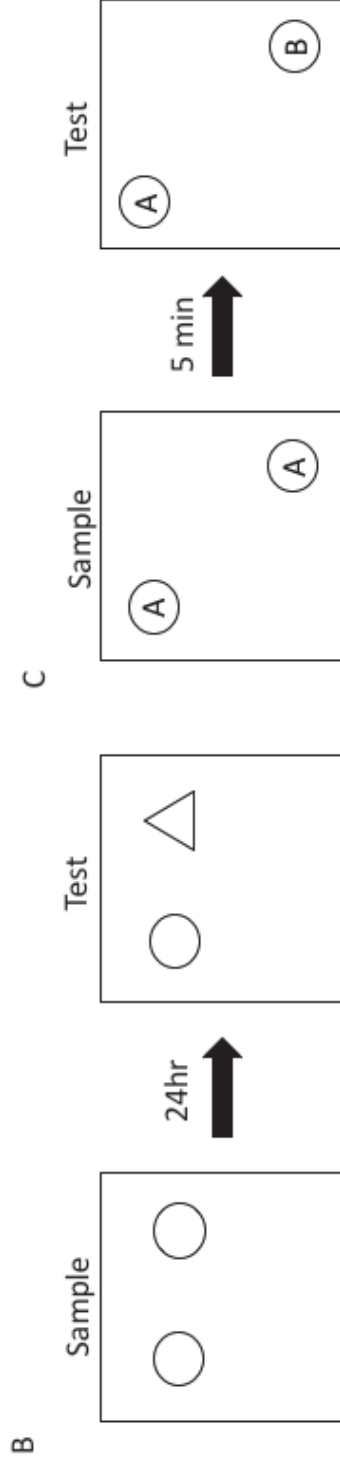
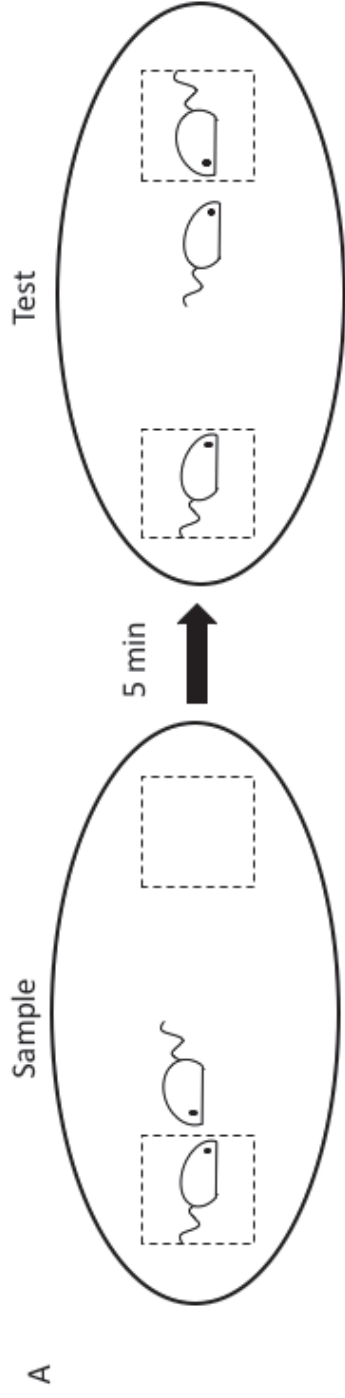
Supplementary Table 2. Effect of diet type on physiological parameters (mean \pm SEM) measured after 28 days of diet exposure. * indicates $P \leq 0.05$.

Diet group	Body Weight /g	rpWAT/g	gnWAT/g	Liver score	Liver weight /g
Control	318.6 (8.3)	3.3 (0.4)	2.2 (0.2)	0	13.7 (0.5)
HFHS	366.4 (20.1)*	5.0 (0.6)*	3.6 (0.6)*	1*	16.5 (1.4)*

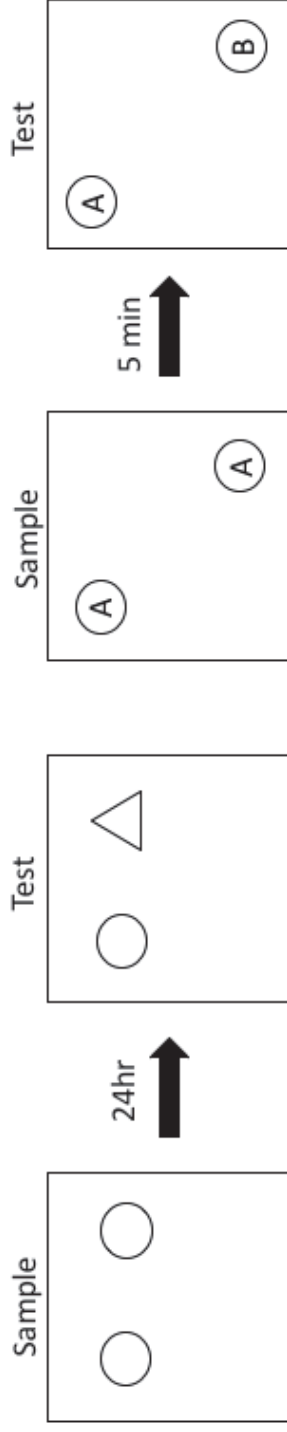
Supplementary Table 3. Analyses of differences in relative abundance differences in faecal microbiota of HFHS group relative to Control ($q < 0.05$).

Phylum	Class	Order	Family	Genus	log2foldchange	q
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	5.89	<0.01
			Bifidobacteriaceae	Bifidobacterium	3.28	0.02
					3.03	0.04
					3.00	0.03
Bacteroidetes	Bacteroidia	Bacteroidales	Unspecified	Unspecified	2.84	<0.01
					3.35	0.01
					2.90	0.03
					3.38	0.05
					1.86	0.05
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	2.51	0.05
					5.16	0.01
					4.30	0.04
					5.38	0.00
					5.53	0.00
					4.13	0.03
					6.26	0.00
					5.55	0.00
					4.28	0.04
					3.37	0.03
	4.28	0.02				
	4.99	0.00				
	5.03	0.01				
	5.00	0.01				

			5.37	0.01
	Lachnospiraceae	Unspecified	4.36	0.03
			4.81	0.05
	Ruminococcaceae	Unspecified	5.18	0.00
			4.90	0.01
			6.98	0.00
	Veillonellaceae	Phascolarctobacterium	4.16	0.02
			4.47	0.02
			4.07	0.04
	Unspecified	Unspecified	5.29	0.02
			9.68	0.00
Tenericutes	Erysipelotrichi	Erysipelotrichales	2.31	0.04
		Erysipelotrichaceae	3.31	0.00
		Allobaculum		



C



Supplementary Figure 1 A) Schematic of social memory testing procedure. B) Schematic of novel odour recognition procedure, where A and B are different odours contained in identical containers. C) Schematic of novel object recognition procedure.