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

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Abstract

Excessive consumption of high fat and high sugar (HFHS) diets are known to alter reward processing and aspects of behaviour, and change microbiota profiles. Studies in gnotobiotic mice also provide evidence that gut microorganisms influence social behaviour. To further investigate these interactions, the impact of intermittent access to a HFHS diet on social behaviour, gene expression and microbiota composition was examined. Rats were permitted intermittent daily access (2h / day) to a palatable HFHS diet for 28 days across the adolescent period. Social interaction, social memory and novel object recognition were assessed during this period. Following testing, RT-PCR was conducted on hippocampal and prefrontal cortex (PFC) samples. 16S ribosomal RNA amplicon sequencing was used for identification and relative quantification of bacterial taxa. Reduced social interaction behaviours, and impaired social memory and novel object recognition were observed in HFHS diet rats. Reduced levels of monoamine oxidase A (Maoa), catechol-O-methyltransferase (Comt) and brain derived neurotrophic factor (Bdnf) mRNA were observed in the PFC of HFHS diet rats. The relative abundance of a number of specific taxa differed significantly between the two diet groups, in particular, *Lachnospiraceae* and *Ruminoccoceae* bacteria, which also predicted social behaviours, novel object recognition performance and Maoa expression. This is the first study to show that limited daily access to HFHS diet alters social behaviour and cognition in rats. Furthermore, behavioural changes are associated with alterations to cortical gene expression of enzymes involved in monoamine synthesis and neuroplasticity, and microbiota profiles predicted diet-induced changes to behaviour and gene expression.

List of abbreviations

ACTB - Actin, beta
ANOVA – Analysis of variance
BDNF – Brain derived neurotrophic factor
CD11b - cluster of differentiation molecule 11b
Comt - catechol-O-methyltransferase
DRD1 - Dopamine receptor D1
DRD2 - Dopamine receptor D2
FB ratio - *Firmicutes* to *Bacteroidetes* ratio
GAD1 - Glutamate decarboxylase 1
GF - Germ free
gnWAT – Gonadal white adipose tissues
HFHS – High fat and high sugar
HTR4 - 5-hydroxytryptamine (serotonin) receptor 4, G-coupled
IL6 - Interleukin 6
ITGAM - Integrin, alpha M
PFC – Prefrontal cortex
MANOVA - Multivariate analysis of variance
Maoa – Monoamine Oxidase
mRNA – messenger ribonucleic acid
Nlrp3 - NLR family, pyrin domain containing 3
OTU - Operational taxonomic units
P – Postnatal day
PCA - Principle Component Analysis
PLS-DA - Partial least-squares discriminant analysis
QIIME - Quantitative Insights Into Microbial Ecology
rpWAT – Retroperitoneal white adipose tissues
Tnf-α - Tumour necrosis factor alpha

1. Introduction

The global rate of obesity is rapidly growing, and the incidence of overweight and obesity is particularly increasing amongst young people and children (Ogden et al., 2014). Epidemiological studies indicate that adolescents and young adults most frequently consume hypercaloric high fat and high sucrose (HFHS) "junk" foods (Braithwaite et al., 2014), increasing negative health outcome risks.

Chronic exposure to hypercaloric diets causes multiple changes to behavioural processes and neuromodulation within the brain, which has been linked to decreased dopamine turnover in the mesolimbic system (Davis et al., 2008). However, the effects of chronic HFHS diet consumption may mask critical periods in development whereby intermittent consumption of this diet leads to lasting biological and behavioural changes in adulthood. Indeed, emerging data suggests that adolescence may be a sensitive period for susceptibility to diet-induced behavioural changes in mood (Baker et al., 2017), reward seeking (Naneix et al., 2017; Reichelt, 2016) and cognition (Labouesse et al., 2017).

Beyond a role in cognition, recent studies have suggested links to obesity, hypercaloric diet consumption and changes in social behaviour in rodents (Carvalho et al., 2016; Teixeira et al., 2017; Yaseen et al., 2018). High fat diet consumption increased social interaction in adult male mice, but impaired recognition memory of a novel mouse (Takase et al., 2016), and social recognition has also been recently shown to be altered in juvenile rats following short term exposure to high fat diets (Yaseen et al., 2018). Social play is a characteristic adolescent social behaviour that decreases into adulthood (Trezza et al., 2010). Social play was reduced following neonatal overfeeding, suggesting that nutrition may

impact the expression of this behaviour (Carvalho et al., 2016); however litter size manipulations may contribute to altered social repertoires.

There are links between the brain regions that support social behaviour and those that are altered by HFHS diet. The prefrontal cortex (PFC) matures across adolescence (Spear, 2000) and represents a critical period of vulnerability to diet evoked cognitive deficits (Baker et al., 2017; Reichelt and Rank, 2017). The PFC has an important role in social processing (Bicks et al., 2015; Kolb, 1974), and appropriate maturation is fundamental for the development of social cognition (Kim et al., 2015). Experimental evidence highlights that the rodent homologue of the medial PFC and the hippocampus are important for social behaviour, including social memory and sociability (Kogan et al., 2000; Okuyama et al., 2016; Rudebeck et al., 2007). Aspects of social interaction are rewarding (Trezza et al., 2010), and the increased dopamine neurotransmission and refinement of reward-associated neural connections within the PFC across adolescence is proposed to invigorate this behaviour.

Moreover, previous observations noted that intermittent access to a HFHS diet (Baker and Reichelt, 2016), or high fat diet (Labouesse et al., 2017) across adolescence evoked PFC dysregulation, complementing research demonstrating that PFC excitation/inhibitory imbalance underpins social deficits (Selimbeyoglu et al., 2017), thus providing rationale for exploring the impact of an intermittent HFHS diet on social behaviours. Restricted access to palatable foods has been shown to impact on reward neurocircuitry (Bocarsly et al., 2014; Furlong et al., 2014), and furthermore allows behavioural examination both immediately following palatable food consumption and when animals have not had access to palatable foods.

Diet influences the gut microbial composition (Albenberg and Wu, 2014) and alterations to gut flora has been linked to changes in cognition, mood and behaviour (Desbonnet et al., 2015; Frohlich et al., 2016). Studies utilising germ-free (GF) mice demonstrate that the presence, composition, and functionality of the gut microbiota is crucial for normal social behaviours, which are reduced in GF mice (Desbonnet et al., 2015). Moreover, GF mice and antibiotic-induced gut dysbiosis rodent models have demonstrated associations between disruption of the gut microbial community and cognitive, social and emotional alterations (Desbonnet et al., 2015; Frohlich et al., 2016).

To further the evidence that intermittent exposure to a HFHS diet during the juvenile developmental phase alters cognitive control and neurotransmitter systems within the brain, we examined the effects of intermittent HFHS food consumption on social interaction and social memory in young rats. We highlight putative molecular pathways by examination of the expression of genes associated with neuroplasticity, monoamine signalling, and neuroinflammation in the PFC and hippocampus, and faecal microbiota composition to explore diet-induced alterations. Spontaneous novel object recognition and odour recognition memory were examined to assess HFHS diet effects on memory and olfaction. Exploratory analyses through linear modelling were performed to determine associations between faecal microbiota composition, behaviour and cortical gene expression.

2. Methods

2.1. Animals

Male (*n*=32) albino Sprague Dawley rats (Animal Resources Centre, Western Australia) arrived at postnatal day (P)21 (~50g) and were housed in groups of four in climate

(21°C ± 2°C; humidity 55 ± 5%) and light (12 h cycle lights on at 07:00h) controlled colony room. Standard laboratory rat chow (Meat Free Rat and Mouse Diet, Specialty Feeds, Western Australia; energy composition of 14 KJ/g, 23% protein, 12% fat, 65% carbohydrates) and water was available *ad libitum* throughout the experiment. Behavioural tests were performed between 08:00 and 14:00h and procedures were approved by the Animal Care and Ethics Committee at RMIT University.

2.2. Diet administration

Rats were allocated to diet conditions: Control (chow fed, *n*=8) or HFHS condition (*n*=8), or were allocated as age/weight matched sample for social memory and social interaction (*n*=16). Body weights were standardized in all treatment groups prior to the commencement of the diet (Control: 75.5 ± 2.0g; HFHS: 76.4 ± 2.0 g), and rats were handled for 7 days prior to manipulations. Group-housing was used to negate social isolation stress (Skelly et al., 2015). Rats in the HFHS diet condition were provided with 2 h daily homecage access (between 09:00-11:00h) to semi-pure HFHS pellets (Specialty Feeds, Western Australia, SP04-025; 18.4kJ/g digestible energy; composed of 20% fat (lard), 39.6% sucrose, 19.4% protein, providing 36% energy from lipids and 55% from sucrose), in addition to *ad libitum* chow (Specialty Feeds, Western Australia, Meat Free Rat and Mouse Diet, energy composition of 14 KJ/g, 23% protein, 12% fat, 65% carbohydrates) and water access. Consumption of HFHS diet was calculated from the weight (g) between the HFHS pellets allocated and that collected after 2 h access per cage. Body weight was recorded at baseline before the diet began, and thereafter twice per week. Chow consumption over a 24 h period was measured twice per week in conjunction with HFHS pellet intake to calculate total energy intake per cage of four rats (Del Rio et al., 2016).

182 **2.3. Behavioural analysis**

183 A timeline of the general experimental procedures is illustrated in Figure 1A. Diet
184 administration began on P28, coinciding with definitions of adolescence in male rats (Spear,
185 2000). Behavioural tests were conducted in a room illuminated to 30 lux, rats were assessed
186 for social interaction, social memory, social odour preference, novel object recognition and
187 odour recognition memory. All behavioural data were scored by an observer who was blind
188 to the group allocations using ODLog (version 2.7, Macropod Software, Australia).
189
190 2.3.1. *Social interaction*

191 Social interaction tests were conducted in a square test arena (dimensions: 50 cm [length] x
192 50 cm [width] x 60 cm [height]) constructed from black Perspex. A camera mounted above
193 the test area recorded all the social interaction tests to a computer for subsequent scoring. All
194 rats were habituated to the arena 24 h prior to testing by being placed individually into the
195 arena for 10 minutes.
196 Prior to social interaction testing, rats were isolated from their cage mates in individual
197 holding cages for 15 minutes. In the social interaction test, one rat from either the control or
198 HFHS diet condition rat was placed in the arena with an unfamiliar partner matched for body
199 weight (+/- 10g). Test sessions were 10 min duration. To differentiate between animals, one
200 rat was marked on its back with a black odourless fabric pen marker 24 h prior to testing.
201 The two rats were placed into the test arena simultaneously so that they were facing each
202 other in opposing corners. Rats in the HFHS diet condition were tested 1 h after access to the
203 HFHS pellets “post”, and 23 h after HFHS pellet access “pre”, counterbalanced across days
204 and animals. Between tests the arena was cleaned with 70% ethanol to eliminate odour cues.
205 As social behaviour in rats has been shown to depend on the playfulness of its partner,
206 both animals in a sample pair were considered as one experimental unit (Trezza et al., 2010).

Pinning and pouncing frequencies were quantified and considered the most characteristic parameters of social play behaviour in rats. Social play behaviours usually occur very rapidly and they are of short duration thus, individual frequency was scored. Videos were scored to measure i) the total time (sec) spent in social interaction, ii) frequency of social investigation behaviour (sniffing, licking, grooming), iii) frequency of social play behaviour (pinning, pouncing), iv) frequency of aggressive-like behaviour (rump biting, boxing, overt physical harm).

2.3.2. *Social memory*

Social memory was tested in two phases (see Fig 1D). As HFHS rats showed differences in social interaction pre HFHS food consumption, social memory testing and other behavioural tests were conducted after HFHS access to ensure that any memory deficits observed were not due to reduced social contact in the HFHS diet rats. Social memory tests were conducted in a circular arena (dimensions: 100 cm diameter, 50 cm high) constructed from grey Perspex. The arena contained two wire chambers with plastic bases (dimensions: 18 cm [length] x 20 cm [width] x 22 cm [height]). The wires were interspaced 1cm apart which allows the test rats to interact with the novel sample rats but not physically contact them. A camera mounted above the test area recorded the tests as described above. Control and HFHS diet rats were habituated to the testing apparatus 24 h prior to testing by being placed individually into the arena with the empty chambers for 10 minutes. Sample rats were also habituated to the individual chambers for 10 minutes 24 h prior to testing.

Social memory was tested in two phases. In Phase 1, rats were placed in the arena for 5 min with one sample rat in a chamber and the other chamber empty. Time exploring the chamber containing the sample rat versus the empty chamber was considered a measure of sociability (Crawley et al., 2007). The experimental rat was then removed and placed into

individual holding cages for a 5 min inter-trial interval (ITI) period. In Phase 2, the arena contained the original sample rat (familiar) in a chamber and the previously empty chamber contained a novel rat. The experimental rat was returned to the arena to explore for a 3 min period. Between test phases the arena was cleaned with 70% ethanol to eliminate odour cues. Videos were scored to measure the duration of time the rat spent exploring the chambers during each phase. Sociability was quantified as the time spent exploring the chamber containing the sample rat as opposed to the empty chamber, and social recognition memory was measured as the time spent in proximity to the chamber containing the novel rat versus the familiar sample rat.

2.3.3. Social odour preference

The chambers used for social recognition were filled with soiled bedding from a cage of young male rats (approximately 5 weeks of age) housed in an adjacent holding room, or clean bedding. Rats were allowed to freely explore the arena for 5 min and the amount of time spent exploring empty chambers containing either soiled or clean bedding was recorded.

2.3.4. Odour memory

Odour memory was conducted in the square test arena (as described in 2.3.1). Identical cylindrical stainless steel containers (10cm [height] x 6cm [width]) with perforated stainless steel lids were filled with corncob bedding and then scented with 3 ml of peppermint or almond extract (Queen, Australia) to serve as odour stimuli (see Fig 1E). The odour memory test consisted of 2 phases – sample and test. Pilot testing determined that these odours were equally explored by the rats. During the sample phase two of the same scented containers were placed in opposite corners of the arena. The rat was allowed to freely explore the arena for 5 min. The rat was then removed from the arena and placed in a holding cage for a 5 min

retention period. The arena was thoroughly cleaned with 70% ethanol and one of the scented containers was replaced with an identical container filled with a novel odour. The rat was then returned to the arena for a 3 min test phase. The duration of time the rat spent exploring each of the odour containers during each phase was measured.

2.3.5. Object recognition memory

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

2.4. Sample collection

Rats were anaesthetised (sodium pentobarbital 100 mg/kg, intraperitoneal), brains removed and the PFC and hippocampus (composed of dorsal and ventral poles) dissected and snap frozen in liquid nitrogen and stored at -80˚C for analysis by RT-PCR. Retroperitoneal and gonadal white adipose tissues (rpWAT; gnWAT) and liver were dissected and weighed. Livers were visually scored for markers of hepatic steatosis based on previous criteria (Velkoska et al., 2010). One faecal bolus was collected from the terminal caecum, snap frozen and stored at -80°C prior to microbiota analysis.

2.5. *Quantitative RT-PCR*

RNA was extracted using Tri-Reagent (Sigma-Aldrich) and RNeasy Mini kit (Qiagen), and quantity and purity of RNA determined by nanodrop. RNA was converted to cDNA (RT^2 First Strand Kit Qiagen). Gene expression was quantified by Custom RT^2 Profiler PCR Arrays (Qiagen) with RT^2 SYBR Green Mastermix (Qiagen, Australia), real-time PCR was then performed using a QuantStudioTM 7 Flex Real-Time PCR System (Applied Biosystems). Genes of interest were NLR family, pyrin domain containing 3 (Nlrp3), Glutamate decarboxylase 1 (Gad1), Brain-derived neurotrophic factor (Bdnf), Dopamine receptor D1 (Drd1), Dopamine receptor D2 (Drd2), Monoamine oxidase A (Maoa), Catechol-O-methyltransferase (Comt), 5-hydroxytryptamine (serotonin) receptor 4, G-coupled (Htr4), Tumour necrosis factor alpha (Tnf-α), Interleukin 6 (Il6), Integrin, alpha M (Itgam) and Actin, beta (Actb) from Qiagen (See Supplementary Table 1 for reference sequences). Analysis of relative gene expression was normalized to the housekeeping gene beta actin, via the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001).

2.6. 16S rRNA gene amplicon sequencing and bioinformatics

Total DNA was isolated using the Bioline ISOLATE Faecal DNA Kit (Bioline). PCR was performed using Q5 DNA polymerase (New England Biolabs) with a primer set selected to amplify V3-V4 region of 16S rRNA gene (forward: ACTCCTACGGGAGGCAGCAG and reverse: GGACTACHVGGGTWTCTAAT). Sequencing was performed on an Illumina MiSeq instrument (2 x 300bp paired-end sequencing), following the method detailed by Fadrosh et al. (2014). Sequences were joined in Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 (http://qiime.org) using the fastq-join method. Maximum allowed percent differences within the overlapping region was zero. Sequences were de-multiplexed using the QIIME split library protocol, keeping only sequences with Phred quality score higher than 20. The dataset was inspected for chimeric sequences using Pintail (Ashelford et al., 2005).

Operational taxonomic units (OTUs) were clustered at 97% sequence identity using UCLUST (Edgar, 2010). Taxonomic assignments were performed against the GreenGenes database (DeSantis et al., 2006). OTUs with a relative abundance of less than 0.01% were removed.

2.7. ***Statistical analyses***

2.7.1. Behaviour, physiological parameters and brain mRNA expression

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

Social recognition memory and novel object recognition performance were converted to Exploration Ratios [Ratio = Time(novel-familiar) / Time(novel+familiar)] to permit bivariate analysis using correlations (Pearson's R, 1-tail) to examine associations between mRNA expression and behaviours found to differ between diet groups. Liver scores were analysed using the Kruskal-Wallis test. Data were analysed with IBM SPSS Statistics 24, GraphPad Prism 7 and R.

2.7.2. Microbiota

Visualisation, alpha diversity and distance measures of microbiota were conducted using the R packages *phyloseq* and *MixOmics*. Data were total-sum scaled (i.e. relative abundance of operational taxonomic units – OTUs) and centre-log ratio transformed where

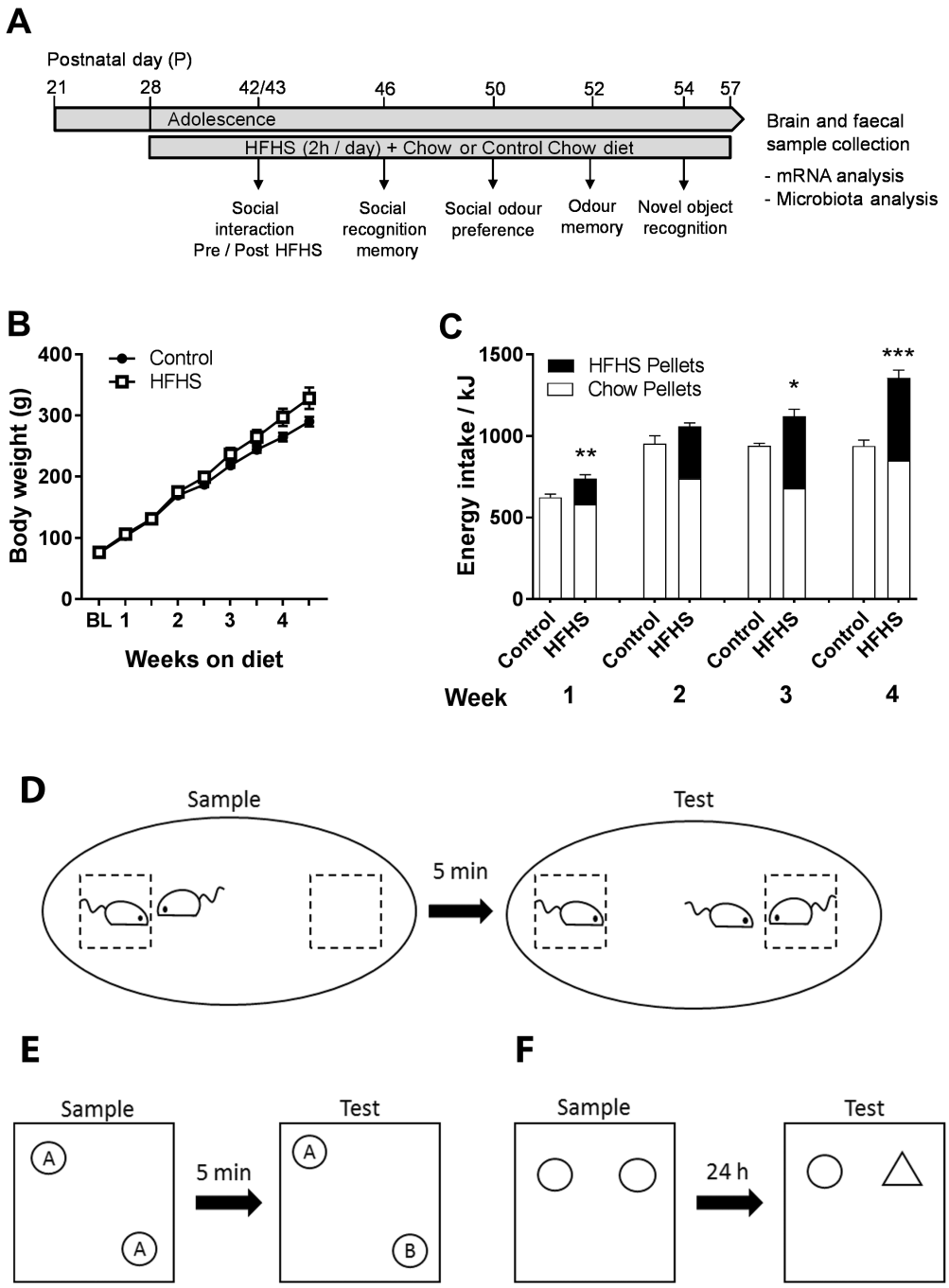
appropriate. The *DESeq2* package was used to undertake differential abundance testing (Love et al., 2014), and multivariate analysis of variance (MANOVA) was used to test associations between *Firmicutes* to *Bacteroidetes* (FB) ratio, behaviour and gene expression.

Significance for differential abundance analyses was assessed on the basis of a threshold q-value of 0.05 (i.e. p-value adjusted using the False Discovery Rate approach; Benjamini et al. (2001)). Bivariate correlations were calculated using Pearson's R, 2-tailed.

3. Results

3.1. Body Weight, energy consumption and physiological measurements

All rats gained weight across the experiment, however HFHS diet rats gained more weight than controls (Fig 1B, time x diet group $F(8,112)=5.07$, $P<0.001$). Overall, rats consumed increasing amounts of energy across the 4 week period ($F(3,18)=81.4$, $P<0.001$), and HFHS diet rats consumed more energy than control rats (Fig 1C, diet group x time $F(1,6)=10.8$, $P<0.001$). At the experimental end point, HFHS diet rats were heavier than chow fed animals ($F(1,14)=4.516$, $P=0.05$), had greater rpWAT ($F(1,14)=5.54$, $P=0.034$), gnWAT ($F(1,14)=4.71$, $P=0.048$), and increased liver scores ($U=5$, $P<0.01$) (Supplementary Table 2).



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349 ***Figure 1.*** A) Timeline of experimental procedures showing the ages of the rats at each
350 behavioural test (conducted at the same age in all animals) and at sacrifice. Social interaction
351 testing was conducted both before and after access to palatable HFHS food in the HFHS rats.
352 As HFHS rats showed differences in social interaction pre HFHS food, social memory
353 testing, and other behavioural tests were conducted after HFHS access, to ensure that any

memory or behavioural deficits observed were not due to reduced social contact in the HFHS diet rats. B) Mean body weights of control and HFHS rats across the 4-week diet exposure period. C) Mean energy consumption (kJ) per cage of rats across the 4-week diet exposure period. D) Schematic of social memory testing procedure. E) Schematic of novel odour recognition procedure, where A and B are different odours contained in identical containers. F) Schematic of novel object recognition procedure. Error bars represent +SEM. * indicates $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.2. Effect of HFHS diet on social interaction

To assess the effect of what and when HFHS diet consumption has upon social behaviour, we tested the total social exploration time one hour prior ("pre") or one hour following ("post") HFHS food access. Social interaction duration did not differ in the control (normal chow fed) animals. However, HFHS diet rats spent less time engaged in social interaction "pre" HFHS food access, compared to "post" HFHS food access (diet access x diet group $F(1,14)=5.66$, $P<0.05$, effect of diet group "pre" $F(1,14)=9.271$, $P<0.01$, but not "post" $F<1$, Fig 2A). The microstructure of social behaviour was also examined. Social investigation frequency was increased in the HFHS rats post-consumption (diet access x diet group $F(1,14) = 8.6$, $P<0.05$; HFHS $F(1,14)=21.59$, $P<0.001$, control $F<1$, Fig 2B). No differences were observed in the frequency of social play behaviours (Fig 2C), and aggressive behaviours were not observed. Together, this data suggests that for those rats on the intermittent HFHS diet, social motivation is decreased the longer the period is since diet consumption.

3.3. Effect of HFHS diets on social recognition memory

Mouse social behaviour has been typically examined using the 'three-chamber' social approach test. We adapted this protocol for use in rats to assay whether changes in social recognition memory could be altered by HFHS diet. During the social approach phase of the sociability test (Fig 2D), both control and HFHS rats preferentially explored the novel rat, "sample", compared to the empty cage ($F(1,14)=275.5$, $P<0.001$), no significant between group or interaction effect, Fs<1). However, HFHS rats showed impaired social recognition, exploring the familiar and novel rat equally, contrasting to the strong preference of control rats to explore the novel rat (chamber x diet group $F(1,14)=39.15$, $P<0.001$, control $F(1,14)=109.3$, $P<0.001$, HFHS $F(1,14)=2.6$, $P=0.13$, Fig 2E). Exploration ratios calculated from the test data [Mean (SEM): Control = 0.80 (0.03); HFHS = 0.56 (0.03)] differed significantly between groups ($F(1,14)=33.2$, $P<0.001$).

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

To confirm that the lack of social recognition memory in the HFHS rats was not due to a lack of olfactory sensitivity, we tested their ability to discriminate between clean and soiled bedding and between two non-social odours. Control and HFHS diet rats preferentially explored the chamber containing a social odour ($F(1,14)=217.8$, $P<0.001$, Fig 2F). During odour recognition testing, control and HFHS diet rats preferentially explored the novel odour container, demonstrating odour recognition memory (odour x diet group $F(1,14)=3.0$, $P=0.105$, Fig 2G). Together, HFHS rats were unimpaired in odour discrimination, suggesting that the social recognition deficit cannot be explained by a lack of sensitivity to social olfactory cues.

3.5. Effects of HFHS diet on novel object recognition

To confirm a role in cognition, we tested HFHS diet rats on their ability to explore novel compared to previously explored objects. Control rats showed intact object recognition memory by preferentially exploring the novel object; however, HFHS rats explored the familiar and novel objects equally, indicating impaired object recognition (object x diet group, $F(1,14)=50.7$, $P<0.001$; control $F(1,14)=120.5$, $P<0.001$, HFHS $F<1$, Fig 2H). Exploration ratios calculated from the test data [Mean (SEM): Control = 0.73 (0.01); HFHS = 0.52 (0.02)] differed significantly between groups ($F(1,14)=60.8$, $P<0.001$).

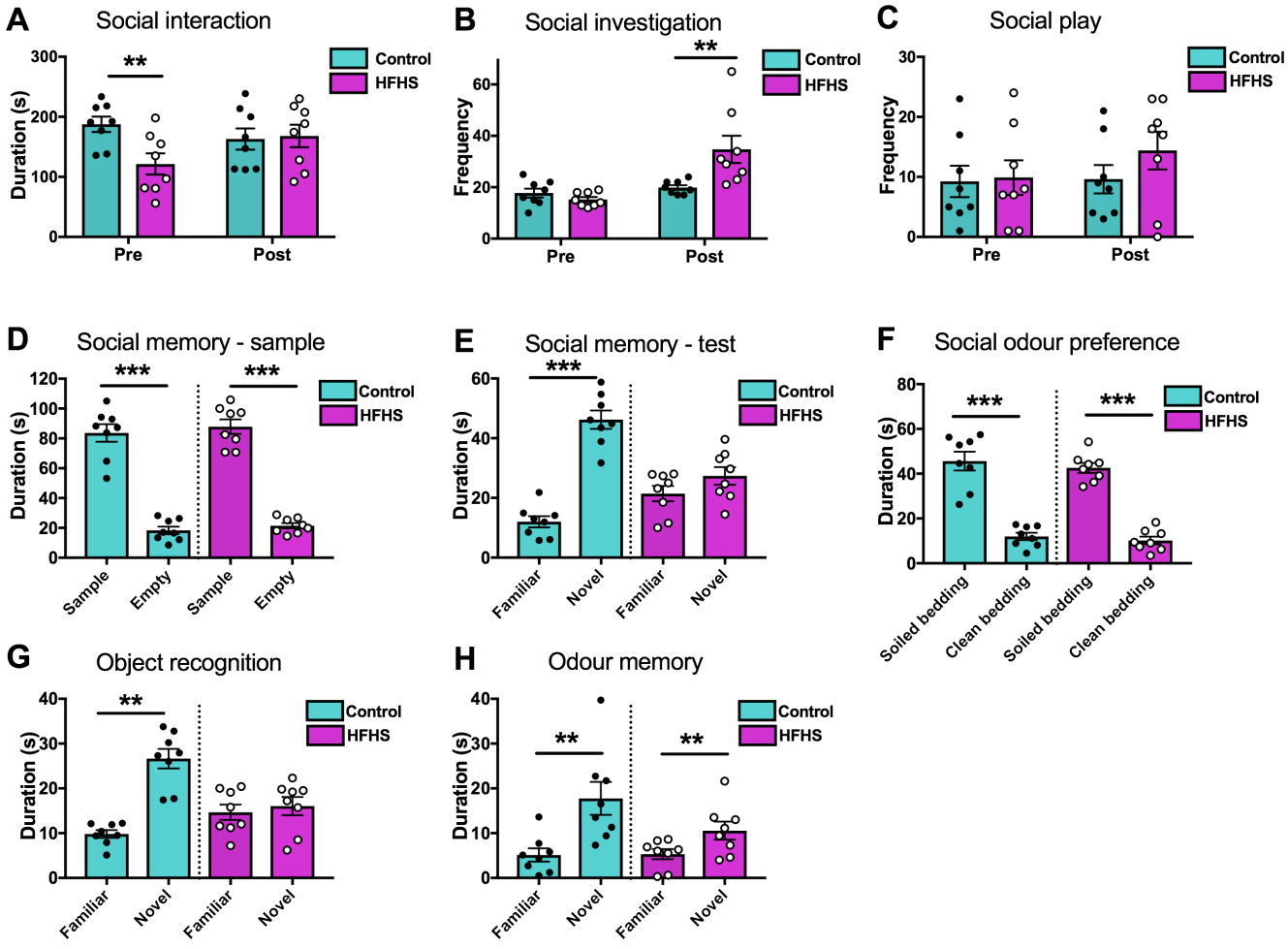


Figure 2. Social behaviours between control / HFHS diet exposed rats and a novel weight / age matched conspecific. HFHS diet rats were tested either 23h after HFHS pellet access "pre" or 1h after access to the HFHS pellets "post", A) Total duration of social contact

between rats, B) frequency of social interactions, and C) frequency of social play. Performance of HFHS diet and control rats in social recognition memory - D) exploration times of the chamber containing the sample rat "sample" and empty chamber "empty" during the sample phase of social memory testing, E) exploration times of the chamber containing the familiar sample rat and chamber containing a novel sample rat. F) Exploration time of chambers containing soiled bedding "social odour" or clean bedding. G) Novel odour recognition performance in control and HFHS diet rats during the test phase following a 5 min delay. H) Novel object recognition performance during the test phase following a 24h delay**.** Error bars represent +SEM. ** $P<0.01$. Error bars represent +SEM. * indicates $P\leq0.05$, ** $P<0.01$, *** $P<0.001$ between groups comparisons.

3.6. Diet effects on PFC and hippocampal mRNA expression

To determine the whether short, intermittent periods of exposure to HFHS diet changed transcript expression within two brain regions associated with social behaviour, we quantified mRNA expression of genes related neuroplasticity, dopamine and monoamine signalling and inflammation (Table 1). We found the majority of transcript changes occurred in the prefrontal cortex. Consumption of the HFHS diet correlated with reduced expression of genes encoding enzymes involved in monoamine degradation, Comt and Maoa. The HFHS diet fed rats had reduced Maoa expression in the PFC ($F(1,13)=8.50$, $P<0.05$) and hippocampus ($F(1,14)=6.89$, $P<0.05$); Comt expression was reduced in the PFC ($F(1,14)=19.0$, $P<0.001$), but not the hippocampus. The neuroplasticity associated gene Bdnf was reduced in the PFC of HFHS consuming rats ($F(1,13)=4.99$, $P<0.05$). Group differences in PFC expression of the neuroinflammatory genes Nlrp3 ($F(1,14)=4.41$, $P=0.056$) and Il6 ($F(1,14)=4.24$, $P=0.06$) trended towards significance (Table 1).

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

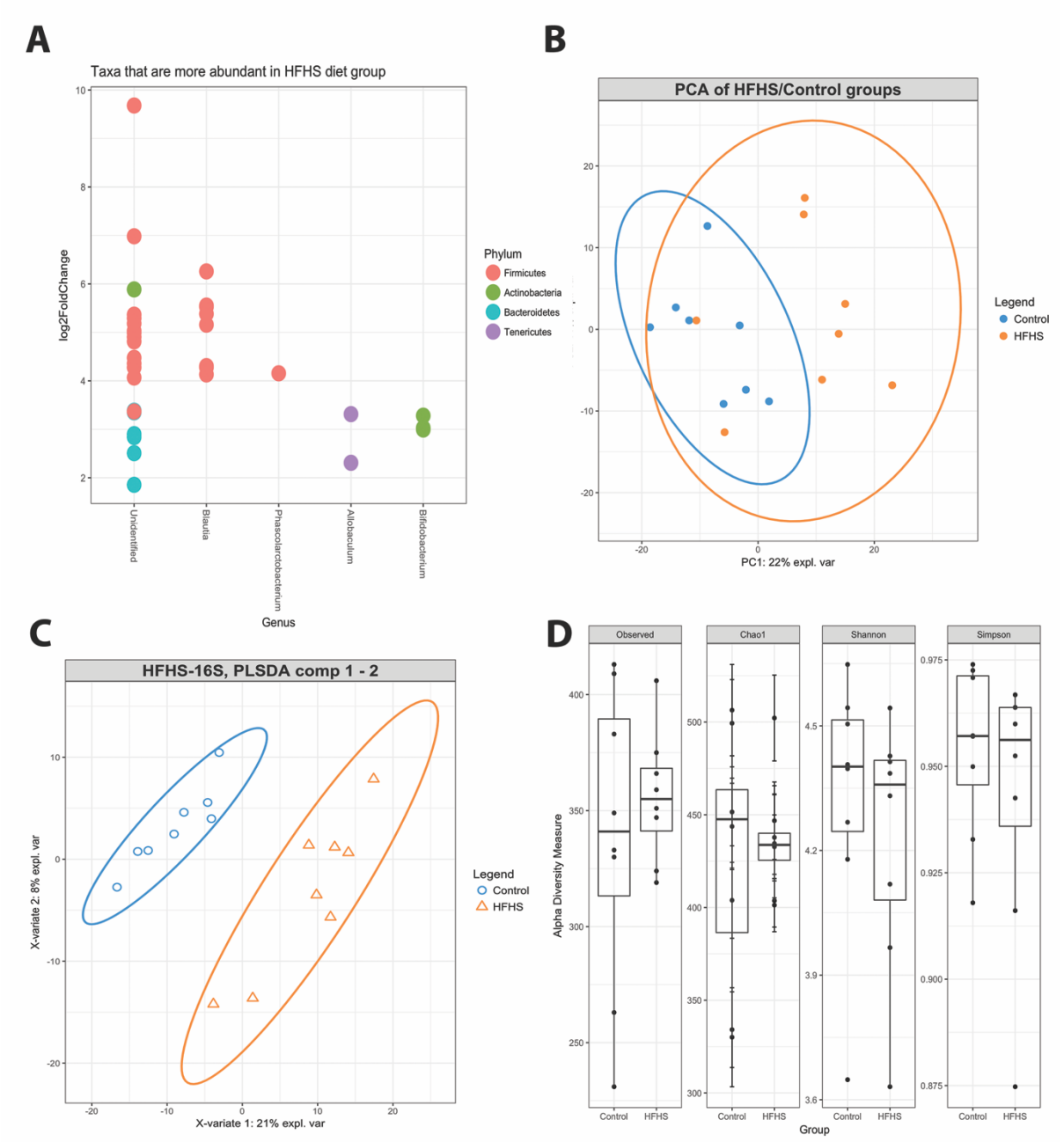
Gene	Prefrontal cortex			Hippocampus		
	Control	HFHS	*p*-value	Control	HFHS	*p*-value
Neuroplasticity						
Gad1	0.84 (0.04)	0.94 (0.07)	*n.s.*	1.00 (0.08)	0.94 (0.06)	*n.s.*
Bdnf	1.00 (0.10)	0.72 (0.03)*	*0.045*	1.00 (0.08)	0.96 (0.17)	*n.s.*
Dopamine receptors						
Drd1a	1.00 (0.23)	0.65 (0.13)	*n.s.*	1.00 (0.11)	0.95 (0.09)	*n.s*
Drd2	1.00 (0.32)	0.56 (0.15)	*n.s.*	0.93 (0.03)	0.85 (0.07)	*n.s.*
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)	*n.s.*
Serotonin receptor						
Htr4	0.85 (0.07)	0.79 (0.08)	*n.s.*	1.00 (0.05)	0.96 (0.04)	*n.s.*
Inflammation						
Tnf-α	1.00 (0.16)	0.79 (0.11)	*n.s.*	1.00 (0.21)	0.65 (0.06)	*n.s.*
Il6	1.00 (0.15)	0.62 (0.08)$^{\#}$	*0.060*	0.81 (0.14)	0.59 (0.11)	*n.s.*
Nlrp3	1.00 (0.06)	0.85 (0.03)$^{\#}$	*0.056*	1.00 (0.18)	1.03 (0.14)	*n.s.*
Itgam	1.00 (0.10)	1.09 (0.09)	*n.s.*	1.00 (0.11)	1.23 (0.19)	*n.s.*

441

442 *3.7. Microbiota composition and analysis*

443 The relative abundance of a number of specific taxa differed significantly between the
444 two diet groups as shown by DESEq2 analysis (Fig 3A, Supplementary Table 3). HFHS diet
445 increased bacteria from *Firmicutes* phylum *Clostridales* family, including *Lachnospiraceae*

446 (Genus *Blauta,* q<0.04; Genus Unspecified q<0.03), *Ruminoccoceae* (Genus Unspecified
447 q<0.01) and *Veillonellaceae* (Genus *Phascolarctobacterium* q<0.02). HFHS diet increased
448 bacteria from *Actinobacteria* phylum, family *Bifidobacteriaceae* (Genus *Bifidobacterium*
449 q<0.04), *Bacteroidetes* phylum, order *Bacteroidales* (Genus Unspecified q<0.05) and
450 *Tenericutes* phylum, order *Erysipelotrichaceae* (Genus *Allobaculum* q<0.05).
451
452 Unsupervised principle component analysis (PCA) revealed overlap between groups,
453 the first component explained 22% of variance; the second component 11% (Fig 3B). Partial
454 least squares discriminant analysis (PLS-DA) identified the two components that discriminate
455 maximally between the HFHS and control diet groups, showing a large proportion of
456 variance accounted for by the first component (21%) and a lesser degree by the second (8%),
457 this demonstrated significant separation of the microbiota community structure between the
458 groups (Fig 3C). Alpha diversity did not differ between the HFHS and control groups
459 measured by observed species, Chao 1, Shannon or Simpson indices (see Fig 3D; *Fs*<1).
460



461

462 ***Figure 3.*** A) Graphical depiction of DESEq2 analysis. Each coloured circle represents one
463 bacterial genus that was more abundant in the HFHS than control group ($q < 0.05$). Log2 fold
464 change refers to the difference abundance of the log2 values between diet groups for each
465 bacterial genus. B) Unsupervised Principle Component Analysis (PCA) plot of microbiome
466 samples from the HFHS diet (orange dots) and control diet (blue dots). The first component
467 explained 22% of variance; the second component 11%.

C) Partial least-squares discriminant analysis (PLS-DA) figure showing a large proportion of variance accounted for by the first component (21%) and a lesser degree by the second (8%). Each point represents a sample. D) No significant differences in alpha diversity of faecal microbiota between HFHS and control diet groups. Each panel represents one alpha diversity measure as follows: Observed = total number of OTU's observed; Chao1 = richness estimator (estimate of the total number of OTU's present in a community); Shannon and Simpson = microbial indexes of diversity. Boxes span the first to third quartiles; the horizontal line inside the boxes represents the median. Whiskers extending vertically from the boxes indicate variability outside the upper and lower quartiles, and the single black circles indicate outliers (all $P>0.05$).

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

Correlations were performed between individual representative values of specific behaviours (social interaction pre consumption of diet, social recognition and novel object recognition) that differed between diet group and biological measurements (adiposity and cortical gene expression).

A number of associations were observed, in particular positive correlations between PFC expression of Maoa and social interaction pre-HFHS diet and object memory. Full bivariate correlation analysis presented in Figure 4.

a) Prefrontal Cortex

Gene	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition	WAT	Body Weight
Nlrp3					
Gad1	*				
Bdnf			*		
Drd1a					
Drd2					
Maoa		*	*		
Comt	*	**	*		
Htr4					
Tnf					
Il6					
Itgam				*	

b) Hippocampus

Gene	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition	WAT	Body Weight
Nlrp3					
Gad1					
Bdnf					
Drd1a					
Drd2					
Maoa	*		*	*	
Comt					
Htr4					
Tnf	**		*		
Il6					
Itgam				**	**

c) Physiological parameters

Parameter	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition
WAT	*	**	**
Body Weight			*

Pearson R^2: 0.6, 0.3, 0, -0.3, -0.6

Figure 4. Heatmap of bivariate correlations (Pearson's R^2) between behavioural assays – social recognition memory, social interaction and novel object recognition performance, and a) prefrontal cortex gene expression, b) hippocampal gene expression and c) physiological parameters. *=P<0.05, **=P<0.01

492 A number of bivariate correlations between physiological parameters (WAT and
493 bodyweight) and gene expression were significant (Figure 4a and b). In particular, PFC and
494 hippocampal Itgam expression was positively correlated with WAT (PFC: r^2=0.52, P<0.05,
495 HPC: r^2=0.66, P<0.01) and bodyweight (HPC: r^2=0.67, P<0.01), and hippocampal Maoa
496 expression was negatively correlated with WAT (r^2=-0.45, P<0.05). Correlations between
497 physiological parameters (WAT and bodyweight) and behavioural performance were
498 observed (Figure 4c), in particular significant negative correlations between WAT and social
499 recognition memory (r^2=-0.56, P<0.05), social interaction pre-HFHS diet (r^2=-0.58, P<0.01)
500 and novel object recognition performance (r^2=-0.65, P<0.01).

501

502 Total WAT was significantly associated with PFC gene expression (F(1,12)=5.4,
503 P<0.05); specifically Tnf-α (adjusted r^2=0.41, P<0.01), Comt (adjusted r^2=0.23, P<0.05),
504 Maoa (adjusted r^2=0.29, P<0.05), and Bdnf (adjusted r^2=0.74, P<0.001). A number of
505 bivariate correlations between bodyweight and gene expression were significant (Figure 4a
506 and b) however these associations did not persist in multivariate linear modelling (overall
507 model F(1,12)=2.1, P=0.17). There were no significant associations between hippocampal
508 gene expression and body weight (F(1,13)<1). WAT weight predicted Il6 expression in the
509 hippocampus (F(1,13)=4.86, P=0.05).

510 Associations between hippocampal and PFC genes differentially expressed in control
511 and HFHS groups (see Table 1, Figure 4) and behavioural performance were examined. No
512 predictive relationships were observed between PFC Bdnf, Comt or Maoa expression and
513 social interaction pre diet consumption, social memory or novel object recognition (P=0.17;
514 P=0.092; P=0.16 for overall model of each gene respectively). There was no evidence for a
515 predictive relationship between hippocampal Maoa expression and behaviours (P=0.35).

3.9. Associations between gut microbiota composition and social behaviour

Scores on pre-diet social behaviour, social recognition memory and novel object recognition tasks respectively were all significantly associated with the relative abundance of a number of bacterial taxa (all associations where q<0.05 presented in Table 2).

Social memory performance was associated with a large number of taxa. Higher social memory scores were associated with a greater abundance of bacteria from the *Bifidobacteriales* and *Bacteroidales* order, *Lachnospiraceae* family (*Blautia* and multiple unspecified genera), *Ruminococcaceae* family and genus *Allobaculum*. Novel object recognition was negatively associated with abundance of *Bacteroidales* and a number of taxa from the *Lachnospiraceae* family. Only three taxa were significantly associated with social behaviour pre HFHS diet: a relative reduction of *Bifidobacteriales* order and two unspecified genera from the *Lachnospiraceae* family.

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

3.10. Firmicutes to Bacteroidetes ratio

There were no significant differences between the diet groups on *Firmicutes* to *Bacteroidetes* ratio (FB ratio; t(9.63)=-1.03, P=0.33). Samples were pooled for subsequent FB ratio analyses, with diet group included to control for potential interaction effects. Multivariate linear modelling demonstrated a significant relationship between FB ratio and the three behavioural dependent variables: social memory, novel object recognition and pre-diet social interaction (F(3,11)=5.26, P=0.02). Post-hoc tests demonstrated strong evidence that FB ratio negatively predicted "pre" diet social behaviour (F(2,13)=11.46, P=0.001), but not object or social recognition memory.

3.11. Associations between gut microbiota and hippocampal / PFC gene expression

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

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

4. Discussion

Here we show that daily limited consumption of a HFHS diet leads to alterations in social interaction and social memory in young rats, and impaired novel object recognition memory. Previous studies have shown that high-energy diets rapidly cause hippocampal-dependent memory deficits (Kanoski et al., 2007), but none have examined the impact of intermittent HFHS diets on social behaviours in rats. This is the first demonstration to associate diet-induced alterations to social behaviour with microbiota and brain changes in reward neurotransmission and neuroplasticity.

Intermittent access to a HFHS diet influenced the presentation of normal social behaviours, including social interaction and preference for social novelty. Consumption of a HFHS diet for 2h/day reduced duration engaged in social interaction prior to diet availability, but not following diet consumption. This indicates that when rats expected to receive the HFHS diet they were less willing to engage in social interaction, potentially underpinned by diet evoked alterations in Maoa and Comt expression, leading to changes in monoamine

566 neurotransmission, or increased anxiety. Thus, limited access to a HFHS diet may influence
567 social interaction, as comparable interaction durations were observed following access to the
568 HFHS food. Moreover, social interaction frequency was increased after rats had access to the
569 HFHS food, suggesting that following consumption of HFHS diet these rats may find
570 interaction more rewarding, or may have reduced anxiety. Social play is important for
571 neurobehavioural development; however, we did not observe differences in frequencies
572 between diet groups. This may be due to the group housing conditions and short period of
573 isolation used prior to behavioural testing; as a recent study reported that isolation amplified
574 social play behaviour (Carvalho et al., 2016). Another possible explanation is that social play
575 declines across adolescence, and that the apparent lack of social play differences was due to
576 the rats age at testing (mid-late adolescence) (Trezza et al., 2010). Further studies should be
577 conducted to examine whether diet manipulations specifically during adolescence endure into
578 adulthood to identify whether adolescence poses as a critical window of vulnerability to
579 social behavioural changes.
580
581 Social recognition memory differed between control and HFHS rats, with HFHS rats
582 showing no observed preference for the chamber containing the novel rat during the test
583 phase, indicative of impaired social memory. This complements a recent study showing that
584 an acute exposure to high fat diet in juvenile rats impaired social memory (Yaseen et al.,
585 2018). As rats showed differences in their duration of time engaged in social interaction prior
586 to consuming the HFHS food, the social memory testing was conducted following HFHS
587 access, to ensure that any memory deficits observed were not due to reduced social contact in
588 the HFHS diet rats. Initial sociability during the sample phase did not differ between HFHS
589 and control diet rats, thus it appears that social memory was impacted specifically by diet.
590 Social memory has been shown to depend upon both PFC and hippocampal function (Kogan

591 et al., 2000; Tanimizu et al., 2017), and our observed alterations to markers of monoamine
592 neurotransmission and neuroplasticity may underpin social changes. This is also
593 complemented by impaired long term novel object recognition, again indicative of
594 hippocampal dysfunction (Warburton and Brown, 2010). Moreover, both HFHS and control
595 diet rats showed preference for a social odour and showed intact odour recognition memory.
596 Thus, intermittent HFHS diet did not impact olfactory discrimination, and highlights that
597 social memory deficits are unlikely to be underpinned by impaired odour discrimination.
598
599 Diet-correlated alterations to mRNA expression of enzymes Maoa and Comt, were
600 observed in the PFC, indicating that HFHS diet consumption impacts on monoamine
601 neurotransmission integral for social behaviour and cognition. Dopamine is a critical
602 neurotransmitter in the regulation of food intake; in particular dopamine activity in the
603 mesocorticolimbic dopamine circuitry is associated with food reward (Volkow et al., 2011).
604 Maoa, the gene for monoamine oxidase, deaminates dopamine and has a key role in
605 controlling the availability of cortical dopamine. Similarly, Comt is involved in the
606 degradation of dopamine. Changes to monoamine signalling may therefore underpin the
607 altered social behaviour and social memory observed in HFHS diet rats, supported by reports
608 of diet-induced alterations to dopamine receptor expression in the striatum (Johnson and
609 Kenny, 2010). However, we observed no dopamine receptor (Drd1a/Drd2) expression
610 changes in the hippocampus or PFC, suggesting that these receptor mRNA changes may be
611 specific to striatal regions following palatable high sugar diets (Naneix et al., 2018; Naneix et
612 al., 2017). Further studies should examine whether other reward associated genes, such as
613 serotonin and mu-opioid receptors are altered by this diet protocol, and also the involvement
614 of oxytocin signalling mechanisms (Yaseen et al., 2018).
615

Reduced PFC Bdnf expression was observed in HFHS consuming rats, which also correlated positively with novel object recognition performance. This diet-induced change may underpin the changes to social behaviours and cognition as BDNF signalling has a critical role in memory encoding (Choi et al., 2010). Decreased levels of BDNF in the hypothalamus, PFC or serum have been shown to correlate with depression-like behaviours in animals and humans (Bocchio-Chiavetto et al., 2010) and high fat diet consumption reduces hippocampal BDNF levels (Molteni et al., 2004; Pistell et al., 2010) linking BDNF to emotional processes. Gut microbiota composition is also linked to alterations in BDNF within regions essential for learning and emotional behaviours, as demonstrated by previous studies indicate reduced cortical and hippocampal Bdnf gene expression in GF mice (Sudo et al., 2004), and antibiotic-induced microbiota dysbiosis altered protein levels of BDNF in the amygdala and hippocampus as well as reduced anxiety-like behaviours in the light-dark box (Bercik et al., 2011). This provides a mechanistic insight into the influence of the microbiome in cognition and emotional regulation via BDNF expression.

Excessive consumption of saturated fats is shown to induce secretion of pro-inflammatory cytokines by adipocytes and macrophages, and affect the integrity of the blood-brain barrier (BBB) (Kanoski et al., 2010), allowing pro-inflammatory cytokines and immune cells to reach the brain (Thaler et al., 2012). Interestingly, no significant changes between groups were observed to inflammatory marker mRNA expression (Il6, TNF-α, Nlrp3, Itgam) were observed in this study, and trends indicated that PFC expression of Il6 and Nlrp3 was lower in HFHS diet rats compared to controls. This may be due to the age of the rats, as emergent evidence suggests that the modulatory effects of obesogenic diets on inflammatory markers occur in an age-dependent manner, with younger rats showing resistance to neuroinflammation (Teixeira et al., 2017). However, Itgam (cluster of differentiation

641 molecule 11b, CD11b) expression in the PFC and hippocampus positively correlated with
642 WAT, indicative that increased adiposity was associated with aspects of neuroinflammation.
643 Itgam is expressed by microglia, and also neutrophils and monocytes in the injured brain
644 (Jeong et al., 2013). More so, evidence indicates that obesity-induced neuroinflammation is
645 dependent on the type of diet in terms of fat and sugar content, the duration of the diet and
646 regional differences in brain structures (Guillemot-Legris and Muccioli, 2017). Future studies
647 utilising immunohistochemistry to examine microglia morphology and astrogliosis and
648 protein markers are needed to validate the region specific impact of obesogenic diets on
649 mPFC and hippocampal neuroinflammatory markers.
650
651 HFHS diet consumption resulted in significantly increased WAT deposits
652 characteristic of diet-induced obesity, and has been previously associated with decreased
653 abundance of *Bacteroidetes* and increases in *Firmicutes* bacteria (Ley et al., 2005); however,
654 we did not observe overall alterations to the *Firmicutes* to *Bacteriodetes* ratio with this
655 intermittent feeding schedule. Our study suggests that members of *Bacteroites* (order
656 *Bacteroidales)* were significantly increased in rats that consumed HFHS diets. Therefore, not
657 all the members of the *Bacteriodetes* family are decreased with adiposity. However, it is
658 possible that *Firmicutes* to *Bacteriodetes* ratio changes become more prominent with the
659 ongoing development of obesity and chronic consumption of HFHS diets, rather than short-
660 term intake of such foods. Differential abundance analyses demonstrated that taxa from
661 *Lachnospiraceae* and *Ruminoccoceae* families of the *Clostridiales* order were the most
662 common bacterial predictors of social behaviour and recognition memory, converging with
663 clinical studies that show similar changes to microbiome populations in neuropsychiatric
664 disorders including major depressive disorder and autism (De Angelis et al., 2013;
665 Naseribafrouei et al., 2014). Moreover, social avoidance behaviour in adult mice is associated

666 with increased abundance of *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiales* in non-
667 obese diabetic mice, and the transfer of intestinal microbiota from these mice to microbiota-
668 depleted recipients evoked similar behavioural phenotypes (Gacias et al., 2016), suggesting a
669 key role of diet and metabolism in microbiota signatures. As such, converging evidence
670 indicates the potential influence of diet on social development and social behaviours via the
671 gut-brain-microbiota axis (Christian et al., 2015; Parashar and Udayabanu, 2016).
672
673 *Future studies*
674 Future studies should examine whether faecal transplants from HFHS diet animals
675 evokes similar behavioural and cortical gene expression changes in Maoa, Comt and Bdnf
676 allowing for increased mechanistic insights into the effects of the microbiome on the brain.
677 Furthermore, predictions of the metagenome functional content from the bacterial
678 communities would further provide insight into the metabolic pathways affected by
679 intermittent HFHS diet consumption. Modulation of the gut-brain axis dynamics has clinical
680 implications for mental health conditions, and as such the use of "psychobiotics" is posited as
681 a novel therapeutic avenue for psychiatric disorders and diet-induced cognitive changes.
682 Treatment strategies that target the gut microbiome should be explored, such as commensal
683 bacteria, which have been shown to ameliorate depressive (Bravo et al., 2011) and anxiety-
684 like behaviour (Bercik et al., 2011), and prebiotics which increase Bdnf mRNA expression in
685 the hippocampus (Burokas et al., 2017). These may provide a route for the attenuation of diet
686 and obesity evoked cognitive and emotional alterations.
687
688 *Conclusions and implications*
689 In conclusion, these results demonstrate that intermittent access to a HFHS diet can
690 rapidly impact social behaviour and cognition, evoke alterations in cortical gene expression,

691 and alter microbiota composition. Modulation of the microbiota may lead to the emergence
692 of novel therapies to combat social, emotional and cognitive deficits, which have been linked
693 with metabolic disorders, and for the treatment of neuropsychiatric disorders.
694

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701

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892 **Tables**

893 **Table 1**: The effects of intermittent high fat and high sucrose (HFHS) diet exposure on
894 prefrontal cortex and hippocampal gene expression, mean (±SEM), * = $P<0.05$, **=$P<0.01$,
895 # = $P<0.10$

896

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

899

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

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

					Social recognition memory		Object recognition memory		Pre-diet social interaction	
Phylum	**Class**	**Order**	**Family**	**Genus**	log2FoldChange	*q*	log2FoldChange	*q*	log2FoldChange	*q*
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	5.13	<0.01			-0.04	0.0
	Bacteroidia	Bacteroidales					-7.27	0.03		
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	8.51	<0.01				
					4.59	0.04				
					4.20	<0.01	-9.99	<0.01		
					-4.87	0.04				
					4.28	0.04				
					4.24	0.05				
					4.66	0.04				
					4.30	0.02				
									-0.04	0.0
									-0.04	0.0
							-8.32	0.02		
							-7.89	0.03		
				Blautia	4.72	0.01				
					4.48	0.04	-8.44	0.02		
					4.51	0.01				
					4.91	0.01				
					5.42	0.01				
							-6.90	0.04		
			Ruminococcaceae	Unspecified	6.06	<0.01				
					4.45	0.02				
					4.13	0.04				
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	2.90	0.04				

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

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

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