

Relationship between the gut microbiome and behaviour at the molecular, organism, population and evolutionary levels



Katerina Vicky-Ann Johnson

University of Oxford

A thesis submitted for the degree of
Doctor of Philosophy in Interdisciplinary Bioscience

Trinity Term 2018



Copyright © Katerina V.-A. Johnson 2018. All rights reserved.

To my mother & father



My heroine and hero. Thank you for giving me your eyes to see the world. And for always being there for me through the amazing times, the less amazing times and all the times in between. You are my greatest supporters, teachers and inspiration. I am the luckiest person alive to have the gift of your everlasting magic.

Με πολλή αγάπη Κατερίνα

To my grandmother



Thank you for teaching me to be happy in the moment, for no second ever comes back, and for patiently going through every page of Heble Martin's Concise British Flora to pick our favourite flowers again and again. Your tea and cake tradition has not been forgotten and remains the best part of the day! I still feel your grace.

To my friends, old & new

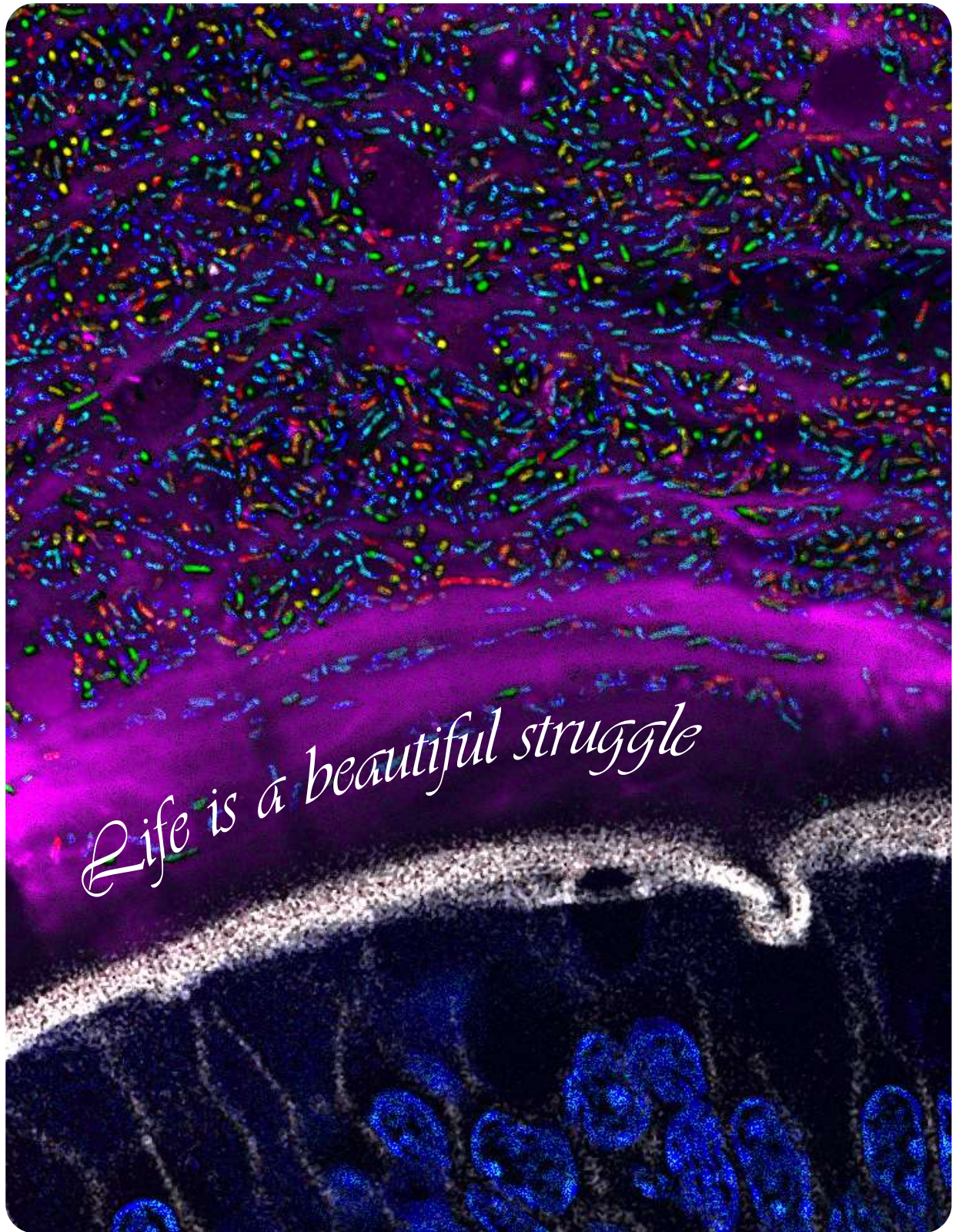


You're amazing! I have had a lot of fun at Oxford on the quiet and it would never have been the same without you. Thank you for dragging me away from my work occasionally...I appreciated it really! I will always remember the laughs, giggles and joy that we shared in this enchanting city.

And of course my microbes



Thanks to my microbial zoo for that gut feeling to pursue this research topic!



Gut microbiome image courtesy of the Sonnenburg Lab, Stanford University

Declaration

I hereby declare that, except where explicitly stated, the contents of this thesis are original and entirely my own work and ideas. This includes designing and performing the research, analysing, interpreting and presenting the data and writing the text. This thesis has not been submitted in whole or in part for any other degree or qualification and contains less than 50,000 words excluding appendices and the list of references.

Katerina Vicky-Ann Johnson

2018

Acknowledgements

Thank you to my supervisors Professor Philip Burnet and Professor Robin Dunbar for their support and guidance during the three years of my DPhil studies. I appreciate the freedom you gave me to design my own DPhil and for allowing me to be independent in my research.

In terms of the sources of data, I am very grateful to uBiome for their generosity in providing the sampling kits to participants for the human study and sequencing the samples. I would also like to thank Dr Lauren Brent (University of Exeter) and Dr Karli Watson (University of Colorado Boulder) for sharing their pre-existing data when I approached them with my research idea for the macaque population, and for their helpful discussions. I am grateful for the social network data and the partly processed microbiome data, on which I based my own research analyses for one of the chapters.

Thank you to the staff and demonstrators at the Doctoral Training Centre (DTC) who provided an engaging and immersive first term of taught modules. I have fond memories from my days at the DTC and although I have not forgotten the sometimes long hours and intense programming courses, the training certainly stood me in good stead for my DPhil research. I would also like to thank the supervisors of my rotation projects during the first year of the DPhil programme with whom I also published my findings: Professor Ben Sheldon and his group in the Department of Zoology's Edward Grey Institute and Professor Robin Dunbar and his Social and Evolutionary Neuroscience Research Group (SENRG). Many thanks to Professor Kevin Foster from the Department of Zoology for our interesting and lively evolutionary discussions on the microbiome–gut–brain axis which ultimately led to our joint *Nature Reviews* publication.

I would like to thank my undergraduate tutor at Pembroke College, Professor Nick Kruger, and all of the teachers from my undergraduate degree here in Biological Sciences whose tutorials and lectures were truly inspiring. Thank you also to my undergraduate project supervisor, Professor Aziz Aboobaker, for the enjoyable research experience I had over the summer in his

lab which confirmed my aspiration to pursue a DPhil. Of course I could not write my acknowledgements without mentioning the lovely staff and lunches that sustained me at Darwin's Café over the years, before it was caught up in the asbestos closure of the Tinbergen Building.

Thank you so much to my wonderful lab friends at the Department of Psychiatry: Shi Yu Chan, Kalina Naidoo, Amy Kao, Jenna Hebert and Marieke Martens. From the day I came to the lab you were always so welcoming and kind. During my long days of experiments you were there to make me smile... and endure my Taylor Swift fandom. And I can definitely say you improved my knowledge of Oxford's eating out scene! I will cherish my happy memories with you all. I would also like to thank Li Chen for her reliable support as our lab technician.

Thank you to my friends from Pembroke College Middle Common Room, the DTC and others from across the University. I have met some incredible people along the way and have made friends for life here... thank you all for always believing in me. And of course infinite thanks to my mother and father who have helped in ways too numerous to mention.

I am grateful to the Biotechnology and Biological Sciences Research Council (BBSRC) for funding this research and to Pembroke College for awarding me a Graduate Scholarship, Senior Studentship and the R. G. Collingwood Prize.

My final thank you goes to the University of Oxford. I feel privileged to have had a wealth of lectures, tutorials and discussions with leaders in their field but all the while treated as an equal. My education here over the last seven years has been unparalleled and certainly the most intellectually exciting and challenging times of my life. You made me who I am today...

Forever dark blue

Abstract

Relationship between the gut microbiome and behaviour at the molecular, organism, population and evolutionary levels

Katerina Vicky-Ann Johnson

Pembroke College

Doctor of Philosophy in Interdisciplinary Bioscience

Trinity Term 2018

Eukaryotes have evolved in the presence of microbial life and so it is unsurprising that microorganisms are functionally integrated with many aspects of animal biology. Research is revealing the numerous ways that the gut microbiome interacts with the host's central nervous system, influencing brain development, neurochemistry, emotion and social behaviour. This microbiome–gut–brain axis is bidirectional since the behaviour and emotional state of the host can in turn alter microbiome composition and function. This thesis investigates these two-way interactions between the gut microbiome and host behaviour at the molecular, organism, population and evolutionary levels.

I demonstrate using animal models that the gut microbiome alters the expression of neuropeptide signalling pathways implicated in social and emotional behaviour, particularly in the frontal cortex. This is the first investigation into the effect of the microbiome on the brain's opioid system. In a large human cohort I find that sociability, neuroticism, anxiety and stress are related to differences in microbiome composition and diversity, revealing a microbial dimension to human personality. I also show in a non-captive macaque population that sociability is related to differential abundances of certain bacterial taxa. From an evolutionary perspective, I find evidence that a change in gut microbiome composition may have facilitated evolution of a larger brain and complex social behaviour. Finally, I apply evolutionary theory to

the microbiome–gut–brain axis and argue against the emerging hypothesis that gut microorganisms manipulate host behaviour.

This multilevel approach provides a holistic view of the microbiome–gut–brain axis, from neurogenetics, to personality, social behaviour and evolution of the social brain. Based on the novel insights from this research, I conclude that the gut microbiome is fundamental to our understanding of the mammalian brain, behaviour and evolution in ways not currently appreciated, but with implications for human psychology and well-being.

Contents

	Declaration	vi
	Acknowledgements	vii
	Abstract	ix
	Contents	xi
	List of figures	xv
	List of tables	xvii
	List of abbreviations	xix
Chapter 1	Introduction	1
1.1	Living in a microbial world	1
1.2	The human gut microbiome	2
1.3	‘That gut feeling’: from past to present	3
1.4	The gut microbiome and behaviour	4
1.5	The gut microbiome in neuropsychiatry	6
1.6	Mechanisms mediating the microbiome–gut–brain axis	8
1.6.1	Neural signalling	10
1.6.2	Immune signalling	12
1.6.3	Endocrine signalling	14
1.7	Bidirectional interactions between the gut microbiome and behaviour	15
1.8	Assessing the gut microbiome	16
1.9	Thesis outline	17
1.9.1	Chapter 2 Neuropeptide pathways	18
1.9.2	Chapter 3 Human personality	18
1.9.3	Chapter 4 Macaque social network	19
1.9.4	Chapter 5 Encephalization	19
1.9.5	Appendix Chapter Evolutionary theory	20
1.10	My DPhil publications	20
Chapter 2	Effect of the gut microbiome on the expression of neuropeptide pathways underpinning social and emotional behaviour	22
2.1	Abstract	22
2.2	Introduction	23
2.3	Materials and methods	27
2.3.1	Animals	27
2.3.2	Antibiotic treatment	27
2.3.3	Tissue dissection	28

2.3.4	RNA extraction and cDNA synthesis	29
2.3.5	Quantitative real-time PCR.....	29
2.3.6	Statistical analyses.....	30
2.4	Results.....	31
2.4.1	Antibiotic-treated mice.....	31
2.4.2	Germ-free mice	33
2.5	Discussion.....	35
Chapter 3	Analysis of the gut microbiome in relation to human personality.....	44
3.1	Abstract.....	44
3.2	Introduction	44
3.3	Materials and methods.....	50
3.3.1	Study population	50
3.3.2	Sample collection, processing and sequencing	51
3.3.3	Questionnaires.....	52
3.3.4	Multiple regression analyses of bacterial abundances.....	53
3.3.5	Analyses of microbiome diversity and community composition.....	55
3.4	Results.....	56
3.4.1	Summary statistics	56
3.4.2	Regression models of bacterial abundances	58
3.4.3	Microbiome diversity	60
3.4.4	Microbial community composition.....	62
3.5	Discussion.....	64
Chapter 4	Behaviour and the gut microbiome in a social network of a macaque population.....	74
4.1	Abstract.....	74
4.2	Introduction	74
4.3	Materials and methods.....	78
4.3.1	Study population and behavioural data collection.....	78
4.3.2	Social network metrics	79
4.3.3	Sample collection, processing and sequencing	79
4.3.4	Multiple regression analyses of bacterial abundances.....	79
4.3.5	Analyses of microbiome diversity and community composition.....	81
4.4	Results.....	82
4.4.1	Taxonomic composition of the microbiome.....	82
4.4.2	Regression models of bacterial abundances	82
4.4.3	Effects of grooming on microbiome diversity and community composition	84
4.5	Discussion.....	85

Chapter 5	Macroevolutionary relationship between encephalization and the mammalian gut microbiome	93
5.1	Abstract	93
5.2	Introduction	94
5.3	Materials and methods	98
5.3.1	Datasets	98
5.3.2	Statistical analyses	99
5.4	Results	99
5.5	Discussion	101
Chapter 6	Discussion	108
6.1	Key findings and significance	108
6.1.1	Chapter 2 Neuropeptide pathways	108
6.1.2	Chapter 3 Human personality	109
6.1.3	Chapter 4 Macaque social network	110
6.1.4	Chapter 5 Encephalization	111
6.1.5	Appendix Chapter Evolutionary theory	112
6.2	Outlook: microbiome, modern-day lifestyles and mental conditions	113
6.3	Limitations	114
6.3.1	Sampling the gut microbiome	114
6.3.2	Assessing the gut microbiome	115
6.3.3	Microbiome sequencing	115
6.4	Future directions	116
6.4.1	Faecal microbiota transplants	116
6.4.2	Psychobiotics	117
6.4.3	Manipulation of the microbiome	118
6.4.4	<i>Ex vivo</i> systems	119
6.4.5	Model organisms	120
6.5	Conclusions	121
References		122
Appendix		158
Chapter 2		158
Figures		158
Tables		159
Chapter 3		159
Figures		159
Tables		162
Supplementary results		184

Chapter 4	185
Figures	185
Tables	186
Chapter 5	190
Tables	190

Appendix

Chapter	Applying evolutionary theory to the microbiome–gut–brain axis	195
A.1	Abstract.....	195
A.2	Introduction	195
A.3	Manipulation of host behaviour.....	198
A.4	Local manipulation in the host.....	206
A.5	By-products of microbial metabolism.....	208
A.6	The evolution of host dependence	210
A.7	Outlook	212

List of figures

Figure 1.1.	Neural, immune and endocrine pathways mediating the bidirectional microbiome–gut–brain axis	9
Figure 2.1.	Effect of antibiotic treatment on body weight.....	28
Figure 2.2.	Effects of post-weaning antibiotic treatment on the expression of neuropeptide receptor genes implicated in social behaviour	32
Figure 2.3.	Effects of post-weaning antibiotic treatment on the gene expression of neuropeptides implicated in social behaviour.....	32
Figure 2.4.	Effects of post-weaning antibiotic treatment on the gene expression of <i>MyD88</i>	33
Figure 2.5.	Effects of germ-free status on the expression of neuropeptide receptor genes implicated in social behaviour	34
Figure 2.6.	Effects of germ-free status on the gene expression of neuropeptides implicated in social behaviour	34
Figure 2.7.	Effects of germ-free status on the gene expression of <i>MyD88</i>	35
Figure 3.1.	Overview of study design.....	51
Figure 3.2.	Stacked bar plots summarizing gut microbiome composition at genus level for the total participant cohort	57
Figure 3.3.	Intercorrelation plot for the study variables, divided into the four categories: behaviour, diet, health and lifestyle, and sociodemographics.....	58
Figure 3.4.	Coefficient plots from regression models predicting abundances of genera in the human gut microbiome.....	59
Figure 3.5.	Bar plot showing results of Kendall's Tau-b correlation analysis between gut microbiome diversity and the study variables.....	61
Figure 3.6.	Bar plot showing explained variance in gut microbiome composition in relation to the study variables	63
Figure 4.1.	Rhesus macaques on Cayo Santiago Island.....	77
Figure 4.2.	Stacked bar plot summarizing gut microbiome composition at genus level for the macaque study population	83
Figure 4.3.	Coefficient plots from regression models predicting abundances of genera in the macaque gut microbiome	84
Figure 4.4.	Relationship between macaque sociability and abundances of the genera <i>Dialister</i> , <i>Faecalibacterium</i> , <i>Prevotella</i> , <i>Sutterella</i> and <i>Streptococcus</i> , as predicted from negative binomial regression.....	85
Figure 4.5.	Principal coordinates analysis plot of microbiome composition using weighted UniFrac distances	86

Figure 5.1.	Positive relationship between Bacteroidetes: Firmicutes ratio and encephalization slope across mammalian suborders	100
Figure 5.2.	Relationship between fibre intake and Bacteroidetes: Firmicutes ratio across mammalian suborders	101
Figure 5.3.	Asymptotic relationship between fibre intake and gut microbiome diversity for a range of mammalian species	102
Figure A.1.	Effects of post-weaning antibiotic treatment on the gene expression of <i>Bdnf</i> ..	158
Figure A.2.	Effects of germ-free status on the gene expression of <i>Bdnf</i>	158
Figure A.3.	Statistical power analysis for sample size estimation	159
Figure A.4.	Intercorrelation plot for the nutrient variables, divided into the four categories: food groups, macronutrients, minerals and vitamins	160
Figure A.5.	Coefficient plots from regression models predicting abundances of genera in the human gut microbiome	161
Figure A.6.	Coefficient plots from regression models predicting abundances of genera in the macaque gut microbiome	185
Figure A.7.	Examples of parasites affecting host behaviour	197
Figure A.8.	Evolution of microbial effects on the brain	200
Figure A.9.	How neurotransmitters in the gut lumen might influence the central nervous system	209

List of tables

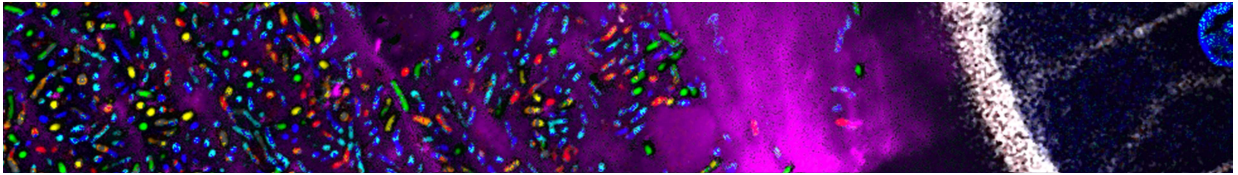
Table 3.1.	Summary of statistically significant associations between bacterial genera in the gut and behavioural or psychiatric traits reported in the literature	47
Table A.1.	Gene expression assays used for quantitative real-time PCR.....	159
Table A.2.	Summary statistics for continuous variables	162
Table A.3.	Data summary for binary variables	163
Table A.4.	Output from regression models predicting abundances of genera in the human gut microbiome	164
Table A.5.	Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and study variables.....	168
Table A.6.	Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and nutrient variables	170
Table A.7.	Results of PERMANOVA to test for an association between gut microbiome composition and study variables	172
Table A.8.	Results of PERMANOVA to test for an association between gut microbiome composition and nutrient variables.....	174
Table A.9.	Output from regression models predicting abundances of genera in the gut microbiome, with sufferers of mental illness excluded from dataset.....	176
Table A.10.	Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and study variables, with sufferers of mental illness excluded from dataset.....	180
Table A.11.	Results of PERMANOVA to test for an association between gut microbiome composition and study variables, with sufferers of mental illness excluded from dataset	182
Table A.12.	Regression statistics for predictors of social network size.....	184
Table A.13.	Summary of statistically significant associations between bacterial genera in the gut and sociability or autistic traits reported in the literature	186
Table A.14.	Output from regression models predicting abundances of genera in the macaque gut microbiome.....	187
Table A.15.	Results of linear mixed model to test the factors predicting gut microbiome diversity	188
Table A.16.	Results of PERMANOVA to test the factors affecting gut microbial community composition	189
Table A.17.	List of the 60 mammalian species (and their taxonomic classification) for which data on gut microbiome composition were available	190

Table A.18.	Data on mammalian encephalization, gut microbiome composition and fibre intake used for linear regression analyses	192
Table A.19.	Data on fibre intake and gut microbiome diversity for the 30 mammalian species.....	193
Table A.20.	Results of linear regression analyses on the relationships between mammalian encephalization, gut microbiome composition and fibre intake.....	194
Table A.21.	Output from non-linear regression model predicting gut microbiome diversity from fibre intake for the 30 mammalian species	194
Table A.22.	Conditions favouring manipulation of a host by a symbiont	201

List of abbreviations

5-HT	5-hydroxytryptamine (serotonin)
Avp	Vasopressin gene
Avpr1a	Arginine vasopressin receptor 1A gene
B2m	Beta-2-microglobulin gene
Bdnf	Brain-derived neurotrophic factor gene
B:F ratio	Bacteroidetes to Firmicutes ratio
cDNA	Complementary DNA
C_T	Threshold cycle number
df	Degrees of freedom
DNA	Deoxyribonucleic acid
FDR	False discovery rate
FFQ	Food frequency questionnaire
GABA	Gamma-amino butyric acid
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase gene
GLP-1	Glucagon-like peptide 1
HPA	Hypothalamus–pituitary–adrenal axis
mRNA	Messenger RNA
MyD88	Myeloid differentiation primary response 88 gene
Oprm1	Opioid receptor μ 1 gene
OTU	Operational taxonomic unit
Oxt	Oxytocin gene
Oxtr	Oxytocin receptor gene
P_{adj}	Adjusted <i>P</i> value
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
Polr2a	RNA polymerase II subunit A gene

<i>Pomc</i>	Pro-opiomelanocortin gene
PYY	Peptide tyrosine–tyrosine
QIIME	Quantitative Insights Into Microbial Ecology
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RT-PCR	Real-time PCR
SCFA	Short-chain fatty acid
SEM	Standard error of the mean



Chapter 1

Introduction

1.1 Living in a microbial world

We inhabit a world teeming with microscopic life (Whitman *et al.*, 1998). Microorganisms live all around us, on us and in us; so much so that bacterial cells make up approximately half the number of cells in the human body (Sender *et al.*, 2016). We also emit an estimated ten million biological particles an hour, forming our own personalized microbial cloud which leaves a microbial signature behind in the environments we occupy (Meadow *et al.*, 2015). The realization that microbial communities inhabit almost every environment on Earth, combined with the evidence that they play key roles in human health, represents one of the most important recent developments in the biological and medical sciences.

Multicellular life has evolved in the presence of microorganisms, resulting in numerous and complex symbiotic interactions between a host and its microbiota (Sachs *et al.*, 2011; Shapira, 2016). Given the long evolutionary history between animals and their associated microbiota, it is unsurprising therefore that the physiological processes of animals are intricately connected to their microbial symbionts (McFall-Ngai *et al.*, 2013). The vast majority of these microorganisms inhabit the gastrointestinal tract which is estimated to contain hundreds to thousands of bacterial species and strains (Bäckhed *et al.*, 2005; Qin *et al.*, 2010). In addition, although microbiome research is currently focused on characterizing bacterial taxa, the microbiome is a complex community consisting of not only bacteria but also archaea, fungi, viruses and eukaryotes such as protozoa (Lloyd-Price *et al.*, 2016). This community of microorganisms inhabiting the gut,

known as the gut microbiome, influences development, digestion, immunity, metabolism and even behaviour (Round & Mazmanian, 2009; Cryan & Dinan, 2012; Brestoff & Artis, 2013; Sommer & Bäckhed, 2013; Sampson & Mazmanian, 2015). The important contributions made by the microbiome to host health are in stark contrast to the widely held view from the antibiotic era when microorganisms were considered largely pathogenic (Aminov, 2010).

1.2 The human gut microbiome

Upon delivery, the newborn is exposed to the mother's vaginal microbiota, and subsequently to an array of microorganisms from its physical and social environment (Sprockett *et al.*, 2018). In fact, although colonization was assumed to begin postnatally, there is now increasing evidence of a microbial presence *in utero* (Collado *et al.*, 2016; Versalovic, 2016). Within the first two years of life, the taxonomic and functional composition of the gut microbiome approximately resembles that of adults (Palmer *et al.*, 2007; Koenig *et al.*, 2011; Bäckhed *et al.*, 2015).

There is striking variation between individuals in gut microbiome composition (Costello *et al.*, 2009; Qin *et al.*, 2010), with the majority of this variation remaining unexplained (Falcony *et al.*, 2016; Zhernakova *et al.*, 2016). While host genetics plays a role in determining its composition (Zoetendal *et al.*, 2001; Benson *et al.*, 2010; Goodrich *et al.*, 2016a), evidence suggests that environmental factors may be more important (Goodrich *et al.*, 2016b; Rothschild *et al.*, 2018). For example, diet, health, lifestyle and age have all been shown to significantly affect the composition of the gut microbial community (Wu *et al.*, 2011; Yatsunenko *et al.*, 2012; David *et al.*, 2014a, 2014b; Falcony *et al.*, 2016; Zhernakova *et al.*, 2016; Bressa *et al.*, 2017). However, longitudinal research has revealed that an individual's gut microbiome is relatively stable over time and resilient to environmentally induced fluctuations (Lozupone *et al.*, 2012; Mehta *et al.*, 2018). Indeed, the gut microbiome is able to recover from perturbations such as dietary shifts (David *et al.*, 2014b) and antibiotic treatment (de la Cochetière *et al.*, 2005), as well as illness and changes in the environment due to travel (David *et al.*, 2014a).

An explosion of research over the past decade has implicated dysbiosis of the gut microbiota in a wide range of conditions including obesity, diabetes, autoimmune illnesses, inflammatory bowel disease and irritable bowel syndrome, as well as neuropsychiatric disorders such as autism, depression and anxiety (Bruce-Keller *et al.*, 2018). While some progress has been made towards identifying the hallmarks of a healthy microbiome in terms of taxonomic composition, functional traits and ecosystem properties (such as stability, resilience and diversity), a better understanding of how to assess health of the microbiome is still required (Lloyd-Price *et al.*, 2016).

1.3 ‘That gut feeling’: from past to present

The realization that our gut and brain are intricately connected is centuries-old. Intestinal auto-intoxication, the idea that gut contents can cause disease, originated in ancient Egypt (Chen & Chen, 1989). The Greek physician Hippocrates also recognized the importance of the gut to systemic health and is claimed to have said that “All disease begins in the gut” (Lyon, 2018). In the nineteenth and early twentieth centuries, scientists postulated that toxins accumulating in the colon were linked to depression and neuroses (Bested *et al.*, 2013). Physicians also proposed the first scientific theory of brain–viscera interactions, known as the James–Lange theory, suggesting that emotional responses may be the result of signalling from the viscera to the brain (Mayer, 2011). Early in the twentieth century, Nobel Prize winner Ilya Mechnikov hypothesized that consumption of bacteria in fermented foods may promote longevity and reduce cognitive decline (Mackowiak, 2013; Scott *et al.*, 2017). Also around this time, dermatologists proposed a theory on the interrelations between the gut, emotional disorders and inflammatory skin conditions (Stokes & Pillsbury, 1930). They hypothesized that changes in the composition of the gut microbiota or alterations in intestinal permeability might mediate the relationship between inflammation and emotions, advocating the use of *Lactobacillus* cultures as a possible treatment. Although largely dismissed by the medical community at the time, these insights laid the foundations for the scientific study of the microbiome–gut–brain axis and

are now gaining ever more prominence, given the current evidence that the gut microbiome plays an important role in both physical and mental health.

This gut–brain connection is even reflected in our everyday language with phrases such as ‘that gut feeling’, ‘gut instinct’ and ‘gutted’. While interactions between the gut and brain have been extensively researched regarding the regulation of food intake and satiety (Konturek *et al.*, 2004), such interactions have largely been ignored with respect to the neuroscience of emotions and behaviour (Mayer, 2011). However, re-examination of this gut–brain connection in light of the complex microbial community of the gut, revealed through advances in sequencing technology, has triggered a paradigm shift in neuroscience (Mayer *et al.*, 2014). This has generated a new dynamic research field investigating the complex bidirectional interactions between the gut, its microbial community and the brain, coined the microbiome–gut–brain axis (Rhee *et al.*, 2009).

1.4 The gut microbiome and behaviour

Research on the microbiome–gut–brain axis has demonstrated the various effects that the gut microbial community can have on brain development and function, neurochemistry and behaviour, influencing both the developing and adult mammalian brain (Cryan & Dinan, 2012; Sampson & Mazmanian, 2015). In the extreme scenario of a total lack of microorganisms, as in germ-free animals, a range of abnormal behaviours is observed, including impaired social behaviour (Crume yrolle-Arias *et al.*, 2014; Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Lu *et al.*, 2018; Luk *et al.*, 2018; Stilling *et al.*, 2018), altered anxiety (Diaz Heijtz *et al.*, 2011; McVey Neufeld *et al.*, 2011; Lu *et al.*, 2018) and a heightened stress response (Sudo *et al.*, 2004) which can be alleviated upon postnatal colonization. Although there are limitations of using germ-free animals, as discussed in Chapter 2, they do provide a useful model to determine the physiological and behavioural processes that are influenced by the gut microbiome. Interestingly, even the nervous system of the cnidarian *Hydra* is modulated by its microbial symbionts, as recent research has shown that the body contractions of germ-free *Hydra* are less frequent and more irregular (Murillo-Rincon *et al.*, 2017). Since the phylum Cnidaria is regarded

as one of the first animal groups to have evolved a nervous system (Bosch *et al.*, 2017), this suggests an evolutionarily ancient origin of interactions between microorganisms and the metazoan nervous system.

While research on germ-free animals indicates that the presence of microorganisms is necessary for normal development of the brain and behaviour, a seminal study adopting faecal microbiota transplantation revealed that the gut microbiota can also influence behavioural traits in adult mice (Bercik *et al.*, 2011a). This research exploited the differences in temperament between mouse strains since Balb/c mice tend to be timid, exhibiting anxiety-like behaviours, whereas NIH Swiss mice are generally more bold and exploratory. Germ-free mice from these two strains were colonized with gut microbiota from specific pathogen-free mice (mice with conventional microbiota but lacking certain pathogens) of either the same strain or the alternative strain. Germ-free mice colonized with the gut microbiota from specific pathogen-free mice of the same strain exhibited similar behaviour to their specific pathogen-free counterparts. However, colonization with the gut microbiota from the alternative mouse strain led to the behaviour of the recipient mouse becoming more like that of the strain from which it received the gut microbiota transplant. This was the first study to show that behavioural phenotypes could be transferred via the gut microbiota, indicating that the gut microbial community contributes to host behaviour. There has subsequently been a wealth of animal studies, and more recently a number of human studies, using techniques to manipulate the gut microbiome to investigate how it can influence the brain and behaviour, as described in detail in subsequent chapters.

Notably, studies in rodents have repeatedly demonstrated that the gut microbiome can affect numerous aspects of social behaviour (Vuong *et al.*, 2017). For example, germ-free rodents typically show reduced interest in social interactions (Crumevolle-Arias *et al.*, 2014; Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Luk *et al.*, 2018; Stilling *et al.*, 2018) and social novelty (Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Lu *et al.*, 2018). Antibiotic exposure has also been found to induce behavioural impairments, including social deficits (Bercik *et al.*, 2011a; Desbonnet *et al.*, 2015; Wang *et al.*, 2015; Hoban *et al.*, 2016a; Leclercq *et al.*, 2017; Guida *et*

al., 2018; Kayyal *et al.*, 2018). While the neuropeptide systems of μ -opioid, oxytocin and vasopressin are integral to mammalian social behaviour (Young *et al.*, 2001; Depue & Morrone-Strupinsky, 2005), little research has yet been conducted into how changes in the gut microbiome may influence the expression of both neuropeptides and their receptors in these three pathways (Desbonnet *et al.*, 2015; Buffington *et al.*, 2016).

1.5 The gut microbiome in neuropsychiatry

Since animal studies have revealed that the gut microbiome can affect social behaviour, the stress response, anxiety and depressive-like symptoms (Cryan & Dinan, 2012; Sharon *et al.*, 2016), this suggests that the human gut microbiome may play a role in neuropsychiatric disorders including autism, anxiety and depression. Indeed, it is an interesting observation that neuropsychiatric conditions are often comorbid with gastrointestinal problems. It is estimated that 50% of cases of psychiatric disorders may be accompanied by irritable bowel syndrome (Folks, 2004). Results of a recent study reveal that a third of individuals suffer from depression or anxiety prior to the onset of a gastrointestinal disorder such as irritable bowel syndrome, while in two-thirds of cases the gastrointestinal disorder precedes psychological distress (Koloski *et al.*, 2016), thereby highlighting the bidirectional nature of gut–brain interactions. Notably, two separate studies have shown that rodents colonized with the faecal microbiota from humans suffering with depression, but not from healthy individuals, display both physiological and behavioural symptoms characteristic of depression, including anhedonia and anxiety-like behaviours (Kelly *et al.*, 2016; Zheng *et al.*, 2016). In addition, a new study has reported that faecal microbiota transplantation in patients with gastrointestinal disease can significantly improve depressive symptoms, anxiety and sleep (Kurokawa *et al.*, 2018). There is also evidence that probiotics (live bacteria) can ameliorate symptoms of depression, anxiety and stress in humans (Messaoudi *et al.*, 2011; Allen *et al.*, 2016; Pinto-Sanchez *et al.*, 2017). Indeed, the term psychobiotics was coined to describe any probiotic with a beneficial effect on psychiatric symptoms (Dinan *et al.*, 2013).

Gastrointestinal comorbidities are also common in individuals with autism (Hsiao, 2014). In fact, up to 70% of children with autism may also suffer from gastrointestinal symptoms (Adams *et al.*, 2011). Unsurprisingly, therefore, there has been growing interest in assessing the aetiology of autism with respect to the gut microbiome (Krajmalnik-Brown *et al.*, 2015; Needham *et al.*, 2018). Indeed, a study involving faecal microbiota transplantation in autistic children found that it not only ameliorated gastrointestinal symptoms but also improved behavioural traits including social behaviour and communication (Kang *et al.*, 2017), indicating that the gut microbial community may contribute to autistic behaviours. There is also some evidence that probiotics may have a beneficial effect on the behaviour of children with autism (Shaaban *et al.*, 2017). Furthermore, a longitudinal study found that probiotic supplementation during the first six months of life may reduce the risk of developing autism or attention deficit hyperactivity disorder by the age of thirteen (Pärtty *et al.*, 2015), suggesting that the state of the gut microbiome during development may be an important factor influencing the likelihood of neuropsychiatric disorders in later life.

Despite the heterogeneity of autism, a key symptom of this disorder is impaired social behaviour (American Psychiatric Association, 2013). Social behaviour is essential for the survival of many animals and is of particular adaptive value in mammals (Silk, 2007), including humans (Holt-Lunstad *et al.*, 2010; Yang *et al.*, 2016b). It is important for activities such as foraging, protection and breeding, and is also integral to mental health in higher-order species (Filiano *et al.*, 2016). Indeed, reduced motivation to engage in and maintain social relationships is a symptom of various neuropsychiatric conditions such as autism and depression (Kennedy & Adolphs, 2012). Studies on the microbiome–gut–brain axis have repeatedly shown that alterations to the gut microbiome, such as antibiotic treatment, germ-free status or a maternal high-fat diet, can all result in social deficits which may be ameliorated by a gut microbiota transplant or the administration of probiotics (Vuong *et al.*, 2017), as discussed in detail in Chapter 2. In addition, research with germ-free animals has found that males exhibit greater impairments in their social behaviour (Desbonnet *et al.*, 2014), as well as more pronounced changes at the neurochemical level, compared to females (Clarke *et al.*, 2013; Hoban *et al.*, 2016b). This observation is

particularly relevant given the notable male bias in autism prevalence (Werling & Geschwind, 2013).

There is also some evidence that neuropsychiatric conditions may be associated with increased permeability of the gut wall. This is often referred to as 'leaky gut', whereby the overgrowth of pathogenic bacteria can compromise the integrity of the intestinal epithelium. This can allow toxins produced by the gut bacteria, along with the bacteria themselves, to leak through the intestinal barrier into the bloodstream, triggering systemic inflammation (Borre *et al.*, 2014). Interestingly, this bears close correspondence to the earlier notion of intestinal auto-intoxication, proposing that toxins originating from the gut can disrupt mental health. Several studies have now implicated a leaky gut in depression (Maes *et al.*, 2008, 2012) and it is also hypothesized to contribute to the pathophysiology of autism (Rao & Gershon, 2016).

1.6 Mechanisms mediating the microbiome–gut–brain axis

Although the main focus of my research is investigating the microbiome–gut–brain axis from a behavioural perspective, the underlying signalling pathways are fundamental to the observed effects of the gut microbiome on behaviour. In this section I therefore summarize the possible mechanisms mediating the microbiome–gut–brain axis in light of current experimental evidence.

The gut contains the largest number of endocrine and immune cells in the body, together with a complex network of neurones forming the enteric nervous system and trillions of bacteria, fungi, viruses and other microorganisms (Forsythe *et al.*, 2016). Interactions between the gut microbiome and the brain are bidirectional and may be mediated via neural, immune and endocrine signalling (Figure 1.1). While there is evidence to support the role of each of these signalling systems, as described below, their relative importance remains to be determined. Furthermore, given the extensive crosstalk between these systems (Procaccini *et al.*, 2014; O'Malley, 2016), it is likely that complex interactions between all three are key to the functioning of the microbiome–gut–brain axis.

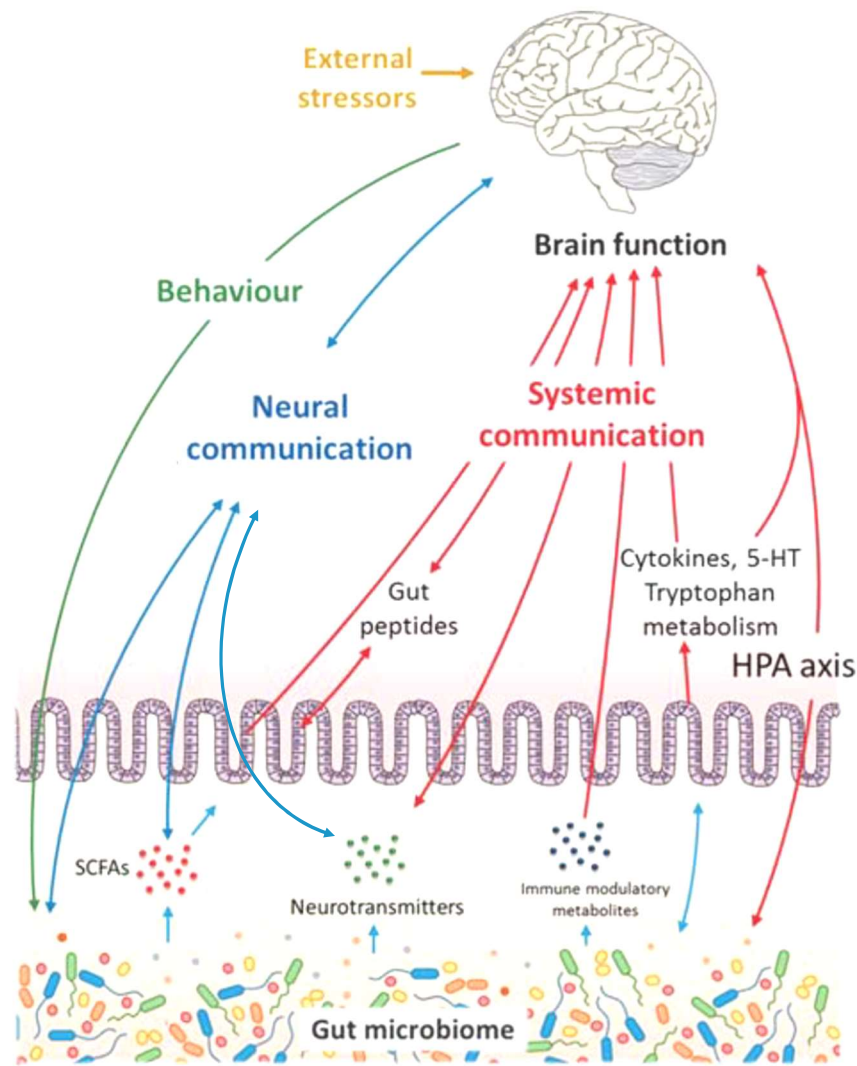


Figure 1.1. Neural, immune and endocrine pathways mediating the bidirectional microbiome–gut–brain axis. There are various direct and indirect pathways via which the gut microbiome and the brain may communicate. Neural communication includes the vagus nerve and enteric nervous system. Neural signalling may be mediated via neuroactive chemicals produced by gut bacteria, such as serotonin (5-HT), or their precursors (tryptophan in the case of serotonin). Gut bacteria may also modulate host production of neurotransmitters. In addition to any direct effect on the nervous system, neuroactive chemicals or their precursors may also influence brain function via systemic communication, such as through the circulatory or immune system. Detection of gut microorganisms and their metabolites by the immune system can influence the immune response through cytokine signalling. Endocrine signalling may also mediate communication between the gut microbiome and the brain since gut bacteria can modulate levels of gut peptides. Short-chain fatty acids (SCFAs) produced by bacterial fermentation can interact with the nervous, immune and endocrine systems. Given the bidirectional nature of the microbiome–gut–brain axis, signals from the brain can in turn affect the gut microbiome via the hypothalamus–pituitary–adrenal (HPA) axis. Stress and emotions can trigger the release of stress hormones which change the physiology and microbial composition of the gut, as well as affecting immune function such as cytokine secretion. Other aspects of host behaviour such as social behaviour may also influence the gut microbiome. Diagram adapted from Rogers *et al.* (2016).

1.6.1 Neural signalling

1.6.1.1 Vagus nerve

The vagus nerve serves as a direct link between the gut and the brain. Although the vagus nerve consists of both motor and sensory nerve fibres, approximately 80% of the nerve fibres are sensory, conveying information to the brain about the state of visceral organs such as the gut (Bonaz *et al.*, 2018). Several animal studies have demonstrated the role of the vagus nerve in mediating the microbiome–gut–brain axis. For example, the probiotic *Lactobacillus rhamnosus* was found to reduce anxiety and depressive-like behaviours in mice only when the vagus nerve was functionally intact (Bravo *et al.*, 2011) and this probiotic has since been shown to increase the firing rate of vagal afferents (Pérez-Burgos *et al.*, 2013). Furthermore, research published this month has reported that activation of vagal afferents innervating the gut is integral to the neuronal reward circuitry, leading to increased levels of striatal dopamine and resulting in reward behaviours (Han *et al.*, 2018). Since the reward system plays a key role in social motivation (Krach *et al.*, 2010), this vagal-mediated pathway may provide a mechanism via which the gut microbiome can affect social behaviour.

Although some studies have shown that an intact vagus nerve is required for gut microorganisms to affect the brain and behaviour (Lyte *et al.*, 2006; Bercik *et al.*, 2011b; Bravo *et al.*, 2011; O’Leary *et al.*, 2018), others find that effects of the gut microbiome can be independent of the vagus nerve (Bercik *et al.*, 2010, 2011a). In addition, since the vagus nerve enables bidirectional communication, the brain can also send signals to the gut via efferent fibres of the vagus nerve, modulating motility and immune homeostasis of the gut, as well as its neurochemistry (Fung *et al.*, 2017).

1.6.1.2 Enteric nervous system

The enteric nervous system is estimated to contain over 200 million neurones and so is often referred to as the ‘second brain’ (Furness, 2006). It plays an important role in sensing and responding to the microbial community by sending neural signals throughout the gut and to other

organs, including the brain (Yoo & Mazmanian, 2017). Both gut microorganisms and their metabolites, such as short-chain fatty acids, can activate enteric neurones which can then influence gut motility (Yoo & Mazmanian, 2017). Metabolites produced by the microbiota can also upregulate the synthesis of serotonin by enterochromaffin cells, resulting in increased stimulation of enteric neurones (Yano *et al.*, 2015). In addition, the gut microbiome has recently been shown to regulate the maturation of the enteric nervous system through the serotonin system (de Vadder *et al.*, 2018). Activity of the enteric nervous system can also be affected by the microbiota via the Toll-like receptors of the immune system (Brun *et al.*, 2013) and secretory products of macrophages (Muller *et al.*, 2014). Thus there are numerous ways in which the gut microbial community can alter activity of the enteric nervous system and these changes may then influence the central nervous system via the intimate connections between the two (Rao & Gershon, 2016).

1.6.1.3 Microorganism-derived neuroactive chemicals

Chemicals that have neuroactive properties in animals, such as dopamine, serotonin and gamma-amino butyric acid (GABA), are also produced by bacteria (Iyer *et al.*, 2004). In fact, while these chemicals are known as neurotransmitters in animals, they serve as cell signalling messengers across the biological kingdoms and are also found in plants and fungi, as well as bacteria. The presence of these cell signalling messengers in eukaryotes is hypothesized to be due to horizontal gene transfer from bacteria (Iyer *et al.*, 2004) and points to the intriguing possibility of inter-kingdom communication between the host and its associated microbiota. This has therefore been proposed as a possible mechanism mediating the microbiome–gut–brain axis (Lyte, 2013). However, while numerous bacterial species known to inhabit the human gut have been shown to produce neuroactive chemicals (Wall *et al.*, 2014; Strandwitz, 2018), this has mainly been observed *in vitro* and the degree to which this occurs *in vivo* in the gut remains unclear (Sampson & Mazmanian, 2015). Even if such microorganism-derived neuroactive chemicals are produced in substantial quantities in the gut, it is unknown whether they have a measurable effect on brain function and behaviour (Johnson & Foster, 2018 [Appendix]).

There is also evidence that certain bacteria have uptake mechanisms for neurotransmitters, potentially modulating host levels. For example, it has recently been reported that *Lactobacillus salivarius* has a biogenic amine transport system which may affect levels of serotonin, dopamine and noradrenaline in the gut (Lyte & Brown, 2018). In addition, the gut microbiota plays a role in generating biologically active forms of these three neurotransmitters from their biologically inactive forms in the gut lumen, via deconjugation by bacterial enzymes (Asano *et al.*, 2012; Hata *et al.*, 2017). Bacteria also produce short-chain fatty acids as a result of fermentation, which have both anti-inflammatory and neuroactive properties (Koh *et al.*, 2016; Stilling *et al.*, 2016a) and are able to cross the blood–brain barrier (Sampson & Mazmanian, 2015). For example, acetate can be taken up by the brain, and subsequently alters levels of the neurotransmitters glutamate and GABA (Frost *et al.*, 2014).

1.6.2 Immune signalling

There is increasing evidence to support the role of the immune system as an important mediator of the microbiome–gut–brain axis (Fung *et al.*, 2017). The gut microbiome has a profound influence on the host's immune system and this is particularly evident in the case of germ-free animals whose immune function is severely compromised (Round & Mazmanian, 2009; Hooper *et al.*, 2012; Belkaid & Hand, 2014). Gut microorganisms interact with the host's innate immune system via stimulating pattern-recognition receptors, such as Toll-like receptors, on intestinal epithelial cells (Chu & Mazmanian, 2013). Microbial signalling via pattern-recognition receptors is important for development of the immune system and also maintains immune homeostasis through secretion of anti-inflammatory cytokines (Chu & Mazmanian, 2013). Indeed, probiotic studies have repeatedly reported the anti-inflammatory effects of bacteria such as *Bifidobacterium* and *Lactobacillus* (Round & Mazmanian, 2009). However, in the presence of pathogenic microorganisms, a pro-inflammatory response is triggered via pattern-recognition receptors (Chu & Mazmanian, 2013). Both pro-inflammatory and anti-inflammatory cytokines released by immune cells can then be transported in the blood to reach the brain (Powell *et al.*, 2017). Interestingly, administration of heat-killed bacteria has also been shown to affect both

the pain response (Kamiya *et al.*, 2006) and gene expression of intestinal epithelial cells (Rousseaux *et al.*, 2007). Such effects are likely due to components of the peptidoglycan cell wall, such as polysaccharide A and lipopolysaccharides, which can interact with Toll-like receptors and thus modulate immune signalling (McDonald *et al.*, 2005; Mazmanian & Kasper, 2006).

In addition, microbial metabolites, most notably short-chain fatty acids, modulate the differentiation and functioning of regulatory T cells which are integral to maintaining immune homeostasis of the gut and suppressing inflammation (Furusawa *et al.*, 2013; Smith *et al.*, 2013; Rooks & Garrett, 2016). Microbial metabolites can also cross the intestinal barrier and enter the circulatory system where they are detected by the host's immune cells (Dorrestein *et al.*, 2014). Furthermore, the gut microbiome can directly affect the immune response of the central nervous system by influencing microglial maturation and function (Erny *et al.*, 2015; Thion *et al.*, 2018).

Such changes to the immune system may then affect brain function and behaviour through the intricate connections between the immune and nervous systems (Steinman, 2004; Baganz & Blakely, 2013; Louveau *et al.*, 2015). For example, cytokines can affect neurotransmitter pathways (Miller *et al.*, 2013) and have been implicated in mood regulation (Pollak & Yirmiya, 2002; Dunn *et al.*, 2005). Indeed, the state of the immune system is increasingly being linked to behaviour (McCusker & Kelley, 2013) and neuropsychiatric conditions such as depression (Dantzer *et al.*, 2008; Wohleb *et al.*, 2016) and autism (Hsiao, 2013; Meltzer & van de Water, 2017). Notably, probiotic administration leads to significant attenuation of pro-inflammatory cytokines (Desbonnet *et al.*, 2008) and since probiotics can alleviate depressive symptoms, it is suggested that their primary mechanism of action may be through reducing systemic inflammation (Park *et al.*, 2018). The immune system is therefore thought to play an important role in integrating the bidirectional signals between the gut microbiome and the central nervous system (Fung *et al.*, 2017; Powell *et al.*, 2017). However, some studies have reported effects of gut bacteria on the brain that appear to be independent of changes in inflammatory state (Lyte *et al.*, 2006; Bercik *et al.*, 2011a; Pinto-Sanchez *et al.*, 2017), indicating that other communication pathways also mediate the microbiome–gut–brain axis.

1.6.3 Endocrine signalling

Gut microorganisms are in close proximity to enteroendocrine cells and can influence the production of various hormones in the gut (Collins *et al.*, 2012; Neuman *et al.*, 2015). While the mechanisms involved in signalling between the microbiome and endocrine system are yet to be elucidated, manipulations of the gut microbial community have been shown to alter hormone levels (Neuman *et al.*, 2015). For example, probiotic treatment reduces corticosterone concentration in rodents (Gareau *et al.*, 2007; Bravo *et al.*, 2011). There is also evidence in humans that both probiotics and prebiotics (selectively fermented indigestible carbohydrates such as fructo- and galacto-oligosaccharides which promote the growth and/or activity of certain gut bacteria) can decrease cortisol levels (Messaoudi *et al.*, 2011; Schmidt *et al.*, 2015). This suggests that interactions between the gut microbiome and the endocrine system may play an important role in influencing behaviours associated with stress and anxiety (Dinan & Cryan, 2012; Neuman *et al.*, 2015). Interestingly, a new study has reported evidence that enteroendocrine cells are directly connected to the brainstem in one synapse via vagal neurones (Kaelberer *et al.*, 2018) and so this may provide another route via which gut microorganisms and their metabolites can affect the brain by interacting with these cells lining the intestinal epithelium.

Experiments with germ-free mice have revealed that the gut microbiome is involved in programming the hypothalamus–pituitary–adrenal axis. Specifically, germ-free mice show an exaggerated hypothalamus–pituitary–adrenal axis response to stress which can be normalized by colonization with *Bifidobacterium infantis* (Sudo *et al.*, 2004). A microbiota transplant from specific pathogen-free mice can partly correct the elevated hypothalamus–pituitary–adrenal axis response but only at an early developmental stage, highlighting the role played by the microbiota during postnatal development (Sudo *et al.*, 2004).

In addition to stress hormones, the gut microbiome also affects levels of hormones involved in metabolism regulation and appetite control, as well as sex hormones and growth hormones (Neuman *et al.*, 2015). Microbial metabolites including short-chain fatty acids can regulate the

secretion of gut hormones, such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine–tyrosine (PYY). These hormones are integral to host appetite and satiety (Fetissov, 2016) but can also have wider effects, influencing behaviour and cognition (Stadlbauer *et al.*, 2015). Moreover, a recent study showed that a high-fat diet leads to changes in the gut microbiome which can induce anxiety and depressive-like symptoms in mice via the action of insulin (Soto *et al.*, 2018). Specifically, these behavioural abnormalities were associated with decreased insulin signalling and increased inflammation in the brain, as well as altered levels of metabolites and neurotransmitters in the brain and plasma. In fact, this effect of the gut microbiome on behaviour via changes in insulin sensitivity and metabolism may be one of the reasons why diabetes is associated with an increased prevalence of anxiety and depression (Soto *et al.*, 2018).

1.7 Bidirectional interactions between the gut microbiome and behaviour

In addition to the ways in which the gut microbiome can communicate with the brain to affect behaviour, the emotional state and behaviour of the host can in turn influence the microbiome. Such reciprocal interactions have predominantly been studied with respect to the stress response. As well as the gut microbiome's role in regulating the hypothalamus–pituitary–adrenal axis (Rea *et al.*, 2016), the secretion of cortisol can influence gut permeability and leads to an altered composition and reduced diversity of the microbial community, as well as affecting immune cell function (Collins *et al.*, 2012).

There is also increasing support for the role of social behaviour in shaping the composition and diversity of the gut microbiome (Archie & Tung, 2015), as discussed in detail in Chapter 4. Studies in primates have found that different social groups have distinct gut microbial communities, with social interactions promoting microbial similarity between individuals (Tung *et al.*, 2015; Moeller *et al.*, 2016; Raulo *et al.*, 2017). In addition, it has also been shown that social transmission of microorganisms contributes to gut microbiome diversity in both insect (Billiet *et al.*, 2017) and mammalian populations (Li *et al.*, 2016a; Moeller *et al.*, 2016; Perofsky *et al.*, 2017).

Furthermore, it is interesting to consider the potential for feedback loops to operate between the gut microbiome and host behaviour. For example, stress may negatively alter the composition of the gut microbial community, reducing the abundance of beneficial members of the gut microbiota such as *Lactobacillus* (Bailey & Coe, 1999; Bailey *et al.*, 2011; Galley *et al.*, 2014a; Marin *et al.*, 2017; Yu *et al.*, 2017). This disrupted gut microbiome composition may then dysregulate the stress response further, given the bidirectional communication between the gut microbiome and the hypothalamus–pituitary–adrenal axis. On the other hand, social behaviour may promote a diverse microbial ecosystem in the gut, limiting the growth of potentially pathogenic bacteria and thus helping to maintain a healthy abundance of beneficial gut microorganisms such as *Lactobacillus* which may have a positive effect on social behaviour (Buffington *et al.*, 2016; Leclercq *et al.*, 2017) and emotional behaviour (Bravo *et al.*, 2011; Takada *et al.*, 2016). Such feedback loops may also be pertinent to the observed association between gastrointestinal and neuropsychiatric disorders.

Thus, as well as the evidence that the gut microbiome can modulate host sociability, stress, anxiety and depression, social and emotional behaviour of the host may in turn alter the gut microbial community. This highlights the intricate bidirectional nature of interactions between the gut microbiome and behaviour.

1.8 Assessing the gut microbiome

The gut microbiome is typically assessed non-invasively through collection of a faecal sample. The sample is processed to extract the DNA, which is usually amplified prior to sequencing (Goodrich *et al.*, 2014). Sequencing is a particularly suitable technique for determining gut microbiome composition since most gut bacteria are anaerobic and therefore cannot easily be detected using traditional culturing methods (Morgan & Huttenhower, 2012). The most commonly used and economical sequencing method to assess microbiome composition is 16S ribosomal RNA (rRNA) gene sequencing. The 16S rRNA gene contains sites that are highly conserved between different species and primers are designed to bind to these conserved regions, meaning that they can target all prokaryotes (Coenye & Vandamme, 2003). The 16S

rRNA gene also contains hypervariable regions, thereby allowing taxonomic identification (Coenye & Vandamme, 2003).

Microbiome composition can be evaluated at different taxonomic levels, ranging from phylum to species. In addition to taxonomic composition, a common technique to assess a microbial community is to estimate its diversity. There are two main ways in which diversity is assessed: alpha diversity measures the diversity within a sample (such that a sample with a greater number of taxa and/or a more even distribution of taxa has a higher diversity score), while beta diversity measures the differences in taxonomic composition between samples (Finotello *et al.*, 2016). Microbiome diversity analyses can be conducted using a range of programmes, including several R software packages or the bioinformatics software Quantitative Insights Into Microbial Ecology (QIIME).

1.9 Thesis outline

The main aim of this thesis is to study the interactions between the gut microbiome and behaviour using a multilevel approach. I begin at the molecular level with experiments in antibiotic-treated and germ-free mice to determine how manipulating the gut microbial community affects the expression of neuropeptide systems implicated in social and emotional behaviour (Chapter 2). At the organism level, I investigate whether the composition and diversity of the gut microbiome is related to behavioural traits such as sociability, neuroticism, anxiety and stress in a large human cohort (Chapter 3). I then study a social network of rhesus macaques from a non-captive population to investigate whether they also show variation in the gut microbiome with respect to differences in sociability (Chapter 4). In the following chapter, I examine the relationship between the gut microbiome and the brain from an evolutionary perspective and test my hypothesis that a more efficient gut microbial community may have facilitated evolution of mammalian brain size and thus the development of complex social behaviour (Chapter 5). Finally, I consider the evolution of the microbiome–gut–brain axis through the application of evolutionary theory (Appendix).

Primarily, my research lies at the interface between the gut microbiome and the behaviour of animals, including humans. Together, these chapters aim to develop an integrated view of the microbiome–gut–brain axis and its relationship with host behaviour. My thesis adopts an interdisciplinary approach, spanning microbiology, neuroscience, metagenomics, molecular genetics, human psychology, animal behaviour and evolutionary biology. This allowed me to use a range of techniques to address my research from different perspectives. I summarize each of these chapters below, outlining their content and scope, discussing the knowledge gaps that they address and their significance and contribution to the field.

1.9.1 Chapter 2 | Neuropeptide pathways

Here I study the microbiome–gut–brain axis at the molecular level, using a bottom-up approach to investigate the causal relationship between the gut microbiome and the neurogenetics of social and emotional behaviour. Specifically, I examine how experimentally manipulating the gut microbiome, using either antibiotic treatment or germ-free conditions, affects the expression of neuropeptide systems in the brain. This complements previous research which has focused on how the gut microbiome influences neurotransmitter systems. Numerous animal studies have implicated the microbiome in social behaviour since both antibiotic-treated and germ-free animals display social deficits. As neuropeptide signalling is integral to mammalian social behaviour, I examine the effects of manipulating the gut microbiome on the expression of both receptors and peptides of the μ -opioid, oxytocin and vasopressin signalling systems. The impact of germ-free status and antibiotic treatment on the expression of these neuroreceptors has not previously been investigated. Furthermore, this is the first study to examine whether the gut microbiome interacts with the brain's opioid system, which could have important implications not only for social and emotional behaviour but also pain perception.

1.9.2 Chapter 3 | Human personality

Most research on the microbiome–gut–brain axis has so far been conducted in animal models. The limited number of human studies has focused on psychiatric traits, revealing that gut

microbiome composition and diversity are altered in neuropsychiatric conditions such as depression and autism. While the gut microbiome has been shown to influence stress, anxiety, depressive symptoms and social behaviour, it remains unknown whether the microbiome–gut–brain axis may also be relevant to personality traits in the general population. Here I combine metagenomic analysis of the human gut microbiome with personality phenotyping and collection of metadata (relating to diet, health, lifestyle and sociodemographics) to investigate whether personality traits are related to the composition and diversity of the gut microbial community in a large human population.

1.9.3 Chapter 4 | Macaque social network

As in the human study, this chapter provides a top-down approach to investigate the relationship between the gut microbiome and behaviour. Here I examine in a free-ranging macaque population how differences between individuals in gut microbiome composition and diversity are related to behavioural variation. While the microbiome–gut–brain axis has been well studied in model organisms and clinical populations, it is yet to be explored with respect to the behaviour of non-captive animals. Social network analysis of this macaque population provides a measure of an individual's tendency to engage in social interactions. The main focus of this chapter is to test whether the abundances of certain bacterial genera (namely those previously associated with sociability or autistic-like behaviours in rodents and humans) are related to individual differences in social behaviour. I also examine whether social interactions influence both gut microbiome diversity and compositional similarity between individuals.

1.9.4 Chapter 5 | Encephalization

Here I study the macroevolutionary relationship between encephalization and the composition of the mammalian gut microbiome. The primary motivation for this research is the well-known theory in anthropology, called the 'expensive tissue' hypothesis, proposing that evolution of increased brain size was accompanied by a compensatory reduction in the size of other metabolically costly organs, particularly the gut. Since this theory has received limited empirical

support, here I propose that rather than a reduction in gut size, perhaps a change in its microbial community may have helped to meet the cost of encephalization through increasing the efficiency of energy extraction from the diet. In particular, I examine the relationship between encephalization and the relative abundances of Bacteroidetes and Firmicutes, since these are the two dominant phyla of the mammalian gut microbiome and have been extensively linked to energy harvest efficiency. While research on the relationship between the gut microbiome and the brain has focused on host health, this represents the first investigation into the possible role of the gut microbiome in mammalian brain evolution.

1.9.5 Appendix Chapter | Evolutionary theory

The final aspect of my thesis is theoretical and considers the selective pressures that may have led to the observed effects of the gut microbiome on the brain and behaviour by applying evolutionary theory to the microbiome–gut–brain axis. In particular, I examine the validity of the emerging hypothesis that members of the gut microbiota may actively manipulate host behaviour to enhance their own transmission, such as increasing host sociability. I combine the evolutionary theory of parasite manipulation with recent empirical studies of the microbiome–gut–brain axis to determine whether the conditions required for manipulation are satisfied by the mammalian gut microbiome and propose alternative explanations for the evolution of microbial effects on the brain.

1.10 My DPhil publications

Johnson, K.V.-A. & Foster, K.R. (2018) Why does the microbiome affect behaviour? *Nat. Rev. Microbiol.* **16**, 647–655.

Selected to be published in print in special 15th anniversary issue of Nature Reviews Microbiology and ranked 1st out of all articles ever published in this journal by Altmetric. Notable media coverage including interview on BBC Radio 4 PM programme and Fun Kids Science Weekly (most popular UK children's podcast).



Johnson, K.V.-A. & Burnet, P.W.J. (2016) Microbiome: should we diversify from diversity? *Gut Microbes* **7**, 455–458.

Johnson, K.V.-A. Gut microbiome composition and diversity are related to human personality traits (submitted).

Johnson, K.V.-A. & Burnet, P.W.J. Opposing effects of antibiotics and germ-free status on the neurogenetics of social behaviour (written for submission).

Johnson, K.V.-A. & Dunbar, R.I.M. Cost of encephalization may have been met by changes in the mammalian gut microbiome (submitted).

Johnson, K.V.-A., Brent, L.J.N., Watson, K.K., Dunbar, R.I.M. & Burnet, P.W.J. Sociability in a non-captive macaque population is linked to beneficial gut bacteria (in preparation).

Sarkar, A., Harty, S., **Johnson, K.V.-A.** *et al.* Microbial transmission in animal social networks and the emergence of the social microbiome [review article] (joint second author, submitted).

Sarkar, A., Harty, S., **Johnson, K.V.-A.** *et al.* The role of the microbiome in the neurobiology of social behaviour [review article] (joint second author, in preparation).

Oxford Interdisciplinary Microbiome Team, **Johnson, K. V.-A.** *et al.* Setting the agenda for social science research in the human microbiome (submitted).

I also published my findings from both three-month rotation projects that I conducted as part of the first year of the Doctoral Training Programme:

Johnson, K.V.-A. & Dunbar, R.I.M. (2016) Pain tolerance predicts human social network size. *Sci. Rep.* **6**, 25267.

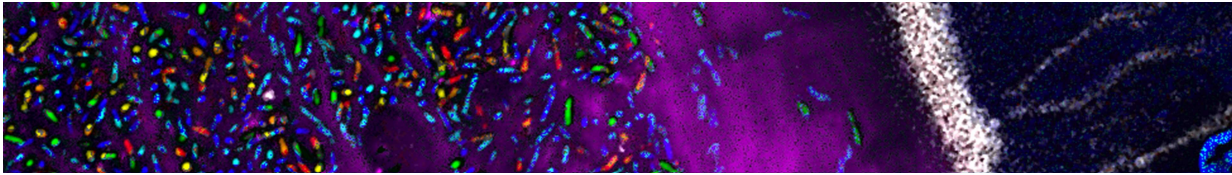
Worldwide media coverage including live filming for Sky and CTV News; ITV News feature filming; BBC World Service and international radio interviews; all main newspapers (e.g. The Times, Telegraph, Guardian) and online magazines like Time. Ranked 16th by Altmetric out of all articles published in this journal and in top 1,000 out of over 11 million articles ever published in any journal.



Johnson, K.V.-A., Aplin, L.M., Cole, E.F. *et al.* (2017) Male great tits assort by personality during the breeding season. *Anim. Behav.* **128**, 21–32.

Considerable media coverage, most notably an interview on BBC Radio 4 Inside Science. Ranked 11th by Altmetric out of all articles ever published in this journal.





Chapter 2

Effect of the gut microbiome on the expression of neuropeptide pathways underpinning social and emotional behaviour

2.1 Abstract

Recent research has revealed that the community of microorganisms inhabiting the gut affects brain development, function and behaviour. In particular, disruption of the gut microbiome during critical developmental windows can have lasting effects on host physiology. Both antibiotic exposure and germ-free conditions impact the central nervous system and can alter multiple aspects of behaviour. Social impairments are typically displayed by antibiotic-treated and germ-free animals, yet there is a lack of understanding of the underlying neurobiological changes. Since the μ -opioid, oxytocin and vasopressin systems are key modulators of mammalian social behaviour, here I investigate the effect of experimentally manipulating the gut microbiome on the expression of these pathways. I show that social neuropeptide signalling is disrupted in germ-free and antibiotic-treated mice, which may contribute to the behavioural deficits observed in these animal models. The most notable finding is the reduction in neuroreceptor gene expression in the frontal cortex of mice administered an antibiotic cocktail post-weaning. While antibiotic exposure is often considered a more amenable alternative to germ-free conditions, my contrasting results for these two treatments suggest that they should be viewed as distinct models. Since antibiotic administration is common in childhood and adolescence, my findings highlight the potential adverse effects that antibiotic exposure during these key neurodevelopmental periods may have on the human brain, including the possible increased risk of neuropsychiatric conditions later in life.

2.2 Introduction

The regulation of behaviour and emotion is complex, influenced by both genes and the environment. The microbial environment within the gut, known as the gut microbiome, has recently been shown to affect various aspects of brain development and behaviour (Cryan & Dinan, 2012; Sampson & Mazmanian, 2015; Sharon *et al.*, 2016). The brain is particularly sensitive to perturbations throughout childhood and adolescence when its structure is undergoing rapid change (Lenroot & Giedd, 2006; Lamblin *et al.*, 2017). During this time, environmental disruptions may permanently impact brain function and increase susceptibility to neuropsychiatric conditions. These include changes to the gut microbiome which can affect neurodevelopment via gut–brain signalling. The microbial community of the gut may influence the functioning of the central nervous system through various mechanisms, including communication via the nervous, immune and endocrine systems (Collins *et al.*, 2012; Cryan & Dinan, 2012; Johnson & Foster, 2018 [Appendix]).

Numerous studies in rodents have revealed that manipulation of the gut microbiome can affect the brain's anatomy and physiology, as well as behaviour (Cryan & Dinan, 2012; Sampson & Mazmanian, 2015; Sharon *et al.*, 2016). In particular, multiple facets of social behaviour are influenced by the gut microbial community (Vuong *et al.*, 2017). Germ-free animals provide a useful model to directly investigate which aspects of neurodevelopment and behaviour are modulated by the gut microbiome since they are raised in a sterile environment with no associated microorganisms. The germ-free phenotype exhibits behavioural traits reflective of autism, such as a reduced preference for social interactions (Crume yrolle-Arias *et al.*, 2014; Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Luk *et al.*, 2018; Stilling *et al.*, 2018) and social novelty (Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Lu *et al.*, 2018), as well as repetitive behaviours (Desbonnet *et al.*, 2014). However, if germ-free mice are colonized at weaning with gut microbiota from conventionally raised mice, their deficits in social interaction and repetitive behaviours can be reversed (Desbonnet *et al.*, 2014). While the majority of studies using the standard three-chamber social interaction test (Silverman *et al.*, 2010) have found that germ-free mice are less sociable (Desbonnet *et al.*, 2014; Buffington *et al.*, 2016; Luk *et al.*, 2018;

Stilling *et al.*, 2018), one study has reported an increase in sociability (Arentsen *et al.*, 2015). This may be due to different experimental conditions such as the age of the mice being tested, the strain of stimulus mouse used in the social interaction test and animal husbandry practices. Nonetheless, germ-free studies reveal that the gut microbial community is important for normal social development.

In addition, recent research provides strong support for the causal relationship between a dysbiotic gut microbiome and altered social behaviour (Buffington *et al.*, 2016). Offspring of mice which had been fed a high-fat diet exhibited autistic-like behaviours including social impairments, anxiety and repetitive behaviours, as well as fewer oxytocin-expressing neurones in the hypothalamus. Notably, offspring were found to have an altered gut microbial community but supplementation with the bacterial species *Lactobacillus reuteri* restored oxytocin levels and reversed social deficits in this mouse model of autism.

Another useful approach to investigate the effect of the gut microbiome on the brain and behaviour is the use of antibiotic treatment to deplete the gut microbial community during specific developmental periods (Hoban *et al.*, 2016a). Antibiotics alter the composition and function of the gut microbiome, as well as reducing its diversity (Chapter 3; Modi *et al.*, 2014). Studies in rodents have demonstrated that antibiotic treatment can cause visceral pain (Verdú *et al.*, 2006; O'Mahony *et al.*, 2014; Hoban *et al.*, 2016a), cognitive deficits (Desbonnet *et al.*, 2015; Fröhlich *et al.*, 2015; Hoban *et al.*, 2016a) and behavioural changes, including impairments in social behaviour (Bercik *et al.*, 2011a; Desbonnet *et al.*, 2015; Wang *et al.*, 2015; Hoban *et al.*, 2016a; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Kayyal *et al.*, 2018). For example, early-life antibiotic exposure to low-dose penicillin can have lasting effects on both gut microbiome composition and behaviour, with mice showing decreased sociability and a reduced preference for social novelty (Leclercq *et al.*, 2017; Kayyal *et al.*, 2018). However in both studies, supplementation with *Lactobacillus rhamnosus* at the same time as antibiotic treatment protected animals from these social impairments. Similarly, mice administered an antibiotic cocktail during the key developmental stage of early adolescence have an altered microbiome composition and exhibit cognitive and social deficits (Desbonnet *et al.*, 2015). They also show reduced expression of

the neuropeptides vasopressin and oxytocin, with the latter only reduced when the animals have been exposed to stress (Desbonnet *et al.*, 2015). In addition, administration of *L. reuteri* in mice has been found to increase plasma oxytocin through vagal signalling (Poutahidis *et al.*, 2013).

While studies have frequently reported the impact of an altered gut microbiome on host social behaviour, there is little understanding of the underlying neurochemical changes. I therefore aimed to address this by investigating the effect of antibiotic treatment and germ-free status on the gene expression of neuropeptides and their receptors implicated in social behaviour. Mammalian social behaviour is underpinned by multiple neuropeptide signalling pathways, namely the μ -opioid, oxytocin and vasopressin systems (Young *et al.*, 2001; Depue & Morrone-Strupinsky, 2005), which have evolutionarily conserved functions in regulating social behaviour (Donaldson & Young, 2008). Specifically, both oxytocin and vasopressin are well known for their roles in social cognition, pair bonding and sociosexual behaviours (Donaldson & Young, 2008; McCall & Singer, 2012). The endogenous opioid system is also involved in the modulation of social behaviour. As well as mediating physical pain (Zubieta *et al.*, 2001; Holden *et al.*, 2005), the opioid system plays a key role in social attachment, affiliative behaviour and emotion regulation. Indeed, opioid receptors are abundantly expressed in brain regions central to the regulation of social and emotional behaviour (Kennedy *et al.*, 2006; le Merrer *et al.*, 2009; Nummenmaa & Tuominen, 2018). Specifically, β -endorphin, which binds to μ -opioid receptors, modulates social motivation and is important for the formation and maintenance of social bonds (Burkett *et al.*, 2011; Johnson & Dunbar, 2016; Pearce *et al.*, 2017; Pellissier *et al.*, 2018). Activation of the μ -opioid system is associated with increased sociability (Trezza *et al.*, 2011; Loseth *et al.*, 2014), while the μ -opioid antagonist naltrexone diminishes feelings of social connection in people (Inagaki *et al.*, 2015) and inhibits social behaviour in rodents (Loseth *et al.*, 2014; Pellissier *et al.*, 2018). Notably, μ -opioid receptor knockout mice display symptoms characteristic of autism, with impairments in social interactions, communication and attachment behaviour (Moles *et al.*, 2004; Becker *et al.*, 2014; Gigliucci *et al.*, 2014). Furthermore, the endogenous opioid system regulates both oxytocin and vasopressin release and there is

considerable interaction between these three neuropeptide systems (van de Heijning *et al.*, 1991; Csiffáry *et al.*, 1992; Gao & Yu, 2004; Schorscher-Petcu *et al.*, 2010; Gigliucci *et al.*, 2014).

Neuropeptides are postulated to play an important part in communication between the gut microbiome and the brain, especially since they interact with both the immune system and the vagus nerve (Lach *et al.*, 2018). Interestingly, μ -opioid receptors are not only widely expressed in the brain but also in the gut, where they regulate the gut–brain neural circuitry involved in satiety (Durauffourd *et al.*, 2012). In fact, opioids, oxytocin and vasopressin can all influence gut physiology, such as motility (Li *et al.*, 2007; Monstein *et al.*, 2008; Holzer, 2011; Welch *et al.*, 2014). Certain *Lactobacillus* species can induce μ -opioid receptor expression in the gut via the nuclear factor- κ B immune response (Rousseaux *et al.*, 2007), while antibiotic treatment has been found to reduce expression of this receptor in the gut (Aguilera *et al.*, 2015). However, the interaction between the gut microbiome and the μ -opioid system in the central nervous system has not previously been investigated. Furthermore, there have been few studies on the relationship between the gut microbiome and brain neuropeptide systems (Desbonnet *et al.*, 2015; Buffington *et al.*, 2016), with research focusing on the expression of the peptides rather than their receptors.

Here I examine the influence of the gut microbiome on neuropeptide pathways implicated in social and emotional behaviour by measuring gene expression of both the peptides and their corresponding receptors. The experiments were conducted in mice since they are a naturally social mammalian species and therefore represent a suitable model organism (Shemesh *et al.*, 2014). I adopt two different approaches, using mice treated with antibiotics post-weaning and germ-free mice. This also provides the opportunity to compare results between the two, since both models are commonly used to ascertain the role of the microbiome in host development and behaviour. Given the behavioural impairments typically exhibited by both antibiotic-treated and germ-free mice, I hypothesize that the neuropeptide pathways of the brain will be dysregulated in both cases. Specifically, I hypothesize that the expression of the receptors and/or peptides may be downregulated in both treatments.

2.3 Materials and methods

2.3.1 Animals

For the antibiotic experiment, suckling male NIH Swiss mice (Charles River) at postnatal day 16 were housed with dams in cages maintained under standard controlled laboratory conditions with a 12 h light–dark cycle at $21 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity. At postnatal day 21, mice were weaned and housed three per cage under identical environmental conditions and fed a standard mouse chow diet *ad libitum*. The animals were allocated randomly to the treatment and control groups and were provided with either drinking water containing a cocktail of antibiotics or drinking water alone, respectively. For the germ-free experiment, Swiss Webster mice were housed by the supplier (Taconic Biosciences) with three to five mice per cage in a gnotobiotic isolator under a 12 h light–dark cycle at $21 \pm 1^\circ\text{C}$ and an average relative humidity of 50%. Mice were fed an autoclaved chow diet *ad libitum* and specific pathogen-free mice received chlorinated water, while germ-free mice received water that had been chlorinated and then autoclaved. The same strain, supplier and age (8 weeks) of germ-free and specific pathogen-free mice were used as in the seminal study showing reduced sociability in germ-free mice (Desbonnet *et al.*, 2014). In both the antibiotic and germ-free experiments there were eight male animals in each of the treatment and control groups ($n = 8$). This research was carried out with local ethical approval and a UK Home Office licence granted under the Animals (Scientific Procedures) Act 1986.

2.3.2 Antibiotic treatment

This method followed that of a previous study which had shown that an antibiotic cocktail administered in early adolescence altered cognitive, social and emotional behaviour (Desbonnet *et al.*, 2015). Mice were treated with antibiotics from postnatal day 21 to deplete the microbiota. This high-dose antibiotic cocktail administered in the drinking water comprised ampicillin (1 mg/ml), vancomycin (5 mg/ml), neomycin (10 mg/ml), metronidazole (10 mg/ml) and amphotericin-B (0.1 mg/ml). This specific mixture has been shown to reduce faecal bacterial

DNA load by 400 fold without causing morbidity (Reikvam *et al.*, 2011). Fluid intake was monitored every two days and the concentrations of antibiotics were adjusted according to the volume of fluid consumed. Mice were weighed routinely to monitor their general health and check that antibiotic treatment did not significantly affect body mass (Figure 2.1). Animals were treated with antibiotics until postnatal day 55, when they were sacrificed.

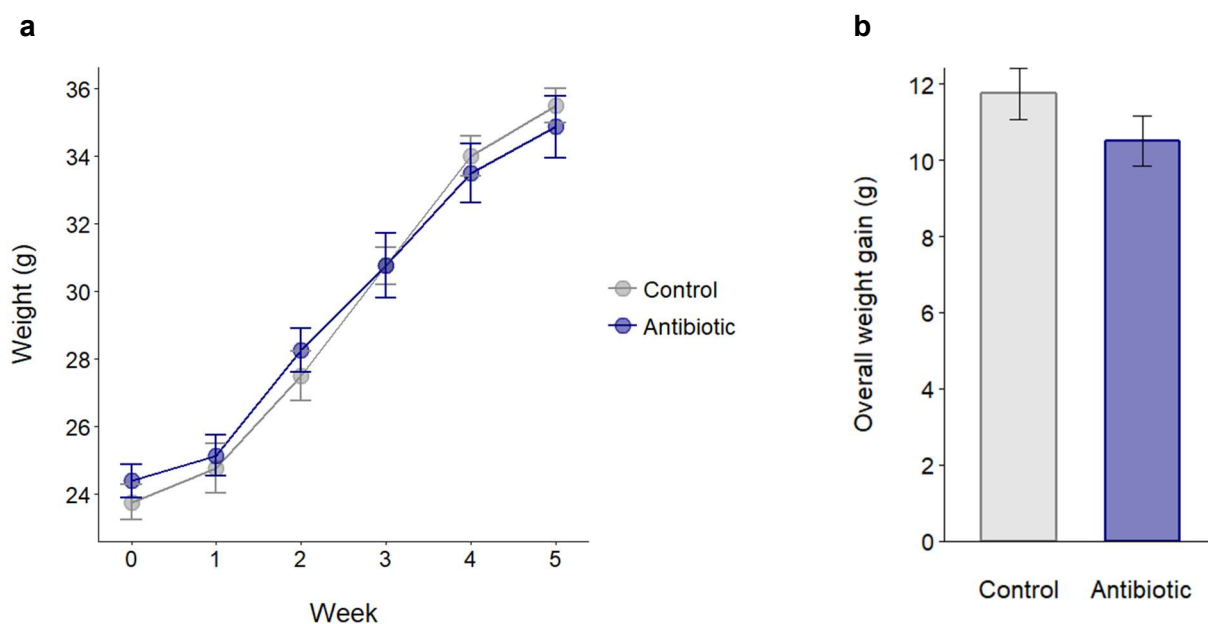


Figure 2.1. Effect of antibiotic treatment on body weight. Data are plotted as mean weight change $\pm$ SEM, with $n = 8$ per group. (a) Line graph depicts weekly changes in weight for each group throughout the experiment. (b) Bar plot shows overall weight gain from prior to the start of antibiotic treatment (week 0) to the end of treatment (week 5). There was no significant difference in weight gain between groups (Mann–Whitney U test, $P = 0.284$).

2.3.3 Tissue dissection

The brains of the antibiotic-treated and control mice were dissected into the frontal cortex, hippocampus, hypothalamus and brainstem and frozen at -80°C . The brain samples from germ-free and specific pathogen-free mice were placed in RNAlater (Sigma-Aldrich), transported on dry ice and then frozen at -80°C , prior to dissection into the same regions.

2.3.4 RNA extraction and cDNA synthesis

The mass of each brain region was measured (to the nearest mg) prior to homogenizing the tissue in TRI Reagent (Sigma-Aldrich) for RNA extraction. The RNA concentration of each sample was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and each RNA extract was assessed for purity by ensuring the 260/280 nm absorbance ratio was approximately two. Each RNA sample was diluted in nuclease-free water (Millipore) and stored at -20°C . In preparation for reverse transcription, the RNA was treated with DNase (Promega) and heated to 37°C for 20 min, followed by 5 min at 75°C . Complementary DNA (cDNA) was synthesized from 1 μg RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and incubated at 37°C for 1 h, along with a corresponding negative control for each sample. The cDNA samples were then diluted in nuclease-free water to a concentration of 20 ng/ μl , prior to storage at -20°C .

2.3.5 Quantitative real-time PCR

Quantitative real-time polymerase chain reaction (RT-PCR) was performed on the 7900HT Fast Real-Time PCR System (Applied Biosystems), with all cDNA samples diluted in nuclease-free water to a concentration of 5 ng/ μl . Each RT-PCR reaction consisted of 3 μl cDNA, 6 μl TaqMan Universal PCR Master Mix (no AmpErase UNG) and 0.6 μl TaqMan probes, and was made up to a total volume of 12 μl with nuclease-free water. The same thermal cycling conditions were used for all runs: 5 min at 95°C (initial step), 15 s at 95°C (denaturation step) and 45 s at 60°C (annealing and extension step), with the latter two steps repeated for 40 cycles. TaqMan gene expression assays (Table A.1) were used to quantify messenger RNA (mRNA) expression of genes encoding the μ -opioid (*Oprm1*), oxytocin (*Oxtr*) and vasopressin (*Avpr1a*) receptors and their corresponding peptides, namely pro-opiomelanocortin (*Pomc*), oxytocin (*Oxt*) and vasopressin (*Avp*) respectively (note that pro-opiomelanocortin is cleaved into multiple peptides, including β -endorphin which binds to μ -opioid receptors). While there are several opioid and vasopressin receptor genes, I measured the expression of *Oprm1* and *Avpr1a* since these are

most strongly associated with social behaviour (Koshimizu *et al.*, 2012; Freeman *et al.*, 2014; Loseth *et al.*, 2014; Pellissier *et al.*, 2018).

In addition, gene expression levels were also determined for myeloid differentiation factor 88 (*MyD88*) which is a key mediator of host–microorganism communication and integral to the host immune response and intestinal homeostasis (Rakoff-Nahoum *et al.*, 2004; Chu & Mazmanian, 2013). Expression of the gene encoding brain-derived neurotrophic growth factor (*Bdnf*), important for neuronal growth and survival, was measured as a positive control since several studies have reported its downregulation in both antibiotic-treated mice (Desbonnet *et al.*, 2015; Fröhlich *et al.*, 2015; Guida *et al.*, 2018) and germ-free mice (Sudo *et al.*, 2004; Diaz Heijtz *et al.*, 2011; Clarke *et al.*, 2013). Negative controls (both no-template and nuclease-free water) were also included in each run for the treatment and control groups. All samples were run in triplicate and the average threshold cycle number (C_T) recorded. As recommended for accurate normalization of RT-PCR data (Vandesompele *et al.*, 2002), C_T values for the target genes were normalized to the geometric mean of three endogenous control genes (*Gapdh*, *B2m* and *Polr2a*) whose expression did not differ significantly between the control and treatment groups.

2.3.6 Statistical analyses

Data were analysed using the comparative C_T method (Livak & Schmittgen, 2001; Schmittgen & Livak, 2008) and two-sample t-tests were performed on the $2^{-\Delta\Delta C_T}$ values to test for statistically significant differences in gene expression between the control and treatment groups. Where necessary, Welch's t-test was conducted when the variances of the two groups were significantly different (determined using Levene's test for homogeneity of variances) and the non-parametric Mann–Whitney U test was carried out when the assumption of normality was not met (determined using the Shapiro–Wilk test).

2.4 Results

2.4.1 Antibiotic-treated mice

The most striking effect of antibiotic administration was in the frontal cortex, where all three receptor genes showed a reduction in expression (Figure 2.2). This downregulation in the treatment group was significant for both *Oprm1* ($P = 0.021$) and *Oxtr* ($P = 0.016$), with a trend towards reduced expression for *Avpr1a* ($P = 0.079$). While there were no significant differences in receptor gene expression in the hippocampus or hypothalamus, there was a significant increase in *Oprm1* expression in the brainstem ($P = 0.046$).

In terms of the corresponding peptides (Figure 2.3), the expression of *Pomc* and *Avp* was reduced in the frontal cortex and hippocampus of the antibiotic-treated mice. However, these differences were not significant due to the considerable within-group variation in peptide gene expression. In the hypothalamus there was increased expression of both *Oxt* ($P = 0.065$) and *Avp* ($P = 0.037$), which was statistically significant in the case of *Avp*. In addition, there was a consistent trend towards reduced *Pomc* expression in each of the four brain regions, and when the results from all regions were combined, this downregulation was statistically significant ($P = 0.010$).

MyD88 expression was reduced in all four brain regions of antibiotic-treated mice (Figure 2.4), with a significant downregulation in the frontal cortex ($P = 0.021$) and brainstem ($P = 0.043$). Antibiotic administration also led to reduced expression of *Bdnf* (included as the positive control gene) in the frontal cortex ($P = 0.013$, Figure A.1). This is in accordance with the majority of previous research showing a decrease in *Bdnf* expression in antibiotic-treated mice (Desbonnet *et al.*, 2015; Fröhlich *et al.*, 2015; Guida *et al.*, 2018), thereby validating the treatment effects.

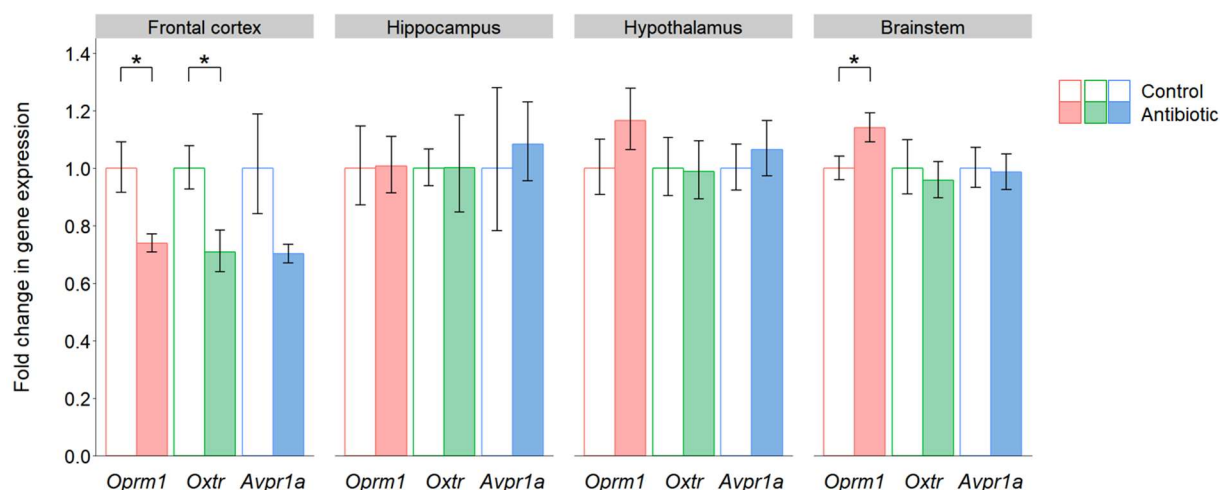


Figure 2.2. Effects of post-weaning antibiotic treatment on the expression of neuropeptide receptor genes implicated in social behaviour. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Oprm1*, *Oxtr* and *Avpr1a* (encoding the μ -opioid, oxytocin and vasopressin receptors respectively) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

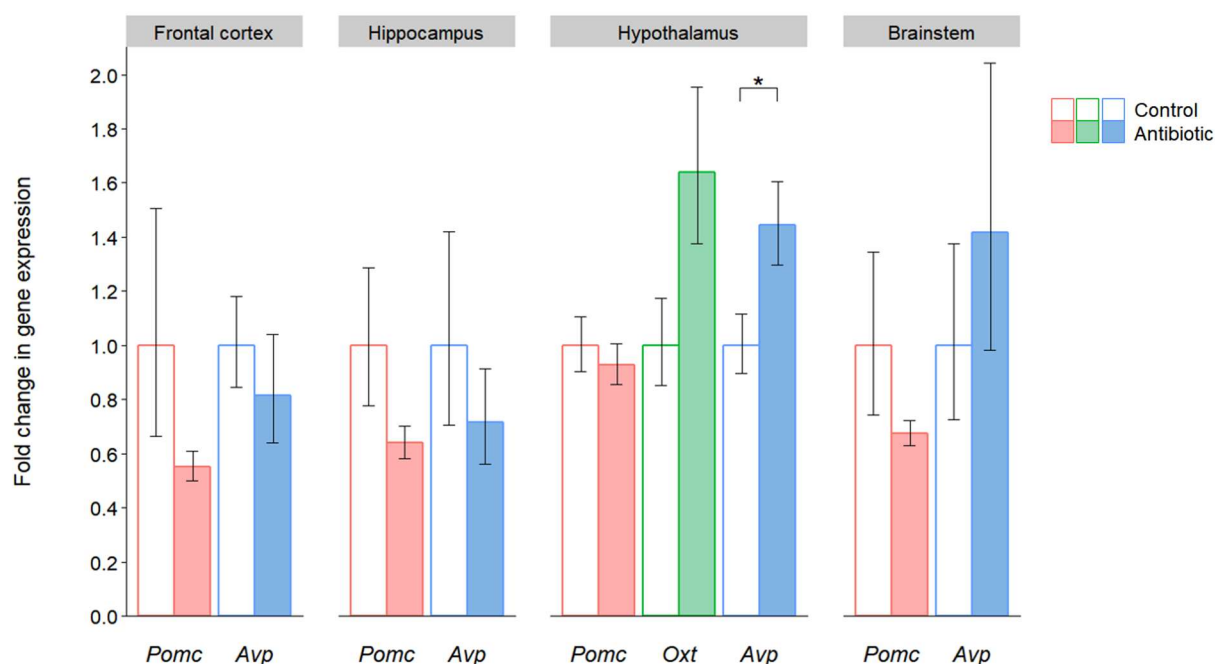


Figure 2.3. Effects of post-weaning antibiotic treatment on the gene expression of neuropeptides implicated in social behaviour. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Pomc*, *Oxt* and *Avp* (encoding the peptides pro-opiomelanocortin, oxytocin and vasopressin respectively) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$. Note that *Oxt* was only detectable in the hypothalamus, its site of production.

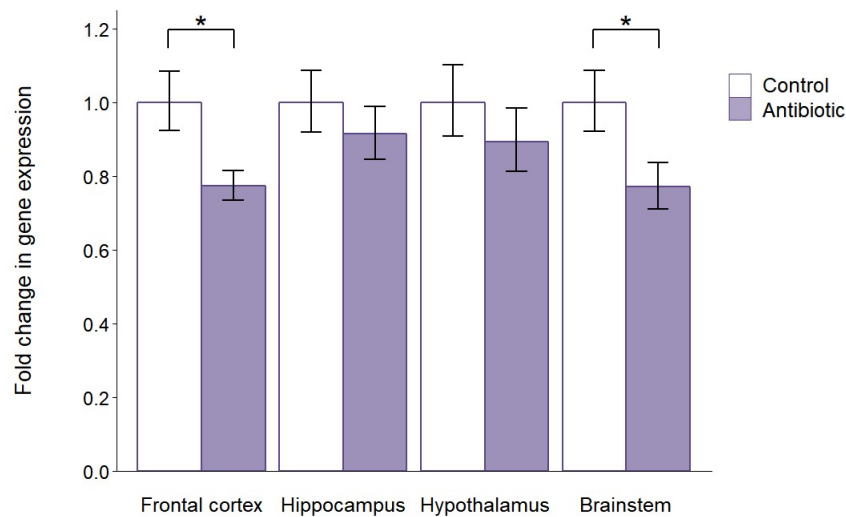


Figure 2.4. Effects of post-weaning antibiotic treatment on the gene expression of *MyD88*. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *MyD88* (encoding myeloid differentiation factor 88) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

2.4.2 Germ-free mice

The only significant change in receptor gene expression was for *Oprm1* in the frontal cortex (Figure 2.5). Notably the treatment effect was in the opposite direction to antibiotic-treated mice since *Oprm1* expression was upregulated in the frontal cortex of germ-free mice ($P = 0.018$).

Regarding peptide gene expression (Figure 2.6), *Avp* was downregulated in the hypothalamus of germ-free mice ($P = 0.046$), which again contrasted the direction of change in antibiotic-treated mice. *Pomc* expression was significantly reduced in the frontal cortex ($P = 0.030$) and combining the expression results from all four brain regions also revealed a significant overall downregulation of *Pomc* ($P = 0.008$), as was the case in the antibiotic-treated animals.

MyD88 expression was significantly reduced in the frontal cortex ($P = 0.019$) and brainstem ($P = 0.012$) of germ-free mice (Figure 2.7), as in the antibiotic treatment group. Similarly, *Bdnf* expression in germ-free mice (Figure A.2) was also reduced in the frontal cortex ($P = 0.050$) and brainstem ($P = 0.005$). These changes in the positive control gene are consistent with the majority of previous findings showing downregulation of *Bdnf* in germ-free mice (Sudo *et al.*, 2004; Diaz Heijtz *et al.*, 2011; Clarke *et al.*, 2013; Desbonnet *et al.*, 2015).

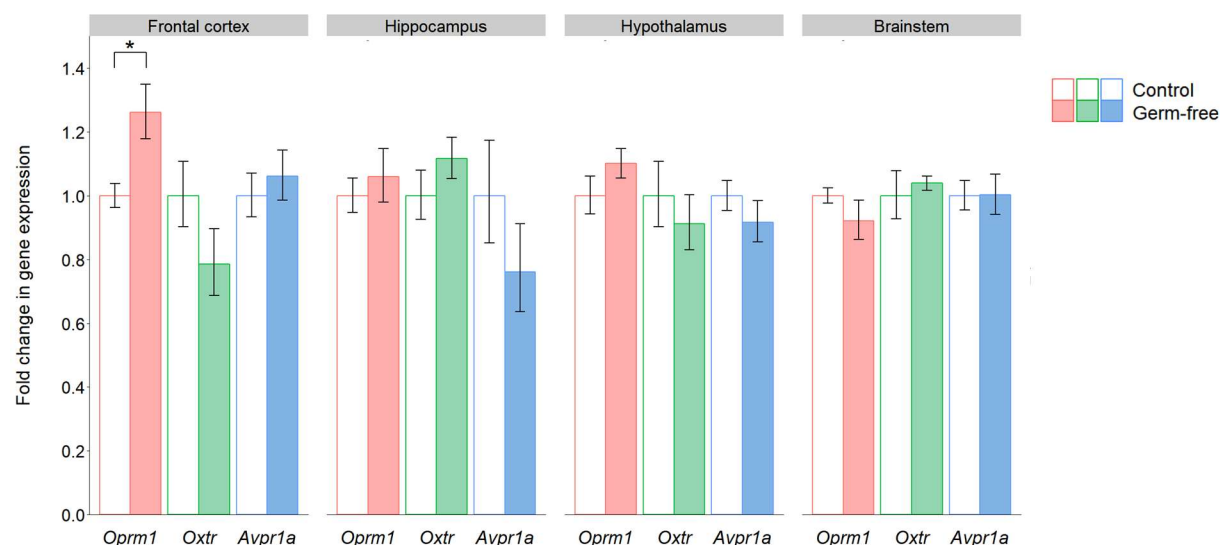


Figure 2.5. Effects of germ-free status on the expression of neuropeptide receptor genes implicated in social behaviour. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Oprm1*, *Oxt* and *Avpr1a* (encoding the μ -opioid, oxytocin and vasopressin receptors respectively) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

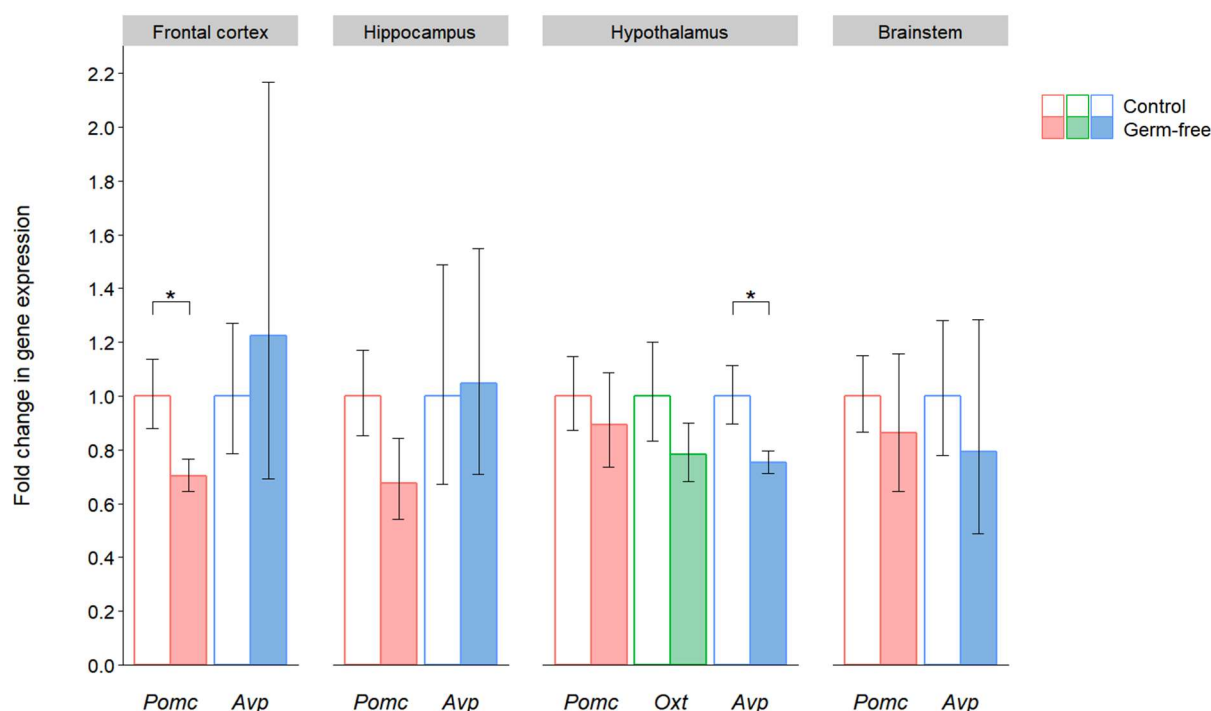


Figure 2.6. Effects of germ-free status on the gene expression of neuropeptides implicated in social behaviour. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Pomc*, *Oxt* and *Avp* (encoding the peptides pro-opiomelanocortin, oxytocin and vasopressin respectively) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

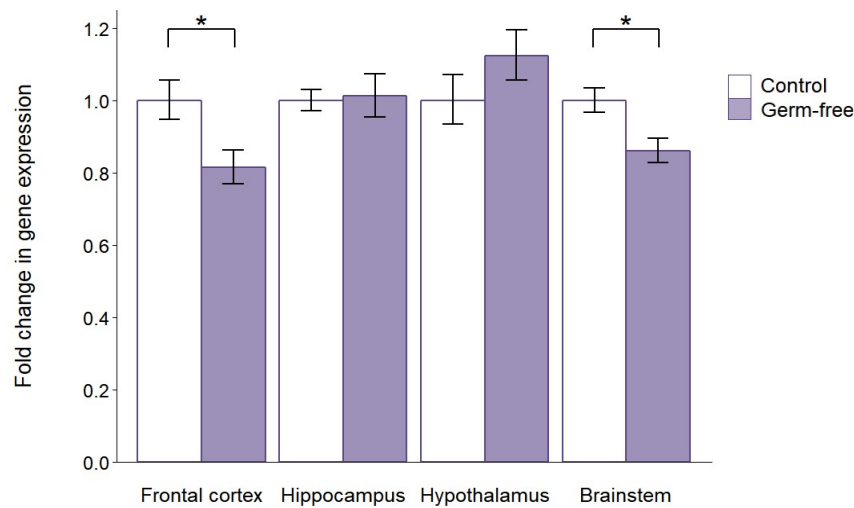


Figure 2.7. Effects of germ-free status on the gene expression of *MyD88*. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *MyD88* (encoding myeloid differentiation factor 88) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

2.5 Discussion

Here I show that the gene expression of social neuropeptides and their receptors is altered in both antibiotic-treated and germ-free mice. This is the first study that directly compares the effects in mice of antibiotic treatment and germ-free status on the brain, and the contrasting results reported here have implications for future research in this field. The most notable finding is the reduction in neuropeptide receptor gene expression in the frontal cortex of mice administered the antibiotic cocktail, which is particularly relevant given the prominent role of this brain region in social behaviour (Franklin *et al.*, 2017). Downregulation of these receptors may partly reflect the considerable interaction between the μ -opioid, oxytocin and vasopressin systems (van de Heijning *et al.*, 1991; Csiff  ry *et al.*, 1992; Gao & Yu, 2004; Schorscher-Petcu *et al.*, 2010; Gigliucci *et al.*, 2014). The behavioural impairments reported in antibiotic-treated rodents (Bercik *et al.*, 2011a; O'Mahony *et al.*, 2014; Desbonnet *et al.*, 2015; Wang *et al.*, 2015; Hoban *et al.*, 2016a; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Kayyal *et al.*, 2018), including mice receiving the identical combination of antibiotics used in this study (Desbonnet *et al.*, 2015), may therefore partially be a result of reduced activation of these neuropeptide pathways.

In contrast to the downregulation of *Oprm1* (encoding the μ -opioid receptor) in the frontal cortex of antibiotic-treated mice, this gene was significantly upregulated in the brainstem. Notably, research on the serotonergic system has also reported elevated signalling in the brainstem and reduced serotonin turnover in the frontal cortex of suicides with a psychiatric diagnosis (Bach *et al.*, 2014). This similar pattern of regional differences in the μ -opioid system may therefore reflect the known interaction between serotonin and opioid signalling (Tao & Auerbach, 2002; Lopez-Gimenez *et al.*, 2008; Manzke *et al.*, 2009). The antibiotic-treated mice also showed an increase in expression of *Avp* (encoding vasopressin) and a trend towards upregulation of *Oxt* (encoding oxytocin) in the hypothalamus, their site of synthesis, perhaps compensating for the corresponding reduction in receptor gene expression in the frontal cortex. Interestingly, a similar pattern of increased gene expression of the peptide but downregulation of its receptor was also reported for the neuropeptide Y pathway in antibiotic-treated mice (Fröhlich *et al.*, 2015). The upregulation of *Avp* found here is in the opposite direction to a previous study using the same antibiotic treatment and mouse strain, where *Avp* expression was reduced in the hypothalamus (Desbonnet *et al.*, 2015). One important difference, however, is that our animals were behaviourally naïve rather than having been subjected to a series of behavioural tests prior to measuring gene expression. In contrast to the increased expression of *Oxt* and *Avp* in the antibiotic treatment group, *Pomc* showed an overall reduction in expression when the results of all four brain regions were combined. This suggests reduced endorphin levels in the brains of antibiotic-treated mice, since the peptide encoded by *Pomc* is cleaved to produce β -endorphin. Combined with reduced expression of the corresponding receptor gene *Oprm1*, these data indicate that μ -opioid signalling may be attenuated in these animals. In fact, reduced activation of the μ -opioid system has been associated with social deficits (Loseth *et al.*, 2014; Pellissier *et al.*, 2018), and such deficits are typically observed in rodents administered antibiotics (Desbonnet *et al.*, 2015; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Kayyal *et al.*, 2018).

The one previous study looking at the effect of antibiotic treatment on the neuropeptides oxytocin and vasopressin only measured gene expression of the peptide and not its corresponding receptor (Desbonnet *et al.*, 2015). My results indicate that quantifying mRNA abundance for the

receptors, rather than the neuropeptides, may be more informative since they showed considerably less variation in gene expression. Perhaps this is because receptor transcript levels are more stable, with a lower turnover than signalling peptides. Thus, measuring both peptide and receptor gene expression may allow a more holistic assessment of the disruption of brain signalling pathways and the possible compensatory mechanisms involved.

It might be expected that germ-free conditions would have similar effects on gene expression as antibiotic exposure, and perhaps more extreme given the complete lack of microorganisms, rather than just a severe depletion. Surprisingly, the significant changes in expression were generally in the opposite direction to the antibiotic-treated mice. Notably, microRNAs have recently been shown to be differentially expressed in the brains of germ-free mice and antibiotic-treated adult rats (Hoban *et al.*, 2017). In my study, *Oprm1* was upregulated in the frontal cortex of germ-free mice, in contrast to its decreased expression in antibiotic-treated mice. Interestingly, germ-free mice showed reduced *Pomc* expression in the frontal cortex and this pattern of peptide downregulation and receptor upregulation in germ-free mice may reflect a homeostatic mechanism. In fact, there is evidence of feedback inhibition in the opioid system whereby high levels of receptor activation inhibit pro-opiomelanocortin neurone activity, resulting in lower concentrations of the peptide (Pennock & Hentges, 2011).

In light of the known interactions between these neuropeptides and the immune system (Chikanza & Grossman, 1998; Ninković & Roy, 2013; Li *et al.*, 2017) and the increasing evidence that the immune system is an important mediator of the microbiome–gut–brain axis (Fung *et al.*, 2017), I was also interested to investigate the effect of both antibiotic treatment and germ-free status on *MyD88* expression. This gene encodes an immune system protein which is a key mediator of host–microorganism communication in vertebrates. Specifically, MyD88 functions as an adaptor protein for Toll-like receptors, which detect and respond to gut bacteria in the intestinal epithelium (Rakoff-Nahoum *et al.*, 2004; Moynagh, 2005; Chu & Mazmanian, 2013). MyD88 signalling therefore plays an integral role in the host’s immune response (Koedel *et al.*, 2004; Pietras *et al.*, 2011; Chu & Mazmanian, 2013; Karmarkar & Rock, 2013) and is also important for homeostasis of the intestinal barrier (Rakoff-Nahoum *et al.*, 2004; Asquith *et al.*,

2010; Kubinak *et al.*, 2015). Both antibiotic treatment and germ-free status had the same effect on *MyD88* expression, with a significant reduction in both the frontal cortex and brainstem. Since commensal microbiota drive stimulation of *MyD88*, its downregulation may reflect the reduction in microbial interactions with Toll-like receptors due to the lack of microorganisms in antibiotic-treated and germ-free mice. In fact, there is evidence that both probiotics and prebiotics benefit gut health by stimulating the immune system through *MyD88*-dependent NF- κ B activation (van Baarlen *et al.*, 2009, 2011; Pagnini *et al.*, 2010; Ortega-González *et al.*, 2014; Hughes *et al.*, 2017). Moreover, *MyD88* is expressed in many different organs and cell types beyond the gut, including neurones (Liu *et al.*, 2016) and microglia (Dean *et al.*, 2010), suggesting that it may also be involved in communicating the state of the gut to distant organs such as the brain. Future studies using immunodeficient animals, such as *MyD88* conditional knockout mice, will be required to experimentally determine its role in the microbiome–gut–brain axis.

Overall, my findings suggest that the gut microbiome plays an important role in the correct development and functioning of neuropeptide pathways implicated in social and emotional behaviour. This is in line with previous studies showing social impairments in antibiotic-treated and germ-free rodents (Crume yrolle-Arias *et al.*, 2014; Desbonnet *et al.*, 2014, 2015; Buffington *et al.*, 2016; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Kayyal *et al.*, 2018; Lu *et al.*, 2018; Stilling *et al.*, 2018). However, while these animal models may be useful for establishing causality, we cannot necessarily extrapolate from these findings the role of the gut microbiome in the social behaviour of healthy animals. Future studies involving more fine-scale manipulations of the gut microbial community, such as probiotic or prebiotic feeding or narrow-spectrum antibiotic administration, are required to determine the contribution of specific members of the gut microbiota. While the use of antibiotics to deplete the gut microbiota is often seen as a more accessible alternative to germ-free models (Reikvam *et al.*, 2011; Desbonnet *et al.*, 2015), my results have implications for future research since the differences between antibiotic-treated and germ-free mice suggest that they should be viewed as distinct models of gut microbiome manipulation. Indeed, antibiotic-treated mice have a normal gut microbiome during the early postnatal period whereas germ-free mice represent a rather different biological case since these

animals are never exposed to microorganisms and so exhibit impaired physiology and immune development from birth. However, it cannot be eliminated that differences between the antibiotic-treated and germ-free mice may be partly due to the different mouse strains, though both were outbred strains. The antibiotic experiment was conducted with NIH Swiss mice since this was the same strain used by a previous study which had demonstrated effects of antibiotic treatment on the brain and behaviour (Desbonnet *et al.*, 2015), and whose methodology was followed here. The germ-free experiment was carried out with Swiss Webster mice since this is the strain most commonly available as a germ-free model and is the primary strain in studies reporting impaired sociability in germ-free mice (Desbonnet *et al.*, 2014; Arentsen *et al.*, 2015; Luk *et al.*, 2018; Stilling *et al.*, 2018).

To date, there have been few studies investigating the effects of antibiotics on the brain during early life (Diaz Heijtz, 2016), yet antibiotic treatment is arguably more clinically relevant than germ-free conditions. In fact, antibiotics are the most commonly prescribed drug to children (Chai *et al.*, 2012) and human population studies have linked antibiotic exposure during early life with increased risk of allergy, inflammatory bowel disease and obesity, as well as poorer neurocognitive outcomes (Neuman *et al.*, 2018). The results here, particularly the salient reduction in neuroreceptor gene expression in the frontal cortex, have potential implications for understanding the impact of antibiotic administration on human brain function. Furthermore, the interaction between the gut microbiome and the brain's endogenous opioid system has not previously been investigated, with the results of this study indicating reduced μ -opioid signalling in mice treated with antibiotics in early adolescence. This may at least partly explain the behavioural deficits in animals exposed to antibiotics (Desbonnet *et al.*, 2015; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Kayyal *et al.*, 2018) since activation of the μ -opioid system is integral to social motivation and social bonding (Loseth *et al.*, 2014; Pellissier *et al.*, 2018), as well as regulating stress and emotion (Ribeiro *et al.*, 2005; Bali *et al.*, 2015; Nummenmaa & Tuominen, 2018). While caution must be exercised when considering the possible impact of antibiotics during childhood and adolescent years in humans, I hypothesize that the μ -opioid system may also be downregulated, with implications for behaviour. Notably, μ -opioid receptor density in

the human frontal cortex is positively associated with motivation to seek close social relationships (Nummenmaa *et al.*, 2015), which is especially relevant given the reduced gene expression of this receptor in the frontal cortex of antibiotic-treated mice. Human brain imaging studies have also revealed that activation of the μ -opioid system helps to alleviate the experience of social pain and is positively correlated with social motivation and psychological resilience (Hsu *et al.*, 2013, 2015). Thus a decrease in μ -opioid signalling may have consequences for mental health and well-being. In fact, dysregulation of the endogenous opioid system has been implicated in depression and schizophrenia, with the majority of human studies reporting a reduction in β -endorphin levels (Bernstein *et al.*, 2002; Hegadoren *et al.*, 2009) or decreased activity of the μ -opioid system (Kennedy *et al.*, 2006; Hsu *et al.*, 2015). It is an interesting observation that antibiotic exposure in humans is associated with increased risk of depression and anxiety (Lurie *et al.*, 2015) and treatment with antibiotics during the first year of life has also been linked to an increased risk of depression and behavioural difficulties in childhood (Slykerman *et al.*, 2017). However, further research is required to determine the changes in brain function that may underlie these associations and whether the effects are long-lasting.

As well as its role in social behaviour, the endogenous opioid system is a key regulator of pain (Zubieta *et al.*, 2001; Holden *et al.*, 2005). These findings therefore have potential clinical relevance since they show that the gut microbiome affects the expression of opioid receptors in the central nervous system. In fact, antibiotic treatment in rodents increases visceral pain (Verdú *et al.*, 2006; Hoban *et al.*, 2016a), including early-life antibiotic exposure (O'Mahony *et al.*, 2014). The mechanisms by which the gut microbiome modulates pain are yet to be elucidated (O'Mahony *et al.*, 2017) but my findings suggest that the effect of the microbiome on both pain and social behaviour may be underpinned at least in part by its interaction with the μ -opioid system. In support of this, a recent study showed that decreased expression of *Oprm1* in the prefrontal cortex was associated with increased pain sensitivity in male and female rats (Yan & Kentner, 2017). A reduction in μ -opioid receptor availability in the brain has been linked to increased pain sensitivity in healthy volunteers (Prossin *et al.*, 2015) and has also been found in

sufferers of chronic pain (Harris *et al.*, 2007). In addition, since the opioid system is central to addiction (Darcq & Kieffer, 2018) and I show that the gut microbiome interacts with this neuropeptide system, my findings are also relevant to the observation that antibiotic treatment enhances addictive behaviour in mice (Kiraly *et al.*, 2016).

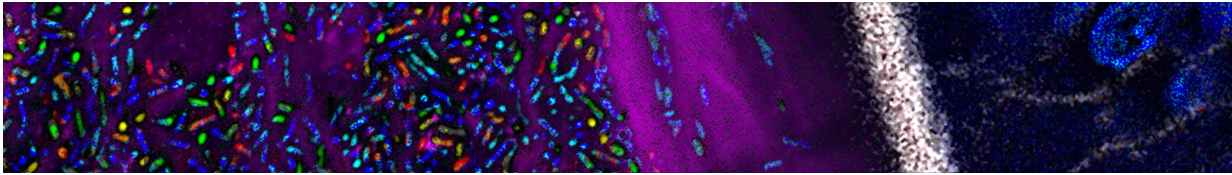
In terms of further research directions, it would be interesting to investigate whether prebiotic or probiotic supplementation in antibiotic-treated animals can rescue the disrupted social neuropeptide signalling, and whether any effects of probiotics are species-specific or strain-specific. Since antibiotic treatment can deplete the production of short-chain fatty acids, particularly butyrate (Dominguez-Bello *et al.*, 2015; Zarrinpar *et al.*, 2018), it would also be informative to test whether administration of butyrate helps to reduce the impact of antibiotic treatment. If so, this would suggest that the influence of the gut microbiome on the expression of these neuropeptide pathways is at least partly mediated by butyrate. Future research should also examine the impact of antibiotic administration on the neurogenetics of adult mice to determine whether the effect is less marked than antibiotic treatment immediately following weaning when the developing brain is particularly vulnerable to perturbation. To avoid introducing additional variation, this study was only conducted on male mice but it would be interesting to investigate whether females show similar changes in gene expression since there are some notable sex differences in the microbiome–gut–brain axis (Davis *et al.*, 2016a; Coretti *et al.*, 2017; Leclercq *et al.*, 2017; Sylvia *et al.*, 2017). Indeed, a recently reported study found that early-life antibiotic exposure induced social deficits only in male mice (Kayyal *et al.*, 2018). Similarly, male germ-free mice show more pronounced social impairments (Desbonnet *et al.*, 2014) and alterations in neurochemistry (Clarke *et al.*, 2013; Hoban *et al.*, 2016b) compared to their female counterparts, which may be reflective of the higher incidence of autism among males. It would also be informative to investigate whether my findings hold across different mouse strains since the effects of the gut microbiota can depend on the host strain (Fujisaka *et al.*, 2016; Felice & Moloney, 2018). This study focused on quantifying mRNA expression which is considered to provide a more accurate indication of treatment-induced changes in neuronal gene expression than measuring protein levels which may reflect homeostatic balance between

neuronal release, translation and storage (Fröhlich *et al.*, 2015). However, while there is a degree of correspondence between mRNA and protein levels (Vogel & Marcotte, 2012; Koussounadis *et al.*, 2015), protein abundance cannot necessarily be inferred from quantifying mRNA transcripts.

While antibiotic treatment and germ-free conditions are two helpful approaches to investigate causality, both models have limitations. Germ-free animals show numerous defects, including in their development, gut morphology and physiology, nervous system, immune response and metabolism (Smith *et al.*, 2007; Round & Mazmanian, 2009; Forsythe *et al.*, 2016; Rooks & Garrett, 2016). Notably, they also have a more permeable blood–brain barrier (Braniste *et al.*, 2014). Although this artificial model is useful for investigating which processes are modulated by the gut microbiome, the findings are not easily translatable to human physiology and disease. In terms of the high-dose antibiotic treatment used in this study, it not only dramatically reduces the bacterial load but also significantly restructures the gut microbial community, decreasing its diversity and altering its composition (Desbonnet *et al.*, 2015). While the relative abundances of the phyla Firmicutes and Bacteroidetes are reduced, Cyanobacteria and Proteobacteria increase in relative abundance with this antibiotic cocktail (Desbonnet *et al.*, 2015). Indeed, the phylum Proteobacteria includes a wide variety of pathogens such as *Escherichia*, *Helicobacter*, *Pseudomonas*, *Salmonella* and *Vibrio* (Williams *et al.*, 2010) and increased abundance of this phylum is associated with psychological distress in patients with irritable bowel syndrome (Peter *et al.*, 2018). In addition, members of the phylum Cyanobacteria produce toxins including lipopolysaccharides which can have pathological effects (Stewart *et al.*, 2006). Thus, the effects of antibiotic treatment may not necessarily be through depleting the gut microbiota but also by significantly changing its composition. Furthermore, antibiotics can have systemic effects in addition to their antimicrobial actions (Cryan & Dinan, 2012; Forsythe *et al.*, 2016). Antibiotics may even act directly on the brain and there is evidence that they can modulate the enteric nervous system, influencing intestinal motility (Delungahawatta *et al.*, 2017). Such antibiotic-induced changes may also be involved in mediating gut–brain communication, in addition to the direct effects of antibiotics on reducing bacterial load. Finally, it is important to bear in mind that

although mice represent a useful model for understanding mammalian physiology, with many similarities between the mouse and human gut microbiome, there are also notable differences and caution is needed when extrapolating the potential significance of these findings to humans (Loan *et al.*, 2015; Forsythe *et al.*, 2016).

The results of this study reveal that disruption of the gut microbiome in early life can significantly impact social neuropeptide signalling. The reduced activation of these pathways in mice treated with antibiotics post-weaning likely contributes to their social impairments. This is the first study to carry out the same experiments in both antibiotic-treated and germ-free mice, revealing opposing effects of these two conditions on gene expression in the brain. My findings indicate the possible effects that early-life exposure to antibiotics may have on pathways in the brain mediating social and emotional behaviour, with potential implications for the risk of developing neuropsychiatric conditions such as autism, depression and anxiety.



Chapter 3

Analysis of the gut microbiome in relation to human personality

3.1 Abstract

The gut microbiome has a measurable impact on the brain, influencing stress, anxiety, depressive symptoms and social behaviour. This microbiome–gut–brain axis may be mediated by various mechanisms including neural, immune and endocrine signalling. To date, the majority of research has been conducted in animal models, while the limited number of human studies has focused on psychiatric conditions. Here I investigate whether the composition and diversity of the gut microbiome is linked to human personality. Using regression models to control for possible confounding factors, I show that abundances of specific bacterial genera are significantly related to personality traits. Diversity analyses of the gut microbiome reveal that people with larger social networks tend to have a more diverse microbiome. In contrast, anxiety and stress are linked to reduced diversity and an altered microbiome composition. Together, these results add a new dimension to our understanding of personality and reveal that the microbiome–gut–brain axis may also be relevant to behavioural variation in the general population as well as to cases of psychiatric disorders.

3.2 Introduction

Personality shapes our world. It influences our health, our friendships, how we deal with stress, what jobs we succeed in and how we like to spend our time. It is approximately 50% heritable in human populations (Power & Pluess, 2015), suggesting that environmental factors also contribute significantly to our personality. In addition to our external environment, the

burgeoning field of the microbiome is revealing the many ways that our 'environment within' can affect our body's physiology, including our digestion, immunity, metabolism, development and even our behaviour (Round & Mazmanian, 2009; Cryan & Dinan, 2012; Brestoff & Artis, 2013; Sommer & Bäckhed, 2013; Sampson & Mazmanian, 2015). In fact, the number of microbial cells in our bodies is estimated to be roughly equal to our own human cells (Sender *et al.*, 2016), with the majority of these microorganisms inhabiting the gut. Studies in the past decade provide evidence that the gut microbiome interacts with the central nervous system (Eisenstein, 2016) and such findings have the exciting potential to aid the development of new treatments for conditions such as autism (Gilbert *et al.*, 2013) and depression (Dinan *et al.*, 2013). However, research has largely been conducted in animal models and it is unknown how translatable these findings are to humans (Foster & McVey Neufeld, 2013; Dinan & Cryan, 2017; Bruce-Keller *et al.*, 2018). While there is some indication that dysbiosis of the gut microbiota may be implicated in neurological and psychiatric disorders (Rogers *et al.*, 2016), an open question remains as to whether variation in the gut microbial community is related to personality, that is behavioural differences between individuals that are consistent over time and different situations and therefore broadly predictable.

Animal studies have demonstrated that the gut microbiome can influence the stress response, anxiety and depressive-like behaviours, as well as social behaviour and communication (Cryan & Dinan, 2012; Sharon *et al.*, 2016). Some of the most convincing findings stem from faecal microbiota transplantation whereby behavioural traits can be transferred between mouse strains when their gut microbiota are swapped (Bercik *et al.*, 2011a; Collins *et al.*, 2013). For example, when the more anxious and timid Balb/c mice are colonized with the gut microbiota of NIH Swiss mice, their temperament becomes more bold and exploratory like that of the donor NIH Swiss mice, and vice versa (Bercik *et al.*, 2011a). Further support comes from the induction of anxiety and depressive-like behaviours in rodents colonized with the gut microbiota of humans suffering from these symptoms (Kelly *et al.*, 2016; Zheng *et al.*, 2016; de Palma *et al.*, 2017). The transmission of such behaviours via the microbiota therefore suggests that gut microorganisms can contribute causally to behavioural traits. In fact, a recent human study reported

improvement in psychiatric symptoms following faecal microbiota transplantation in patients with gastrointestinal disease (Kurokawa *et al.*, 2018).

There are numerous possible mechanisms that may mediate this interaction between the gut microbial community and the brain, including communication via neural, immune and endocrine pathways (Collins *et al.*, 2012; Cryan & Dinan, 2012; Johnson & Foster, 2018 [Appendix]). Microorganisms can also produce various neuroactive chemicals (Wall *et al.*, 2014; Strandwitz, 2018) and can modulate host neurotransmitter levels (Yano *et al.*, 2015). Gut dysbiosis might therefore lead to imbalances in neurotransmitters, inflammation or heightened activity of the hypothalamus–pituitary–adrenal axis that regulates the stress response (Bruce-Keller *et al.*, 2018). The observation that psychiatric illnesses are often comorbid with gastrointestinal problems (Folks, 2004; Mussell *et al.*, 2008) supports the role of the microbiome–gut–brain axis in human biology and psychology. A number of studies has reported associations between the composition of the gut microbiome and conditions such as autism, depression and schizophrenia (Table 3.1). However, results sometimes differ with regard to changes in the abundances of specific bacterial taxa, likely due to individual variability in gut microbiome composition and underpowered studies.

Intervention studies in humans have started to investigate whether the gut microbiome makes a detectable contribution to the functioning of the central nervous system. Although studies administering probiotics have yielded mixed results in humans, meta-analyses do provide support for their beneficial effect on stress, anxiety and depressive symptoms, including in healthy humans as well as those suffering from psychiatric conditions (Pirbaglou *et al.*, 2016; Wang *et al.*, 2016; McKean *et al.*, 2017; Wallace & Milev, 2017). In addition, brain imaging studies have revealed that consumption of probiotics in healthy volunteers can affect neural signatures of emotion (Tillisch *et al.*, 2013; Bagga *et al.*, 2018). For example, probiotic administration over a period of four weeks was found to modulate brain activity, resulting in reduced activity of brain regions involved in emotional processing when participants were presented with negative facial expressions (Tillisch *et al.*, 2013). In studies involving prebiotics, healthy humans show a reduction in salivary cortisol levels after three weeks of taking the

supplement (Schmidt *et al.*, 2015) and patients with irritable bowel syndrome have reported reduced anxiety following one month of prebiotic supplementation (Azpiroz *et al.*, 2017).

Since the gut microbiome has been implicated in social development and behaviour (Hsiao *et al.*, 2013; de Theije *et al.*, 2014; Desbonnet *et al.*, 2014; Buffington *et al.*, 2016), recent research has investigated whether it may modulate behavioural symptoms of autism (Krajmalnik-Brown *et al.*, 2015). Autism is not only characterized by impaired social behaviour but is frequently comorbid with gastrointestinal issues (Hsiao, 2014) and immune dysfunction (Hsiao, 2013; Meltzer & van de Water, 2017). Studies comparing autistic individuals with neurotypical controls often report significant differences in gut microbiome composition (Table 3.1) and interestingly, a recent trial of faecal microbiota transplantation in children with autism found that it improved not only gastrointestinal symptoms but also social behaviour and communication (Kang *et al.*, 2017). This suggests that the gut microbial community may play a part in the expression of autistic behavioural traits. Given the evidence that autistic traits are normally distributed across the population (Wing, 1988; Constantino & Todd, 2003; Ruzich *et al.*, 2015), gut microbiome composition may also be related to variation in sociability in the general population.

Table 3.1. Summary of statistically significant associations between bacterial genera in the gut and behavioural or psychiatric traits reported in the literature. Table lists findings from research in both animal models and human populations and includes all genera (23 in total) associated with these traits in at least two independent studies.

Genus	Change in abundance	Behavioural trait/psychiatric condition	Study subject	References
<i>Akkermansia</i>	↓	Autism	Children	Wang <i>et al.</i> , 2011
	↑	Autism	Children	de Angelis <i>et al.</i> , 2013
	↓	Stress	Mice	Bharwani <i>et al.</i> , 2016; Maltz <i>et al.</i> , 2017
<i>Alistipes</i>	↓	Autism	Children	Strati <i>et al.</i> , 2017
	↑	Stress	Mice	Bangsgaard Bendtsen <i>et al.</i> , 2012
	↑	Depression	Adults	Naseribafrouei <i>et al.</i> , 2014; Jiang <i>et al.</i> , 2015
<i>Bacteroides</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
	↓	Stress	Mice	Bailey <i>et al.</i> , 2011; Maltz <i>et al.</i> , 2017
	↑	Negative mood	Adults	Li <i>et al.</i> , 2016b
	↑	Psychosis	Adults	Schwarz <i>et al.</i> , 2017

Table 3.1. (cont.)

Genus	Change in abundance	Behavioural trait/psychiatric condition	Study subject	References
<i>Bifidobacterium</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; Adams <i>et al.</i> , 2011; Wang <i>et al.</i> , 2011, 2013; de Angelis <i>et al.</i> , 2013; Son <i>et al.</i> , 2015; Grimaldi <i>et al.</i> , 2017
	↑	Autism	Children	Pulikkan <i>et al.</i> , 2018
	↓	Autism	Mice	Golubeva <i>et al.</i> , 2017
	↓	Stress	Mice	Wong <i>et al.</i> , 2016; Yang <i>et al.</i> , 2017
	↑	Stress	Mice	Marin <i>et al.</i> , 2017
	↑	Negative mood	Adults	Li <i>et al.</i> , 2016b
	↓	Depression	Adults	Aizawa <i>et al.</i> , 2016
<i>Blautia</i>	↓	Autism	Infants	Inoue <i>et al.</i> , 2016
	↓	Autism	Children	Luna <i>et al.</i> , 2017
	↓	Autism	Mice	Golubeva <i>et al.</i> , 2017
	↓	Schizophrenia	Adults	Shen <i>et al.</i> , 2018
<i>Clostridium</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2002; Song <i>et al.</i> , 2004; Parracho <i>et al.</i> , 2005; de Angelis <i>et al.</i> , 2013; Tomova <i>et al.</i> , 2015; Grimaldi <i>et al.</i> , 2017; Luna <i>et al.</i> , 2017; Strati <i>et al.</i> , 2017
	↓	Autism	Children	Finegold <i>et al.</i> , 2010
	↑	Stress	Mice	Bailey <i>et al.</i> , 2011; Arase <i>et al.</i> , 2016; Marin <i>et al.</i> , 2017
	↑	Depression	Adults	Jiang <i>et al.</i> , 2015; Lin <i>et al.</i> , 2017
	↑	Schizophrenia	Adults	Shen <i>et al.</i> , 2018
<i>Collinsella</i>	↑	Autism	Children	Strati <i>et al.</i> , 2017
	↓	Autism	Children	Finegold <i>et al.</i> , 2010
	↑	Schizophrenia	Adults	Shen <i>et al.</i> , 2018
<i>Corynebacterium</i>	↑	Autism	Children	Strati <i>et al.</i> , 2017
	↓	Stress	Rats	Yu <i>et al.</i> , 2017
<i>Desulfovibrio</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2010; Tomova <i>et al.</i> , 2015
<i>Dialister</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; Strati <i>et al.</i> , 2017
	↑	Sociability	Infants	Christian <i>et al.</i> , 2015
	↓	Depression	Adults	Jiang <i>et al.</i> , 2015; Kelly <i>et al.</i> , 2016
<i>Dorea</i>	↑	Autism	Children	de Angelis <i>et al.</i> , 2013; Strati <i>et al.</i> , 2017
	↓	Autism	Children	Luna <i>et al.</i> , 2017
	↓	Stress	Mice	Bailey <i>et al.</i> , 2011
<i>Faecalibacterium</i>	↑	Autism	Infants	Inoue <i>et al.</i> , 2016
	↓	Autism	Children	de Angelis <i>et al.</i> , 2013; Kang <i>et al.</i> , 2018
	↓	Negative mood	Adults	Turroni <i>et al.</i> , 2017
	↑	Negative mood	Adults	Li <i>et al.</i> , 2016
	↓	Depression	Adults	Jiang <i>et al.</i> , 2015
	↑	Depression (prenatal)	Adults	Togher <i>et al.</i> , 2017
	↓	Bipolar disorder	Adults	Evans <i>et al.</i> , 2017; Painold <i>et al.</i> , 2018
<i>Flavonifractor</i>	↑	Autism	Children	Luna <i>et al.</i> , 2017
	↑	Depression	Adults	Jiang <i>et al.</i> , 2015

Table 3.1. (cont.)

Genus	Change in abundance	Behavioural trait/ psychiatric condition	Study subject	References
<i>Lactobacillus</i>	↑	Autism	Children	Adams <i>et al.</i> , 2011; Kang <i>et al.</i> , 2013; Tomova <i>et al.</i> , 2015; Strati <i>et al.</i> , 2017; Pulikkan <i>et al.</i> , 2018
	↓	Autism	Children	de Angelis <i>et al.</i> , 2013
	↓	Stress	Mice	Bailey <i>et al.</i> , 2011; Galley <i>et al.</i> , 2014a; Marin <i>et al.</i> , 2017
	↓	Stress	Rats	Yu <i>et al.</i> , 2017
	↓	Stress	Macaques	Bailey & Coe, 1999
	↓	Depression	Adults	Aizawa <i>et al.</i> , 2016
	↑	Psychosis	Adults	Schwarz <i>et al.</i> , 2017
<i>Lactococcus</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
<i>Oscillibacter</i>	↑	Depression	Adults	Naseribafrouei <i>et al.</i> , 2014
	↑	Stress	Rats	Yu <i>et al.</i> , 2017
<i>Oscillospira</i>	↓	Autism	Children	de Angelis <i>et al.</i> , 2013
	↑	Sociability	Mice	Szyszkowicz <i>et al.</i> , 2017
	↓	Stress	Mice	Maltz <i>et al.</i> , 2017; Szyszkowicz <i>et al.</i> , 2017; Zhang <i>et al.</i> , 2017
<i>Parabacteroides</i>	↓	Autism	Children	Strati <i>et al.</i> , 2017
	↑	Autism	Children	Finegold <i>et al.</i> , 2010
	↑	Sociability	Infants	Christian <i>et al.</i> , 2015
	↓	Stress	Mice	Bailey <i>et al.</i> , 2011; Galley <i>et al.</i> , 2014a; Maltz <i>et al.</i> , 2017; Szyszkowicz <i>et al.</i> , 2017
	↑	Negative mood	Adults	Li <i>et al.</i> , 2016
	↑	Depression	Adults	Jiang <i>et al.</i> , 2015
<i>Prevotella</i>	↑	Autism	Children	de Angelis <i>et al.</i> , 2013
	↓	Autism	Children	Kang <i>et al.</i> , 2013
	↑	Stress	Mice	Szyszkowicz <i>et al.</i> , 2017
	↓	Negative mood	Adults	Li <i>et al.</i> , 2016
	↑	Depression	Adults	Lin <i>et al.</i> , 2017
	↓	Depression	Adults	Kelly <i>et al.</i> , 2016
<i>Roseburia</i>	↑	Autism	Children	de Angelis <i>et al.</i> , 2013
	↑	Stress	Mice	Bailey <i>et al.</i> , 2011
	↓	Stress	Mice	Maltz <i>et al.</i> , 2017
	↓	Negative mood	Adults	Li <i>et al.</i> , 2016
	↓	Schizophrenia	Adults	Shen <i>et al.</i> , 2018
<i>Streptococcus</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
	↑	Depression	Adults	Lin <i>et al.</i> , 2017
<i>Sutterella</i>	↑	Autism	Children	Wang <i>et al.</i> , 2011, 2013, Williams <i>et al.</i> , 2011, 2012
	↓	Autism	Children	Luna <i>et al.</i> , 2017
	↑	Stress	Mice	Maltz <i>et al.</i> , 2017
<i>Turicibacter</i>	↓	Autism	Children	de Angelis <i>et al.</i> , 2013
	↑	Sociability	Mice	Szyszkowicz <i>et al.</i> , 2017
	↓	Stress	Mice	Wong <i>et al.</i> , 2016; Marin <i>et al.</i> , 2017; Szyszkowicz <i>et al.</i> , 2017
	↑	Depression	Adults	Kelly <i>et al.</i> , 2016

The main aim of this research was to determine whether individual variation in the composition and diversity of the human gut microbiome is related to differences in personality. Previous research has focused on animal models or examined broadscale associations between microbiome composition and psychiatric symptoms. In contrast, here I conduct targeted regression analyses to investigate the relationship between personality traits and the abundances of specific bacterial genera identified in the microbiome–gut–brain axis literature (Table 3.1). I hypothesize that genera previously associated with autism may be related to differences in sociability in the general population, while genera previously linked to depression and stress in animal models or psychiatric populations may be differentially abundant in the general population with respect to neurotic traits. As well as assessing genus-level composition, I also investigate the relationship between personality and measures of microbiome diversity, hypothesizing that variables assessing sociability may be positively related to diversity and those assessing neurotic traits may be negatively related.

3.3 Materials and methods

3.3.1 Study population

Adults (over 18 years) were invited to take part in the study (Figure 3.1) via advertising to uBiome customers and through social media. A faecal sample was received from 671 individuals but sixteen samples were removed ($n = 655$) since they did not meet the read quality control threshold set at 10,000 reads to ensure detection of low abundance taxa (Almonacid *et al.*, 2017). The research was conducted under a Human Subjects Protocol provided by an institutional review board (Ethical and Independent Review Services, IRB Study #13044-03). All participants provided informed consent and data were analysed anonymously. While all efforts were made to maximize the number of participants involved in the study, ultimately the sample size was restricted by those who volunteered within a reasonable time period and provided adequate responses to the questionnaire. The sample size is considerably greater than the majority of human microbiome studies and was considered sufficient based on a statistical power analysis (Figure A.3). The sample was 71% female and 29% male, with a mean age of

42 years. Participants resided in twenty different countries and four continents, predominantly North America (83%), with participants from USA accounting for 77% of the sample. 11% of the study participants were from Europe (4% from UK), 5% from Australasia and 2% from Asia.

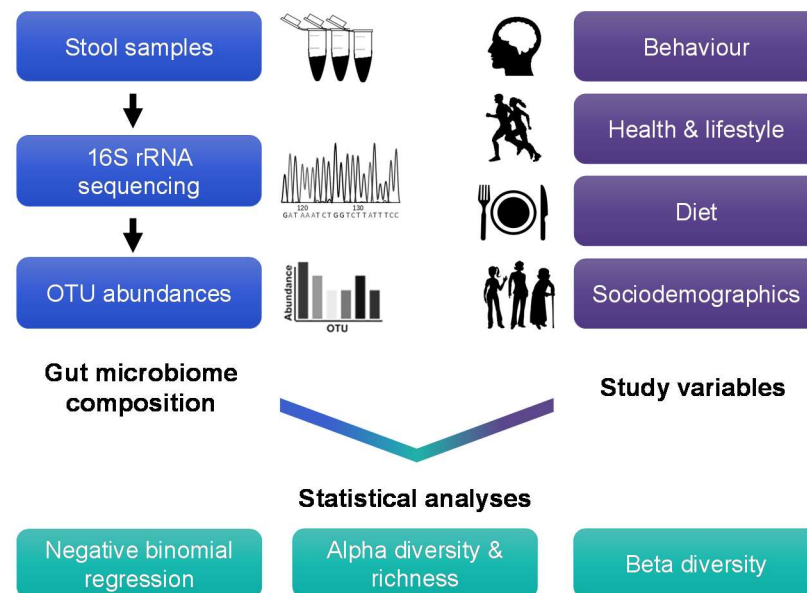


Figure 3.1. Overview of study design. A faecal sample was provided by each participant using the commercially available uBiome Gut Kit and sample processing and sequencing were carried out by uBiome to generate OTU (operational taxonomic unit) abundances. Participants were asked to complete a comprehensive study questionnaire assessing their behaviour, diet, health, lifestyle and sociodemographics (see Figure 3.3 for variables tested within each of these categories). A range of statistical analyses were conducted to determine the relationships between gut microbiome composition and the study variables, with a primary interest in the variables assessing behavioural traits.

3.3.2 Sample collection, processing and sequencing

The microbiome sequencing company uBiome provided participants with their commercially available Gut Kit. Each participant was supplied with one kit to be used for faecal sample collection at home. Analysis of faecal samples is the standard procedure in microbiome research since they are sufficiently representative of the composition of the gut microbiome (Eckburg *et al.*, 2005). The sampling procedure followed the guidelines of the National Institutes of Health Human Microbiome Project (McInnes & Cutting, 2010). Following sample collection by the participant, the sample was posted back to uBiome in their sample collection tube containing a lysis and stabilization buffer for DNA preservation during transport at ambient temperatures (Almonacid *et al.*, 2017). The 16S rRNA gene was amplified using universal

primers for the V4 hypervariable region and sequencing was performed on the Illumina NextSeq 500 platform, alongside positive and negative controls. Standardized methodology (Almonacid *et al.*, 2017) for sample processing and sequencing was conducted by uBiome and has been shown to generate reproducible microbiome profiles (Vera-Wolf *et al.*, 2018).

3.3.3 Questionnaires

In total, 44 study variables (continuous and categorical) were analysed relating to behavioural traits, diet, health and lifestyle and also sociodemographics. While the majority of variables were assessed through binary questions or point scales that I had designed, personality and social behaviour were evaluated with widely used and validated questionnaires from psychological research. The 50-item International Personality Item Pool (Goldberg, 2001) was used to assess personality traits according to the five-factor model of personality. A large number of studies find that individual differences in human behaviour can broadly be divided into five key domains, known as the 'Big Five' (Digman, 1990; McCrae & John, 1992), comprising extraversion (propensity to seek and enjoy others' company), agreeableness (trust and cooperation in social interactions), conscientiousness (attention to detail and focus), neuroticism (tendency to feel negative emotions) and openness (creativity, intellectual curiosity and willingness to seek new experiences). These traits are temporally stable within individuals (McCrae *et al.*, 1999) and are found in both sexes, all races and across cultures (McCrae & Costa, 1997). In addition, the State–Trait Anxiety Inventory (Spielberger *et al.*, 1983), specifically the trait subscale, was used to determine the participants' general tendency to feel anxious. Participants were also scored for two subscales (social skill and communication) from the Autism Spectrum Quotient, which measures the degree to which any neurotypical adult lies on the continuum between normality and autistic-like behaviours (Baron-Cohen *et al.*, 2001). Social network size was evaluated based on the number of people in their two innermost layers, approximately corresponding to those individuals contacted on a weekly and monthly basis respectively (Roberts *et al.*, 2008).

Detailed dietary habits of the participants were assessed with the EPIC-Norfolk Food Frequency Questionnaire (Bingham *et al.*, 2001). The associated software, Food Frequency Questionnaire

EPIC Tool for Analysis (Mulligan *et al.*, 2014), was used to estimate total calorie consumption and intake of food groups, macronutrients, minerals and vitamins (56 variables in total). Food frequency questionnaires with more than ten missing entries were excluded in accordance with the guidelines.

3.3.4 Multiple regression analyses of bacterial abundances

Microbiome count data are often overdispersed, with a high proportion of zeros (Xu *et al.*, 2015; Weiss *et al.*, 2017) and heavily right-skewed (Xia & Sun, 2017). This is because there are relatively few taxa that are shared by the majority of samples, since most taxa are rare and only detected in a small proportion of samples (Xu *et al.*, 2015). Given this overdispersion, count data cannot be normalized via transformation and so negative binomial regression was conducted with genus abundance as the response variable (or zero-inflated negative binomial regression in cases where the count data contained an excess of zeros). When modelling microbiome data, it is necessary to account for variation in sequencing read depth between samples. However, rarefying count data or transforming it into proportions can lead to a high rate of false positives and is not recommended for analyses of taxon abundance (McMurdie & Holmes, 2014). Instead, the logarithm of the total read number was included as an offset in the regression models, thereby preserving statistical power by retaining all the count data (Goodrich *et al.*, 2014; Xu *et al.*, 2015).

As opposed to the majority of microbiome studies which only conduct bivariate correlations between variables and microbial taxon abundances, key variables known to influence the gut microbiome were included in the regression models to avoid potential confounding effects. These variables were sex (Arumugam *et al.*, 2011; Ding & Schloss, 2014; Jašarević *et al.*, 2016; Martin *et al.*, 2016), age (Arumugam *et al.*, 2011; Yatsunenko *et al.*, 2012), body mass index (Arumugam *et al.*, 2011), birth delivery mode (Dominguez-Bello *et al.*, 2010; Bokulich *et al.*, 2016; Martin *et al.*, 2016), type of infant feeding method (Ding & Schloss, 2014; Praveen *et al.*, 2015; Martin *et al.*, 2016), whether participants had been treated with oral antibiotics within the last six months (since studies suggest the gut microbial community largely recovers after this

time (de la Cochetière *et al.*, 2005; Dethlefsen *et al.*, 2008)), whether they suffered from a gut condition (Bhattarai *et al.*, 2017; Halfvarson *et al.*, 2017) and if they took probiotic supplements (Hemarajata & Versalovic, 2013; Bagga *et al.*, 2018), though evidence is somewhat lacking as to the degree to which probiotic supplementation can alter faecal microbiota composition (Kristensen *et al.*, 2016; Zmora *et al.*, 2018). These regression analyses could only be conducted on a subset of the population cohort ($n = 261$) who supplied the necessary data for all these variables.

The variables of primary interest were those relating to personality traits. Pairwise Kendall's Tau-b correlation coefficients were computed to assess the correlation structure of all variables in the study, revealing considerable intercorrelation between extraversion, social skill and communication and also between neuroticism, anxiety and stress, as expected from the literature (Austin, 2005; Uliaszek *et al.*, 2010). To avoid multicollinearity in the regression models, two new variables were therefore created by summing the z-scores for the individual variables: sociability (a combined score of an individual's extraversion, social skill and communication) and neurotic tendencies (a combined score of an individual's neuroticism, anxiety and stress). These two new variables were used in the regression analyses since social behaviour, anxiety, stress and depressive-like behaviours are the main behavioural traits that have been linked to the gut microbiome (Vuong *et al.*, 2017).

Negative binomial regression analyses were conducted using the R package VGAM to model microbiome count data. All statistical analyses in this study were performed using R 3.2.3 software (R Development Core Team, 2015). I carried out an extensive literature review to identify putative taxa that have been associated with behaviour or psychiatric traits in animal research or human population studies (Table 3.1). Genera were included in these regression analyses if they had been significantly associated with behaviour or psychiatric measures in at least two independent studies. Genus-level count data were used for the regression models since 16S rRNA sequencing provides an estimated 96% accuracy for genus identification (Srinivasan *et al.*, 2015) but has limited phylogenetic resolution at the species level (Janda & Abbott, 2007).

3.3.5 Analyses of microbiome diversity and community composition

Analyses were also conducted to determine how the overall compositional profile of the microbiome, measured with alpha and beta diversity metrics, was related to the study variables. Alpha diversity is the ecological diversity of a single sample, taking into account the number of different taxa and their relative abundances, while beta diversity measures differences in microbial community composition between individuals (Finotello *et al.*, 2016). The R package phyloseq was used to determine richness and alpha diversity at the genus level (McMurdie & Holmes, 2013). Since the choice of diversity index can significantly influence results (Johnson & Burnet, 2016), both Shannon's diversity index and the inverse Simpson's diversity index were calculated (the inverse of Simpson's index being more intuitive since its value increases as diversity increases). While these two alpha diversity indices take into account both richness and evenness, Shannon's diversity index is more sensitive to changes in abundance of rare taxa, whereas the inverse Simpson's diversity index gives more weight to common taxa (Hill *et al.*, 2003).

Since the correlation between sequencing depth and diversity was not negligible, microbiome count data were rarefied prior to diversity analyses by randomly sampling counts without replacement such that all samples had an equal number of total counts. Although rarefying the count data was avoided for the regression models, it is still considered a useful normalization technique when conducting diversity analyses as uneven sequence counts across samples can significantly impact diversity estimates (Goodrich *et al.*, 2014; Weiss *et al.*, 2017). In terms of alpha diversity, associations between each variable and either diversity or richness were measured using Kendall's Tau-b correlation coefficient. This method is appropriate since it is a non-parametric measure of correlation and also accounts for tied values.

To assess beta diversity, permutational multivariate analysis of variance (PERMANOVA) was conducted to determine which variables significantly affect the overall composition of the gut microbiome. Distance matrices were computed using the distance function in the R package phyloseq (McMurdie & Holmes, 2013) to assess the pairwise similarity of gut microbiome

composition between individuals. The Bray–Curtis index provides a quantitative measure of dissimilarity between microbial communities, while the Jaccard index is qualitative since it only takes into account presence versus absence of taxa and not their abundances (Goodrich *et al.*, 2014). PERMANOVA was carried out on both Bray–Curtis and Jaccard distance matrices using the *adonis* function from the R package *vegan* with 1,000 permutations. This function estimates the variance in the distance matrix that is attributable to the variable of interest, that is, the extent to which each variable may explain differences between individuals in microbial community composition. The *P* values from these analyses were then adjusted for multiple comparisons using the Benjamini–Hochberg method to control for the false discovery rate (FDR), with $P_{\text{adj}} < 0.1$ considered statistically significant.

3.4 Results

3.4.1 Summary statistics

Gut microbiome composition differed markedly between individuals (Figure 3.2), as has repeatedly been shown in human populations (Lozupone *et al.*, 2012). Out of all the genera detected across the gut microbiome samples, the top twenty most abundant genera showed close correspondence with previous research (Riedel *et al.*, 2014). *Bacteroides* was the most abundant genus, as found in other studies (The Human Microbiome Project Consortium, 2012; Faith *et al.*, 2013). There was also considerable variation among the study population in the variables assessed in the questionnaire (Tables A.2 and A.3). The majority of study variables from the questionnaire showed little to modest intercorrelation (Figure 3.3), though as expected there was a high degree of correlation between the nutrient variables assessed in the food frequency questionnaire (Figure A.4).

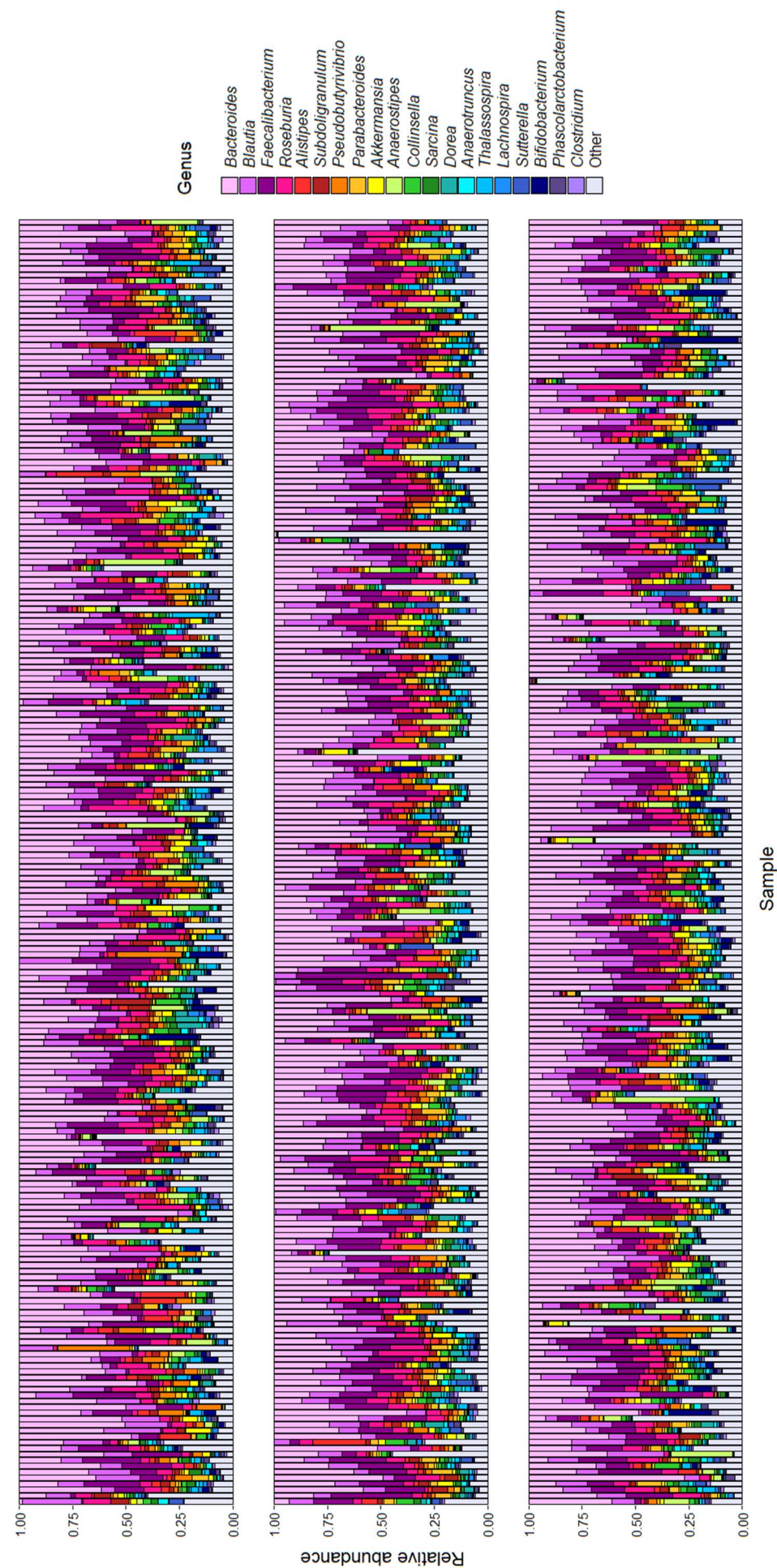


Figure 3.2. Stacked bar plots summarizing gut microbiome composition at genus level for the total participant cohort. Microbiome data are plotted showing relative abundance of the top twenty most abundant genera across all samples, with the remaining genera grouped into 'Other'. The twenty genera in the key are ordered from most to least abundant.

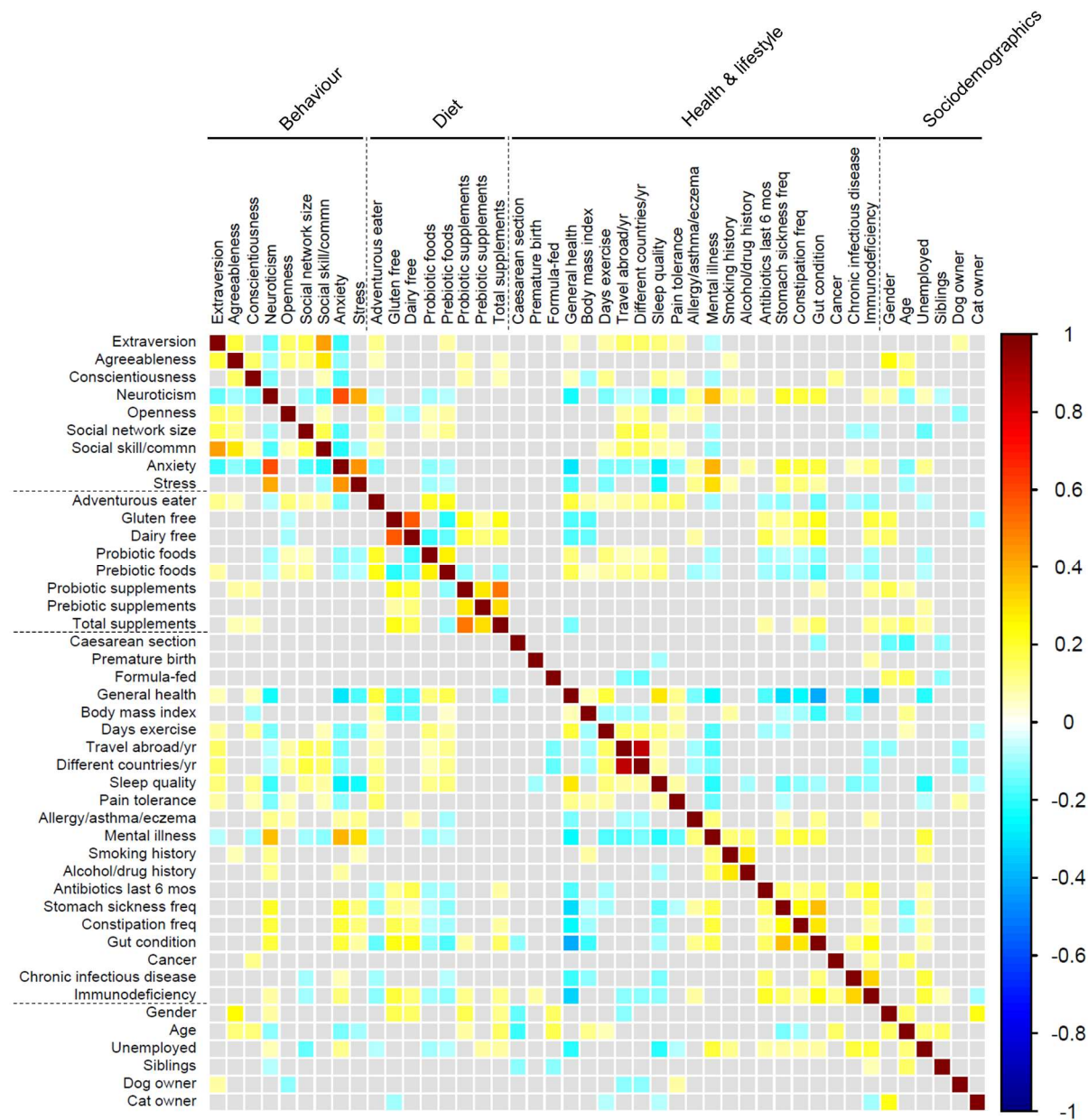


Figure 3.3. Inter-correlation plot for the study variables, divided into the four categories: **behaviour, diet, health and lifestyle, and sociodemographics**. Plot depicts strength and direction of pairwise correlations, as measured by Kendall's Tau-b correlation coefficient. Grey squares denote associations that were not statistically significant at FDR < 0.1.

3.4.2 Regression models of bacterial abundances

Negative binomial regression analyses revealed that the abundances for seven of the 23 genera were significantly predicted by individual variation in behavioural traits (Figure 3.4; Table A.4). Sociability (a combined measure of the participant’s extraversion, social skill and communication) was a positive predictor of genus abundance for *Akkermansia* ($P = 0.038$),

Lactococcus ($P = 0.002$) and *Oscillospira* ($P < 0.001$), and a negative predictor of the abundances of *Desulfovibrio* ($P = 0.019$) and *Sutterella* ($P = 0.028$). The variable assessing neurotic tendencies (a combined measure of neuroticism, anxiety and stress) negatively predicted genus abundance for *Corynebacterium* ($P < 0.001$) and *Streptococcus* ($P = 0.029$).

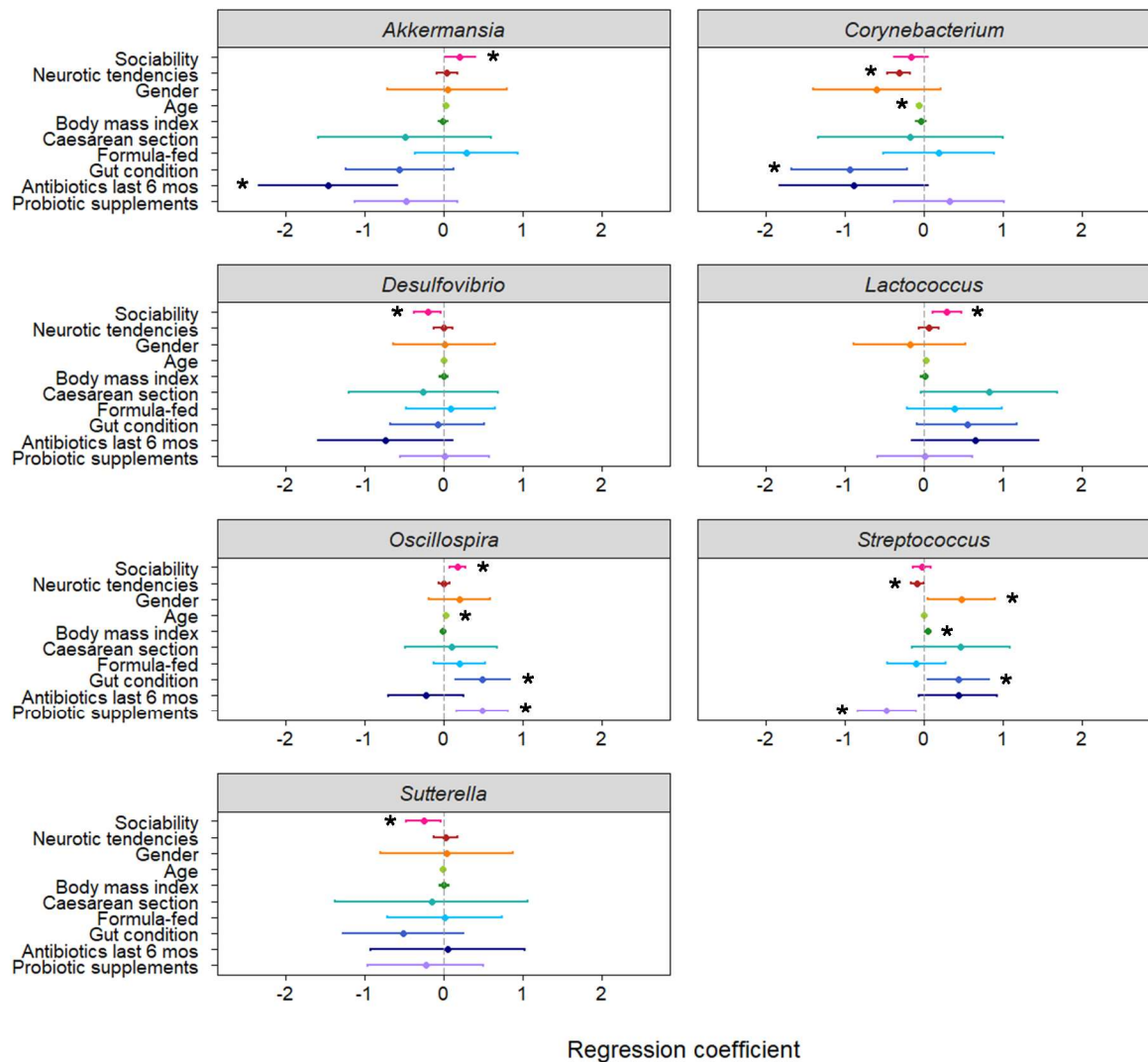


Figure 3.4. Coefficient plots from regression models predicting abundances of genera in the human gut microbiome. Asterisks denote significant predictors of genus abundance where $P < 0.05$ and bars indicate 95% confidence intervals. The key variables of interest for this study were those relating to behavioural traits, namely sociability (extraversion, social skill and communication) and neurotic tendencies (neuroticism, anxiety and stress), while other variables were also included to avoid potential confounding effects. A positive relationship with gender indicates a higher abundance of the genus in females. Plots displayed here depict genera whose abundances were significantly related to differences in behavioural traits (for remaining regression coefficient plots see Figure A.5).

There were also notable results in terms of the possible confounding factors included in the models. Age and body mass index were common predictors of abundance, significantly predicting the abundances of eight and seven genera, respectively. In contrast, birth delivery mode and infant feeding method had little influence on the abundances of most genera. Delivery mode was only a significant predictor of the abundance of one genus, *Flavonifractor*, with a higher abundance in individuals born by caesarean section. Notably, *Flavonifractor* has also been found to be more abundant in infants with food allergies (Ling *et al.*, 2014) and birth by caesarean section is often linked to an increased risk of allergies (Papathoma *et al.*, 2016). Formula feeding during infancy was a positive predictor of *Lactobacillus* abundance, in agreement with previous research findings (Penders *et al.*, 2006). Surprisingly, abundances of only three of the 23 genera were significantly altered by antibiotic treatment in the past six months, indicative of the differential effects antibiotics can have on bacterial taxa. Three genera, *Lactobacillus*, *Prevotella* and *Streptococcus*, were more abundant in females. In fact, *Lactobacillus* has previously been found to be more prevalent in infant girls than boys (Martin *et al.*, 2016) and also more abundant in female mice (Fransen *et al.*, 2017).

3.4.3 Microbiome diversity

Overall, 25 of the 44 study variables were significantly related to gut microbiome diversity ($P_{\text{adj}} < 0.1$), as measured by Shannon's diversity index, with the majority of these (22 variables) also retaining significance at $\text{FDR} < 0.05$ (Figure 3.5; Table A.5). For the primary variables of interest relating to behaviour, both stress ($P_{\text{adj}} = 0.045$) and anxiety ($P_{\text{adj}} = 0.077$) were negatively correlated with Shannon's diversity index, and agreeableness was also negatively related ($P_{\text{adj}} = 0.048$). There was a positive correlation between Shannon's diversity index and social network size ($P_{\text{adj}} = 0.043$), such that individuals with a larger social network tended to have a more diverse gut microbiome.

In terms of the other variables in the study, individuals with a more adventurous diet and those who ate more foods with naturally occurring probiotics (fermented foods) or prebiotics (non-digestible fibre) tended to have a more diverse gut microbial community. In contrast,

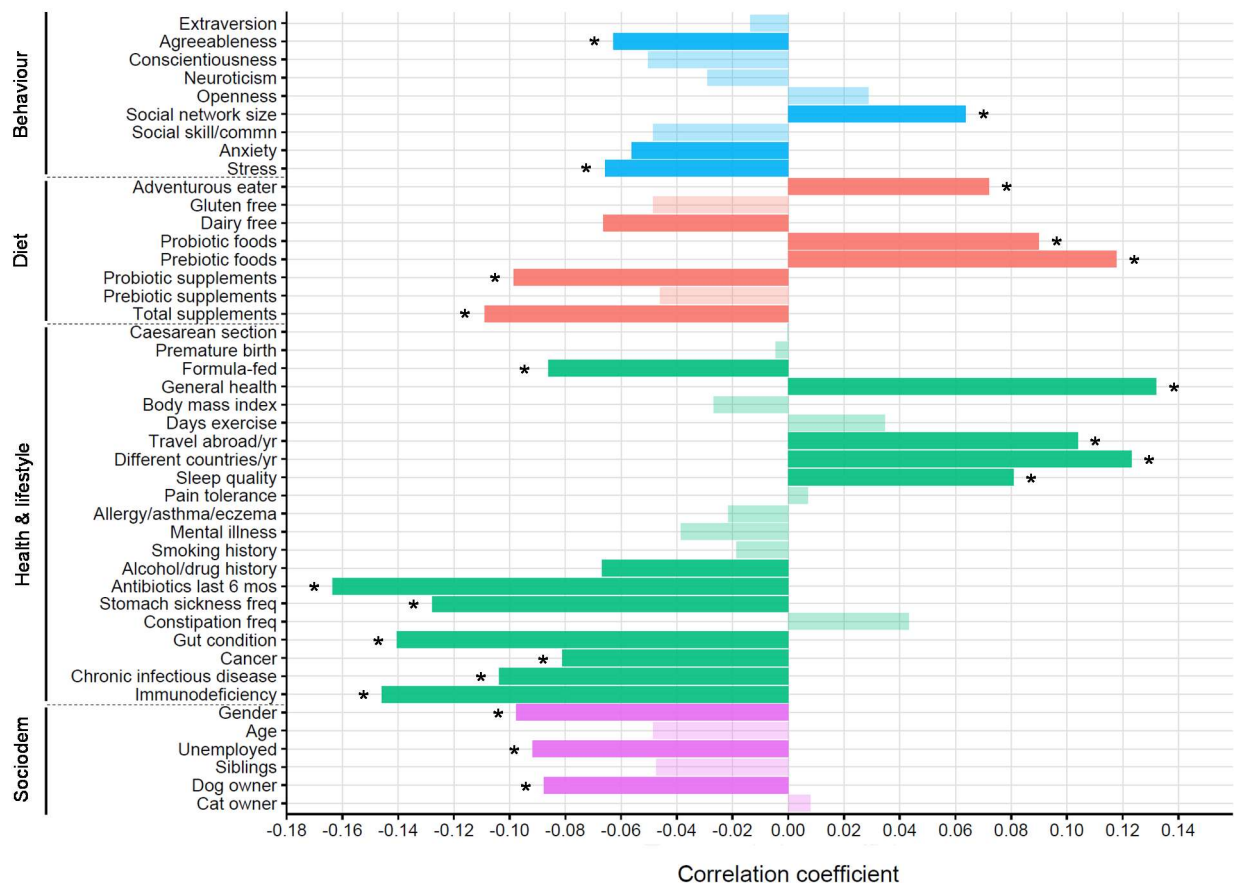


Figure 3.5. Bar plot showing results of Kendall's Tau-b correlation analysis between gut microbiome diversity and the study variables. Opaque shading indicates a significant correlation at $FDR < 0.1$ and asterisks denote significance also at $FDR < 0.05$. The negative relationship with gender reflects a lower diversity in females compared with males. Plot depicts results with Shannon's diversity index (for results using the inverse Simpson's diversity index and genus richness see Table A.5).

consumption of probiotic supplements was significantly correlated with lower diversity. Variables relating to ill health were generally associated with reduced diversity and, not surprisingly, the strongest negative relationship was with antibiotic treatment. Adults who were formula-fed as infants had a significantly less diverse microbiome compared with those that were breast-fed. There was also evidence that females had a lower gut microbiome diversity than males. Diversity was not related to exercise frequency or body mass index, while the strongest positive correlation was with general health. Microbiome diversity was positively correlated with travel, while it was negatively related to dog ownership and also unemployment.

Comparing the correlation results of Shannon's diversity index and the inverse Simpson's diversity index revealed good consistency between the two indices (Table A.5). Most of these

relationships were also present when correlating the variables with the observed number of genera (Table A.5), though as expected there were some differences between the results since this is a measure of community richness rather than diversity. Notably, there was a significant negative correlation between conscientiousness and genus richness ($P_{\text{adj}} = 0.014$), which was in the same direction as the non-significant trend with diversity.

With respect to nutrient intake estimated from the food frequency questionnaire, the consumption of fruit and cereals, and therefore fibre (and more generally carbohydrates), was positively related to diversity (Table A.6). This was as expected since the metabolism of carbohydrates, particularly fibre, is known to be an important factor driving microbial diversity (Koropatkin *et al.*, 2012; Sonnenburg & Sonnenburg, 2014; Sonnenburg *et al.*, 2016). Diversity was also significantly correlated with the dietary abundance of a range of minerals and vitamins (Table A.6). There was little relationship between genus richness and nutrient intake with the exception of fish consumption which was positively related, perhaps due to exposure to different bacterial genera inhabiting marine ecosystems.

3.4.4 Microbial community composition

Differences in the composition of the gut microbiome, as quantified by the Bray–Curtis index, were significantly related to 28 of the 44 variables ($P_{\text{adj}} < 0.1$), with most of these (22 variables) retaining significance at $\text{FDR} < 0.05$ (Figure 3.6; Table A.7). While the P values reported below refer to results with the Bray–Curtis index, the same variables were also significant when using the Jaccard index to measure differences in microbial community composition (Table A.7).

Regarding behavioural traits, anxiety ($P_{\text{adj}} = 0.094$) and stress ($P_{\text{adj}} = 0.052$) were significantly related to an altered microbiome composition. Another factor influencing composition was whether the participant suffered from a mental illness ($P_{\text{adj}} = 0.020$). The majority of variables assessing health and lifestyle were also significantly related to composition. In accordance with previous findings (Falcony *et al.*, 2016), there was no effect of early-life events (mode of delivery, premature birth and infant feeding), though these factors may still influence establishment of the microbiome during infancy, with potential consequences for development of the immune system.

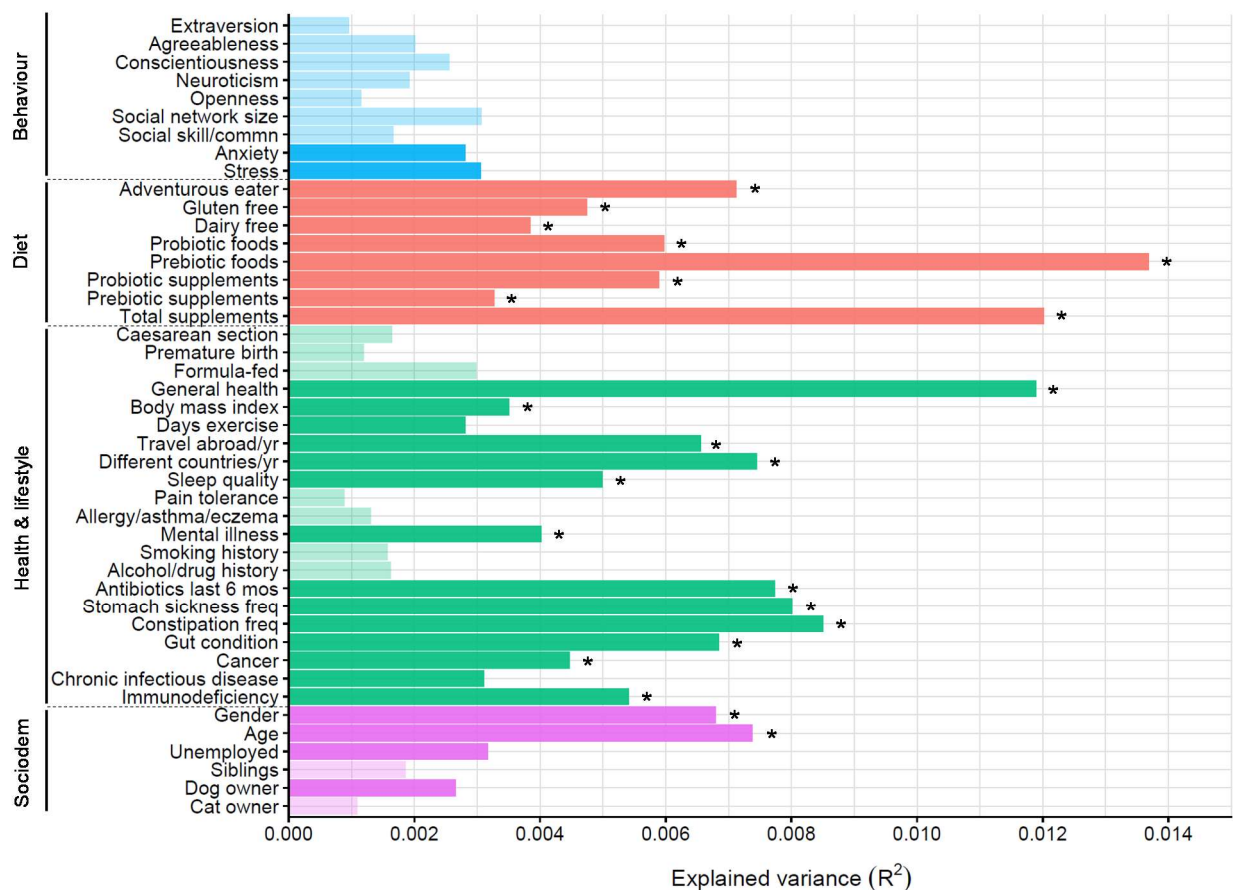


Figure 3.6. Bar plot showing explained variance in gut microbiome composition in relation to the study variables. Opaque shading indicates variables explaining significant inter-individual variation at FDR < 0.1 and asterisks denote significance also at FDR < 0.05. Plot depicts results using the Bray–Curtis distance matrix (for results with the Jaccard distance matrix see Table A.7).

While exercise frequency did not significantly influence diversity, it did affect overall composition of the gut microbiome, as has been shown in other studies (Mach & Fuster-Botella, 2017). As was the case with diversity, unemployment was a significant factor influencing microbial community composition. Composition also differed by age and gender, in line with previous research (Yatsunenکو *et al.*, 2012).

All the main dietary variables were significantly related to composition (Figure 3.6). Both gluten-free and dairy-free diets were associated with an altered microbial community, in accordance with other research findings (David *et al.*, 2014b; Zhu *et al.*, 2015; Bonder *et al.*, 2016). The consumption of naturally occurring prebiotic fibre showed the strongest relationship with gut microbiome composition, highlighting the important role of fibre in shaping the microbiome (Koropatkin *et al.*, 2012; Sonnenburg & Sonnenburg, 2014; Sonnenburg *et al.*, 2016). In terms

of the results of the food frequency questionnaire, consumption of fruit, vegetables and cereals, and therefore the total intake of carbohydrates including fibre, were all significantly related to microbiome composition, as well as certain minerals and vitamins (Table A.8).

3.5 Discussion

Investigations of the microbiome–gut–brain axis have predominantly been conducted in animal models or clinical populations, with few studies exploring whether these findings are translatable to the general population. Here I show that both gut microbiome composition and diversity are related to differences in personality. This is the first time that abundances of microbial genera have been modelled with respect to human personality, while also controlling for the possible confounding effects of key variables known to influence the gut microbiome. The regression results revealed that abundances of particular genera are significantly related to behavioural traits, suggesting that studying the gut microbiome may be relevant to understanding variation in human personality. I also found that people with larger social networks tend to have more diverse microbial communities, indicating that social behaviour may promote diversity of the human gut microbiome. By contrast, lower diversity was associated with increased levels of stress and anxiety, and these traits were also related to differences in overall composition of the microbial community. In addition, the intercorrelation analysis (Figure 3.3) revealed that people who ate more foods with naturally occurring probiotics or prebiotics had significantly lower levels of anxiety, stress and neuroticism and were also less likely to suffer from a mental illness, but this relationship was not found in those consuming probiotics or prebiotics in supplement form. Consistent with this, a previous study reported that females consuming more fermented foods (a source of natural probiotics) had fewer symptoms of social anxiety (Hilimire *et al.*, 2015) and similarly a traditional Japanese diet rich in fermented soy products has been linked to reduced depressive symptoms (Selhub *et al.*, 2014).

The significant relationships between personality traits and the abundances of particular genera targeted in the regression analyses were largely in the expected direction based on studies in humans with psychiatric conditions and in laboratory animals (Table 3.1). The genera

Akkermansia, *Lactococcus* and *Oscillospira* were found to be more abundant in individuals with a higher sociability score. In accordance with these results, previous studies in children have reported a reduction in *Lactococcus* and *Oscillospira* in autism, while one study found decreased abundance of *Akkermansia* (Table 3.1). *Oscillospira* has also been positively related to sociability in mice (Table 3.1). Two genera, *Desulfovibrio* and *Sutterella*, were more abundant in less sociable individuals. This is in line with those studies finding that their abundances are also elevated in people suffering from autism (Table 3.1) and in fact, it has been hypothesized that *Desulfovibrio* species may play an important role in the pathophysiology of autism (Finegold, 2011). The variable measuring neurotic tendencies was a significant negative predictor of the abundances of *Streptococcus* and *Corynebacterium*. Therefore, individuals that were more neurotic tended to have lower levels of these genera. Since higher neuroticism is a known risk factor for developing depression (Klein *et al.*, 2009), this result is in agreement with a study in a rat model of stress-induced depression reporting a reduction in *Corynebacterium* (Table 3.1). However, in contrast to the findings here, *Streptococcus* has previously been found to be more prevalent in adults suffering from depression (Table 3.1). Although *Streptococcus* species are part of a healthy microbiome, members of this genus can be opportunistic pathogens (Henriques-Normark & Normark, 2010). The finding here that *Streptococcus* abundance is negatively related to neurotic tendencies is therefore somewhat surprising, especially since this genus showed elevated levels in sufferers of a gut condition in this study, in agreement with previous research reporting increased abundance in inflammatory bowel disease (Heidarian *et al.*, 2017) and irritable bowel syndrome (Rajilić-Stojanović *et al.*, 2011).

Notably, not all of the 23 genera targeted in these regression analyses were significantly predicted by behavioural traits (Figure A.5; Table A.4), though this was expected given the somewhat inconsistent results reported in the literature (Table 3.1). In particular, despite extensive findings from animal studies that species belonging to *Bifidobacterium* and *Lactobacillus* can alleviate symptoms of anxiety, stress and depression, and also improve social behaviour (Desbonnet *et al.*, 2010; Bercik *et al.*, 2011b; Bravo *et al.*, 2011; Messaoudi *et al.*, 2011; Savignac *et al.*, 2014; Buffington *et al.*, 2016; Davis *et al.*, 2016b; Bharwani *et al.*, 2017;

Jang *et al.*, 2018), abundances of these genera were not significantly related to either sociability or neurotic tendencies in this study. This may be because these regression analyses were conducted at the genus level, yet there is some evidence that the behavioural effects of *Bifidobacterium* and *Lactobacillus* may be species-specific and even strain-specific (Savignac *et al.*, 2014; Buffington *et al.*, 2016).

Interestingly, the genera targeted in the regression analyses for their putative links with behaviour (Table 3.1) also tend to have high representation in the gut microbiome, with approximately half in the top twenty most abundant genera (Figure 3.2). Perhaps their relatively high abundance helps these taxa to exert a substantial effect on the body's physiology (though some bacteria may still be able to significantly affect the host with only a modest abundance). While many factors may influence microbiome composition, any one factor typically has a relatively small effect (Falcony *et al.*, 2016). Thus, associations between bacterial taxa and host phenotypes are often modest, even with clearly defined phenotypes such as gender and body mass index (The Human Microbiome Project Consortium, 2012; Fodor, 2014). In contrast, the multifaceted nature of personality makes it harder to objectively quantify so it is noteworthy that numerous significant relationships were still found in these analyses, and it is likely therefore that these results underestimate true effects. The results of this study suggest that some bacterial genera may be more strongly linked to behaviour and, in light of my findings, perhaps future research may benefit from investigating the effects of the genera *Akkermansia*, *Desulfovibrio*, *Lactococcus*, *Oscillospira* and *Sutterella* on social behaviour and autistic-like symptoms in animal models, with a view to developing potential new therapies for autism.

Interpreting results from this study also requires consideration of the bidirectional interactions between the gut microbiome and behaviour. As well as the behavioural effects of gut microorganisms, behaviour can in turn shape the composition of the gut microbiome. For example, the gut microbiome can affect the stress response and stress also disrupts the gut microbiome (Cryan & Dinan, 2012; Sharon *et al.*, 2016). In addition, there is growing evidence that how an animal interacts socially can significantly influence its gut microbial community, whose composition is the product of microbial immigration and competition throughout life via

transmission of microorganisms, primarily through the faecal–oral route (Browne *et al.*, 2017). Research in wild primate populations has revealed that social contact shapes gut microbiome composition (Tung *et al.*, 2015; Moeller *et al.*, 2016; Amato *et al.*, 2017; Grieneisen *et al.*, 2017; Perofsky *et al.*, 2017; Raulo *et al.*, 2017) and studies in humans find that we share gut microbiota with household members (Yatsunenko *et al.*, 2012; Song *et al.*, 2013; Lax *et al.*, 2014; Schloss *et al.*, 2014; Odamaki *et al.*, 2018). A key research avenue is untangling the contributions of specific microorganisms to social behaviour versus the influence of social behaviour on microbial colonization. In at least some cases, microbial abundances may differ in relation to autistic traits or sociability because certain taxa are better adapted for transmission between hosts. Notably, the genus *Oscillospira*, whose abundance was positively predicted by sociability in this study, has also been found to be positively related to host density in a wild animal population (Li *et al.*, 2016a). It is important to bear in mind, therefore, that some genera may be efficiently transmitted socially, rather than having a causal effect on social behaviour. Indeed, *Oscillospira* belongs to the family Ruminococcaceae, many of which are intestinal spore-forming bacteria, thereby facilitating transmission between hosts (Browne *et al.*, 2016). In fact, numerous members of the microbiota are well adapted for transmission and it is estimated that at least half the bacterial genera in the gut are able to produce resilient spores adapted for survival and dispersal (Browne *et al.*, 2016).

The evidence of social transmission of microorganisms is particularly relevant to the finding here that people with larger social networks tend to have a more diverse gut microbiome. Indeed, animal studies have revealed that social interactions are positively associated with microbiome diversity (Li *et al.*, 2016a; Moeller *et al.*, 2016; Billiet *et al.*, 2017; Perofsky *et al.*, 2017). For example, reduced social contact in a bumblebee colony results in lower gut microbiome diversity (Billiet *et al.*, 2017) and chimpanzees that interact more socially have a more diverse gut microbiome (Moeller *et al.*, 2016). In fact, horizontal transmission of gut microorganisms through chimpanzee social interactions appears to play a greater role than vertical transmission from the mother in shaping gut microbiome composition (Moeller *et al.*, 2016). This suggests that although the mother provides the primary inoculum for the newborn's microbiome, the

composition of the microbiome through the individual's lifetime may be more strongly influenced by social interactions. The relationship between gut microbiome diversity and human social networks has not previously been explored but the positive relationship found here suggests that social interactions may also influence the microbiota of human societies. Interestingly, a study of gut microbiome composition and temperament in infants reported an association between gut microbiome diversity and sociability (Christian *et al.*, 2015). By maintaining diversity of the gut microbial community, social transmission of microorganisms may benefit host health in numerous ways (Browne *et al.*, 2017). For example, diversity may help to promote stability and resilience of the gut microbiome (Lozupone *et al.*, 2012), and is often linked to good health, though this is not always the case (Johnson & Burnet, 2016). Diversity can provide resistance against infection (Dillon *et al.*, 2005; Koch & Schmid-Hempel, 2011; Lawley *et al.*, 2012; Abt & Pamer, 2014), improve immune function (Browne *et al.*, 2017) and may reduce the risk of allergies (Bloomfield *et al.*, 2016). It is well known that people with more social ties are healthier and live longer, and reduced inflammation is postulated to play a role in this relationship (Holt-Lunstad *et al.*, 2010; Yang *et al.*, 2016b). It is interesting therefore to speculate whether the microbiome may mediate this association between social integration and health, particularly since it is a key regulator of the host immune response (Rooks & Garrett, 2016).

Participants who were more stressed or anxious, and also those reporting poorer sleep quality, tended to have both a less diverse gut microbiome and an altered composition. In line with these results, a recent study found that anxiety status was related to stool consistency, suggesting that anxiety may be associated with differences in gut microbiome composition, perhaps through inducing dysbiosis (Shiro *et al.*, 2017). Depression has also been associated with differences in microbial community composition (Zhernakova *et al.*, 2016). In addition, it has previously been shown in animal studies that stress not only alters the abundances of various microbial taxa but also reduces the diversity of the gut microbial community (Bailey *et al.*, 2011; Partrick *et al.*, 2018). In fact, part of the reason why individuals with a larger social network have a more diverse microbiome may be because social support can help buffer the adverse effects of stress on diversity (Johnson & Dunbar, 2016). However, social network size

was not significantly correlated with stress in this study (Figure 3.3) and the positive relationship between network size and microbiome diversity is consistent with evidence from animal populations that members of the microbiota are transmitted through interactions with the social environment. Further research should attempt to validate this finding and investigate the various microbial transmission routes and their relative importance. Indeed, modern lifestyle choices are geared towards preventing pathogen transmission but maybe we should instead consider ways to promote the spread of beneficial microorganisms (Browne *et al.*, 2017). Notably, the negative relationship found here between conscientiousness and genus richness of the gut microbiome may be because conscientious people are more likely to engage in hygienic behaviour (Aunger *et al.*, 2016), which may result in a smaller number of microbial genera inhabiting the gut. The observed relationship between sleep quality and gut microbiome composition and diversity may be due to its intercorrelation with stress, anxiety and neuroticism (Figure 3.3) but may also partly reflect the known relationship between the gut microbiome and host circadian rhythms (Paschos & FitzGerald, 2017).

Although the focus of this research was on personality traits, there are also other novel findings of considerable interest from this study. People who travelled frequently or visited more countries tended to have a more diverse gut microbiome, suggesting that our interaction with the environment does play a considerable role in influencing our gut microbial community. This increased diversity may also partly reflect the different foods people tend to eat when travelling. However, holidays abroad are also positively associated with the prevalence of antibiotic resistance genes in the gut (von Wintersdorff *et al.*, 2014). This suggests that although travelling may increase the taxonomic diversity of the gut, it may also increase the risk of acquiring antibiotic resistance. Another interesting result not previously shown was that more adventurous eaters also had a greater gut microbiome diversity, supporting the idea that microbiome health may be improved through a diverse diet (Heiman & Greenway, 2016). This finding is particularly pertinent given the increasingly restrictive dietary habits of Western cultures and is also in agreement with a recent study on a wild primate population which reported a positive association between dietary diversity and microbiome richness (Perofsky *et al.*, 2017). Diversity was also

related to the amount of food people consumed containing natural probiotics and prebiotics. Indeed, fermentation has been practised by human civilization for over 9,000 years (McGovern *et al.*, 2004) and is traditionally used in many cultures in the production of foods such as cheese, yogurt, sauerkraut and kimchi. From an evolutionary perspective, fermented foods may represent an adaptation passed on through generations, given their potential beneficial effects on health. However, probiotic supplementation was significantly associated with reduced microbiome diversity. A likely explanation for this finding is that people with reduced microbiome diversity (for example, due to a course of antibiotics or gut dysbiosis) may be more inclined to take probiotic supplements. Indeed, while there was no evidence of a relationship between probiotic consumption and either antibiotic treatment or general health, probiotic supplementation was positively correlated with having a gut condition (Figure 3.3). Somewhat surprisingly, dog ownership was also linked to lower diversity, which contrasts the finding that infants living with household pets have a more diverse gut microbiome (Azad *et al.*, 2013).

Other notable results include females having a significantly lower gut microbiome diversity compared to males. This contrasts a previous human study which found no significant differences in diversity between the sexes (Haro *et al.*, 2016) but is in the same direction as recent research in a wild baboon population (Grieneisen *et al.*, 2017). As expected, antibiotic treatment was associated with reduced diversity of the microbiome and an altered composition, in accordance with previous findings (Jernberg *et al.*, 2007; Dethlefsen *et al.*, 2008; Dethlefsen & Relman, 2010; Jakobsson *et al.*, 2010; Dominguez-Bello *et al.*, 2015). Although there is some evidence that birth by caesarean section can affect the diversity and composition of the infant gut microbiome (Dominguez-Bello *et al.*, 2010; Bokulich *et al.*, 2016; Martin *et al.*, 2016), other studies find that delivery mode does not significantly influence development of the microbiome (Chu *et al.*, 2017; Stewart *et al.*, 2017) and no lasting differences were detected here in the adult population. However, individuals that were formula-fed as infants did have a lower gut microbiome diversity. This is the first time the effect of infant diet has been explored with respect to the adult gut microbiome. Most research in infants actually finds that formula feeding is associated with increased diversity compared to breastfeeding (Praveen *et al.*, 2015), but results

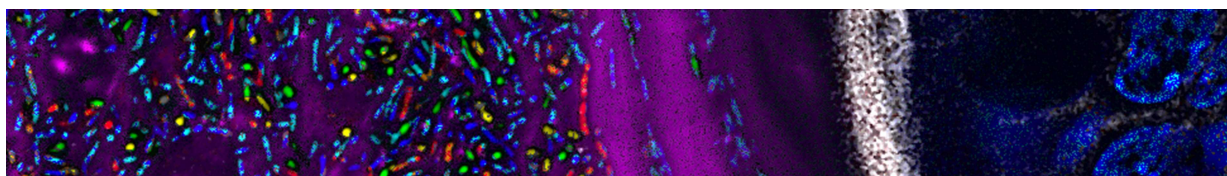
of a two-year longitudinal study have shown that while formula-fed infants in their first year of life may have higher gut microbiome diversity, breast-fed infants tend to have higher diversity between one and two years of age (Bokulich *et al.*, 2016). The reduced microbiome diversity of people in unemployment may partly be a reflection of socioeconomic status which can influence health through lifestyle behaviours, diet, access to medical care and psychosocial factors such as stress (Findley *et al.*, 2016). With respect to other comprehensive population studies of the human gut microbiome (Falcony *et al.*, 2016; Zhernakova *et al.*, 2016), the results here replicate previous findings that age, gender, body mass index, sleep, constipation, gut conditions, antibiotic treatment and consumption of fruit, vegetables and cereals are related to gut microbiome variation.

As this study was cross-sectional, causation cannot be proved, particularly given the bidirectional nature of the microbiome–gut–brain axis. As discussed, gut bacteria can affect behaviour and behaviour can in turn influence the composition of the gut microbiome. Despite this limitation, the findings here represent an important step in understanding the relationships between the gut microbiome and personality traits, and the potential consequences for mental health. Since previous studies have demonstrated that colonizing mice with the faecal microbiota of depressed patients can induce symptoms of depression (Kelly *et al.*, 2016; Zheng *et al.*, 2016), it would therefore be interesting to investigate whether colonizing mice with the faecal microbiota from people with a range of different personality types also leads to behavioural differences in the animals. It would also be informative to assess how inflammatory markers are related to human personality traits and gut microbiome composition, given the link between personality and immunological traits (Segerstrom, 2000; Vedhara *et al.*, 2015; D'Acquisto, 2017) and the increasing evidence that the immune system is an important mediator of the microbiome–gut–brain axis (Fung *et al.*, 2017). In addition, since this research assessed taxonomic composition and diversity of the microbiome, further insight could be gained by applying methods such as metabolomics and metatranscriptomics to also determine how microbiome function is related to behavioural variation.

A key benefit of this study is that rather than examining the expression of extreme traits, as seen in psychiatric disorders, this research focused on behavioural variation in the general population. The findings reported here challenge the results of a recently published study concluding a lack of significant relationships between psychiatric measures in healthy humans and microbiome composition and diversity (Kleiman *et al.*, 2017). However, the research was based on a comparatively small sample size and only involved female participants. Given the very exploratory nature of their study, the researchers FDR-corrected their P values for almost 4,000 hypotheses which may have led to numerous false negative results. As opposed to their conclusions that the gut microbiome may only be relevant in cases of psychiatric illness, my findings suggest that the microbiome may also be related to personality traits in the healthy population. Indeed, nearly all the results were replicated when anyone suffering from a mental illness was excluded from the dataset (Tables A.9 to A.11), showing that my findings were not driven by those scoring towards the extreme end for the personality traits. The only notable exception was for the beta diversity analyses, where stress and anxiety were no longer significantly associated with differences in the overall community composition of the gut microbiome. The majority of the regression results were also replicated, although the positive relationship between sociability and *Akkermansia* abundance was not detected. Additionally, there was evidence in this dataset that *Blautia* was less abundant in more sociable individuals ($P = 0.043$) and *Prevotella* was less abundant in more neurotic individuals ($P = 0.002$). Similarly, some previous studies have found *Prevotella* to be reduced in people with low mood or those suffering from depression (Table 3.1).

In conclusion, I show that differences in gut microbiome composition and diversity are linked to personality traits in the general population. The results of this study add a new dimension to our understanding of personality and are in line with accumulating evidence that the gut microbiome can influence the central nervous system in humans, with effects on behaviour. Such findings may inform the development of probiotic or prebiotic therapies to help improve mood and treat conditions such as autism, anxiety and depression. Discovering new and effective interventions for mental health conditions is of pressing concern, given the declines in psychological health of

our modern society. Finally, it is pertinent to reflect on the ways in which our modern-day living may provide a perfect storm for dysbiosis of the gut. We lead stressful lives with fewer social interactions and less time spent with nature, our diets are typically deficient in fibre, we inhabit oversanitized environments and are dependent on antibiotic treatments. All these factors can influence the gut microbiome and so may be affecting our behaviour and psychological well-being in currently unknown ways.



Chapter 4

Behaviour and the gut microbiome in a social network of a macaque population

4.1 Abstract

Studies in laboratory animals have demonstrated that the gut microbiome can affect multiple aspects of behaviour, including social behaviour. Here, I explore for the first time whether the microbiome–gut–brain axis is also relevant to behavioural variation in a non-captive animal population. Social behaviour is assessed in a population of rhesus macaques (*Macaca mulatta*), based on grooming interactions, and combined with gut microbiome data. I focus my analyses on bacterial genera previously linked to sociability and autistic behaviours in rodents and humans. I show in this macaque population that some of these genera are also related to an individual's propensity to engage in social interactions. Interestingly, I find that several of these genera positively related to sociability are well known for their beneficial effects on health, such as *Faecalibacterium*. Overall, my findings highlight the connections between social behaviour, microbiome composition and health in a non-captive animal population.

4.2 Introduction

The emergence of social systems is considered one of the major evolutionary transitions (Maynard Smith & Szathmáry, 1997). Social organization is a fundamental characteristic of many animal populations. In particular, most primate species, including humans, live in complex

social groups. While group living comes with numerous benefits, such as the acquisition of resources, access to mates and protection from predation (Silk, 2007), social networks can also facilitate the spread of disease (Krause & Ruxton, 2002). To date, most research has focused on the transmission of parasites and pathogens within animal social groups (Altizer *et al.*, 2003; Craft, 2015), which may occur either directly, via interactions between individuals, or indirectly through their shared environment. However, the advent of microbiome research has revealed that the majority of host-associated symbionts are harmless and many may even be beneficial to health and survival. This has prompted studies exploring how the social transmission of microorganisms may influence microbiome composition, and the possible consequences for host fitness (Archie & Tung, 2015).

Research over the past few years has started to reveal the role of the host's social environment in shaping an individual's microbiome (Archie & Tung, 2015). For example, in a wild baboon population, social group membership was found to be the strongest predictor of gut microbiome composition, with individuals that interacted more frequently through grooming having more similar gut microbial communities (Tung *et al.*, 2015). This was true for both core and non-core microbial taxa, the former constituting taxa considered to provide primary functions to host health (Grieneisen *et al.*, 2017). Studies on various other primates, notably chimpanzees, lemurs and howler monkeys, have also shown that different social groups within a species have distinct gut microbial communities (Degnan *et al.*, 2012; Bennett *et al.*, 2016; Amato *et al.*, 2017; Perofsky *et al.*, 2017) and that social interactions, such as grooming, promote microbial similarity between individuals (Moeller *et al.*, 2016; Raulo *et al.*, 2017). In addition, increased social contact has been associated with a more diverse gut microbiome in a range of animal populations (Li *et al.*, 2016a; Moeller *et al.*, 2016; Billiet *et al.*, 2017; Perofsky *et al.*, 2017). For example, chimpanzees engage in more frequent social interactions during the wet season and this is correlated with increased species richness of the gut microbiome, independent of dietary variability (Moeller *et al.*, 2016). In fact, social transmission between chimpanzees was found to play a more important role than vertical transmission from the mother in shaping the gut microbiome composition of infants. Further evidence of socially mediated microbial transmission comes from a study of

wild baboons showing that the gut microbiome of immigrant males in a social group changes over time to resemble the microbiome composition of long-term residents of the group (Grieneisen *et al.*, 2017). There is also evidence that microorganisms are socially transmitted between humans since people living in the same household have more similar gut microbial communities (Yatsunenko *et al.*, 2012; Song *et al.*, 2013; Lax *et al.*, 2014; Schloss *et al.*, 2014; Odamaki *et al.*, 2018) and individuals with a larger social network have a more diverse gut microbiome (Chapter 3). The role of social behaviour in influencing microbiome composition may therefore have implications for host health and fitness, since transmission of beneficial microorganisms and a more diverse microbiome may help to protect hosts from pathogens (Dillon *et al.*, 2005; Koch & Schmid-Hempel, 2011; Lawley *et al.*, 2012; Abt & Pamer, 2014) and improve the stability and resilience of the gut microbiome (Lozupone *et al.*, 2012).

The reciprocal interaction between host behaviour and infection status is well known in parasitology (Ezenwa *et al.*, 2016), whereby behaviour can influence parasite transmission (Altizer *et al.*, 2003) and parasites can in turn alter the behaviour of their host (Moore, 2013; Johnson & Foster, 2018 [Appendix]). Similarly, in addition to the role of social interactions in microbial transmission, recent research in the field of the microbiome–gut–brain axis has demonstrated that the mammalian gut microbiome can affect various aspects of host behaviour, including anxiety, depressive-like behaviour and sociability (Cryan & Dinan, 2012; Sharon *et al.*, 2016). In particular, rodent studies manipulating the gut microbiome (using germ-free conditions, antibiotic treatment or probiotic supplementation) have revealed that the gut microbiome influences social development and behaviour (Hsiao *et al.*, 2013; Crumeyrolle-Arias *et al.*, 2014; de Theije *et al.*, 2014; Desbonnet *et al.*, 2014, 2015; Buffington *et al.*, 2016; Leclercq *et al.*, 2017; Guida *et al.*, 2018; Stilling *et al.*, 2018). These findings have motivated research investigating whether the gut microbiome also contributes to the social deficits seen in autism (Krajmalnik-Brown *et al.*, 2015), especially since autism is frequently comorbid with gastrointestinal issues (Hsiao, 2014). Differences in gut microbiome composition between autistic and neurotypical controls have been found repeatedly in human studies, indicating that autism is associated with an altered gut microbial community (Vuong & Hsiao, 2017). In fact, a

recent study involving faecal microbiota transplantation in children with autism showed that it improved multiple aspects of behaviour, including social behaviour, as well as ameliorating gastrointestinal symptoms (Kang *et al.*, 2017). However, so far this link between the gut microbiome and autistic behaviours has only been investigated in laboratory animals or human populations.

Here I examine the relationship between social behaviour and gut microbiome composition in a non-captive population of rhesus macaques on the island of Cayo Santiago, off Puerto Rico. Since autistic traits are continuously distributed across the human population (Wing, 1988; Constantino & Todd, 2003; Ruzich *et al.*, 2015), it is likely that the same is true for non-human primates and indeed, macaques in this population show variation in social motivation and behaviour (Watson & Platt, 2012; Madlon-Kay *et al.*, 2017). Social network analysis was used to assess variation in social behaviour, measured through grooming interactions. Rhesus macaques live in large mixed-sex groups and the social structure of this study population is well characterized (Maestriperi & Hoffman, 2012). They are highly social animals and grooming is their primary means of making and maintaining relationships, providing a good indicator of social interactions between individuals (Figure 4.1). Since rhesus macaques live in dominance hierarchies, rank can influence grooming interactions but only explains a minority of the variation in their grooming network (Brent *et al.*, 2013, 2017). Thus, other factors also likely affect



Figure 4.1. Rhesus macaques on Cayo Santiago Island. Affiliative interactions such as grooming help to form and maintain social bonds. Image courtesy of Lauren Brent, University of Exeter.

individual motivation to engage in social interactions. I hypothesize that inter-individual variation in macaque social behaviour may be associated with differential abundances of gut bacteria previously linked to autistic and sociability traits in rodent studies and clinical populations (Table A.13). Such differences in gut microbiome composition may result in some individuals showing more interest to engage in affiliative grooming interactions than others, thereby contributing to the differences in social behaviour observed in the population. I also consider the role of social relationships in influencing gut microbiome composition and diversity in this macaque population.

4.3 Materials and methods

4.3.1 Study population and behavioural data collection

Study subjects were adult rhesus macaques from a population on the island of Cayo Santiago, off the coast of Puerto Rico. This free-ranging population stems from a founding population introduced to the island from India in 1938 (Rawlings & Kessler, 1986). Although the animals forage for natural vegetation, their diet is also supplemented with daily feed and water but there is no regular medical intervention. Macaques are individually recognized by observers who record their behaviours using 10 min focal animal observations (Altmann, 1974). Behavioural data collection is ongoing in this population and is distributed evenly during the day, both between and within subjects. Grooming interactions between individuals were recorded in terms of both the duration of the interaction and the identities of the giver and receiver, while dominance rank was calculated based on the direction of submissive interactions. Behavioural data relating to grooming interactions and social rank from years 2012 to 2013 for one social group from the population were used in this study since this is the period over which the faecal samples were collected for microbiome sequencing. Sample size was limited by the number of individuals for whom gut microbiome sequence data were available, with 50 samples in total representing 38 individuals (since faecal samples for some individuals were collected on more than one occasion).

4.3.2 Social network metrics

Social networks based on grooming interactions were constructed for each year separately, using the software UCINET (Borgatti *et al.*, 2002). Social networks included all members of the group, regardless of whether microbiome data were available for that individual. Network metrics were normalized by expressing each individual's score relative to the mean score in each year for the social group. A sociability index was calculated for each individual by taking their cumulative score of the normalized network metrics for degree (number of different grooming partners) and strength (amount of time spent both giving and receiving grooming, relative to the amount of time for which the individual was observed).

4.3.3 Sample collection, processing and sequencing

During behavioural observation of the animals, faecal samples (uncontaminated by urine, water or other faeces) were collected opportunistically from both sexes over the two-year period. Samples were taken shortly after defecation and then stored at -20°C . DNA was extracted from the samples according to the Earth Microbiome Project standard protocols (Gilbert *et al.*, 2014) using the PowerSoil-HTP Kit (Qiagen). The 16S rRNA gene was amplified via PCR using universal primers for the V4 hypervariable region and the resulting amplicons were sequenced on the Illumina MiSeq platform, according to a previously described protocol (Caporaso *et al.*, 2012). Blanks were also incorporated in sequencing runs to control for contamination. Sequencing data were processed and analysed using the QIIME software version 1.9.1 (Caporaso *et al.*, 2010). Sequences were demultiplexed and quality filtered in QIIME and then assigned to operational taxonomic units (OTUs) with open-reference OTU picking against the Greengenes database (DeSantis *et al.*, 2006). This OTU picking method was used since it preserves sequence data and therefore taxonomic diversity (Navas-Molina *et al.*, 2013).

4.3.4 Multiple regression analyses of bacterial abundances

Microbiome count data contain a high proportion of zeros (Xu *et al.*, 2015; Weiss *et al.*, 2017) and their frequency distributions are heavily right-skewed (Xia & Sun, 2017). This

overdispersion arises because there are relatively few taxa that are found in all samples as most taxa are rare (Xu *et al.*, 2015). Count data cannot typically be normalized using transformation and so negative binomial regression was conducted with genus abundance as the response variable (or zero-inflated negative binomial regression when the count data contained an excess of zeros). When modelling microbiome data, it is also important to take into account variation in sequencing read depth between samples. Rarefying count data or expressing the data as a proportion can result in a high rate of false positives and so is not recommended for models of taxon abundance (McMurdie & Holmes, 2014). Instead, the total read number was included as a covariate in the regression models, thereby preserving all sequence data (Goodrich *et al.*, 2014; Xu *et al.*, 2015).

Negative binomial regression was conducted using the R package glmmADMB to model microbiome count data. All statistical analyses in this study were performed using R 3.2.3 software (R Development Core Team, 2015). Regression models were constructed to predict genus abundance as 16S rRNA sequencing is estimated to be 96% accurate for genus identification (Srinivasan *et al.*, 2015), whereas its phylogenetic resolution at the species level is more limited (Janda & Abbott, 2007). The main variable of interest, the sociability index, was included in each model, as well as the total read number. Individual identity was added as a random effect to control for repeated sampling of some individuals. Effects of rank, sex, age, season and sampling year were assessed as potential confounding variables since these factors may vary with both primate microbiome composition (Degnan *et al.*, 2012; Fogel, 2015; Bennett *et al.*, 2016; Ren *et al.*, 2016; Sun *et al.*, 2016; Perofsky *et al.*, 2017; Raulo *et al.*, 2017; Springer *et al.*, 2017; Hicks *et al.*, 2018; Trosvik *et al.*, 2018) and sociability (Schino, 2001; Krause & Ruxton, 2002; Shimizu *et al.*, 2012; Almeling *et al.*, 2016; Perofsky *et al.*, 2017). Although rank can influence grooming relationships (Schino, 2001), it was not significantly correlated with the sociability index in this population ($P = 0.827$) and so could be included in the regression models without resulting in multicollinearity. Given the lack of existing knowledge about how particular genera may vary in abundance in relation to these variables, the inclusion of variables in the regression models was guided by the Akaike information criterion (Akaike, 1974). The main

reason for this selective inclusion of variables was to avoid losing degrees of freedom that would reduce the statistical power, given the relatively small sample size.

4.3.5 Analyses of microbiome diversity and community composition

Alpha and beta diversity analyses were conducted to investigate how the overall composition of the gut microbiome was related to grooming interactions, while controlling for the effects of rank, sex, age, season and sampling year. While alpha diversity considers the ecological diversity of each sample individually, beta diversity measures differences in community composition between microbiome samples (Finotello *et al.*, 2016). For these diversity analyses, microbiome count data were rarefied by randomly sampling counts without replacement so that the number of total counts for each sample was equal. This is an advisable normalization method as uneven sequence counts across samples can significantly affect diversity estimates (Goodrich *et al.*, 2014; Weiss *et al.*, 2017).

A linear mixed model was constructed using the `lme` function in the R package `nlme` to predict alpha diversity, estimated with the Chao1 index based on genus-level OTUs. The variables rank, sex, age, season and year were also incorporated in the model and individual identity was included as a random effect to account for pseudoreplication.

To assess beta diversity, PERMANOVA was performed to determine which variables significantly affect the overall composition of the gut microbiome. Gut microbial similarity between individuals based on genus-level OTUs was calculated in QIIME using both weighted UniFrac and unweighted UniFrac distance matrices (Lozupone & Knight, 2005) and visualized using EMPeror (Vázquez-Baeza *et al.*, 2013). UniFrac distances account for the phylogenetic relatedness of microbial taxa; unweighted distances only consider presence versus absence of taxa, whereas weighted distances also take into account their abundances. PERMANOVA was carried out on these matrices using the `adonis` function from the R package `vegan` with 1,000 permutations, incorporating the same variables as the model predicting alpha diversity. This function estimates the variance in the distance matrix attributable to each variable.

4.4 Results

4.4.1 Taxonomic composition of the microbiome

Gut microbiome composition differed considerably between individuals (Figure 4.2), as has been found in numerous other studies of primate social groups (Fogel, 2015; Tung *et al.*, 2015; Ren *et al.*, 2016; Perofsky *et al.*, 2017). The most abundant genera across all samples were similar to previous research on the gut microbiome of captive rhesus macaques (Yasuda *et al.*, 2015; Amaral *et al.*, 2017).

4.4.2 Regression models of bacterial abundances

Negative binomial regression analyses revealed that the abundances of five of the sixteen genera targeted in the regression analyses were significantly predicted by host sociability (Figures 4.3 and 4.4; Table A.14). Sociability positively predicted genus abundance for *Dialister* ($P = 0.008$), *Faecalibacterium* ($P = 0.002$), *Prevotella* ($P = 0.019$) and *Sutterella* ($P = 0.006$). On the other hand, the abundance of *Streptococcus* was negatively predicted by sociability ($P = 0.001$). In terms of the other variables included in the models, sex, age, season and sampling year were also significantly related to the abundances of certain genera (Table A.14). Notably, the abundance of *Faecalibacterium* was lower in females compared to males, in accordance with findings in humans (Aguirre de Cárcer *et al.*, 2011). Older animals showed reduced abundances of *Prevotella* and *Streptococcus*. Similarly, in humans *Prevotella* has been found to be more prevalent in infants than adults (Yatsunenkeno *et al.*, 2012) and its abundance is negatively predicted by age (Chapter 3).

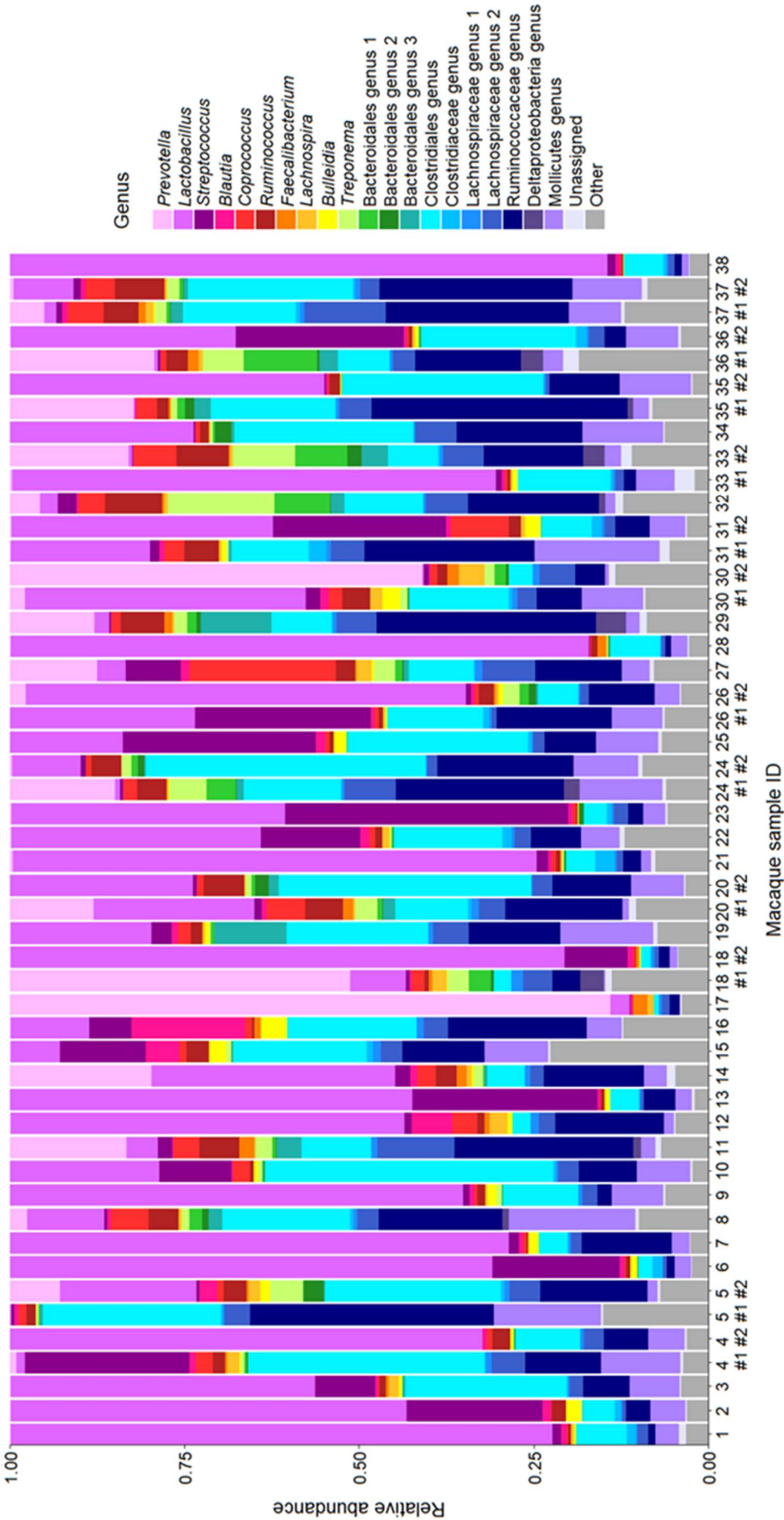


Figure 4.2. Stacked bar plot summarizing gut microbiome composition at genus level for the macaque study population. Microbiome data are plotted showing relative abundance of the top twenty most abundant genera across all samples, with the remaining genera grouped into 'Other'. The twenty genera in the key are ordered from most to least abundant. As labelled in the chart, some individuals from the population were sampled twice on different occasions (such as a different year or season). Microbial taxa that could not be identified to genus level were instead numbered at the taxonomic rank to which they could be resolved. By convention, 'Unassigned' denotes reads not sufficiently similar to any previously classified microbial taxa in the Greengenes database.

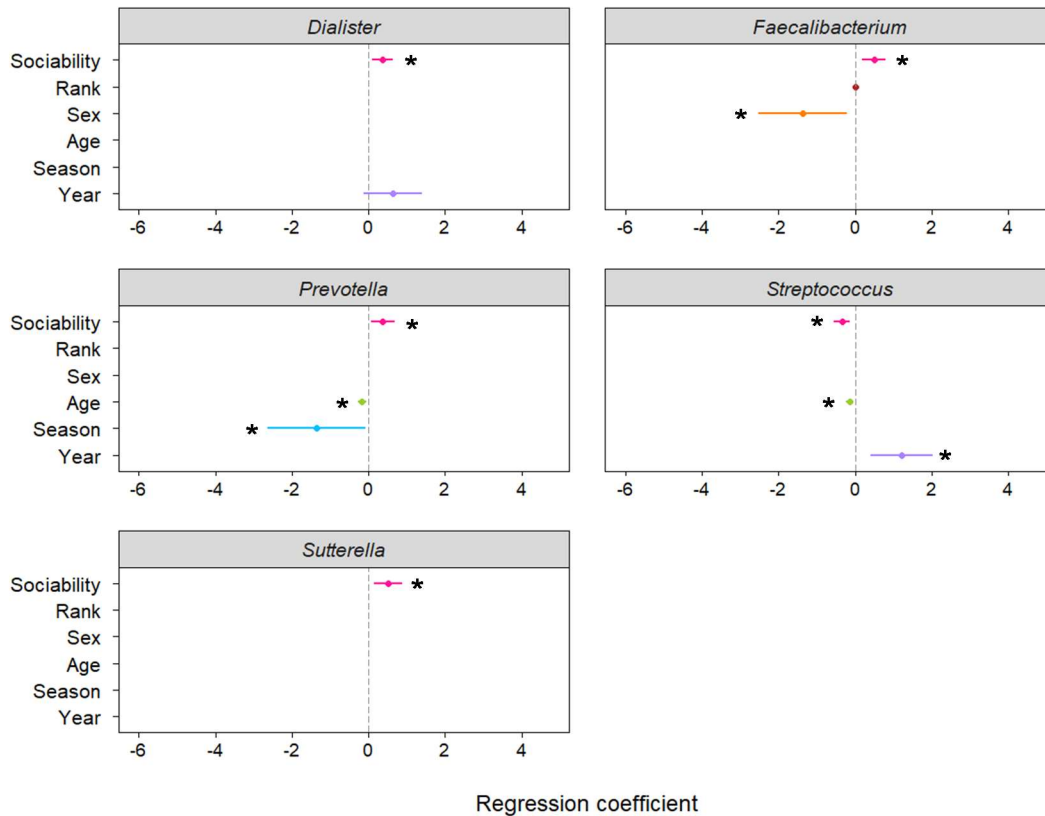


Figure 4.3. Coefficient plots from regression models predicting abundances of genera in the macaque gut microbiome. Asterisks denote significant predictors of genus abundance where $P < 0.05$ and bars indicate 95% confidence intervals. The main variable of interest was the sociability index (as measured by the number of grooming partners and duration of grooming interactions), while other variables were also included where appropriate, as determined using Akaike information criterion. A positive relationship with sex indicates a higher abundance of the genus in females. Plots displayed here depict genera whose abundances were significantly related to sociability (for remaining regression coefficient plots see Figure A.6).

4.4.3 Effects of grooming on microbiome diversity and community composition

Neither grooming interactions nor any of the other variables included in the linear mixed model significantly predicted alpha diversity of the gut microbiome (Table A.15). In terms of beta diversity, the amount of grooming received by an individual explained 5% of the variation in gut microbiome composition ($P = 0.022$), as revealed by PERMANOVA using weighted UniFrac distances (Figure 4.5; Table A.16). Age ($P = 0.039$) and season ($P = 0.007$) were also significant factors influencing microbiome composition. However, for unweighted UniFrac distances (Table A.16), only season had a significant effect ($P = 0.015$).

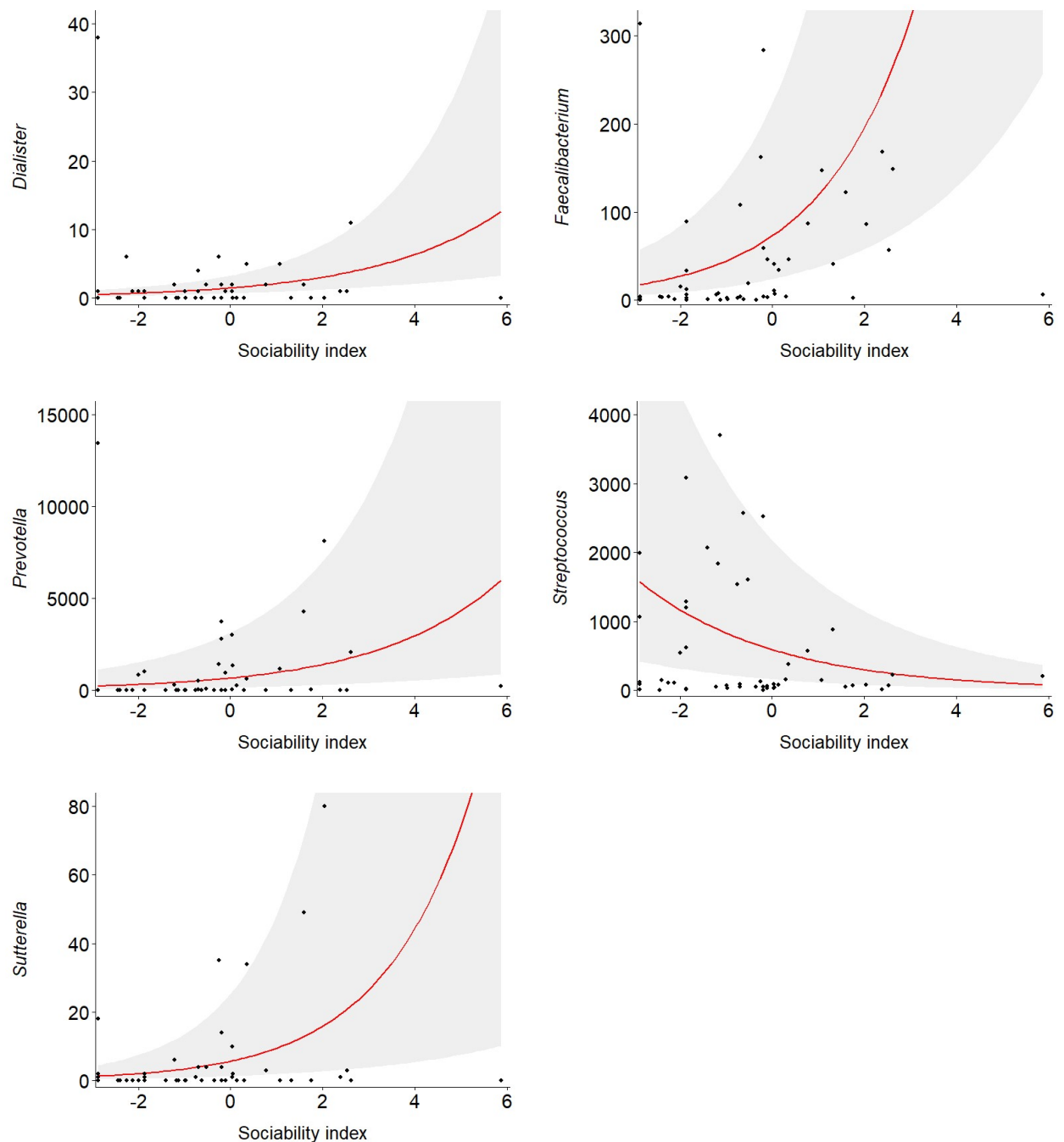


Figure 4.4. Relationship between macaque sociability and abundances of the genera *Dialister*, *Faecalibacterium*, *Prevotella*, *Sutterella* and *Streptococcus*, as predicted from negative binomial regression. Shaded region denotes 75% confidence interval for the regression line.

4.5 Discussion

Investigations of the relationship between the gut microbiome and host social behaviour in primates have so far focused on understanding the role of social relationships in microbial transmission within social groups. Here I consider gut microbiome composition from the perspective of the microbiome–gut–brain axis and show that a number of genera previously

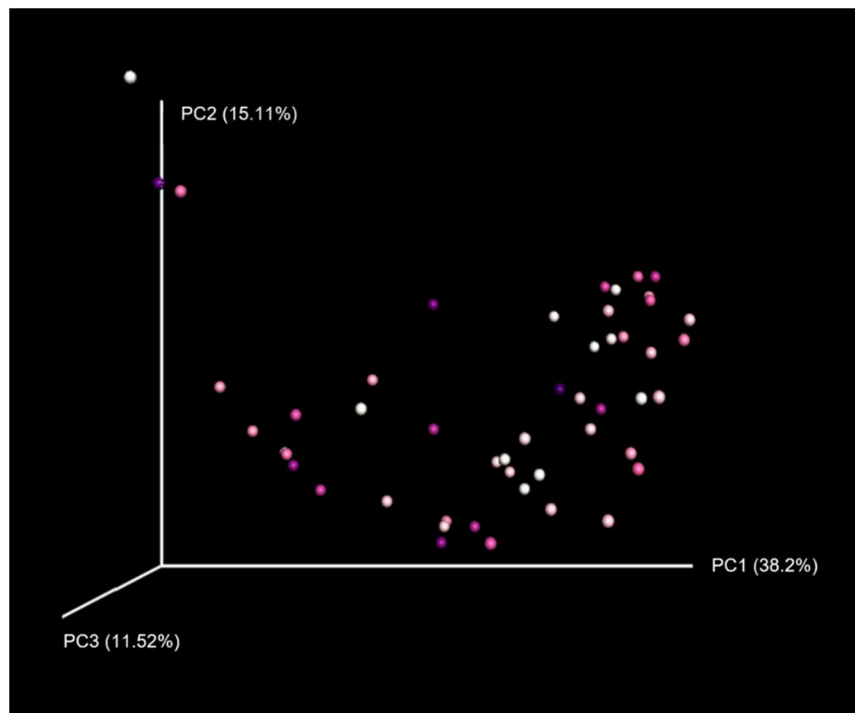


Figure 4.5. Principal coordinates analysis plot of microbiome composition using weighted UniFrac distances. Principal coordinates analysis reduces the dimensionality of microbiome data so that differences in microbiome composition between samples can be visualized, with points clustered together indicating more similar gut microbial communities. Points are coloured on a gradient from white (individuals receiving the least grooming) to dark purple (those receiving the most grooming).

linked to individual differences in sociability or autistic traits are also significantly related to macaque social behaviour in this population. The majority of significant relationships were in the expected direction based on the literature (Table A.13). The genera *Dialister*, *Faecalibacterium*, *Prevotella* and *Sutterella* were more abundant in individuals scoring higher on the sociability index, that is, those engaging in grooming interactions for a longer duration and with more partners.

In line with these findings, some previous studies have reported a reduction in abundance of these genera in autism and *Dialister* has also been positively related to sociability in infants (Table A.13). However, the results of studies comparing the gut microbiota of autistic and neurotypical individuals are not always consistent. For example, while one study found a lower abundance of *Sutterella* in autism (in accordance with the finding here), the majority of studies have reported an increased abundance of this genus in autism (Table A.13). Indeed, the abundance of *Sutterella* was also greater in humans with a lower sociability score (Chapter 3).

In contrast to the two studies reporting reduced levels of *Streptococcus* in the gut microbiota of autistic children (Table A.13), in this macaque population *Streptococcus* was found to be more abundant in less sociable individuals. Notably, some members of this genus can be opportunistic pathogens (Henriques-Normark & Normark, 2010), producing pro-inflammatory cytokines (van den Bogert *et al.*, 2014), and thus increased *Streptococcus* abundance in less sociable animals may reflect their poorer social integration and health. In fact, there is evidence that this genus is also more prevalent in humans suffering from depression (Lin *et al.*, 2017), though somewhat surprisingly *Streptococcus* was less abundant in more neurotic humans (Chapter 3). One possible explanation for this may be that *Streptococcus thermophilus* is a common constituent of fermented food products such as yoghurts (Burton *et al.*, 2017) and there was evidence in the human cohort that neuroticism was negatively related to the consumption of probiotic foods. In comparison, since the macaques are not exposed to probiotics, a higher abundance of *Streptococcus* in less sociable macaques may reflect the presence of potentially pathogenic members of this genus, though this cannot be confirmed given the genus-level analysis. However, direct comparison of the microbiome composition of humans and macaques may provide limited insight since the human gut microbiome is known to differ from that of non-human primates (Davenport *et al.*, 2017). In addition, the macaques were from a semi-wild population which is very different from the modern human state since western diets, antibiotic intake and sanitized environments can all impact composition and diversity of the gut microbial community (Segata, 2015).

Given the novel and exploratory nature of these analyses, a significant relationship between sociability and genus abundance was not found for every genus included in this study (Figure A.6). Notably, there was no relationship between *Lactobacillus* abundance and engagement in social interactions, despite numerous animal studies reporting behavioural effects of *Lactobacillus* species (Bravo *et al.*, 2011; Buffington *et al.*, 2016; Davis *et al.*, 2016b; Bharwani *et al.*, 2017; Jang *et al.*, 2018). One reason for this may be because the analyses were only performed at the genus level, yet behavioural effects of *Lactobacillus* may be species-specific (Buffington *et al.*, 2016). No regression analysis was conducted for *Bifidobacterium* as this

genus was too zero-inflated to be informative, with the majority of macaque microbiome samples lacking bacteria belonging to this genus. Interestingly, *Bifidobacterium* is also undetected in the gut microbiota of Hadza hunter-gatherers but is commonly found in the microbiota of industrialized populations (Schnorr *et al.*, 2014).

It is compelling that some of the gut bacterial genera that are generally reduced in abundance in autism also have a lower abundance in less sociable monkeys. While this may reflect an effect of gut microorganisms on the brain and behaviour, as has been repeatedly demonstrated in animal models (Cryan & Dinan, 2012; Sampson & Mazmanian, 2015; Sharon *et al.*, 2016), it is important to consider the reciprocal interactions that may be involved. Research in the autism field largely investigates the gut microbiome from the viewpoint that dysbiosis may give rise to behavioural impairments (Li & Zhou, 2016). However, people suffering from autism may also have limited exposure to the outside environment, including the social environment, and this in turn may influence the composition of their gut microbiome. Similarly, at least some of the relationships reported here between macaque social behaviour and bacterial abundances may reflect the fact that certain microorganisms are better adapted for social transmission than others. For example, the genus *Prevotella* was previously found to be positively associated with host density in a wild animal population (Li *et al.*, 2016a), suggesting that it may be transmitted effectively through social contact, rather than necessarily having a causal effect on host social behaviour. In fact, it is estimated that at least half the bacterial genera living in the gut can produce resilient spores and are therefore well adapted for transmission between hosts (Browne *et al.*, 2016). In addition to the social transmission of microorganisms, another factor that can influence the gut microbiome is exposure to stress (Bailey & Coe, 1999; Bailey *et al.*, 2011; Bangsgaard Bendtsen *et al.*, 2012; Bharwani *et al.*, 2016). Grooming interactions have been shown to reduce heart rate and cortisol levels, thereby buffering the detrimental effects of stress (Brent *et al.*, 2014). This may therefore represent another route through which social behaviour may be linked to gut microbiome composition.

Regardless of the direction of these relationships, it is notable that several of these bacterial genera related to sociability have also been associated with beneficial effects on host immunity,

such as *Faecalibacterium* and *Prevotella*. The positive relationship between sociability and the abundance of *Faecalibacterium* is particularly interesting since this genus has potent anti-inflammatory properties (Qiu *et al.*, 2013; Quévrain *et al.*, 2015) and is often associated with good health (Miquel *et al.*, 2013). Indeed, its abundance is typically reduced in illnesses such as inflammatory bowel disease (Sokol *et al.*, 2008; Martín *et al.*, 2015), chronic fatigue syndrome (Giloteaux *et al.*, 2016), depression (Jiang *et al.*, 2015) and bipolar disorder (Evans *et al.*, 2017). This genus is only known to contain one species, *F. prausnitzii*, which is one of the most abundant bacterial species in the human gut microbiome (Chapter 3; Walker *et al.*, 2011; Lopez-Siles *et al.*, 2012), accounting for approximately 5% of the total bacterial population (Hold *et al.*, 2003). It is a prolific producer of the short-chain fatty acid butyrate (Louis & Flint, 2009) which has beneficial effects on the immune system and can also cross the blood–brain barrier and influence brain development and behaviour (Rogers *et al.*, 2016; Stilling *et al.*, 2016a). In addition, a higher abundance of *Prevotella* in young macaques has been associated with an increased number of regulatory T cells, which are important for host defence against pathogens (Ardeshir *et al.*, 2014). Indeed, *Prevotella* can produce the anti-inflammatory short-chain fatty acid propionate (Cosorich *et al.*, 2017) and this genus has been shown to have beneficial anti-inflammatory effects in animal models of disease (Marietta *et al.*, 2016; Mangalam *et al.*, 2017). In fact, many of the genera targeted in these regression models due to their associations with sociability or autistic traits are important producers of short-chain fatty acids that can affect host physiology, including the brain (Koh *et al.*, 2016).

The finding in this study that engagement in social interactions is positively related to the abundances of gut microorganisms with beneficial immunological functions is pertinent to the well-known association between social relationships and health, which is postulated to be mediated at least in part by the immune system (Holt-Lunstad *et al.*, 2010; Yang *et al.*, 2016b). In fact, recent research which manipulated the social status of female macaques found that a lower rank and reduced engagement in grooming interactions resulted in a heightened inflammatory response (Snyder-Mackler *et al.*, 2016). Given the important anti-inflammatory effects of certain members of the microbiota such as *Faecalibacterium*, the gut microbial

community may play a role in mediating the relationship between social integration and health via its regulation of the host immune response. It would be informative therefore for future research to assess inflammatory markers in this study population and test whether immune status is related to the abundances of these bacterial genera that are associated with host social behaviour. Indeed, examining the potential links between the microbiome, health and sociality has been identified as an important research direction (Nunn *et al.*, 2015) and the findings here provide some insight into these interrelations.

In terms of the diversity analyses, none of the variables significantly predicted alpha diversity of the gut microbiome. This contrasts some previous findings that gut microbiome diversity is positively related to sociability in primate social groups (Li *et al.*, 2016a; Moeller *et al.*, 2016; Billiet *et al.*, 2017; Perofsky *et al.*, 2017) and also to social network size in humans (Chapter 3). Perhaps this result reflects the limited sample size, although other primate studies have likewise reported no relationship between sociability and diversity (Bennett *et al.*, 2016), or even a negative relationship (Raulo *et al.*, 2017). Previous research in primates has also found no effect of age or sex on gut microbiome composition (Raulo *et al.*, 2017) or diversity (Bridgewater *et al.*, 2017), while studies that have found an effect typically report small effect sizes (Amato *et al.*, 2014; Tung *et al.*, 2015; Bennett *et al.*, 2016). Although microbiome diversity has been linked to seasonal differences in primates (Sun *et al.*, 2016; Raulo *et al.*, 2017; Springer *et al.*, 2017; Trosvik *et al.*, 2018) and Hadza hunter-gatherers (Schnorr *et al.*, 2014), there was no evidence that season significantly influenced diversity in this macaque population.

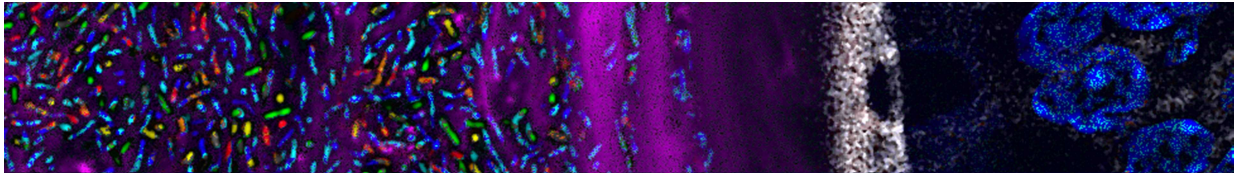
In contrast to alpha diversity, social relationships were found to predict beta diversity of the gut microbiome in this population. Specifically, the amount of grooming an individual received was a significant factor influencing the overall composition of their gut microbial community. This is consistent with previous research showing that social interactions such as grooming can promote gut microbiome similarity within social groups (Tung *et al.*, 2015; Moeller *et al.*, 2016; Raulo *et al.*, 2017). Rhesus macaques are not coprophagic and so grooming is likely the primary method of microbial transmission between individuals through hand-to-mouth actions. The results revealed that the amount of grooming an individual received can explain approximately

5% of the variation in gut microbiome composition between individuals, which is not negligible given the many different factors that can influence the microbiome. However, this result was only significant using weighted, and not unweighted, UniFrac distances. Previous research has also shown that beta diversity analyses on weighted versus unweighted UniFrac distances can yield significantly different results (Lozupone *et al.*, 2007). In particular, weighted UniFrac is effective at detecting differences in community composition that are due to differential microbial abundance, rather than merely the presence or absence of certain taxa as measured with unweighted UniFrac (Navas-Molina *et al.*, 2013). This therefore suggests that grooming may have a greater effect on the abundances of particular microbial taxa, rather than which taxa are present in the gut. I also found evidence that season and age can significantly influence the composition of the microbial community, in accordance with previous research (Sun *et al.*, 2016; Springer *et al.*, 2017; Hicks *et al.*, 2018; Trosvik *et al.*, 2018). Notably, there was no effect of dominance rank on gut microbiome composition or diversity. This is somewhat surprising as social status is known to impact primate health and immunity (Sapolsky, 2005; Archie *et al.*, 2012; Tung & Barreiro, 2012; Snyder-Mackler *et al.*, 2016) and so may also be expected to influence the host's microbiome. This suggests that an individual's engagement in social interactions may be more important than social rank in shaping its gut microbial community and adds to current evidence that social contact represents an important route for microbial transmission.

To date, research on the gut microbial communities of primate populations has focused on social transmission of the microbiota, revealing the role of social organization and interactions in shaping the gut microbiota of individuals living in social groups. Like parasites, microorganisms can be transmitted via social contact and can also affect host behaviour, both of which are likely to occur simultaneously (Ezenwa *et al.*, 2016). This reciprocal interaction between host behaviour and the gut microbiome presents a challenging case to disentangle cause from effect. While experiments with laboratory animals are useful for manipulating the gut microbiome to examine the causal relationships involved (Chapter 2), the very hygienic nature of laboratory environments has implications for host physiology, most notably a weakened immune system

(Beura *et al.*, 2016; Abolins *et al.*, 2017; Rosshart *et al.*, 2017). Thus, one benefit of research in non-captive animal populations is that it represents a more ecologically valid system for understanding how behaviour might be influenced by the microbiome and vice versa (Allen *et al.*, 2017).

This work adds to the growing research at the interface of microbial ecology and animal behaviour (Archie & Theis, 2011; Archie & Tung, 2015) but also extends it in a novel direction. This is the first study to investigate whether microbial taxa previously associated with sociability or autistic traits are differentially abundant in an animal population with respect to individual variation in social behaviour. Given the demonstrated effects of the gut microbiome on rodent behaviour, it is certainly plausible, if not expected, that the gut microbiome may influence behaviour of other mammalian species, with implications for behavioural ecology and evolution (Amato, 2016). However, further research is required to determine the causal relationships between the gut microbiome and social behaviour in non-captive animal populations. In fact, since probiotic supplementation has been shown to increase sociability in rodent models (Buffington *et al.*, 2016; Bharwani *et al.*, 2017), it would be interesting to test whether probiotics increase social motivation and engagement in grooming interactions in the macaque population. My findings represent the initial step towards understanding how gut microbial communities may affect animal behaviour and reveal that social relationships may be intertwined with health via the microbiota.



Chapter 5

Macroevolutionary relationship between encephalization and the mammalian gut microbiome

5.1 Abstract

Encephalization trends vary across mammals, with an increase in relative brain size associated with sociality. While having a larger brain brings clear advantages, it also increases energy requirements since brains are very metabolically demanding. The ‘expensive tissue’ hypothesis proposes that other energetically expensive organs, such as the gut, shrank in size to compensate for the cost of encephalization. However, this theory has garnered limited support and so here I put forward and test the hypothesis that rather than a reduction in gut size, a change in composition of the microbial community within the gut may have helped to meet the increased energetic demands of a larger brain. Recent research has demonstrated that the gut microbiome plays a key role in host metabolism. A common finding from animal and human studies is that a greater abundance of the gut bacterial phylum Firmicutes relative to the phylum Bacteroidetes is associated with increased energy harvest and adiposity. I therefore predicted that mammals with a greater brain size relative to body size may have a lower Bacteroidetes to Firmicutes ratio, reflecting a gut microbiome more efficient at extracting energy from the host’s diet. On the contrary, I find that the ratio of Bacteroidetes to Firmicutes positively predicts encephalization across mammalian taxa. This result does however support the previously proposed trade-off between relative brain size and mass of adipose depots in mammals. Thus, a change in microbiome composition may have favoured a reduction in adiposity, increasing energy availability for the brain. In addition, since many members of the Bacteroidetes phylum

harbour enzymes for carbohydrate metabolism not encoded within the mammalian genome, the increased abundance of this phylum may have facilitated an increase in brain size by making nutrients more accessible to the host from otherwise indigestible carbohydrates. The gut microbiome may therefore have been an important factor in mammalian encephalization, and ultimately the development of complex cognition and social behaviour.

5.2 Introduction

Mammals tend to have a larger brain-to-body size ratio compared to most other vertebrates (Jerison & Barlow, 1985). An increase in brain size relative to body size is not a general macroevolutionary trend across mammalian taxa but is specifically associated with sociality and group living (Dunbar, 1995; Pérez-Barbería *et al.*, 2007; Shultz & Dunbar, 2010). Since brain tissue is very energetically costly, the benefits of evolving a larger brain must be considerable to surpass such costs (Dunbar, 2009). The 'social brain' hypothesis proposes that an increase in relative brain size, particularly neocortex size, is an adaptation to a more complex social environment involving cooperative and deceptive behaviours (Dunbar, 1998; Dunbar & Shultz, 2007a). Relative brain size correlates with various indices of social complexity including group size (Dunbar, 1992), grooming clique size (Kudo & Dunbar, 2001), the prevalence of social play (Lewis, 2001) and the frequency of coalitions (Dunbar & Shultz, 2007a), deception (Byrne & Corp, 2004) and social learning (Reader & Laland, 2002).

Although the 'social brain' hypothesis was originally put forward to explain primate encephalization, sociality has since generally been associated with relative brain size in a range of mammalian taxa, suggesting that living in social groups may play an important role in the evolution of large brains in mammals more generally (Gordon & Pérez-Barbería, 2005; Shultz & Dunbar, 2006, 2010; Pérez-Barbería *et al.*, 2007; Fox *et al.*, 2017). For example, relative brain size is greater in species that exhibit social monogamy, and this relationship holds not only across four orders of mammals but also in birds (Dunbar & Shultz, 2007b). However, other studies suggest that brain size evolution may be better explained by ecological factors (González-Forero & Gardner, 2018) such as a switch to frugivory (DeCasien *et al.*, 2017). In

fact, the 'social brain' hypothesis can also be considered an ecological hypothesis since it proposes that social groups can more effectively solve ecological problems (Dunbar & Shultz, 2007a). In reality, it is likely that both social and ecological factors played a role in mammalian brain evolution. However, path analysis suggests that they may have played different roles, with sociality being the functional cause of brain size evolution, while ecological factors such as diet acted as constraints. Specifically, path analysis based on data from primate species, combining both social and ecological variables, indicates that living in social groups was the key selective pressure for a larger neocortex size (Dunbar, 2009), which in turn required improvements in dietary quality for increased energy extraction (Dunbar & Shultz, 2007a).

An increase in brain size relative to body size represents a metabolically expensive adaptation (Mink *et al.*, 1981; Karbowski, 2007). For example, the human brain accounts for only 2% of total body mass but uses around 20% of the body's energy (Raichle, 2006). In addition, other organs in mammals including the heart, liver and intestines also require considerable energy resources (Roth & Dicke, 2005). A long-standing hypothesis in anthropology is the 'expensive tissue' hypothesis which proposes that the energy demand of our disproportionately large brain was compensated for by a reduction in other energetically expensive parts of the body such as the gut (Aiello & Wheeler, 1995). Although this hypothesis was originally put forward to explain human encephalization, subsequent studies have tested the idea of a gut–brain trade-off in other taxa with mixed results (Isler & van Schaik, 2006; Warren & Iglesias, 2012; Kotrschal *et al.*, 2013; Tsuboi *et al.*, 2015; Liao *et al.*, 2016). Moreover, a re-analysis of the original anthropoid dataset in light of current phylogenetic methods and using more accurate brain and body measurements did not find support for this trade-off (Navarrete *et al.*, 2011).

Given the numerous challenges to the 'expensive tissue' hypothesis, perhaps it is not so much a reduction in gut size that might enable the host to meet these increased energy requirements but rather changes in the gut's microbial ecosystem. Indeed, there is growing evidence that the gut microbiome plays a key role in host metabolism and digestion (Brestoff & Artis, 2013; Sonnenburg & Bäckhed, 2016). It is interesting to consider, therefore, whether changes in the composition of the gut microbiome and consequent alterations in its metabolic properties may

have helped to fulfil the energetic demands of a larger brain by improving digestive efficiency and the host's ability to extract energy from its diet.

The gut microbial community breaks down dietary components undigested by the host into absorbable nutrients and can thereby influence the host's energy balance (Postler & Ghosh, 2017). In fact, germ-free rats require 30% more calories to maintain body weight (Wostmann *et al.*, 1983). Furthermore, when germ-free mice are colonized with the gut microbiota from conventionally raised animals, they show a 60% increase in body fat despite reduced food intake (Ding *et al.*, 2004). These germ-free studies highlight the role of the gut microbiome in energy extraction and adipose deposition. Indeed, microbial metabolism produces simplified carbohydrates, amino acids and vitamins that can be used by the host (Rowland *et al.*, 2018). Since microbial taxa vary in their ability to extract energy from nutrients, here I hypothesize that encephalization is associated with a more efficient gut microbial community, which may help to meet the increased energy requirements of a larger brain. In fact, evidence suggests that the gut microbiome contributes to phenotypic plasticity of the host, and thus individuals with a more favourable microbiome may undergo positive selection (Alberdi *et al.*, 2016). From an evolutionary perspective, alterations in the gut microbial community may therefore provide a faster route for host adaptation to selective pressures.

Two major bacterial phyla that dominate the gut microbiota of mammals are Bacteroidetes and Firmicutes (Eckburg *et al.*, 2005; Ley *et al.*, 2008). Numerous studies have linked an enrichment of Firmicutes over Bacteroidetes to obesity and increased energy harvest by the gut microbiome in humans (Ley *et al.*, 2006a; Turnbaugh *et al.*, 2009; Koliada *et al.*, 2017), rodents (Turnbaugh *et al.*, 2005, 2006; Fulton *et al.*, 2008) and domesticated animals (Guo *et al.*, 2008; Hou *et al.*, 2016; Yang *et al.*, 2016a). For example, in a mouse model of obesity, a higher abundance of Firmicutes relative to Bacteroidetes, and thus a reduction in the ratio of Bacteroidetes to Firmicutes (B:F ratio), is associated with more efficient energy extraction from the diet (Turnbaugh *et al.*, 2006). This improved capacity for energy harvest can be transferred to germ-free mice on receiving a faecal microbiota transplant from an obese mouse, resulting in an increase in body fat (Turnbaugh *et al.*, 2006). There has also been a case report in humans

documenting the onset of obesity following receipt of a faecal microbiota transplant from an overweight donor (Campbell *et al.*, 2015). These transplant studies therefore indicate that the gut microbial community contributes to weight gain and adiposity. Notably, a negative relationship between B:F ratio and body mass index is not always found in human studies, perhaps due to methodological variation such as different clinical criteria, population sizes and microbiome profiling techniques (Tremaroli, 2012). However, in support of the association between B:F ratio and obesity, a study in humans found that a 20% increase in the proportion of Firmicutes (and concomitant decrease in Bacteroidetes) in the gut microbiome was associated with an increased energy yield of approximately 150 kcal, whereas a 20% increase in Bacteroidetes was linked to a reduction in energy by 150 kcal (Jumpertz *et al.*, 2011). Consistent with this, the Hadza hunter-gatherers have a higher B:F ratio (Schnorr *et al.*, 2014) and also a lower percent body fat and lower body mass index compared to both farming and western populations (Pontzer *et al.*, 2012).

Further evidence in support of this relationship between B:F ratio and energy extraction comes from the finding in human populations that latitude is positively correlated with Firmicutes abundance and negatively correlated with Bacteroidetes abundance (Suzuki & Worobey, 2014). This is in agreement with the hypothesis that an increased ability to extract energy from nutrients represents an adaptation to cooler climes (Suzuki & Worobey, 2014). Gut microbiome composition also differs between hibernation and active periods in mammals, with a lower B:F ratio typically observed during the active summer months (Carey *et al.*, 2013; Stevenson *et al.*, 2014; Sommer *et al.*, 2016). For example, brown bears have a lower B:F ratio in the summer when increased energy extraction from the diet is important to build up energy reserves for winter hibernation (Sommer *et al.*, 2016). Transplanting the summer gut microbiota from the bear into germ-free mice promotes adiposity compared to transplanting the gut microbiota from hibernating bears (Sommer *et al.*, 2016). In contrast, a loss of weight and body fat during bear hibernation is associated with a higher B:F ratio (Sommer *et al.*, 2016), as is weight loss in humans (Ley *et al.*, 2006a; Remely *et al.*, 2015). These findings are therefore consistent with

the theory that differences in gut microbiome composition, and specifically the B:F ratio, are associated with variation in energy extraction in mammals (Suzuki, 2017).

Given the energy trade-offs implicated in encephalization and the role of the gut microbiome in energy extraction, a plausible hypothesis is that a change in the composition of the gut microbial community helped to increase the energy available for brain expansion. Metagenomic analysis provides the opportunity to explore this energy trade-off hypothesis at the microbial level. In light of the evidence from rodent models, human populations and wild animals that the B:F ratio may be used as an estimate of the energy harvest capacity of the microbiome, I predicted that a greater relative abundance of Firmicutes and lesser relative abundance of Bacteroidetes may be associated with encephalization.

5.3 Materials and methods

5.3.1 Datasets

Data for these analyses were obtained from published datasets on mammalian encephalization (Shultz & Dunbar, 2010); microbiome composition, together with fibre intake (Ley *et al.*, 2008); and microbiome diversity (Muegge *et al.*, 2011). The encephalization slope, representing the rate at which brain size changes against body size across evolutionary time, had been measured at the suborder level, using phylogenetic generalized least squares analysis to account for phylogeny. For a range of 60 mammalian species (Table A.17), data on gut microbiome composition (specifically the relative abundances of the bacterial phyla Bacteroidetes and Firmicutes) were summarized at the suborder level so that they could be collated with the encephalization data (Table A.18). Dietary fibre index (available for 51 of these species) was also taken into account since this is known to influence gut microbiome composition, particularly by increasing the B:F ratio (de Filippo *et al.*, 2010; de Vadder *et al.*, 2014; Trompette *et al.*, 2014). This fibre index was calculated from the percentage acid detergent fibre (a measure of hemicellulose) and neutral detergent fibre (a measure of cell wall content) in the animal's diet (Ley *et al.*, 2008). For a separate analysis relating to gut microbiome diversity at the species

level, data on both Shannon's diversity index and fibre intake were available for 30 mammalian species (Table A.19).

5.3.2 Statistical analyses

All statistical analyses were performed using R 3.2.3 software (R Development Core Team, 2015). The main relationship assessed was between B:F ratio and encephalization slope, modelled using linear regression, with B:F ratio as the independent variable. Linear regression was also conducted to investigate the relationship between fibre intake and B:F ratio since studies in both humans and animal models have shown that fibre can influence this ratio. These regression models were also repeated with relative abundance of Bacteroidetes instead of B:F ratio, and also with relative abundance of Firmicutes. All these analyses were performed at the suborder level ($n = 7$), ensuring residuals were normally distributed (Shapiro–Wilk test, $P > 0.05$) and met the assumption of homoscedasticity (non-constant variance test, $P > 0.05$).

The relationship between fibre intake and gut microbiome diversity was examined at the species level ($n = 30$) using non-linear regression, since transformation of the data was not sufficient to meet the assumptions of linear regression. The hyperbolic function $y = \frac{ax}{b + x}$ was fitted to the data. A likelihood-ratio test was conducted to determine whether this hyperbolic function fitted the data better than a null model containing only the intercept term.

5.4 Results

Linear regression analyses revealed a significant relationship between microbiome composition and encephalization among mammalian suborders (Table A.20). Specifically, encephalization was positively predicted by B:F ratio ($P = 0.010$; Figure 5.1) and also by the relative abundance of Bacteroidetes ($P = 0.030$). However, there was no relationship between the relative abundance of Firmicutes and encephalization ($P = 0.218$). In terms of fibre intake, this was not significantly related to B:F ratio nor relative abundance of either phylum (Table A.20). However, plotting the data indicated that there may be a more nuanced relationship between fibre intake and microbiome composition, suggesting that a more fibrous diet may be positively related to

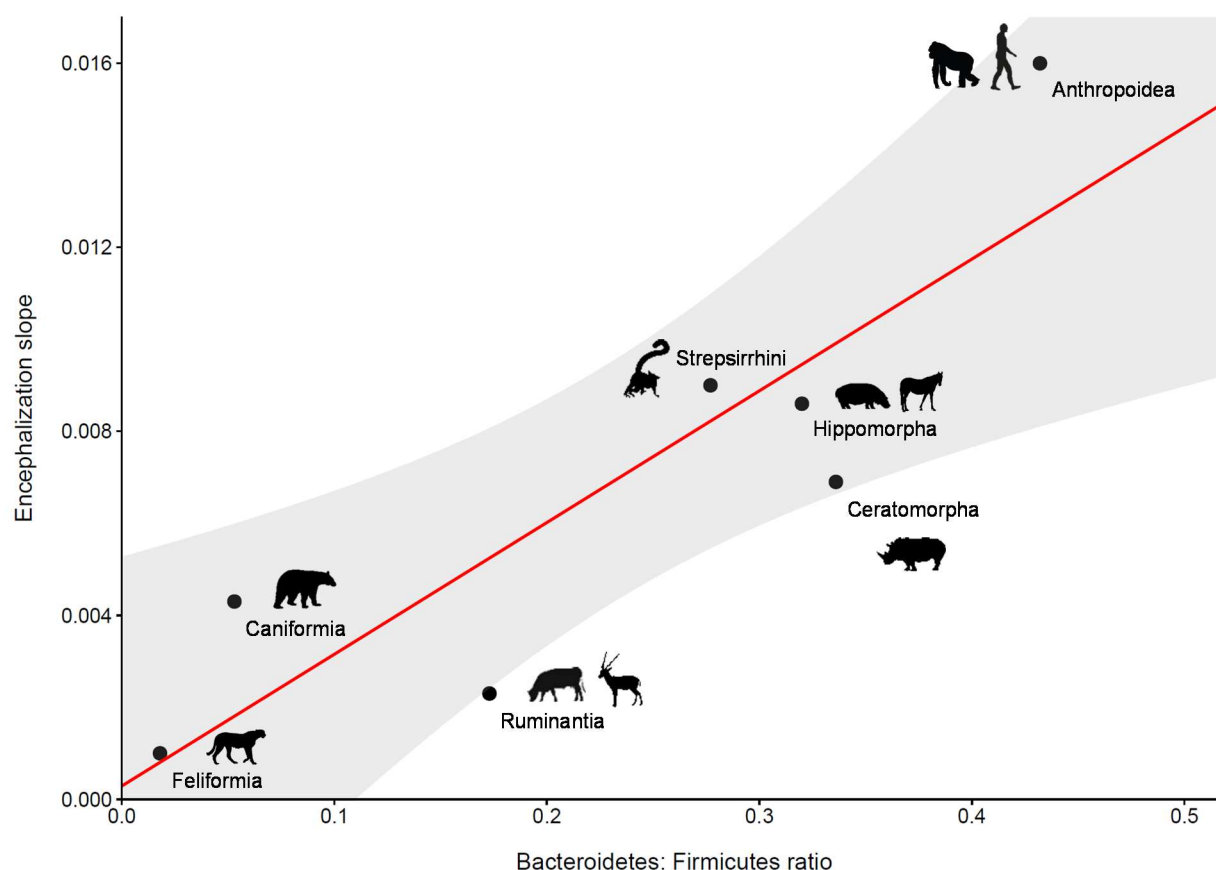


Figure 5.1. Positive relationship between Bacteroidetes: Firmicutes ratio and encephalization slope across mammalian suborders. Shaded region denotes 95% confidence interval for the regression line.

B:F ratio across mammalian suborders, with the exception of primates (Figure 5.2). Notably, there was no evidence of a relationship between fibre intake and encephalization (Table A.20), indicating that the positive relationship between B:F ratio and encephalization is not driven by differences in dietary fibre.

The non-linear regression between fibre intake and microbiome diversity (Figure 5.3; Table A.21) predicted that Shannon's diversity index asymptotes at a value of 7.1 (95% confidence interval = 6.3 to 7.9). A likelihood-ratio test confirmed that this non-linear model fitted the data significantly better than the null model ($\chi^2_{(1)} = 14.265$, $P < 0.001$).

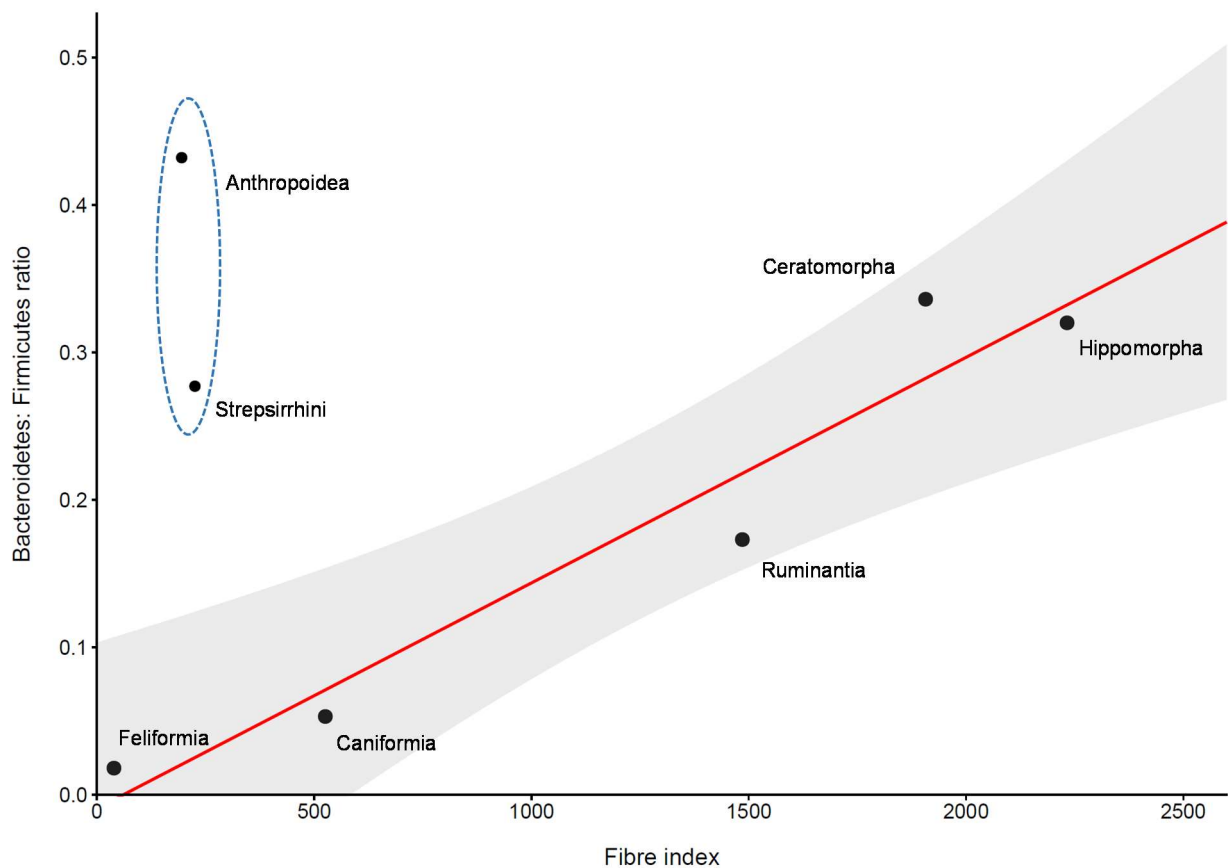


Figure 5.2. Relationship between fibre intake and Bacteroidetes: Firmicutes ratio across mammalian suborders. Oval marks the two primate suborders as potential outliers. Shaded region denotes 95% confidence interval for the regression line if the two primate suborders are excluded from the model.

5.5 Discussion

I expected that there may be a negative relationship between the ratio of Bacteroidetes to Firmicutes and mammalian encephalization, reflecting an increased capacity of the gut microbiome to extract energy from the diet. In fact, the relationship was highly significant in the opposite direction, such that B:F ratio positively predicted encephalization. Notably, a lower abundance of Bacteroidetes relative to Firmicutes is linked to increased energy harvest and adiposity in animals, including humans, although the mechanisms mediating this association remain unexplained (Turnbaugh *et al.*, 2006).

This positive relationship between B:F ratio and encephalization is, however, in agreement with the proposed fat–brain trade-off in mammals, based on a study across 100 species (Navarrete

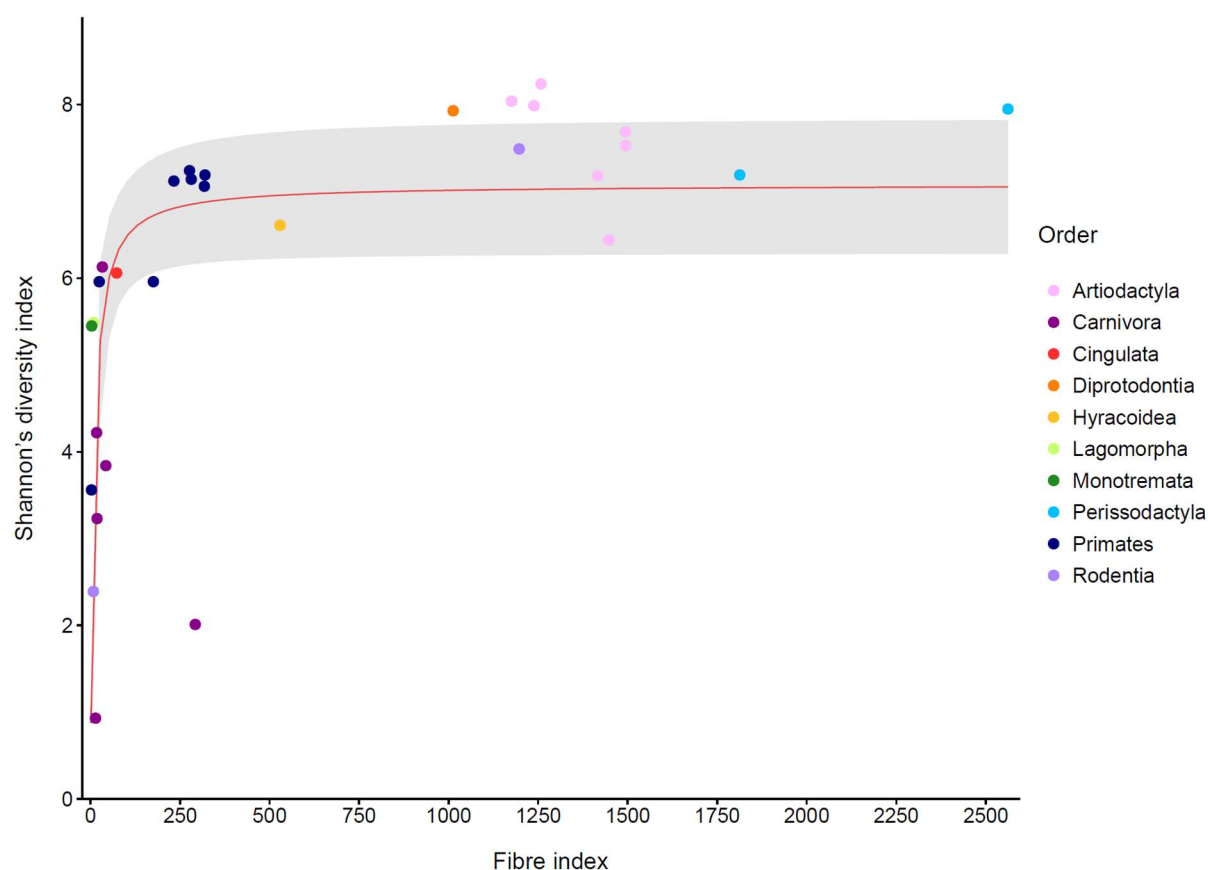


Figure 5.3. Asymptotic relationship between fibre intake and gut microbiome diversity for a range of mammalian species. Shaded region denotes 95% confidence interval for this non-linear function.

et al., 2011). The study measured brain size, the masses of other energetically expensive organs and adipose depots, and found that the strongest trade-off was between encephalization and adiposity. The authors suggest that this fat–brain trade-off may reflect alternative strategies to deal with the risk of starvation, providing either a cognitive or physiological buffer. Although fat deposits are not metabolically expensive *per se*, the energetic cost associated with adiposity derives from the additional energy required to carry around an increased body mass (Navarrete *et al.*, 2011). As opposed to the ‘expensive tissue’ hypothesis which was proposed to explain the evolution of human brain size, this fat–brain trade-off was found to operate among mammals in general (Navarrete *et al.*, 2011). My analysis suggests that this trade-off between brain size and adiposity may also be reflected at the level of the gut microbiome, since B:F ratio positively predicts degree of encephalization in mammals. These results therefore support the hypothesis that evolution favoured lean mammals with a large brain.

While an increase in B:F ratio may be linked to reduced adiposity and thus increased energy allocation to brain growth, the finding that the relative abundance of Bacteroidetes (irrespective of Firmicutes abundance) also significantly predicted encephalization is worthy of discussion. Most notably, members of the phylum Bacteroidetes possess an extensive repertoire of enzymes, not encoded by the mammalian genome, that are capable of breaking down complex plant polysaccharides (Flint *et al.*, 2012). In fact, polysaccharides are the most abundant biological polymer (Wexler, 2007) and therefore represent a key energy source, yet the majority cannot be degraded by the host (Flint *et al.*, 2008). Metagenomic analysis of fibrolytic bacteria from Bacteroidetes and Firmicutes has revealed that members of Bacteroidetes have a greater number of enzymes for degrading polysaccharides (Flint *et al.*, 2012). In particular, bacteria from the *Bacteroides* genus (within the Bacteroidetes phylum) have been found to efficiently degrade a wide range of polysaccharides (Xu & Gordon, 2003; Xu *et al.*, 2003; Sonnenburg *et al.*, 2005; Ley *et al.*, 2006b; Martens *et al.*, 2011; Flint *et al.*, 2012; Larsbrink *et al.*, 2014). Since the amount of dietary fibre that can be metabolized by the host is influenced by the composition of its gut microbiome (Sonnenburg & Bäckhed, 2016), an increased abundance of Bacteroidetes may therefore signify a gut microbial community more efficient at extracting energy from otherwise indigestible fibre. In addition, when members of the Bacteroidetes phylum break down fibre, they produce the short-chain fatty acids acetate and propionate (Macfarlane & Macfarlane, 2003; de Vadder *et al.*, 2014; Koh *et al.*, 2016). These short-chain fatty acids can then be absorbed by the large intestine and used for energy (Hooper *et al.*, 2002); propionate in particular is an important substrate for gluconeogenesis (Wong *et al.*, 2006). In fact, short-chain fatty acids provide a significant proportion of the host's energy needs and in humans account for at least 10% of calories absorbed from the diet (Hooper *et al.*, 2002).

However, it may not be purely energetics that limits brain size (Gibbons, 1998), but rather certain nutrients or metabolites that are important for brain growth and function. The relationship reported here between gut microbiome composition and encephalization may therefore reflect a more direct interaction between the gut microbiome and the brain since the microbiome is known to affect multiple aspects of brain development and function (Sampson & Mazmanian,

2015). Indeed, microorganisms produce a diverse range of metabolites, including short-chain fatty acids that can have direct effects on the brain (Bourassa *et al.*, 2015; Stilling *et al.*, 2016a). Gut bacteria can also produce neurotransmitters (Wall *et al.*, 2014; Strandwitz, 2018) and modulate host neurotransmitter levels in the gut lumen (Asano *et al.*, 2012; Yano *et al.*, 2015; Hata *et al.*, 2017).

Interestingly, several recent human studies have linked Bacteroidetes abundance to cognitive function and brain structure. For example, relative abundance of Bacteroidetes and B:F ratio were both shown to be positively correlated with cognitive performance in adult females, independent of percent body fat or dietary fibre intake (Taylor *et al.*, 2017). Another study in healthy women found that those with a higher abundance of the genus *Bacteroides*, the most abundant genus of the human gut microbiota (Chapter 3; The Human Microbiome Project Consortium, 2012; Faith *et al.*, 2013), had a larger cerebellum and hippocampus and a greater cortical thickness of the frontomarginal gyrus and anterior insula (Tillisch *et al.*, 2017). Research on females with irritable bowel syndrome also reported that those with higher levels of Bacteroidetes tended to have thicker grey matter in the frontal cortex and insula, two key brain regions involved in processing complex information, whereas Firmicutes abundance was negatively related to grey matter volume of these brain regions (Labus *et al.*, 2017). These observations do suggest that there may be a selective advantage to having a higher abundance of members of Bacteroidetes. However, whether Bacteroidetes plays a causal role in this relationship with brain structure and function is yet to be determined. Notably, a recent transcriptomic analysis of faecal samples found that bacterial genes involved in producing the neurotransmitter GABA are encoded by members of the *Bacteroides* genus (Terekhova *et al.*, 2018). Furthermore, bioinformatics analysis of the human microbiome has revealed that Bacteroidetes is the main phylum encoding glutamate decarboxylase enzymes which synthesize GABA from glutamate (Yunes *et al.*, 2016). Since lower levels of GABA in the frontal cortex have been associated with poorer cognition in healthy adults (Porges *et al.*, 2017), this may be one possible explanation for the link between Bacteroidetes abundance and cognition, though further research would be required to test this hypothesis. Indeed, this relies on the assumption

that GABA produced in the gut can have a measurable impact on the brain, which remains unclear.

While the regression analysis found no evidence that fibre intake predicts B:F ratio, visualization of the data (Figure 5.2) suggests that fibre may be positively related to B:F ratio among mammalian suborders with the exception of primates. This observation suggests that primates are somehow able to maintain a high B:F ratio despite their comparatively low fibre intake. This may partly be due to their diet since many primate species are frugivores (dependent on seasonal availability) and a study in a wild population of frugi-folivorous lemurs found that fruit consumption was positively correlated with relative abundance of Bacteroidetes and negatively correlated with relative abundance of Firmicutes (Springer *et al.*, 2017). Similarly, capuchin monkeys show a reduced abundance of Bacteroidetes in the gut during seasonal declines in fruit availability (Orkin *et al.*, 2018). Although the data indicate that there may be a positive relationship between fibre intake and B:F ratio among mammalian suborders (not including primates), data for additional mammalian taxa would need to be collected to confirm any such relationship. Notably, a positive relationship between fibre intake and B:F ratio (or relative abundance of Bacteroidetes) has been reported in intraspecific studies in humans and animal models (de Filippo *et al.*, 2010; de Vadder *et al.*, 2014; Trompette *et al.*, 2014; Sonnenburg *et al.*, 2016) and so it may be expected that this relationship also holds at a macroevolutionary level across mammalian taxa.

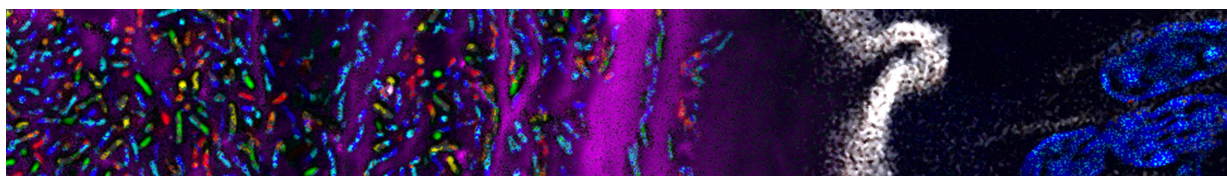
The non-linear positive relationship between fibre intake and gut microbiome diversity across mammalian species is a new finding since previous research has only shown that fibre intake promotes diversity within a species. For example, mice fed a diet lacking in fibre over generations show irreversible loss of gut microbiome diversity (Sonnenburg *et al.*, 2016) and dietary fibre in humans is associated with a more diverse gut microbiome (Chapter 3; de Filippo *et al.*, 2010). The asymptotic relationship reported here between fibre and microbiome diversity in mammals suggests that there is a level of fibre intake beyond which diversity no longer increases. Interestingly, the graph approaches maximum diversity around a fibre index of 250 which is similar to the dietary fibre intake of primates. While previous research has simply shown

that the gut microbiome of herbivores has the highest phylum-level and genus-level richness, followed by omnivores and then carnivores (Ley *et al.*, 2008), my analysis adds to this observation by revealing that an asymptotic relationship between fibre and gut microbiome diversity operates across mammalian taxa.

A limitation of this study is that the causal factors mediating the relationship between gut microbiome composition and mammalian encephalization cannot be determined. Indeed, it may be that changes in the gut microbiome are correlated with some other aspect of mammalian physiology. However, as discussed, it is plausible that alterations in the composition of the gut microbiome may have facilitated brain size evolution. For example, the reduction in adiposity associated with an increased B:F ratio may have allowed more energy to be allocated to brain growth. An alternative explanation is that an increased abundance of Bacteroidetes may have helped to meet the energetic demands associated with encephalization through improving energy extraction from indigestible fibre. It should also be noted that the regression analysis of encephalization against microbiome composition combines two datasets, and while the mammalian species in each dataset are not identical, there is a good degree of overlap between the two. The implicit assumption, therefore, is that variation between species within a suborder is smaller than the differences between suborders. A further consideration is that the gut microbiome data for the majority of mammals included in this research were from animals in captivity (Table A.17). However, several studies have reported differences in gut microbiome composition between wild and zoo animals (Schwab *et al.*, 2009; Nakamura *et al.*, 2011; Nelson *et al.*, 2013; Delsuc *et al.*, 2014; Clayton *et al.*, 2016), which may be due to different diets and environmental conditions.

In conclusion, these analyses of gut microbiome composition in a range of mammalian taxa support my hypothesis that encephalization is associated with differences in the gut microbial community. I propose that such changes are likely to have had consequences for the host's metabolism and energy balance, helping to meet the increased energy demands of sustaining a larger brain. There has so far been limited investigation into the role of the microbiome in evolution, yet this represents an important avenue for future research (Waldor *et al.*, 2015).

Most research on the gut microbiome has been concerned with health, either through studying human populations or using animal models. However, investigating the links between the microbiome and trends in mammalian evolution has the potential to provide insights into the relationship between host-associated microbial communities and host development and physiology. My findings point towards a possible role of the gut microbiome in facilitating mammalian, and ultimately human, brain evolution, and along with it the evolution of complex cognition and social behaviour.



Chapter 6

Discussion

This concluding chapter integrates my key findings and their significance. I also consider the general limitations of research on the microbiome–gut–brain axis and how I see the field progressing in the future to advance our understanding of the relationship between the gut microbiome and behaviour. I conclude with a personal perspective on my research and its contribution.

6.1 Key findings and significance

6.1.1 Chapter 2 | Neuropeptide pathways

By manipulating the gut microbial community using antibiotic treatment or germ-free conditions, these experiments provide causal evidence that the microbiome affects the expression of neuropeptide systems underlying mammalian social and emotional behaviour. In particular, the marked reduction in gene expression of the μ -opioid and oxytocin receptors (and a trend towards downregulation of the vasopressin receptor) in the frontal cortex of antibiotic-treated mice may partly account for their reported social deficits, especially given the important role of the frontal cortex in social behaviour.

This is the first study to show that the microbiome interacts with the brain's opioid system. The evidence of reduced opioid signalling in antibiotic-treated mice is particularly pertinent given the role of the endogenous opioid system in modulating social motivation and behaviour. Since the opioid system is also a key regulator of pain, this demonstrated interaction between the gut

microbiome and opioid signalling may be relevant to the aetiology of pain disorders that have been associated with the microbiome such as irritable bowel syndrome. These findings also have potential implications for medical practice, revealing the adverse effects of antibiotics during early adolescence on neuropeptide signalling. This may potentially increase the risk of developing neuropsychiatric disorders in later life, though more research is necessary to determine whether such neurobiological changes during development may be reversible.

In addition, the downregulation of the gene encoding the immune system protein MyD88 in the brain of both antibiotic-treated and germ-free mice is an interesting finding to investigate further. Given the role of this protein in mediating communication between the host and its microbiota, this suggests that a MyD88-dependent pathway may be involved in relaying the state of the gut microbial community to the brain.

The notable differences in the effects of antibiotic treatment and germ-free status on these neuropeptide systems represent an important finding relevant to current practices in the field. Antibiotic treatment is sometimes viewed as a more accessible alternative to using germ-free animals, but the results here demonstrate that they represent two distinct manipulations of the gut microbial community, with differing effects on gene expression in the brain.

6.1.2 Chapter 3 | Human personality

I show in a large human cohort that sociability, neuroticism, anxiety and stress are related to differences in the composition and diversity of the gut microbiome. I find that the abundances of numerous bacterial genera previously associated with behaviour in laboratory animals or with psychiatric symptoms in humans, are also related to variation in personality traits in the general population. In particular, it is interesting that multiple genera associated with autism in the literature are significantly related to individual differences in sociability in the neurotypical population in the same direction as is typically found in autistic populations. This indicates that the gut microbiome may contribute not only to the behavioural traits seen in autism but also to variation in social behaviour in the general population. Since this is a cross-sectional study, the direction of causality in the relationship between behaviour and the gut microbiome cannot be

confirmed. However, the results of Chapter 2 do reveal that the gut microbiome can affect the expression of neuropeptide pathways implicated in social and emotional behaviour. Experiments involving fine-scale manipulations of the gut microbial community, specifically with respect to the bacterial genera found to be significantly related to human personality traits in this chapter, may provide a means to determine which of these genera have a causal effect on behaviour. Nonetheless, these findings represent a key step to understanding the relationship between the gut microbiome and human psychology and well-being.

In addition, I report the important finding that human social network size is positively related to gut microbiome diversity. This is the first study in humans providing evidence that sociability is associated with microbiome diversity, suggesting that social interactions may represent a key route for microbial transmission. This has implications for our modern-day living practices, particularly regarding the tendency towards oversanitation in industrialized societies which likely impedes transmission of members of the microbiota and thus may result in reduced diversity of the gut microbiome. This is also the first demonstration in humans that stress is negatively associated with microbiome diversity, in accordance with previous findings in animal models. This is particularly relevant given the increasing stress of modern life and the numerous studies showing the benefit of a diverse microbiome for health.

As well as my primary findings relating to behaviour, I also investigated the relationship between the gut microbiome and diet, health, lifestyle and sociodemographics. I report a number of novel relationships, most notably that adults who were formula-fed as children have a less diverse microbiome and that females tend to have a lower diversity than males. I also show that diversity is positively related to international travel, a more diverse diet and a diet high in natural sources of probiotics and prebiotics.

6.1.3 Chapter 4 | Macaque social network

In this study of a social network in a macaque population, I show that the abundances of multiple bacterial genera previously associated with autism or sociability are also related to individual motivation to engage in social interactions. The most striking, and novel, finding is that several

of the bacterial genera whose abundances were significantly related to sociability also have important immunomodulatory properties. This is particularly pertinent given the established link between social ties and both health and longevity, which is considered to be mediated, at least in part, by the immune system. Since the gut microbiome is a key regulator of the immune response, the gut microbial community may play an important, but so far unrecognized, role in this relationship between social integration and well-being. In this chapter I also find that social interactions through grooming influence gut microbiome composition. This is in accordance with the evidence that gut microorganisms are socially transmitted, deriving both from primate studies and my finding in Chapter 3 that human social network size is associated with gut microbiome diversity.

6.1.4 Chapter 5 | Encephalization

In comparison to the extensive research investigating the role of the gut microbiome in host health and behaviour, this chapter provides a novel perspective on the relationship between the gut microbiome and social behaviour from a macroevolutionary viewpoint. I find evidence to support my hypothesis that a change in the gut microbial community during mammalian evolution may have facilitated encephalization by helping to meet the increased energy demands of a larger brain through improved energy extraction from the diet. In light of the ‘social brain’ hypothesis and the evidence that trends in encephalization are associated with sociality, this finding suggests that the gut microbiome may not only influence individual social behaviour but may also have played a role in the evolution of the social brain and thus the development of complex social behaviour in mammals.

The positive relationship between relative abundance of the bacterial phylum Bacteroidetes and relative brain size among mammalian taxa is particularly interesting in view of recent research findings in humans linking a higher Bacteroidetes abundance to better cognitive function and increased volumes of certain brain regions. In light of the proposal that evolved dependence may be integral to the microbiome–gut–brain axis (Johnson & Foster, 2018 [Appendix]), perhaps the mammalian brain has come to depend on members of Bacteroidetes that play a role in brain

growth and function, such that a decrease in their abundance in the human gut microbiome may negatively impact cognition. Thus by examining how the gut microbial community has changed over evolutionary time, this may offer a route towards identifying members of the gut microbiota that have been favoured by natural selection during mammalian evolution.

6.1.5 Appendix Chapter | Evolutionary theory

The key outcome of this chapter is the proposal of an evolutionary framework for studying the microbiome–gut–brain axis, by considering the selection pressures acting on the microbiota and the host that may have led to the gut microbiome affecting the brain and behaviour. Through reviewing recent empirical data from the field, combined with the application of evolutionary theory, there is evidence that the conditions required to favour manipulation of host behaviour by the gut microbiota appear rarely satisfied. Most notably, the diversity of this microbial ecosystem is likely to preclude the evolution of bacterial strains that can manipulate host behaviour for their own fitness benefit. This represents an important insight, countering recent suggestions in the field that the observed effects of gut microorganisms on behaviour, such as increased sociability, may be due to manipulation of the host to enhance their own transmission.

While I find that the manipulation hypothesis is poorly supported, the behavioural effects of members of the microbiota can be most simply explained by natural selection on microorganisms to grow and divide in the gut and natural selection on their hosts to depend on their microbiota for particular functions. Since metabolites generated as a result of microbial growth can influence the host's physiology, the host may evolve to depend on, monitor and regulate their gut symbionts. A key corollary, therefore, is that there should be greater focus on the host as we seek to understand the effects of the gut microbiome on the brain and behaviour. Indeed, if we have evolved to depend on members of the microbiota that have a measurable impact on our neurobiology, then their absence may alter brain function and behaviour and affect the risk of neuropsychiatric conditions. This concept of evolved dependence has not previously been considered in relation to the microbiome–gut–brain axis but may prove fundamental to our understanding of behavioural effects of gut microorganisms.

6.2 Outlook: microbiome, modern-day lifestyles and mental conditions

The findings of this thesis are especially pertinent in this era of antibiotic overuse, refined diets lacking in fibre, modern hygiene practices which impede microbial transmission and our stressful lifestyles. Indeed, as summarized above, I report that antibiotic administration in young animals can affect the expression of neuropeptide pathways, with possible adverse effects on behaviour and potential implications for the later development of autism or mood disorders. I show that gut microbiome diversity is negatively related to stress and antibiotic treatment but positively related to fibre intake. I also find that social transmission of microorganisms likely plays a role in shaping the human gut microbiome, given the positive association between microbiome diversity and social network size.

While the focus of this thesis is the relationship between the gut microbiome and behaviour, rather than the expression of extreme behavioural traits as seen in neuropsychiatric conditions, my findings do also have clinical relevance. Indeed, since our microbiome lies at the interface between our external environment and our physiology, environmental changes due to modernization may be expected to have consequences for our physical and mental health. It is notable that cases of autism have increased in recent times, particularly around the 1990s, which is unlikely to be wholly due to developments in diagnosis (Taylor *et al.*, 2013). There is also a trend towards increasing prevalence of mood disorders with modernization (Hidaka, 2012). These recent changes suggest that modern-day lifestyle factors may be increasing the likelihood of such neuropsychiatric conditions.

A theory gaining traction in light of microbiome research is the hygiene hypothesis, which is based on the observation that improved hygiene is linked to a rise in autoimmune conditions (Strachan, 1989). This association is thought to be due to a lack of microbial exposure (Rook, 2012) and there is growing evidence from animal models and human studies that early-life exposure to microorganisms is important for immune system development and function (Stiemsma *et al.*, 2015; de Agüero *et al.*, 2016). Indeed, modern lifestyle factors such as antibiotic use, western diets, oversanitation and stress have the potential to reduce microbiome

diversity and the abundances of particular members of the gut microbiota that may play beneficial roles in immune function (Round & Mazmanian, 2009). Notably, the immune system is commonly dysregulated in autism (Hsiao, 2013) and there is evidence that autism may be associated with autoimmunity (Brimberg *et al.*, 2013). The hygiene hypothesis may too be relevant to psychiatric conditions such as depression and anxiety (Rook & Lowry, 2008), in line with increasing evidence of a link between mood disorders and both inflammation (Dantzer *et al.*, 2008) and autoimmunity (Benros *et al.*, 2013). Thus therapies that target the microbiome may represent key advances in treating a range of neuropsychiatric disorders. Discovering new treatments for psychiatric conditions is of pressing concern since they account for a growing proportion of disability in developed countries, yet their aetiologies often remain unknown and current treatments have limited efficacy (Sampson & Mazmanian, 2015).

6.3 Limitations

In addition to the specific limitations previously discussed within each chapter, here I outline those limitations applicable to much of the research currently conducted in the microbiome field.

6.3.1 Sampling the gut microbiome

The gut is a spatially heterogeneous environment, with differences in microbial community composition both along its cross-section and longitudinally (Galley *et al.*, 2014b; Lu *et al.*, 2014; Suzuki & Nachman, 2016). For example, different microbial communities inhabit the gut lumen, mucosal layers and colonic crypts (Donaldson *et al.*, 2015). It may be that microbial communities located at particular sites in the intestine play a dominant role in the microbiome–gut–brain axis but invasive procedures such as enteroscopy would be required to determine the role of site-specific microbiota (Chen *et al.*, 2013). The majority of studies are therefore currently limited to determining microbiome composition from faecal material. However, research has demonstrated that faecal samples can provide a sufficient representation of the gut microbial community (Eckburg *et al.*, 2005).

6.3.2 Assessing the gut microbiome

My analyses in Chapters 3 and 4 focused on the taxonomic classification of the microbiome. It is relevant to note that while the human microbiome shows a high degree of inter-individual variability in terms of its composition, studies suggest that its functional properties are more conserved between individuals (The Human Microbiome Project Consortium, 2012; Zhernakova *et al.*, 2016). Functional redundancy is a common characteristic of microbial ecosystems (Louca *et al.*, 2018), including the gut microbiome (Moya & Ferrer, 2016). In fact, a recent study of patients with inflammatory bowel disease reported that some abundant microorganisms in the gut may be inactive, showing little or no gene expression (Schirmer *et al.*, 2018).

In addition, the function of the microbiome relies not only on the microbial genes expressed, but perhaps more importantly on the proteins and metabolites produced (Moya & Ferrer, 2016). Integration of microbiome compositional data (metagenomics) with analysis of expressed genes (metatranscriptomics), proteins (metaproteomics) and metabolites (metabolomics) will provide a more holistic view of the role of the gut microbiome and its relationship with the brain and behaviour. Indeed, it may be that variation in microbiome function is more strongly related to differences in brain function and behaviour than variation in microbiome composition. Notably, there has also been a recent shift in macroecology towards examining functional traits, rather than simply taxonomic composition, to assess the health and dynamics of an ecosystem, termed functional-trait ecology (Cernansky, 2017).

6.3.3 Microbiome sequencing

While I used 16S rRNA sequencing to determine microbiome composition, in the past few years there has been a rise in whole-genome shotgun sequencing for microbiome analysis. By sampling all the DNA present in the community, rather than just sequencing the 16S rRNA gene, this method enables researchers to infer the functional traits of microorganisms by comparing the genes to databases such as the Kyoto Encyclopedia of Genes and Genomes (Arnold *et al.*, 2016).

16S rRNA sequencing is still the most commonly used method to determine the composition of microbial communities and has numerous advantages; it is cost effective, suitable for analysing a large number of samples and there are established bioinformatics pipelines such as QIIME (Jovel, 2016; Ranjan *et al.*, 2016). However, whole-genome shotgun sequencing can accurately classify sequences at the species level, whereas 16S rRNA sequencing provides limited taxonomic resolution (Ranjan *et al.*, 2016). Whole-genome shotgun sequencing also has the advantage that it can identify archaea, viruses, fungi and protozoa, as well as bacteria (Jovel, 2016; Ranjan *et al.*, 2016). Although whole-genome shotgun sequencing has the potential for strain-level identification (Brito & Alm, 2016), the determination of strain-level diversity remains challenging and further methodological development is required (Jovel, 2016). The ability to accurately identify bacterial strains within the microbiome would represent a key advance since different strains within a species can have distinct biological effects (Ahern *et al.*, 2014). Indeed, some studies provide evidence of strain-specific effects of probiotics on inflammation (Kekkonen *et al.*, 2008), the brain (Wall *et al.*, 2012) and behaviour (Savignac *et al.*, 2014).

6.4 Future directions

As well as the future research directions previously addressed within each chapter, here I discuss the future of the field more generally in terms of further developments that may advance our understanding of the microbiome–gut–brain axis and the potential opportunities to benefit human health.

6.4.1 Faecal microbiota transplants

Faecal microbiota transplants can effectively treat *Clostridium difficile* infections and there have also been numerous trials investigating their potential to alleviate inflammatory bowel disease (Gianotti & Moss, 2017). In comparison, there has been little investigation to date on the effect of faecal microbiota transplants on human psychiatric traits. Notably, a faecal microbiota transplant study did report improved behaviour in autistic children (Kang *et al.*, 2017) and a recent study found that this procedure can also ameliorate psychiatric symptoms in patients with

gastrointestinal disease (Kurokawa *et al.*, 2018). While faecal microbiota transplants are still a relatively new medical procedure, evidence from preliminary studies such as these indicates the possibility of using such transplants in the future to help treat neuropsychiatric disorders that are resistant to standard treatments. However, a more thorough assessment of the risks, benefits and ethics of microbiota transplants is required, as well as improved regulation (Lagier, 2014; Hoffmann *et al.*, 2017). For example, it is important to screen stool for infectious pathogens, but even then, we do not have a comprehensive knowledge of all the biological agents contained within faeces and their potential to cause disease in recipients.

6.4.2 Psychobiotics

Psychobiotics offer the potential to help treat conditions such as depression and autism (Dinan *et al.*, 2013; Gilbert *et al.*, 2013). Indeed, there is evidence from human studies that probiotics can improve symptoms of depression, anxiety, stress and autism (Messaoudi *et al.*, 2011; Allen *et al.*, 2016; Pinto-Sanchez *et al.*, 2017; Shaaban *et al.*, 2017), and can also change brain activity (Tillisch *et al.*, 2013; Bagga *et al.*, 2018). Although not all studies find a beneficial effect of probiotics on mental health, recent meta-analyses have concluded that they can have a positive impact on mood and improve psychological symptoms, including in healthy humans (Pirbaglou *et al.*, 2016; Wang *et al.*, 2016; McKean *et al.*, 2017; Wallace & Milev, 2017). Currently the majority of bacteria used as probiotics belong to the genera *Lactobacillus* or *Bifidobacterium* (Thomas *et al.*, 2015). Since neither of my studies on the human nor macaque population (Chapters 3 and 4) found a relationship between the abundances of these genera and behaviour, it may also be of interest to consider other bacterial taxa as potential probiotics which I found were related to beneficial behavioural traits. In addition, there is evidence that the effects of probiotics on murine behaviour can depend on host diet and genotype (Ohland *et al.*, 2013) and thus the efficacy of psychobiotics in humans may also differ depending on the individual's genes and environment. Indeed, a growing trend in microbiome research is the development of personalized treatments and diets (Zmora *et al.*, 2016), and this is likely to be applicable to the treatment of mental, as well as physical, health.

Despite the beneficial effects that probiotics may have, evidence is lacking as to whether they have a significant impact on gut microbiome composition (Kristensen *et al.*, 2016), since the intrinsic colonization resistance of a healthy human gut can limit colonization by probiotic strains (Zmora *et al.*, 2018). Other methods to manipulate the human gut microbiome, such as using prebiotics (Gibson & Roberfroid, 1995) or postbiotics which are metabolic products of bacteria (Suez & Elinav, 2017), can circumvent reliance on colonization of the gut. Since microbial metabolites are hypothesized to play an important role in mediating gut–brain communication (Stilling *et al.*, 2016a; Johnson & Foster, 2018 [Appendix]), postbiotics may prove to be an effective treatment for neuropsychiatric conditions, but their therapeutic potential is yet to be explored.

Although psychobiotics may provide a promising new therapy for the treatment of disorders associated with the microbiome–gut–brain axis, a recent study on probiotic administration in rats found that while probiotics may have some cognitive benefits in cases of gut dysbiosis, they can actually have a negative effect on memory in healthy animals (Beilharz *et al.*, 2018). Notably, there is also new evidence in humans of a possible causal link between probiotic use and both brain foggiess and gastrointestinal symptoms (Rao *et al.*, 2018). Like all new medical treatments, psychobiotics should therefore be approached with caution given this nascent field and their poorly defined mechanisms of action.

6.4.3 Manipulation of the microbiome

Most experimental manipulations of the gut microbial community involve antibiotic treatment, administration of probiotics or prebiotics, or in the extreme case, a total lack of microorganisms as in germ-free animals. However, more fine-scale manipulations will help provide insight into the role of specific bacterial species or strains in the gut and how they may influence the brain and behaviour. For example, a new area of research is the development of antimicrobials to eliminate particular members of the microbiota, using technologies such as the CRISPR–Cas gene-editing system (Bikard *et al.*, 2014; Fagen *et al.*, 2017). Bacteriophage treatment also offers a method to remove bacteria in a strain-specific manner (Sheth *et al.*, 2016).

Currently, research is focused on manipulation of the gut microbiome with respect to bacterial taxa. However, the gut microbiota comprises an array of microorganisms including archaea, viruses, bacteriophages, fungi and other microbial eukaryotes (Mirzaei & Maurice, 2017). For instance, archaea are estimated to represent approximately 10% of anaerobic microorganisms living in the large intestine but are yet to be integrated into microbiome research (Pike & Forster, 2018). The mycobiome has also been neglected with respect to the microbiome–gut–brain axis (Enaud *et al.*, 2018). Interestingly, a recent study found that fungi can functionally replace gut bacteria and protect against intestinal injury and infection (Jiang *et al.*, 2017). Similarly, an enteric virus has been shown to replace the beneficial role of gut bacteria in intestinal homeostasis and immunity (Kernbauer *et al.*, 2014). Together, these findings reveal a more intricate picture, highlighting that functional redundancies may operate at a higher taxonomic level than previously considered. Thus it would be interesting to examine the interactions between the brain and other members of the gut microbiota such as fungi and viruses.

6.4.4 Ex vivo systems

A new and increasingly popular technique in microbiome research is the use of *ex vivo* systems which may help to disentangle the complexity of host–microbiome interactions by enabling precise experimental control outside the host (May *et al.*, 2017). For example, the microfluidic gut-on-a-chip allows particular gut microorganisms to be co-cultured with human intestinal epithelial cells, making it possible to simulate interactions between the microbiome and host physiology under different experimental conditions (Kim *et al.*, 2015). This device allows investigation into the effects of treatments such as probiotics, antibiotics and the inflammation-inducing lipopolysaccharide endotoxin. However, a disadvantage of this approach is that the architecture of the gut tissue is not preserved. Researchers therefore recently developed a three-dimensional organ culture system which maintains normal architecture and intercellular connections within the gut tissue, enabling precise investigation of the interactions between the gut microbiome and the immune and nervous systems (Yissachar *et al.*, 2017). Another newly developed *ex vivo* method is the recording of electrophysiological activity of vagal afferent

neurones to examine the effect of stimulating the distal colon with neuromodulatory reagents or bacterial products (Buckley & O'Malley, 2018).

In addition, organoid cultures enable interactions between host tissue and members of the microbiota to be investigated by microinjecting microorganisms into the lumen of organoids (May *et al.*, 2017). Intestinal organoids can also provide insight into communication between cells lining the gut and the enteric nervous system, helping to uncover the possible mechanisms underpinning the gut–brain link. For example, intestinal organoids were used to elucidate the signalling properties of the rare enterochromaffin cells of the gut, revealing that they form synaptic connections with afferent nerve fibres and signal via serotonin release upon activation (Bellono *et al.*, 2017).

Ex vivo research therefore has exciting potential to reveal the key mechanisms involved in signalling between the gut microbiome, the immune system and the nervous system. While it may represent a reductionist approach, *ex vivo* modelling allows researchers to monitor the effects of specific bacterial taxa or bacterial products under a range of conditions, which can subsequently be tested *in vivo*.

6.4.5 Model organisms

The majority of studies on the microbiome–gut–brain axis have used rodent models, particularly mice. Although rodents are generally social species, it would be informative for future research to investigate the relationship between the gut microbiome and social behaviour in highly social species such as the prairie vole which can also be kept in a laboratory setting (Curtis *et al.*, 2018). Indeed, the social neuropeptide signalling systems (opioid, oxytocin and vasopressin) have been well characterized in this species (Young & Wang, 2004; Burkett *et al.*, 2011; Resendez *et al.*, 2013). Thus it would be interesting to determine how altering the gut microbial community, such as using antibiotic or probiotic treatment, influences behaviour and expression of neuropeptide systems in this species.

6.5 Conclusions

In this thesis I have explored the relationship between the gut microbiome and the brain and behaviour using multiple levels of enquiry. By studying animal models, a human population, an animal social network, mammalian encephalization and evolutionary theory, I demonstrate the multiple scales over which interactions between the microbiota and host behaviour operate. My findings reveal that the microbiome–gut–brain axis may not only be relevant to cases of psychiatric illness but also to biological psychology and our understanding of behavioural variation within, and even across, species.

Despite the recent growth of research on the microbiome–gut–brain axis, it is clear that we have only scratched the surface. Indeed, the human brain is considered to be one of the most complex structures in the known universe and similarly, the gut microbiome is also very complex, representing one of the most diverse ecosystems so far described. We are certainly at a very exciting and challenging frontier of both modern medicine and psychology. Future advances in technology and the rise of systems biology may provide key insights into these immensely complex and dynamic systems and the nature of interactions between the two.

In closing, the evidence that microbial communities living within animals can affect their behaviour is captivating, as is the realization that our gut microbiome may even influence something as unique as our own personality. In my view, this microbial contribution to our physiology and psychology, reflecting our intimate symbiotic relationship with microorganisms, does not detract from our sense of self. Rather we should marvel at this integration of microbial and human life, which has opened up a new avenue in neuroscience research, and with it the potential to enhance well-being.

References

- Abbott, N.J., Rönnbäck, L. & Hansson, E. (2006) Astrocyte–endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* **7**, 41–53.
- Abolins, S. *et al.* (2017) The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. *Nat. Commun.* **8**, 14811.
- Abt, M.C. & Pamer, E.G. (2014) Commensal bacteria mediated defenses against pathogens. *Curr. Opin. Immunol.* **29**, 16–22.
- Adamo, S.A. (2002) Modulating the modulators: parasites, neuromodulators and host behavioral change. *Brain Behav. Evol.* **60**, 370–377.
- Adamo, S.A. (2013) Parasites: evolution’s neurobiologists. *J. Exp. Biol.* **216**, 3–10.
- Adams, J.B., Johansen, L.J., Powell, L.D., Quig, D. & Rubin, R.A. (2011) Gastrointestinal flora and gastrointestinal status in children with autism – comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.* **11**, 22.
- de Agüero, M.G. *et al.* (2016) The maternal microbiota drives early postnatal innate immune development. *Science* **351**, 1296–1302.
- Aguilera, M., Cerdà-Cuellar, M. & Martínez, V. (2015) Antibiotic-induced dysbiosis alters host–bacterial interactions and leads to colonic sensory and motor changes in mice. *Gut Microbes* **6**, 10–23.
- Aguirre de Cárcer, D. *et al.* (2011) Numerical ecology validates a biogeographical distribution and gender-based effect on mucosa-associated bacteria along the human colon. *ISME J.* **5**, 801–809.
- Ahern, P.P., Faith, J.J. & Gordon, J.I. (2014) Mining the human gut microbiota for effector strains that shape the immune system. *Immunity* **40**, 819–823.
- Aiello, L.C. & Wheeler, P. (1995) The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Curr. Anthropol.* **36**, 199–221.
- Aizawa, E. *et al.* (2016) Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. *J. Affect. Disord.* **202**, 254–257.
- Akaike, H. (1974) A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* **19**, 716–723.
- Alberdi, A., Aizpurua, O., Bohmann, K., Zepeda-Mendoza, M.L. & Gilbert, M.T.P. (2016) Do vertebrate gut metagenomes confer rapid ecological adaptation? *Trends Ecol. Evol.* **31**, 689–699.
- Alcock, J., Maley, C.C. & Aktipis, C.A. (2014) Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays* **36**, 940–949.
- Allen, A.P. *et al.* (2016) *Bifidobacterium longum* 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. *Transl. Psychiatry* **6**, e939.
- Allen, A.P., Dinan, T.G., Clarke, G. & Cryan, J.F. (2017) A psychology of the human brain–gut–microbiome axis. *Soc. Personal. Psychol. Compass* **11**, e12309.
- Almeling, L. *et al.* (2016) Motivational shifts in aging monkeys and the origins of social selectivity. *Curr. Biol.* **26**, 1744–1749.
- Almonacid, D.E. *et al.* (2017) 16S rRNA gene sequencing and healthy reference ranges for 28 clinically relevant microbial taxa from the human gut microbiome. *PLoS One* **12**, e0176555.

- Altizer, S. *et al.* (2003) Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annu. Rev. Ecol. Evol. Syst.* **34**, 517–547.
- Altmann, J. (1974) Observational study of behavior: sampling methods. *Behaviour* **49**, 227–267.
- Amaral, W.Z. *et al.* (2017) Social influences on *Prevotella* and the gut microbiome of young monkeys. *Psychosom. Med.* **79**, 888–897.
- Amato, K.R. (2016) Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *Am. J. Phys. Anthropol.* **159**, S196–S215.
- Amato, K.R. *et al.* (2014) The role of gut microbes in satisfying the nutritional demands of adult and juvenile wild, black howler monkeys (*Alouatta pigra*). *Am. J. Phys. Anthropol.* **155**, 652–664.
- Amato, K.R. *et al.* (2017) Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate. *Microb. Ecol.* **74**, 250–258.
- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association Publishing, Washington).
- Aminov, R.I. (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.* **1**, 134.
- Andersen, S.B. *et al.* (2009) The life of a dead ant: the expression of an adaptive extended phenotype. *Am. Nat.* **174**, 424–433.
- de Angelis, M. *et al.* (2013) Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* **8**, e76993.
- Arase, S., Watanabe, Y., Setoyama, H., Nagaoka, N. & Kawai, M. (2016) Disturbance in the mucosa-associated commensal bacteria is associated with the exacerbation of chronic colitis by repeated psychological stress; is that the new target of probiotics? *PLoS One* **11**, e0160736.
- Archie, E.A. & Theis, K.R. (2011) Animal behaviour meets microbial ecology. *Anim. Behav.* **82**, 425–436.
- Archie, E.A., Altmann, J. & Alberts, S.C. (2012) Social status predicts wound healing in wild baboons. *Proc. Natl. Acad. Sci. USA* **109**, 9017–9022.
- Archie, E.A. & Tung, J. (2015) Social behavior and the microbiome. *Curr. Opin. Behav. Sci.* **6**, 28–34.
- Ardeshtir, A. *et al.* (2014) Breast-fed and bottle-fed infant rhesus macaques develop distinct gut microbiotas and immune systems. *Sci. Transl. Med.* **6**, 252ra120.
- Arentsen, T., Raith, H., Qian, Y., Forssberg, H. & Diaz Heijtz, R. (2015) Host microbiota modulates development of social preference in mice. *Microb. Ecol. Health Dis.* **26**, 29719.
- Arnold, J.W., Roach, J. & Azcarate-Peril, M.A. (2016) Emerging technologies for gut microbiome research. *Trends Microbiol.* **24**, 887–901.
- Arumugam, M. *et al.* (2011) Enterotypes of the human gut microbiome. *Nature* **473**, 174–180.
- Asano, Y. *et al.* (2012) Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **303**, G1288–G1295.
- Asquith, M.J., Boulard, O., Powrie, F. & Maloy, K.J. (2010) Pathogenic and protective roles of MyD88 in leukocytes and epithelial cells in mouse models of inflammatory bowel disease. *Gastroenterology* **139**, 519–529.
- Aunger, R. *et al.* (2016) The determinants of reported personal and household hygiene behaviour: a multi-country study. *PLoS One* **11**, e0159551.

- Austin, E.J. (2005) Personality correlates of the broader autism phenotype as assessed by the Autism Spectrum Quotient (AQ). *Pers. Individ. Dif.* **38**, 451–460.
- Ayres, J.S. (2016) Cooperative microbial tolerance behaviors in host–microbiota mutualism. *Cell* **165**, 1323–1331.
- Azad, M.B. *et al.* (2013) Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. *Allergy, Asthma Clin. Immunol.* **9**, 15.
- Azpiroz, F. *et al.* (2017) Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. *Neurogastroenterol. Motil.* **29**, e12911.
- van Baarlen, P. *et al.* (2009) Differential NF- κ B pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. *Proc. Natl. Acad. Sci. USA* **106**, 2371–2376.
- van Baarlen, P. *et al.* (2011) Human mucosal *in vivo* transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *Proc. Natl. Acad. Sci. USA* **108** (Suppl. 1), 4562–4569.
- Bach, H. *et al.* (2014) Elevated serotonin and 5-HIAA in the brainstem and lower serotonin turnover in the prefrontal cortex of suicides. *Synapse* **68**, 127–130.
- Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A. & Gordon, J.I. (2005) Host–bacterial mutualism in the human intestine. *Science* **307**, 1915–1920.
- Bäckhed, F. *et al.* (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* **17**, 690–703.
- Baganz, N.L. & Blakely, R.D. (2013) A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem. Neurosci.* **4**, 48–63.
- Bagga, D. *et al.* (2018) Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes* **9**, 486–496.
- Bailey, M.T. & Coe, C.L. (1999) Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev. Psychobiol.* **35**, 146–155.
- Bailey, M.T. *et al.* (2011) Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav. Immun.* **25**, 397–407.
- Bali, A., Randhawa, P.K. & Jaggi, A.S. (2015) Stress and opioids: role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference. *Neurosci. Biobehav. Rev.* **51**, 138–150.
- Balmer, O. & Tanner, M. (2011) Prevalence and implications of multiple-strain infections. *Lancet Infect. Dis.* **11**, 868–878.
- Bangsgaard Bendtsen, K.M. *et al.* (2012) Gut microbiota composition is correlated to grid floor induced stress and behavior in the BALB/c mouse. *PLoS One* **7**, e46231.
- Banks, W.A. (2009) Characteristics of compounds that cross the blood–brain barrier. *BMC Neurol.* **9** (Suppl. 1), S3.
- Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J. & Clubley, E. (2001) The Autism-Spectrum Quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J. Autism Dev. Disord.* **31**, 5–17.
- Barrett, E., Ross, R.P., O'Toole, P.W., Fitzgerald, G.F. & Stanton, C. (2012) γ -Aminobutyric acid production by culturable bacteria from the human intestine. *J. Appl. Microbiol.* **113**, 411–417.

- Becker, J.A.J. *et al.* (2014) Autistic-like syndrome in mu opioid receptor null mice is relieved by facilitated mGluR4 activity. *Neuropsychopharmacology* **39**, 2049–2060.
- Beilharz, J.E., Kaakoush, N.O., Maniam, J. & Morris, M.J. (2018) Cafeteria diet and probiotic therapy: cross talk among memory, neuroplasticity, serotonin receptors and gut microbiota in the rat. *Mol. Psychiatry* **23**, 351–361.
- Belkaid, Y. & Hand, T.W. (2014) Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141.
- Bellono, N.W. *et al.* (2017) Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell* **170**, 185–198.e16.
- Bennett, G. *et al.* (2016) Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *Am. J. Primatol.* **892**, 883–892.
- Benros, M. *et al.* (2013) Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. *JAMA Psychiatry* **70**, 812–820.
- Benson, A.K. *et al.* (2010) Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc. Natl. Acad. Sci. USA* **107**, 18933–18938.
- Bercik, P. *et al.* (2010) Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology* **139**, 2102–2112.
- Bercik, P. *et al.* (2011a) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* **141**, 599–609.
- Bercik, P. *et al.* (2011b) The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut–brain communication. *Neurogastroenterol. Motil.* **23**, 1132–1139.
- Berdoy, M., Webster, J.P. & Macdonald, D.W. (2000) Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. R. Soc. B Biol. Sci.* **267**, 1591–1594.
- Bernstein, H.G. *et al.* (2002) Fewer beta-endorphin expressing arcuate nucleus neurons and reduced beta-endorphinergic innervation of paraventricular neurons in schizophrenics and patients with depression. *Cell. Mol. Biol.* **48**, OL259–OL265.
- Bested, A.C., Logan, A.C. & Selhub, E.M. (2013) Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part I – autointoxication revisited. *Gut Pathog.* **5**, 5.
- Beura, L.K. *et al.* (2016) Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* **532**, 512–516.
- Bharwani, A. *et al.* (2016) Structural & functional consequences of chronic psychosocial stress on the microbiome & host. *Psychoneuroendocrinology* **63**, 217–227.
- Bharwani, A., Mian, M.F., Surette, M.G., Bienenstock, J. & Forsythe, P. (2017) Oral treatment with *Lactobacillus rhamnosus* attenuates behavioural deficits and immune changes in chronic social stress. *BMC Med.* **15**, 7.
- Bhattarai, Y., Muniz Pedrego, D.A. & Kashyap, P.C. (2017) Irritable bowel syndrome: a gut microbiota-related disorder? *Am. J. Physiol. Gastrointest. Liver Physiol.* **312**, G52–G62.
- Bikard, D. *et al.* (2014) Exploiting CRISPR–Cas nucleases to produce sequence-specific antimicrobials. *Nat. Biotechnol.* **32**, 1146–1150.
- Billiet, A. *et al.* (2017) Colony contact contributes to the diversity of gut bacteria in bumblebees (*Bombus terrestris*). *Insect Sci.* **24**, 270–277.
- Bingham, S.A. *et al.* (2001) Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr.* **4**, 847–858.

- Biron, D.G. *et al.* (2005) Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proc. R. Soc. B Biol. Sci.* **272**, 2117–2126.
- Bloomfield, S.F. *et al.* (2016) Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. *Perspect. Public Health* **136**, 213–224.
- van den Bogert, B., Meijerink, M., Zoetendal, E.G., Wells, J.M. & Kleerebezem, M. (2014) Immunomodulatory properties of *Streptococcus* and *Veillonella* isolates from the human small intestine microbiota. *PLoS One* **9**, e114277.
- Bokulich, N.A. *et al.* (2016) Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Sci. Transl. Med.* **8**, 343ra82.
- Bonaz, B., Bazin, T. & Pellissier, S. (2018) The vagus nerve at the interface of the microbiota–gut–brain axis. *Front. Neurosci.* **12**, 49.
- Bonder, M.J. *et al.* (2016) The influence of a short-term gluten-free diet on the human gut microbiome. *Genome Med.* **8**, 45.
- Borgatti, S.P., Everett, M.G. & Freeman, L.C. (2002) *UCINET for Windows: Software for Social Network Analysis* (Analytic Technologies, Harvard, Massachusetts). Available at: <http://sites.google.com/site/ucinetsoftware>.
- Borre, Y.E. *et al.* (2014) Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol. Med.* **20**, 509–518.
- Bosch, T.C.G. *et al.* (2017) Back to the basics: cnidarians start to fire. *Trends Neurosci.* **40**, 92–105.
- Bourassa, M.W., Alim, I., Bultman, S.J. & Ratan, R.R. (2015) Butyrate, neuroepigenetics and the gut microbiome: can a high fiber diet improve brain health? *Neurosci. Lett.* **625**, 56–63.
- Bourke, A.F.G. (2011) *Principles of Social Evolution* (Oxford University Press, Oxford).
- Braniste, V. *et al.* (2014) The gut microbiota influences blood–brain barrier permeability in mice. *Sci. Transl. Med.* **6**, 263ra158.
- Bravo, J.A. *et al.* (2011) Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* **108**, 16050–16055.
- Brent, L.J.N. *et al.* (2013) Genetic origins of social networks in rhesus macaques. *Sci. Rep.* **3**, 1042.
- Brent, L.J.N., Chang, S.W.C., Gariépy, J.F. & Platt, M.L. (2014) The neuroethology of friendship. *Ann. NY Acad. Sci.* **1316**, 1–17.
- Brent, L.J.N., Ruiz-Lambides, A. & Platt, M.L. (2017) Persistent social isolation reflects identity and social context but not maternal effects or early environment. *Sci. Rep.* **7**, 17791.
- Bressa, C. *et al.* (2017) Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS One* **12**, e0171352.
- Brestoff, J.R. & Artis, D. (2013) Commensal bacteria at the interface of host metabolism and the immune system. *Nat. Immunol.* **14**, 676–684.
- Bridgewater, L.C. *et al.* (2017) Gender-based differences in host behavior and gut microbiota composition in response to high fat diet and stress in a mouse model. *Sci. Rep.* **7**, 10776.
- Brimberg, L., Sadiq, A., Gregersen, P.K. & Diamond, B. (2013) Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Mol. Psychiatry* **18**, 1171–1177.
- Brito, I.L. & Alm, E.J. (2016) Tracking strains in the microbiome: insights from metagenomics and models. *Front. Microbiol.* **7**, 712.

- Brown, S.P. (1999) Cooperation and conflict in host-manipulating parasites. *Proc. R. Soc. B Biol. Sci.* **266**, 1899–1904.
- Brown, S.P. (2005) Do all parasites manipulate their hosts? *Behav. Processes* **68**, 237–240.
- Browne, H.P. *et al.* (2016) Culturing of “unculturable” human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**, 543–546.
- Browne, H.P., Neville, B.A., Forster, S.C. & Lawley, T.D. (2017) Transmission of the gut microbiota: spreading of health. *Nat. Rev. Microbiol.* **15**, 531–543.
- Bruce-Keller, A., Salbaum, J.M. & Berthoud, H.-R. (2018) Harnessing gut microbes for mental health: getting from here to there. *Biol. Psychiatry* **83**, 214–223.
- Brun, P. *et al.* (2013) Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. *Gastroenterology* **145**, 1323–1333.
- Buckley, M.M. & O'Malley, D. (2018) Development of an *ex vivo* method for multi-unit recording of microbiota–colonic–neural signaling in real time. *Front. Neurosci.* **12**, 112.
- Buffington, S.A. *et al.* (2016) Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* **165**, 1762–1775.
- Burkett, J.P., Spiegel, L.L., Inoue, K., Murphy, A.Z. & Young, L.J. (2011) Activation of μ -opioid receptors in the dorsal striatum is necessary for adult social attachment in monogamous prairie voles. *Neuropsychopharmacology* **36**, 2200–2210.
- Burton, J.P., Chanyi, R.M. & Schultz, M. (2017) Common organisms and probiotics: *Streptococcus thermophilus* (*Streptococcus salivarius* subsp. *thermophilus*). *The Microbiota in Gastrointestinal Pathophysiology: Implications for Human Health, Prebiotics, Probiotics, and Dysbiosis*, eds Floch, M.H., Ringel, Y. & Walker, W.A., 165–169 (Academic Press, Amsterdam).
- Byrne, R.W. & Corp, N. (2004) Neocortex size predicts deception rate in primates. *Proc. R. Soc. B Biol. Sci.* **271**, 1693–1699.
- Caballero, S. *et al.* (2017) Cooperating commensals restore colonization resistance to vancomycin-resistant *Enterococcus faecium*. *Cell Host Microbe* **21**, 592–602.
- Campbell, J.I. *et al.* (2015) Weight gain after fecal microbiota transplantation. *Open Forum Infect. Dis.* **2**, ofv004.
- Caporaso, J.G. *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336.
- Caporaso, J.G. *et al.* (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6**, 1621–1624.
- Carey, H.V., Walters, W.A. & Knight, R. (2013) Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **304**, R33–R42.
- Cernansky, R. (2017) The biodiversity revolution. *Nature* **546**, 22–24.
- Chai, G. *et al.* (2012) Trends of outpatient prescription drug utilization in US children, 2002–2010. *Pediatrics* **130**, 23–31.
- Chatzidaki-Livanis, M., Geva-Zatorsky, N. & Comstock, L.E. (2016) *Bacteroides fragilis* type VI secretion systems use novel effector and immunity proteins to antagonize human gut Bacteroidales species. *Proc. Natl. Acad. Sci. USA* **113**, 3627–3632.
- Chen, T.S. & Chen, P.S. (1989) Intestinal autointoxication: a medical leitmotif. *J. Clin. Gastroenterol.* **11**, 434–441.

- Chen, X., D'Souza, R. & Hong, S.T. (2013) The role of gut microbiota in the gut–brain axis: current challenges and perspectives. *Protein Cell* **4**, 403–414.
- Chikanza, I.C. & Grossman, A.S. (1998) Hypothalamic–pituitary-mediated immunomodulation: arginine vasopressin is a neuroendocrine immune mediator. *Br. J. Rheumatol.* **37**, 131–136.
- Choi, E.-Y. *et al.* (2004) Iron chelator triggers inflammatory signals in human intestinal epithelial cells: involvement of p38 and extracellular signal-regulated kinase signaling pathways. *J. Immunol.* **172**, 7069–7077.
- Christian, L.M. *et al.* (2015) Gut microbiome composition is associated with temperament during early childhood. *Brain Behav. Immun.* **45**, 118–127.
- Chu, D.M. *et al.* (2017) Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat. Med.* **23**, 314–326.
- Chu, H. & Mazmanian, S.K. (2013) Innate immune recognition of the microbiota promotes host–microbial symbiosis. *Nat. Immunol.* **14**, 668–675.
- Clarke, G. *et al.* (2013) The microbiome–gut–brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* **18**, 666–673.
- Clayton, J.B. *et al.* (2016) Captivity humanizes the primate microbiome. *Proc. Natl. Acad. Sci. USA* **113**, 10376–10381.
- de la Cochetière, M.F. *et al.* (2005) Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J. Clin. Microbiol.* **43**, 5588–5592.
- Coenye, T. & Vandamme, P. (2003) Intragenomic heterogeneity between multiple 16S ribosomal RNA operons in sequenced bacterial genomes. *FEMS Microbiol. Lett.* **228**, 45–49.
- Collado, M.C., Rautava, S., Aakko, J., Isolauri, E. & Salminen, S. (2016) Human gut colonisation may be initiated *in utero* by distinct microbial communities in the placenta and amniotic fluid. *Sci. Rep.* **6**, 23129.
- Collins, S.M., Surette, M. & Bercik, P. (2012) The interplay between the intestinal microbiota and the brain. *Nat. Rev. Microbiol.* **10**, 735–742.
- Collins, S.M., Kassam, Z. & Bercik, P. (2013) The adoptive transfer of behavioral phenotype via the intestinal microbiota: experimental evidence and clinical implications. *Curr. Opin. Microbiol.* **16**, 240–245.
- Constantino, J.N. & Todd, R.D. (2003) Autistic traits in the general population: a twin study. *Arch. Gen. Psychiatry* **60**, 524–530.
- Coretti, L. *et al.* (2017) Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder. *Sci. Rep.* **7**, 45356.
- Cosorich, I. *et al.* (2017) High frequency of intestinal T_H17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. *Sci. Adv.* **3**, e1700492.
- Costello, E.K. *et al.* (2009) Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694–1697.
- Coyte, K.Z., Schluter, J. & Foster, K.R. (2015) The ecology of the microbiome: networks, competition, and stability. *Science* **350**, 663–666.
- Craft, M.E. (2015) Infectious disease transmission and contact networks in wildlife and livestock. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140107.
- Crumeyrolle-Arias, M. *et al.* (2014) Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology* **42**, 207–217.

- Cryan, J.F. & Dinan, T.G. (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* **13**, 701–712.
- Csiffáry, A., Ruttner, Z., Tóth, Z. & Palkovits, M. (1992) Oxytocin nerve fibers innervate beta-endorphin neurons. *Neuroendocrinology*. **56**, 429–435.
- Curtis, J.T., Assefa, S., Francis, A. & Köhler, G.A. (2018) Fecal microbiota in the female prairie vole (*Microtus ochrogaster*). *PLoS One* **13**, e0190648.
- D'Acquisto, F. (2017) Affective immunology: where emotions and the immune response converge. *Dialogues Clin. Neurosci.* **19**, 395–404.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W. & Kelley, K.W. (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* **9**, 46–57.
- Darcq, E. & Kieffer, B.L. (2018) Opioid receptors: drivers to addiction? *Nat. Rev. Neurosci.* **19**, 499–514.
- Davenport, E.R. *et al.* (2017) The human microbiome in evolution. *BMC Biol.* **15**, 127.
- David, L.A. *et al.* (2014a) Host lifestyle affects human microbiota on daily timescales. *Genome Biol.* **15**, R89.
- David, L.A. *et al.* (2014b) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563.
- Davis, D.J. *et al.* (2016a) Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. *Brain Behav. Immun.* **59**, 38–48.
- Davis, D.J. *et al.* (2016b) *Lactobacillus plantarum* attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. *Sci. Rep.* **6**, 33726.
- Dean, J.M. *et al.* (2010) Microglial *MyD88* signaling regulates acute neuronal toxicity of LPS-stimulated microglia *in vitro*. *Brain Behav. Immun.* **24**, 776–783.
- DeCasien, A.R., Williams, S.A. & Higham, J.P. (2017) Primate brain size is predicted by diet but not sociality. *Nat. Ecol. Evol.* **1**, 0112.
- Degnan, P.H. *et al.* (2012) Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. *Proc. Natl. Acad. Sci. USA* **109**, 13034–13039.
- Delsuc, F. *et al.* (2014) Convergence of gut microbiomes in myrmecophagous mammals. *Mol. Ecol.* **23**, 1301–1317.
- Delungahawatta, T. *et al.* (2017) Antibiotic driven changes in gut motility suggest direct modulation of enteric nervous system. *Front. Neurosci.* **11**, 588.
- Depue, R.A. & Morrone-Strupinsky, J.V. (2005) A neurobehavioral model of affiliative bonding: implications for conceptualizing a human trait of affiliation. *Behav. Brain Sci.* **28**, 313–350.
- DeSantis, T.Z. *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72**, 5069–5072.
- Desbonnet, L., Garrett, L., Clarke, G., Bienenstock, J. & Dinan, T.G. (2008) The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J. Psychiatr. Res.* **43**, 164–174.
- Desbonnet, L. *et al.* (2010) Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* **170**, 1179–1188.
- Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G. & Cryan, J.F. (2014) Microbiota is essential for social development in the mouse. *Mol. Psychiatry* **19**, 146–148.

- Desbonnet, L. *et al.* (2015) Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav. Immun.* **48**, 165–173.
- Dethlefsen, L., Huse, S.M., Sogin, M.L. & Relman, D.A. (2008) The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol.* **6**, e280.
- Dethlefsen, L. & Relman, D.A. (2010) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl. Acad. Sci. USA* **108**, 4554–4561.
- Dey, N. *et al.* (2015) Regulators of gut motility revealed by a gnotobiotic model of diet–microbiome interactions related to travel. *Cell* **163**, 95–107.
- Diaz Heijtz, R. (2016) Fetal, neonatal, and infant microbiome: perturbations and subsequent effects on brain development and behavior. *Semin. Fetal Neonatal Med.* **21**, 410–417.
- Diaz Heijtz, R. *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. USA* **108**, 3047–3052.
- Digman, J.M. (1990) Personality structure: emergence of the five-factor model. *Annu. Rev. Psychol.* **41**, 417–440.
- Dillon, R.J., Vennard, C.T., Buckling, A. & Charnley, A.K. (2005) Diversity of locust gut bacteria protects against pathogen invasion. *Ecol. Lett.* **8**, 1291–1298.
- Dinan, T.G. & Cryan, J.F. (2012) Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. *Psychoneuroendocrinology* **37**, 1369–1378.
- Dinan, T.G., Stanton, C. & Cryan, J.F. (2013) Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry* **74**, 720–726.
- Dinan, T.G. & Cryan, J.F. (2017) Brain–gut–microbiota axis – mood, metabolism and behaviour. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 69–70.
- Ding, H. *et al.* (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* **101**, 15718–15723.
- Ding, T. & Schloss, P.D. (2014) Dynamics and associations of microbial community types across the human body. *Nature* **509**, 357–360.
- Dominguez-Bello, M.G. *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. USA* **107**, 11971–11975.
- Dominguez-Bello, M.G. *et al.* (2015) Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *MBio* **6**, e01693-15.
- Donaldson, G.P., Lee, S.M. & Mazmanian, S.K. (2015) Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32.
- Donaldson, Z.R. & Young, L.J. (2008) Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* **322**, 900–904.
- Dorrestein, P.C., Mazmanian, S.K. & Knight, R. (2014) Finding the missing links among metabolites, microbes, and the host. *Immunity* **40**, 824–832.
- Dunbar, R.I.M. (1992) Neocortex size as a constraint on group size in primates. *J. Hum. Evol.* **20**, 469–493.
- Dunbar, R.I.M. (1995) Neocortex size and group size in primates. *J. Hum. Evol.* **28**, 287–296.
- Dunbar, R.I.M. (1998) The social brain hypothesis. *Evol. Anthropol.* **6**, 178–190.

- Dunbar, R.I.M. (2009) Brain and behaviour in primate evolution. *Mind the Gap: Tracing the Origins of Human Universals*, eds Kappeler, P.H. & Silk, J., 315–330 (Springer, London).
- Dunbar, R.I.M. & Shultz, S. (2007a) Understanding primate brain evolution. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 649–658.
- Dunbar, R.I.M. & Shultz, S. (2007b) Evolution in the social brain. *Science* **317**, 1344–1347.
- Dunn, A.J., Swiergiel, A.H. & de Beaurepaire, R. (2005) Cytokines as mediators of depression: what can we learn from animal studies? *Neurosci. Biobehav. Rev.* **29**, 891–909.
- Duraffourd, C. *et al.* (2012) Mu-opioid receptors and dietary protein stimulate a gut–brain neural circuitry limiting food intake. *Cell* **150**, 377–388.
- Eckburg, P.B. *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638.
- Eisenstein, M. (2016) Microbiome: bacterial broadband. *Nature* **533**, S104–S106.
- Enaud, R. *et al.* (2018) The mycobiome: a neglected component in the microbiota–gut–brain axis. *Microorganisms* **6**, 22.
- Erny, D. *et al.* (2015) Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **18**, 965–977.
- Evans, S.J. *et al.* (2017) The gut microbiome composition associates with bipolar disorder and illness severity. *J. Psychiatr. Res.* **87**, 23–29.
- Ezenwa, V.O. *et al.* (2016) Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. R. Soc. B Biol. Sci.* **283**, 20153078.
- Fagen, J.R., Collias, D., Singh, A.K. & Beisel, C.L. (2017) Advancing the design and delivery of CRISPR antimicrobials. *Curr. Opin. Biomed. Eng.* **4**, 57–64.
- Faith, J.J. *et al.* (2013) The long-term stability of the human gut microbiota. *Science* **341**, 1237439.
- Falcony, G. *et al.* (2016) Population-level analysis of gut microbiome variation. *Science* **352**, 560–564.
- Fanning, S. *et al.* (2012) Bifidobacterial surface-exopolysaccharide facilitates commensal–host interaction through immune modulation and pathogen protection. *Proc. Natl. Acad. Sci. USA* **109**, 2108–2113.
- Felice, D. & Moloney, G. (2018) Strain differences in the susceptibility to the gut–brain axis and neurobehavioural alterations induced by maternal immune activation in mice. *Behav. Pharmacol.* **29**, 181–198.
- Fernstrom, J.D. (1983) Role of precursor availability in control of monoamine biosynthesis in brain. *Physiol. Rev.* **63**, 484–546.
- Fernstrom, J.D. & Wurtman, R.J. (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* **173**, 149–152.
- Fetissov, S.O. (2016) Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. *Nat. Rev. Endocrinol.* **13**, 11–25.
- Filiano, A.J. *et al.* (2016) Unexpected role of interferon- γ in regulating neuronal connectivity and social behaviour. *Nature* **535**, 425–429.
- de Filippo, C. *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. USA* **107**, 14691–14696.
- Findley, K., Williams, D.R., Grice, E.A. & Bonham, V.L. (2016) Health disparities and the microbiome. *Trends Microbiol.* **24**, 847–850.

- Finegold, S.M. (2011) *Desulfovibrio* species are potentially important in regressive autism. *Med. Hypotheses* **77**, 270–274.
- Finegold, S.M. *et al.* (2002) Gastrointestinal microflora studies in late-onset autism. *Clin. Infect. Dis.* **35** (Suppl. 1), S6–S16.
- Finegold, S.M. *et al.* (2010) Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* **16**, 444–453.
- Finotello, F., Mastorilli, E. & Di Camillo, B. (2016) Measuring the diversity of the human microbiota with targeted next-generation sequencing. *Brief. Bioinform.* **19**, 679–692.
- Flint, H.J., Bayer, E.A., Rincon, M.T., Lamed, R. & White, B.A. (2008) Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* **6**, 121–131.
- Flint, H.J., Scott, K.P., Duncan, S.H., Louis, P. & Forano, E. (2012) Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* **3**, 289–306.
- Fodor, R. (2014) Utilizing “omics” tools to study the complex gut ecosystem. *Microbial Endocrinology: The Microbiota–Gut–Brain Axis in Health and Disease*, eds Lyte, M. & Cryan, J.F., 25–38 (Springer, New York).
- Fogel, A.T. (2015) The gut microbiome of wild lemurs: a comparison of sympatric *Lemur catta* and *Propithecus verreauxi*. *Folia Primatol.* **86**, 85–95.
- Folks, D.G. (2004) The interface of psychiatry and irritable bowel syndrome. *Curr. Psychiatry Rep.* **6**, 210–215.
- Forsythe, P. & Kunze, W.A. (2013) Voices from within: gut microbes and the CNS. *Cell. Mol. Life Sci.* **70**, 55–69.
- Forsythe, P., Bienenstock, J. & Kunze, W.A. (2014) Vagal pathways for microbiome–brain–gut axis communication. *Microbial Endocrinology: The Microbiota–Gut–Brain Axis in Health and Disease*, eds Lyte, M. & Cryan, J.F., 115–133 (Springer, New York).
- Forsythe, P., Kunze, W.A. & Bienenstock, J. (2016) Moody microbes or fecal phrenology: what do we know about the microbiota–gut–brain axis? *BMC Med.* **14**, 58.
- Foster, J.A. & McVey Neufeld, K.-A. (2013) Gut–brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* **36**, 305–312.
- Foster, K.R., Schluter, J., Coyte, K.Z. & Rakoff-Nahoum, S. (2017) The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**, 43–51.
- Fox, K.C.R., Muthukrishna, M. & Shultz, S. (2017) The social and cultural roots of whale and dolphin brains. *Nat. Ecol. Evol.* **1**, 1699–1705.
- Franklin, T.B. *et al.* (2017) Prefrontal cortical control of a brainstem social behavior circuit. *Nat. Neurosci.* **20**, 260–270.
- Fransen, F. *et al.* (2017) The impact of gut microbiota on gender-specific differences in immunity. *Front. Immunol.* **8**, 754.
- Franzosa, E.A. *et al.* (2015) Sequencing and beyond: integrating molecular “omics” for microbial community profiling. *Nat. Rev. Microbiol.* **13**, 360–372.
- Freeman, S.M., Inoue, K., Smith, A.L., Goodman, M.M. & Young, L.J. (2014) The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (*Macaca mulatta*). *Psychoneuroendocrinology* **45**, 128–141.
- Fröhlich, E.E. *et al.* (2015) Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota–brain communication. *Brain Behav. Immun.* **56**, 140–155.

- Frost, G. *et al.* (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* **5**, 3611.
- Fujisaka, S. *et al.* (2016) Antibiotic effects on gut microbiota and metabolism are host dependent. *J. Clin. Invest.* **126**, 4430–4443.
- Fukumoto, S. *et al.* (2003) Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **284**, R1269–R1276.
- Fulton, L., Gordon, J.I., Turnbaugh, P.J. & Ba, F. (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* **3**, 213–223.
- Fung, T.C., Olson, C.A. & Hsiao, E.Y. (2017) Interactions between the microbiota, immune and nervous systems in health and disease. *Nat. Neurosci.* **20**, 145–155.
- Furness J.B. (2006) *The Enteric Nervous System* (Blackwell Publishing, Oxford).
- Furusawa, Y. *et al.* (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450.
- Galley, J.D. *et al.* (2014a) Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol.* **14**, 189.
- Galley, J.D. *et al.* (2014b) The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut Microbes* **5**, 748–760.
- Gao, L. & Yu, L.-C. (2004) Involvement of opioid receptors in the oxytocin-induced antinociception in the central nervous system of rats. *Regul. Pept.* **120**, 53–58.
- Gareau, M.G., Jury, J., MacQueen, G., Sherman, P.M. & Perdue, M.H. (2007) Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut* **56**, 1522–1528.
- Gianotti, R.J. & Moss, A.C. (2017) Fecal microbiota transplantation: from *Clostridium difficile* to inflammatory bowel disease. *Gastroenterol. Hepatol.* **13**, 209–213.
- Gibbons, A. (1998) Solving the brain's energy crisis. *Science* **280**, 1345–1347.
- Gibson, G.R. & Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **125**, 1401–1412.
- Gigliucci, V. *et al.* (2014) Region specific up-regulation of oxytocin receptors in the opioid *Oprm1*^{-/-} mouse model of autism. *Front. Pediatr.* **2**, 91.
- Gilbert, J.A., Krajmalnik-Brown, R., Porazinska, D.L., Weiss, S.J. & Knight, R. (2013) Toward effective probiotics for autism and other neurodevelopmental disorders. *Cell* **155**, 1446–1448.
- Gilbert, J.A., Jansson, J.K. & Knight, R. (2014) The Earth Microbiome project: successes and aspirations. *BMC Biol.* **12**, 69.
- Giloteaux, L. *et al.* (2016) Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome* **4**, 30.
- Goldberg, L.R. (2001) *International Personality Item Pool*. Available at: <http://ipip.ori.org>.
- Golubeva, A.V. *et al.* (2017) Microbiota-related changes in bile acid & tryptophan metabolism are associated with gastrointestinal dysfunction in a mouse model of autism. *EBioMedicine* **24**, 166–178.
- González-Forero, M. & Gardner, A. (2018) Inference of ecological and social drivers of human brain-size evolution. *Nature* **557**, 554–557.

- Goodrich, J.K. *et al.* (2014) Conducting a microbiome study. *Cell* **158**, 250–262.
- Goodrich, J.K. *et al.* (2016a) Genetic determinants of the gut microbiome in UK twins. *Cell Host Microbe* **19**, 731–743.
- Goodrich, J.K., Davenport, E.R., Waters, J.L., Clark, A.G. & Ley, R.E. (2016b) Cross-species comparisons of host genetic associations with the microbiome. *Science* **352**, 29–32.
- Gordon, I.J. & Pérez-Barbería, F.J. (2005) Gregariousness increases brain size in ungulates. *Oecologia* **145**, 41–52.
- Grieneisen, L.E., Livermore, J., Alberts, S.C., Tung, J. & Archie, E.A. (2017) Group living and male dispersal predict the core gut microbiome in wild baboons. *Integr. Comp. Biol.* **57**, 770–785.
- Grimaldi, R. *et al.* (2017) *In vitro* fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. *FEMS Microbiol. Ecol.* **93**, fiw23.
- Guida, F. *et al.* (2018) Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain Behav. Immun.* **67**, 230–245.
- Guo, X. *et al.* (2008) Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. *Lett. Appl. Microbiol.* **47**, 367–373.
- Guthrie, G.D. & Nicholson-Guthrie, C.S. (1989) γ -Aminobutyric acid uptake by a bacterial system with neurotransmitter binding characteristics. *Proc. Natl. Acad. Sci. USA* **86**, 7378–7381.
- Halfvarson, J. *et al.* (2017) Dynamics of the human gut microbiome in inflammatory bowel disease. *Nat. Microbiol.* **2**, 17004.
- Hamilton, W.D. (1964a) The genetical evolution of social behaviour I. *J. Theor. Biol.* **7**, 1–16.
- Hamilton, W.D. (1964b) The genetical evolution of social behaviour II. *J. Theor. Biol.* **7**, 17–52.
- Han, W. *et al.* (2018) A neural circuit for gut-induced reward. *Cell* **175**, 665–678.e23.
- Haro, C. *et al.* (2016) Intestinal microbiota is influenced by gender and body mass index. *PLoS One* **11**, e0154090.
- Harris, R.E. *et al.* (2007) Decreased central μ -opioid receptor availability in fibromyalgia. *J. Neurosci.* **27**, 10000–10006.
- Hata, T. *et al.* (2017) Regulation of gut luminal serotonin by commensal microbiota in mice. *PLoS Med.* **12**, e0180745.
- Hegadoren, K.M., O'Donnell, T., Lanius, R., Coupland, N.J. & Lacaze-Masmonteil, N. (2009) The role of b-endorphin in the pathophysiology of major depression. *Neuropeptides* **43**, 341–353.
- Heidarian, F., Noormohammadi, Z., Aghdaei, H.A. & Alebouyeh, M. (2017) Relative abundance of *Streptococcus* spp. and its association with disease activity in inflammatory bowel disease patients compared with controls. **12**, e57291.
- van de Heijning, B.J.M., Koekkoek-van den Herik, I. & van Wimersma Greidanus, T.B. (1991) The opioid receptor subtypes μ and κ , but not δ , are involved in the control of the vasopressin and oxytocin release in the rat. *Eur. J. Pharmacol.* **209**, 199–206.
- Heiman, M.L. & Greenway, F.L. (2016) A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol. Metab.* **5**, 317–320.
- Hemarajata, P. & Versalovic, J. (2013) Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therap. Adv. Gastroenterol.* **6**, 39–51.

- Henriques-Normark, B. & Normark, S. (2010) Commensal pathogens, with a focus on *Streptococcus pneumoniae*, and interactions with the human host. *Exp. Cell Res.* **316**, 1408–1414.
- Hicks, A.L. *et al.* (2018) Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nat. Commun.* **9**, 1786.
- Hidaka, B.H. (2012) Depression as a disease of modernity: explanations for increasing prevalence. *J. Affect. Disord.* **140**, 205–214.
- Hilimire, M.R., DeVlyder, J.E. & Forestell, C.A. (2015) Fermented foods, neuroticism, and social anxiety: an interaction model. *Psychiatry Res.* **228**, 203–208.
- Hill, D.A. & Artis, D. (2010) Intestinal bacteria and the regulation of immune cell homeostasis. *Annu. Rev. Immunol.* **28**, 623–667.
- Hill, T.C.J., Walsh, K.A., Harris, J.A. & Moffett, B.F. (2003) Using ecological diversity measures with bacterial communities. *FEMS Microbiol. Ecol.* **43**, 1–11.
- Hoban, A.E. *et al.* (2016a) Regulation of prefrontal cortex myelination by the microbiota. *Transl. Psychiatry* **6**, e774.
- Hoban, A.E. *et al.* (2016b) Behavioural and neurochemical consequences of chronic gut microbiota depletion during adulthood in the rat. *Neuroscience* **339**, 463–477.
- Hoban, A.E. *et al.* (2017) Microbial regulation of microRNA expression in the amygdala and prefrontal cortex. *Microbiome* **5**, 102.
- Hoffmann, D. *et al.* (2017) Improving regulation of microbiota transplants. *Science* **358**, 1390–1391.
- Hold, G.L., Schwartz, A., Aminov, R.I., Blaut, M. & Flint, H.J. (2003) Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. *Appl. Environ. Microbiol.* **69**, 4320–4324.
- Holden, J.E., Jeong, Y. & Forrest, J.M. (2005) The endogenous opioid system and clinical pain management. *AACN Clin. Issues* **16**, 291–301.
- Holt-Lunstad, J., Smith, T.B. & Layton, J.B. (2010) Social relationships and mortality risk: a meta-analytic review. *PLoS Med.* **7**, e1000316.
- Holzer, P. (2011) Opioid receptors in the gastrointestinal tract. *Regul. Pept.* **155**, 11–17.
- Hooper, L.V. (2009) Do symbiotic bacteria subvert host immunity? *Nat. Rev. Microbiol.* **7**, 367–374.
- Hooper, L.V., Midtvedt, T. & Gordon, J.I. (2002) How host–microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* **22**, 283–307.
- Hooper, L.V., Littman, D.R. & Macpherson, A.J. (2012) Interactions between the microbiota and the immune system. *Science* **336**, 1268–1273.
- Hou, Q. *et al.* (2016) Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. *Sci. Rep.* **6**, 37376.
- Hsiao, E.Y. (2013) Immune dysregulation in autism spectrum disorder. *Int Rev Neurobiol* **113**, 269–302.
- Hsiao, E.Y. (2014) Gastrointestinal issues in autism spectrum disorder. *Harv Rev Psychiatry* **22**, 104–111.
- Hsiao, E.Y. *et al.* (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155**, 1451–1463.
- Hsu, D.T. *et al.* (2013) Response of the μ -opioid system to social rejection and acceptance. *Mol. Psychiatry* **18**, 1211–1217.

- Hsu, D.T. *et al.* (2015) It still hurts: altered endogenous opioid activity in the brain during social rejection and acceptance in major depressive disorder. *Mol. Psychiatry* **20**, 193–200.
- Hughes, D.P. *et al.* (2011) Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. *BMC Ecol.* **11**, 13.
- Hughes, K.R. *et al.* (2017) *Bifidobacterium breve* reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner. *Open Biol.* **7**, 160155.
- Inagaki, T.K., Irwin, M.R. & Eisenberger, N.I. (2015) Blocking opioids attenuates physical warmth-induced feelings of social connection. *Emotion* **15**, 494–500.
- Inoue, R. *et al.* (2016) A preliminary investigation on the relationship between gut microbiota and gene expressions in peripheral mononuclear cells of infants with autism spectrum disorders. *Biosci. Biotechnol. Biochem.* **80**, 2450–2458.
- Isler, K. & van Schaik, C. (2006) Costs of encephalization: the energy trade-off hypothesis tested on birds. *J. Hum. Evol.* **51**, 228–243.
- Iyer, L.M., Aravind, L., Coon, S.L., Klein, D.C. & Koonin, E.V. (2004) Evolution of cell–cell signaling in animals: did late horizontal gene transfer from bacteria have a role? *Trends Genet.* **20**, 292–299.
- Jakobsson, H.E. *et al.* (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* **5**, e9836.
- Janda, J.M. & Abbott, S.L. (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J. Clin. Microbiol.* **45**, 2761–2764.
- Jang, H.-M., Lee, H., Jang, S.-E., Han, M.J. & Kim, D.-H. (2018) Evidence for interplay among antibacterial-induced gut microbiota disturbance, neuro-inflammation, and anxiety in mice. *Mucosal Immunol.* **11**, 1386–1397.
- Jašarević, E., Morrison, K.E. & Bale, T.L. (2016) Sex differences in the gut microbiome–brain axis across the lifespan. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150122.
- Jerison, H.J. & Barlow, H.B. (1985) Animal intelligence as encephalization. *Philos. Trans. R. Soc. B Biol. Sci.* **308**, 21–35.
- Jernberg, C., Löfmark, S., Edlund, C. & Jansson, J.K. (2007) Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J.* **1**, 56–66.
- Jiang, H. *et al.* (2015) Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* **48**, 186–194.
- Jiang, T.T. *et al.* (2017) Commensal fungi recapitulate the protective benefits of intestinal bacteria. *Cell Host Microbe* **22**, 809–816.e4.
- Johnson, K.V.-A. & Burnet, P.W.J. (2016) Microbiome: should we diversify from diversity? *Gut Microbes* **7**, 455–458.
- Johnson, K.V.-A. & Dunbar, R.I.M. (2016) Pain tolerance predicts human social network size. *Sci. Rep.* **6**, 25267.
- Johnson, K.V.-A. & Foster, K.R. (2018) Why does the microbiome affect behaviour? *Nat. Rev. Microbiol.* **16**, 647–655.
- Jovel, J. (2016) Characterization of the gut microbiome using 16S or shotgun metagenomics. *Front. Microbiol.* **7**, 459.
- Jumpertz, R. *et al.* (2011) Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am. J. Clin. Nutr.* **94**, 58–65.

- Kaelberer, M.M. *et al.* (2018) A gut–brain neural circuit for nutrient sensory transduction. *Science* **361**, eaat5236.
- Kamiya, T. *et al.* (2006) Inhibitory effects of *Lactobacillus reuteri* on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut* **55**, 191–196.
- Kang, D.-W. *et al.* (2013) Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLoS One* **8**, e68322.
- Kang, D.-W. *et al.* (2017) Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* **5**, 10.
- Kang, D.-W. *et al.* (2018) Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. *Anaerobe* **49**, 121–131.
- Karbowski, J. (2007) Global and regional brain metabolic scaling and its functional consequences. *BMC Biol.* **5**, 18.
- Karmarkar, D. & Rock, K.L. (2013) Microbiota signalling through MyD88 is necessary for a systemic neutrophilic inflammatory response. *Immunology* **140**, 483–492.
- Kayyal, M., Mian, F., Stanis, A., McVey Neufeld, K.-A. & Forsythe, P. (2018) Sex-dependent effects of postnatal exposure to low dose penicillin on the microbiota–gut–brain axis. *Neurogastroenterol. Motil.* **30** (Suppl. 1), abstract no. 211.
- Kekkonen, R.A. *et al.* (2008) Probiotic intervention has strain-specific anti-inflammatory effects in healthy adults. *World J. Gastroenterol.* **14**, 2029–2036.
- Kelly, D. *et al.* (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear–cytoplasmic shuttling of PPAR- γ and RelA. *Nat. Immunol.* **5**, 104–112.
- Kelly, J.R. *et al.* (2016) Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* **82**, 109–118.
- Kennedy, D.P. & Adolphs, R. (2012) The social brain in psychiatric and neurological disorders. *Trends Cogn. Sci.* **16**, 559–572.
- Kennedy, S.E., Koeppe, R.A., Young, E.A. & Zubieta, J.-K. (2006) Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. *Arch. Gen. Psychiatry* **63**, 1199–1208.
- Kernbauer, E., Ding, Y. & Cadwell, K. (2014) An enteric virus can replace the beneficial function of commensal bacteria. *Nature* **516**, 94–98.
- Kim, H.J. *et al.* (2015) Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc. Natl. Acad. Sci. USA* **113**, E7–E15.
- Kiraly, D.D. *et al.* (2016) Alterations of the host microbiome affect behavioral responses to cocaine. *Sci. Rep.* **6**, 35455.
- Kleiman, S.C. *et al.* (2017) The gut–brain axis in healthy females: lack of significant association between microbial composition and diversity with psychiatric measures. *PLoS One* **12**, e0170208.
- Klein, D.N., Durbin, C.E. & Shankman, S.A. (2009) Personality and mood disorders. *Handbook of Depression*, eds Gotlib, I.H. & Hammen, C.L., 93–112 (Guilford Press, New York).
- Klein, S.L. (2003) Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiol. Behav.* **79**, 441–449.
- Koch, H. & Schmid-Hempel, P. (2011) Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc. Natl. Acad. Sci. USA* **108**, 19288–19292.
- Koedel, U. *et al.* (2004) MyD88 is required for mounting a robust host immune response to *Streptococcus pneumoniae* in the CNS. *Brain* **127**, 1437–1445.

- Koenig, J.E. *et al.* (2011) Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl. Acad. Sci. USA* **108** (Suppl. 1), 4578–4585.
- Koh, A., de Vadder, F., Kovatcheva-Datchary, P. & Bäckhed, F. (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165**, 1332–1345.
- Koliada, A. *et al.* (2017) Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol.* **17**, 120.
- Koloski, N.A., Jones, M. & Talley, N.J. (2016) Evidence that independent gut-to-brain and brain-to-gut pathways operate in the irritable bowel syndrome and functional dyspepsia: a 1-year population-based prospective study. *Aliment. Pharmacol. Ther.* **44**, 592–600.
- Kommineni, S. *et al.* (2015) Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* **526**, 719–722.
- Konturek, S., Konturek, J., Pawlik, T. & Brzozowski, T. (2004) Brain–gut axis and its role in the control of food intake. *J. Physiol. Pharmacol.* **55**, 137–154.
- Koropatkin, N.M., Cameron, E.A. & Martens, E.C. (2012) How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Microbiol.* **10**, 323–335.
- Koshimizu, T.A. *et al.* (2012) Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol. Rev.* **92**, 1813–1864.
- Kotschal, A. *et al.* (2013) Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* **23**, 168–171.
- Koussounadis, A., Langdon, S.P., Um, I.H., Harrison, D.J. & Smith, V.A. (2015) Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci. Rep.* **5**, 10775.
- Krach, S., Paulus, F.M., Bodden, M. & Kircher, T. (2010) The rewarding nature of social interactions. *Front. Behav. Neurosci.* **4**, 22.
- Krajmalnik-Brown, R., Lozupone, C.A., Kang, D.-W. & Adams, J.B. (2015) Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease. *Microb. Ecol. Health Dis.* **26**, 26914.
- Krause, J. & Ruxton, G.D. (2002) *Living in Groups* (Oxford University Press, Oxford).
- Kristensen, N.B. *et al.* (2016) Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Med.* **8**, 52.
- Kubinak, J.L. *et al.* (2015) MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. *Cell Host Microbe* **17**, 153–163.
- Kudo, H. & Dunbar, R.I.M. (2001) Neocortex size and social network size in primates. *Anim. Behav.* **62**, 711–722.
- Kurokawa, S. *et al.* (2018) The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: an open-label observational study. *J. Affect. Disord.* **235**, 506–512.
- Labus, J.S. *et al.* (2017) Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. *Microbiome* **5**, 49.
- Lach, G., Schellekens, H., Dinan, T.G. & Cryan, J.F. (2018) Anxiety, depression, and the microbiome: a role for gut peptides. *Neurotherapeutics* **15**, 36–59.
- Lagier, J.C. (2014) Faecal microbiota transplantation: from practice to legislation before considering industrialization. *Clin. Microbiol. Infect.* **20**, 1112–1118.

- Lamblin, M., Murawski, C., Whittle, S. & Fornito, A. (2017) Social connectedness, mental health and the adolescent brain. *Neurosci. Biobehav. Rev.* **80**, 57–68.
- Larsbrink, J. *et al.* (2014) A discrete genetic locus confers xyloglucan metabolism in select human gut Bacteroidetes. *Nature* **506**, 498–502.
- Laterra, J., Keep, R., Betz, L.A. & Goldstein, G.W. (1999) Blood–brain–cerebrospinal fluid barriers. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, eds Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K. & Uhler, M.D., 671–690, 6th ed. (Lippincott-Raven, Philadelphia).
- Lawley, T.D. *et al.* (2012) Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing *Clostridium difficile* disease in mice. *PLoS Pathog.* **8**, e1002995.
- Lax, S. *et al.* (2014) Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science* **345**, 1048–1052.
- Leclercq, S. *et al.* (2017) Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *Nat. Commun.* **8**, 15062.
- Lenroot, R.K. & Giedd, J.N. (2006) Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci. Biobehav. Rev.* **30**, 718–729.
- Lewin-Epstein, O., Aharonov, R. & Hadany, L. (2017) Microbes can help explain the evolution of host altruism. *Nat. Commun.* **8**, 14040.
- Lewis, K.P. (2001) A comparative study of primate play behaviour: implications for the study of cognition. *Folia Primatol.* **71**, 417–421.
- Ley, R.E., Turnbaugh, P.J., Klein, S. & Gordon, J.I. (2006a) Microbial ecology: human gut microbes associated with obesity. *Nature* **444**, 1022–1023.
- Ley, R.E., Peterson, D.A. & Gordon, J.I. (2006b) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**, 837–848.
- Ley, R.E. *et al.* (2008) Evolution of mammals and their gut microbes. *Science* **320**, 1647–1651.
- Li, H. *et al.* (2016a) Pika population density is associated with the composition and diversity of gut microbiota. *Front. Microbiol.* **7**, 758.
- Li, L., Kong, X., Liu, H. & Liu, C. (2007) Systemic oxytocin and vasopressin excite gastrointestinal motility through oxytocin receptor in rabbits. *Neurogastroenterol. Motil.* **19**, 839–844.
- Li, L. *et al.* (2016b) Gut microbes in correlation with mood: case study in a closed experimental human life support system. *Neurogastroenterol. Motil.* **28**, 1233–1240.
- Li, Q. & Zhou, J.M. (2016) The microbiota–gut–brain axis and its potential therapeutic role in autism spectrum disorder. *Neuroscience* **324**, 131–139.
- Li, T., Wang, P., Wang, S.C. & Wang, Y.-F. (2017) Approaches mediating oxytocin regulation of the immune system. *Front. Immunol.* **7**, 693.
- Liao, W.B., Lou, S.L., Zeng, Y. & Kotschal, A. (2016) Large brains, small guts: the expensive tissue hypothesis supported within anurans. *Am. Nat.* **188**, 693–700.
- Lin, P. *et al.* (2017) *Prevotella* and *Klebsiella* proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. *J. Affect. Disord.* **207**, 300–304.
- Ling, Z. *et al.* (2014) Altered fecal microbiota composition associated with food allergy in infants. *Appl. Environ. Microbiol.* **80**, 2546–2554.
- Liu, X.-J. *et al.* (2016) TLR signaling adaptor protein MyD88 in primary sensory neurons contributes to persistent inflammatory and neuropathic pain and neuroinflammation. *Sci. Rep.* **6**, 28188.

- Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25**, 402–408.
- Lloyd-Price, J., Abu-Ali, G. & Huttenhower, C. (2016) The healthy human microbiome. *Genome Med.* **8**, 51.
- Loan, T., Nguyen, A., Vieira-Silva, S., Liston, A. & Raes, J. (2015) How informative is the mouse for human gut microbiota research? *Dis. Model. Mech.* **8**, 1–16.
- Logan, S.L. *et al.* (2018) The *Vibrio cholerae* type VI secretion system can modulate host intestinal mechanics to displace gut bacterial symbionts. *Proc. Natl. Acad. Sci. USA* **115**, E3779–E3787.
- Lopez-Gimenez, J.F., Vilaró, M.T. & Milligan, G. (2008) Morphine desensitization, internalization, and down-regulation of the mu opioid receptor is facilitated by serotonin 5-hydroxytryptamine_{2A} receptor coactivation. *Mol. Pharmacol.* **74**, 1278–1291.
- Lopez-Siles, M. *et al.* (2012) Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Appl. Environ. Microbiol.* **78**, 420–428.
- Loseth, G.E., Ellingsen, D.-M. & Leknes, S. (2014) State-dependent μ -opioid modulation of social motivation. *Front. Behav. Neurosci.* **8**, 430.
- Louca, S. *et al.* (2018) Function and functional redundancy in microbial systems. *Nat. Ecol. Evol.* **2**, 936–943.
- Louis, P. & Flint, H.J. (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **294**, 1–8.
- Louveau, A. *et al.* (2015) Structural and functional features of central nervous system lymphatic vessels. *Nature* **523**, 337–341.
- Lozupone, C.A. & Knight, R. (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228–8235.
- Lozupone, C.A., Hamady, M., Kelley, S.T. & Knight, R. (2007) Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* **73**, 1576–1585.
- Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. & Knight, R. (2012) Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220–230.
- Lu, H.P. *et al.* (2014) Spatial heterogeneity of gut microbiota reveals multiple bacterial communities with distinct characteristics. *Sci. Rep.* **4**, 6185.
- Lu, J. *et al.* (2018) Microbiota influence the development of the brain and behaviors in C57BL/6J mice. *PLoS One* **13**, e0201829.
- Luk, B. *et al.* (2018) Postnatal colonization with human “infant-type” *Bifidobacterium* species alters behavior of adult gnotobiotic mice. *PLoS One* **13**, e0196510.
- Luna, R.A. *et al.* (2017) Distinct microbiome–neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum disorder. *Cell. Mol. Gastroenterol. Hepatol.* **3**, 218–230.
- Lurie, I., Yang, Y., Haynes, K., Mamtani, R. & Boursi, B. (2015) Antibiotic exposure and the risk for depression, anxiety, or psychosis: a nested case-control study. *J. Clin. Psychiatry* **76**, 1522–1528.
- Lyon, L. (2018) “All disease begins in the gut”: was Hippocrates right? *Brain* **141**, e20.
- Lyte, M. (2013) Microbial endocrinology in the microbiome–gut–brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathog.* **9**, e1003726.

- Lyte, M., Li, W., Opitz, N., Gaykema, R.P.A. & Goehler, L.E. (2006) Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. *Physiol. Behav.* **89**, 350–357.
- Lyte, M. & Brown, D.R. (2018) Evidence for PMAT- and OCT-like biogenic amine transporters in a probiotic strain of *Lactobacillus*: implications for interkingdom communication within the microbiota–gut–brain axis. *PLoS One* **13**, e0191037.
- Macfarlane, S. & Macfarlane, G.T. (2003) Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **62**, 67–72.
- Mach, N. & Fuster-Botella, D. (2017) Endurance exercise and gut microbiota: a review. *J. Sport Health Sci.* **6**, 179–197.
- Mackowiak, P.A. (2013) Recycling Metchnikoff: probiotics, the intestinal microbiome and the quest for long life. *Front. Public Health* **1**, 52.
- Madlon-Kay, S., Brent, L.J.N., Montague, M.J., Heller, K.A. & Platt, M.L. (2017) Using machine learning to discover latent social phenotypes in free-ranging macaques. *Brain Sci.* **7**, 91.
- Maes, M., Kubera, M. & Leunis, J.-C. (2008) The gut–brain barrier in major depression: translocation of LPS from gram negative enterobacteria plays a role in the inflammatory pathophysiology of depression. *Neuroendocrinol. Lett.* **29**, 117–124.
- Maes, M., Kubera, M., Leunis, J.C. & Berk, M. (2012) Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J. Affect. Disord.* **141**, 55–62.
- Maestripieri, D. & Hoffman, C.L. (2012) Behavior and social dynamics of rhesus macaques on Cayo Santiago. *Bones, Genetics, and Behavior of Rhesus Macaques: Macaca mulatta of Cayo Santiago and Beyond*, ed. Wang, Q., 247–262 (Springer, New York).
- Maltz, R. *et al.* (2017) Social stressor exposure in C57BL/6 mice impacts inflammation, microbiota, short chain fatty acids in a murine model of colitis. *Inflamm. Bowel Dis.* **23** (Suppl. 1), S103–S104.
- Mangalam, A. *et al.* (2017) Human gut-derived commensal bacteria suppress CNS inflammatory and demyelinating disease. *Cell Rep.* **20**, 1269–1277.
- Manzke, T. *et al.* (2009) Serotonin targets inhibitory synapses to induce modulation of network functions. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 2589–2602.
- Mao, Y.-K. *et al.* (2013) *Bacteroides fragilis* polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.* **4**, 1465.
- Marchesi, J.R. & Ravel, J. (2015) The vocabulary of microbiome research: a proposal. *Microbiome* **3**, 31.
- Marietta, E.V. *et al.* (2016) Human gut-derived *Prevotella histicola* suppresses inflammatory arthritis in humanized mice. *Arthritis Rheumatol.* **68**, 2878–2888.
- Marin, I.A. *et al.* (2017) Microbiota alteration is associated with the development of stress-induced despair behavior. *Sci. Rep.* **7**, 43859.
- Marino, S., Baxter, N.T., Huffnagle, G.B., Petrosino, J.F. & Schloss, P.D. (2014) Mathematical modeling of primary succession of murine intestinal microbiota. *Proc. Natl. Acad. Sci. USA* **111**, 439–444.
- Markel, T.A. *et al.* (2007) The struggle for iron: gastrointestinal microbes modulate the host immune response during infection. *J. Leukoc. Biol.* **81**, 393–400.
- Martens, E.C. *et al.* (2011) Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biol.* **9**, e1001221.
- Martin, R. *et al.* (2016) Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PLoS One* **11**, e0158498.

- Martín, R. *et al.* (2015) *Faecalibacterium prausnitzii* prevents physiological damages in a chronic low-grade inflammation murine model. *BMC Microbiol.* **15**, 67.
- May, G. & Nelson, P. (2014) Defensive mutualisms: do microbial interactions within hosts drive the evolution of defensive traits? *Funct. Ecol.* **28**, 356–363.
- May, S., Evans, S. & Parry, L. (2017) Organoids, organs-on-chips and other systems, and microbiota. *Emerg. Top. Life Sci.* **1**, 385–400.
- Mayer, E.A. (2011) Gut feelings: the emerging biology of gut–brain communication. *Nat. Rev. Neurosci.* **12**, 453–466.
- Mayer, E.A., Knight, R., Mazmanian, S.K., Cryan, J.F. & Tillisch, K. (2014) Gut microbes and the brain: paradigm shift in neuroscience. *J. Neurosci.* **34**, 15490–15496.
- Maynard Smith, J. & Szathmáry, E. (1997) *The Major Transitions in Evolution* (Oxford University Press, Oxford).
- de Mazancourt, C., Loreau, M. & Dieckmann, U. (2005) Understanding mutualism when there is adaptation to the partner. *J. Ecol.* **93**, 305–314.
- Mazmanian, S.K. & Kasper, D.L. (2006) The love–hate relationship between bacterial polysaccharides and the host immune system. *Nat. Rev. Immunol.* **6**, 849–858.
- McCall, C. & Singer, T. (2012) The animal and human neuroendocrinology of social cognition, motivation and behavior. *Nat. Neurosci.* **15**, 681–688.
- McCrae, R.R. & John, O.P. (1992) An introduction to the five-factor model and its applications. *J. Pers.* **60**, 175–215.
- McCrae, R.R. & Costa, P.T. (1997) Personality trait structure as a human universal. *Am. Psychol.* **52**, 509–516.
- McCrae, R.R., Costa, P., Ostendorf, F. & Angleitner, A. (1999) Age differences in personality across the adult lifespan. *Dev. Psychol.* **35**, 466–477.
- McCusker, R.H. & Kelley, K.W. (2013) Immune–neural connections: how the immune system’s response to infectious agents influences behavior. *J. Exp. Biol.* **216**, 84–98.
- McDonald, C., Inohara, N. & Nuñez, G. (2005) Peptidoglycan signaling in innate immunity and inflammatory disease. *J. Biol. Chem.* **280**, 20177–20180.
- McFall-Ngai, M.J. *et al.* (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. USA* **110**, 3229–3236.
- McGovern, P.E. *et al.* (2004) Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. USA* **101**, 17593–17598.
- McInnes, P. & Cutting, M. (2010) *Manual of Procedures for Human Microbiome Project: Core Microbiome Sampling*, Protocol A, HMP Protocol no. 07-001, Version 11.
- McKean, J., Naug, H., Nikbakht, E., Amiet, B. & Colson, N. (2017) Probiotics and subclinical psychological symptoms in healthy participants: a systematic review and meta-analysis. *J. Altern. Complement. Med.* **23**, 249–258.
- McLoughlin, K., Schluter, J., Rakoff-Nahoum, S., Smith, A.L. & Foster, K.R. (2016) Host selection of microbiota via differential adhesion. *Cell Host Microbe* **19**, 550–559.
- McMurdie, P.J. & Holmes, S. (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**, e61217.
- McMurdie, P.J. & Holmes, S. (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput. Biol.* **10**, e1003531.

- McVey Neufeld, K.-A., Kang, N., Bienenstock, J. & Foster, J.A. (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol. Motil.* **23**, 255–264.
- Meadow, J.F. *et al.* (2015) Humans differ in their personal microbial cloud. *PeerJ* **3**, e1258.
- Mehta, R.S. *et al.* (2018) Stability of the human faecal microbiome in a cohort of adult men. *Nat. Microbiol.* **3**, 347–355.
- Meltzer, A. & van de Water, J. (2017) The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology* **42**, 284–298.
- le Merrer, J., Becker, J.A.J., Befort, K. & Kieffer, B.L. (2009) Reward processing by the opioid system in the brain. *Physiol Rev* **89**, 1379–1412.
- Messaoudi, M. *et al.* (2011) Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br. J. Nutr.* **105**, 755–764.
- Méthot, P.-O. & Alizon, S. (2014) What is a pathogen? Toward a process view of host–parasite interactions. *Virulence* **5**, 775–785.
- Miller, A.H., Haroon, E., Raison, C.L. & Felger, J.C. (2013) Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress. Anxiety* **30**, 297–306.
- Mink, J.W., Blumenshine, R.J. & Adams, D.B. (1981) Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *Am. J. Physiol.* **241**, R203–R212.
- Miquel, S. *et al.* (2013) *Faecalibacterium prausnitzii* and human intestinal health. *Curr. Opin. Microbiol.* **16**, 255–261.
- Mirzaei, M.K. & Maurice, C.F. (2017) Ménage à trois in the human gut: interactions between host, bacteria and phages. *Nat. Rev. Microbiol.* **15**, 397–408.
- Mitri, S. & Foster, K.R. (2013) The genotypic view of social interactions in microbial communities. *Annu. Rev. Genet.* **47**, 247–273.
- Mitri, S. & Foster, K.R. (2016) Pleiotropy and the low cost of individual traits promote cooperation. *Evolution* **70**, 488–494.
- Modi, S.R., Collins, J.J. & Relman, D.A. (2014) Antibiotics and the gut microbiota. *J. Clin. Invest.* **124**, 4212–4218.
- Moeller, A.H. *et al.* (2016) Social behavior shapes the chimpanzee pan-microbiome. *Sci. Adv.* **2**, e1500997.
- Moles, A., Kieffer, B.L. & D’Amato, F.R. (2004) Deficit in attachment behavior in mice lacking the μ -opioid receptor gene. *Science* **304**, 1983–1986.
- Monstein, H.-J., Truedsson, M., Ryberg, A. & Ohlsson, B. (2008) Vasopressin receptor mRNA expression in the human gastrointestinal tract. *Eur. Surg. Res.* **40**, 34–40.
- Moore, J. (2013) An overview of parasite-induced behavioral alterations – and some lessons from bats. *J. Exp. Biol.* **216**, 11–17.
- Morgan, X.C. & Huttenhower, C. (2012) Human microbiome analysis. *PLoS Comput. Biol.* **8**, e1002808.
- Mountfort, D.O. & Pybus, V. (1992) Regulatory influences on the production of gamma-aminobutyric acid by a marine pseudomonad. *Appl. Environ. Microbiol.* **58**, 237–242.
- Moya, A. & Ferrer, M. (2016) Functional redundancy-induced stability of gut microbiota subjected to disturbance. *Trends Microbiol.* **24**, 402–413.

- Moynagh, P.N. (2005) TLR signalling and activation of IRFs: revisiting old friends from the NF- κ B pathway. *Trends Immunol.* **26**, 469–476.
- Muegge, B.D., Fontana, L., Henrissat, B., Knight, R. & Gordon, J.I. (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* **332**, 970–974.
- Muller, P.A. *et al.* (2014) Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* **158**, 300–313.
- Mulligan, A.A. *et al.* (2014) A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. *BMJ Open* **4**, e004503.
- Murillo-Rincon, A.P. *et al.* (2017) Spontaneous body contractions are modulated by the microbiome of *Hydra*. *Sci. Rep.* **7**, 15937.
- Murray, M.J. & Murray, A.B. (1979) Anorexia of infection as a mechanism of host defense. *Am. J. Clin. Nutr.* **32**, 593–596.
- Mussell, M. *et al.* (2008) Gastrointestinal symptoms in primary care: prevalence and association with depression and anxiety. *J. Psychosom. Res.* **64**, 605–612.
- Nadell, C.D., Drescher, K. & Foster, K.R. (2016) Spatial structure, cooperation and competition in biofilms. *Nat. Rev. Microbiol.* **14**, 589–600.
- Nakamura, N. *et al.* (2011) Analysis of the hydrogenotrophic microbiota of wild and captive black howler monkeys (*Alouatta pigra*) in Palenque National Park, Mexico. *Am. J. Primatol.* **73**, 909–919.
- Naseribafrouei, A. *et al.* (2014) Correlation between the human fecal microbiota and depression. *Neurogastroenterol. Motil.* **26**, 1155–1162.
- Navarrete, A., van Schaik, C.P. & Isler, K. (2011) Energetics and the evolution of human brain size. *Nature* **480**, 91–93.
- Navas-Molina, J.A. *et al.* (2013) Advancing our understanding of the human microbiome using QIIME. *Methods Enzymol.* **531**, 371–444.
- Needham, B.D., Tang, W. & Wu, W. (2018) Searching for the gut microbial contributing factors to social behavior in rodent models of autism spectrum disorder. *Dev. Neurobiol.* **78**, 474–499.
- Neish, A.S. *et al.* (2000) Prokaryotic regulation of epithelial responses by inhibition of I κ B- α ubiquitination. *Science* **289**, 1560–1563.
- Nelson, T.M., Rogers, T.L., Carlini, A.R. & Brown, M.V. (2013) Diet and phylogeny shape the gut microbiota of Antarctic seals: a comparison of wild and captive animals. *Environ. Microbiol.* **15**, 1132–1145.
- Neuman, H., Debelius, J.W., Knight, R. & Koren, O. (2015) Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiol. Rev.* **39**, 509–521.
- Neuman, H., Forsythe, P., Uzan, A., Avni, O. & Koren, O. (2018) Antibiotics in early life: dysbiosis and the damage done. *FEMS Microbiol. Rev.* **42**, 489–499.
- Ninković, J. & Roy, S. (2013) Role of the mu-opioid receptor in opioid modulation of immune function. *Amino Acids* **45**, 9–24.
- Nummenmaa, L. *et al.* (2015) Adult attachment style is associated with cerebral μ -opioid receptor availability in humans. *Hum. Brain Mapp.* **36**, 3621–3628.
- Nummenmaa, L. & Tuominen, L. (2018) Opioid system and human emotions. *Br. J. Pharmacol.* **175**, 2737–2749.

- Nunn, C.L., Craft, M.E., Gillespie, T.R., Schaller, M. & Kappeler, P.M. (2015) The sociality–health–fitness nexus: synthesis, conclusions and future directions. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140115.
- O'Mahony, S.M., Dinan, T.G. & Cryan, J.F. (2017) The gut microbiota as a key regulator of visceral pain. *Pain* **158** (Suppl. 1), S19–S28.
- O'Leary, O.F. *et al.* (2018) The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus. *Eur. Neuropsychopharmacol.* **28**, 307–316.
- O'Mahony, S.M. *et al.* (2014) Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience* **277**, 885–901.
- O'Mahony, S.M., Clarke, G., Borre, Y.E., Dinan, T.G. & Cryan, J.F. (2015) Serotonin, tryptophan metabolism and the brain–gut–microbiome axis. *Behav. Brain Res.* **277**, 32–48.
- O'Malley, D. (2016) Neuroendocrine and neuroimmune pathways contribute to the pathophysiology of irritable bowel syndrome. *Am. J. Physiol. Gastrointest. Liver Physiol.* **311**, G934–G941.
- Odamaki, T. *et al.* (2018) Genomic diversity and distribution of *Bifidobacterium longum* subsp. *longum* across the human lifespan. *Sci. Rep.* **8**, 85.
- Ohland, C.L. *et al.* (2013) Effects of *Lactobacillus helveticus* on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology* **38**, 1738–1747.
- Orkin, J.D. *et al.* (2018) Seasonality of the gut microbiota of free-ranging white-faced capuchins in a tropical dry forest. *ISME J.* doi: 10.1038/s41396-018-0256-0.
- Ortega-González, M. *et al.* (2014) Nondigestible oligosaccharides exert nonprebiotic effects on intestinal epithelial cells enhancing the immune response via activation of TLR4-NFκB. *Mol. Nutr. Food Res.* **58**, 384–393.
- Pagnini, C. *et al.* (2010) Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc. Natl. Acad. Sci. USA* **107**, 454–459.
- Painold, A. *et al.* (2018) A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. *Bipolar Disord.* doi: 10.1111/bdi.12682.
- de Palma, G. *et al.* (2017) Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. *Sci. Transl. Med.* **9**, eaaf6397.
- Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A & Brown, P.O. (2007) Development of the human infant intestinal microbiota. *PLoS Biol.* **5**, e177.
- Pannebakker, B.A., Loppin, B., Elemans, C.P.H., Humblot, L. & Vavre, F. (2007) Parasitic inhibition of cell death facilitates symbiosis. *Proc. Natl. Acad. Sci. USA* **104**, 213–215.
- Papathoma, E., Triga, M., Fouzas, S. & Dimitriou, G. (2016) Cesarean section delivery and development of food allergy and atopic dermatitis in early childhood. *Pediatr. Allergy Immunol.* **27**, 419–424.
- Park, C. *et al.* (2018) Probiotics for the treatment of depressive symptoms: an anti-inflammatory mechanism? *Brain Behav. Immun.* **73**, 115–124.
- Parracho, H.M.R.T., Bingham, M.O., Gibson, G.R. & McCartney, A.L. (2005) Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J. Med. Microbiol.* **54**, 987–991.
- Partrick, K.A. *et al.* (2018) Acute and repeated exposure to social stress reduces gut microbiota diversity in Syrian hamsters. *Behav. Brain Res.* **345**, 39–48.

- Pärty, A., Kalliomäki, M., Wacklin, P., Salminen, S. & Isolauri, E. (2015) A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatr. Res.* **77**, 823–828.
- Paschos, G.K. & FitzGerald, G.A. (2017) Circadian clocks and metabolism: implications for microbiome and aging. *Trends Genet.* **33**, 760–769.
- Pearce, E., Wlodarski, R., Machin, A. & Dunbar, R.I.M. (2017) Variation in the β -endorphin, oxytocin, and dopamine receptor genes is associated with different dimensions of human sociality. *Proc. Natl. Acad. Sci. USA* **114**, 5300–5305.
- Pellissier, L.P., Gandía, J., Laboute, T., Becker, J.A.J. & le Merrer, J. (2018) μ opioid receptor, social behaviour and autism spectrum disorder: reward matters. *Br. J. Pharmacol.* **175**, 2750–2769.
- Penders, J. *et al.* (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* **118**, 511–521.
- Pennock, R.L. & Hentges, S.T. (2011) Differential expression and sensitivity of pre- and postsynaptic opioid receptors regulating hypothalamic proopiomelanocortin neurons. *J. Neurosci.* **31**, 281–288.
- Pérez-Barbería, F.J., Shultz, S. & Dunbar, R.I.M. (2007) Evidence for coevolution of sociality and relative brain size in three orders of mammals. *Evolution* **61**, 2811–2821.
- Pérez-Burgos, A. *et al.* (2013) Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304**, G211–G220.
- Perofsky, A.C., Lewis, R.J., Abondano, L.A., di Fiore, A. & Meyers, L.A. (2017) Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka. *Proc. R. Soc. B Biol. Sci.* **284**, 20172274.
- Perrot-Minnot, M.-J. & Cézilly, F. (2013) Investigating candidate neuromodulatory systems underlying parasitic manipulation: concepts, limitations and prospects. *J. Exp. Biol.* **216**, 134–141.
- Peter, J. *et al.* (2018) A microbial signature of psychological distress in irritable bowel syndrome. *Psychosom Med* **80**, 698–709.
- Pietras, E.M. *et al.* (2011) A MyD88-dependent IFN γ R-CCR2 signaling circuit is required for mobilization of monocytes and host defense against systemic bacterial challenge. *Cell Res.* **21**, 1068–1079.
- Pike, L.J. & Forster, S.C. (2018) A new piece in the microbiome puzzle. *Nat. Rev. Microbiol.* **16**, 186.
- Pinto-Sanchez, M.I. *et al.* (2017) Probiotic *Bifidobacterium longum* NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. *Gastroenterology* **153**, 448–459.e8.
- Pirbaglou, M. *et al.* (2016) Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. *Nutr. Res.* **36**, 889–898.
- Pokusaeva, K. *et al.* (2017) GABA-producing *Bifidobacterium dentium* modulates visceral sensitivity in the intestine. *Neurogastroenterol. Motil.* **29**, e12904.
- Pollak, Y. & Yirmiya, R. (2002) Cytokine-induced changes in mood and behaviour: implications for “depression due to a general medical condition”, immunotherapy and antidepressive treatment. *Int. J. Neuropsychopharmacol.* **5**, 389–399.
- Pontzer, H. *et al.* (2012) Hunter-gatherer energetics and human obesity. *PLoS One* **7**, e40503.
- Porges, E.C. *et al.* (2017) Frontal gamma-aminobutyric acid concentrations are associated with cognitive performance in older adults. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **2**, 38–44.
- Postler, T.S. & Ghosh, S. (2017) Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. *Cell Metab.* **26**, 110–130.

- Poulin, R. (1995) “Adaptive” changes in the behaviour of parasitized animals: a critical review. *Int. J. Parasitol.* **25**, 1371–1383.
- Poutahidis, T. *et al.* (2013) Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. *PLoS One* **8**, e78898.
- Powell, N., Walker, M.M. & Talley, N.J. (2017) The mucosal immune system: master regulator of bidirectional gut–brain communications. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 143–159.
- Power, R.A. & Pluess, M. (2015) Heritability estimates of the Big Five personality traits based on common genetic variants. *Transl. Psychiatry* **5**, e604.
- Praveen, P., Jordan, F., Priami, C. & Morine, M.J. (2015) The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome. *Microbiome* **3**, 41.
- Procaccini, C., Pucino, V., de Rosa, V., Marone, G. & Matarese, G. (2014) Neuro-endocrine networks controlling immune system in health and disease. *Front. Immunol.* **5**, 143.
- Prossin, A.R. *et al.* (2015) Dynamic interactions between plasma IL-1 family cytokines and central endogenous opioid neurotransmitter function in humans. *Neuropsychopharmacology* **40**, 554–565.
- Pulikkan, J. *et al.* (2018) Gut microbial dysbiosis in Indian children with autism spectrum disorders. *Microb. Ecol.* **76**, 1102–1114.
- Qin, J. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.
- Qiu, X., Zhang, M., Yang, X., Hong, N. & Yu, C. (2013) *Faecalibacterium prausnitzii* upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. *J. Crohn's Colitis* **7**, e558–e568.
- Quévrain, E. *et al.* (2015) Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut* **65**, 415–425.
- Quigley, E.M.M. (2011) Microflora modulation of motility. *J. Neurogastroenterol. Motil.* **17**, 140–147.
- R Development Core Team (2015) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna). Available at: <http://www.R-project.org>.
- Raichle, M.E. (2006) The brain's dark energy. *Science* **314**, 1249–1250.
- Rajilić-Stojanović, M. *et al.* (2011) Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* **141**, 1792–1801.
- Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. (2004) Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241.
- Ranjan, R., Rani, A., Metwally, A., McGee, H.S. & Perkins, D.L. (2016) Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem. Biophys. Res. Commun.* **469**, 967–977.
- Rao, M. & Gershon, M.D. (2016) The bowel and beyond: the enteric nervous system in neurological disorders. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 517–528.
- Rao, S. *et al.* (2017) Pathogen-mediated inhibition of anorexia promotes host survival and transmission. *Cell* **168**, 503–516.
- Rao, S.S.C., Rehman, A., Yu, S. & de Andino, N.M. (2018) Brain foggiess, gas and bloating: a link between SIBO, probiotics and metabolic acidosis. *Clin. Transl. Gastroenterol.* **9**, 162.
- Raulo, A. *et al.* (2017) Social behavior and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): in search of the role of immunity in the evolution of sociality. *J. Anim. Ecol.* **87**, 388–399.

- Rawlings, R.G. & Kessler, M.J. (1986) *The Cayo Santiago Macaques: History, Behavior & Biology* (State University of New York Press, New York).
- Rea, K., Dinan, T.G. & Cryan, J.F. (2016) The microbiome: a key regulator of stress and neuroinflammation. *Neurobiol. Stress* **4**, 23–33.
- Reader, S.M. & Laland, K.N. (2002) Social intelligence, innovation, and enhanced brain size in primates. *Proc. Natl. Acad. Sci. USA* **99**, 4436–4441.
- Reigstad, C.S. *et al.* (2015) Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* **29**, 1395–1403.
- Reikvam, D.H. *et al.* (2011) Depletion of murine intestinal microbiota: effects on gut mucosa and epithelial gene expression. *PLoS One* **6**, e17996.
- Remely, M. *et al.* (2015) Gut microbiota composition correlates with changes in body fat content due to weight loss. *Benef. Microbes* **6**, 431–439.
- Ren, T., Grieneisen, L.E., Alberts, S.C., Archie, E.A. & Wu, M. (2016) Development, diet and dynamism: longitudinal and cross-sectional predictors of gut microbial communities in wild baboons. *Environ. Microbiol.* **18**, 1312–1325.
- Resendez, S.L. *et al.* (2013) μ -Opioid receptors within subregions of the striatum mediate pair bond formation through parallel yet distinct reward mechanisms. *J. Neurosci.* **33**, 9140–9149.
- Rhee, S.H., Pothoulakis, C. & Mayer, E.A. (2009) Principles and clinical implications of the brain–gut–enteric microbiota axis. *Nat. Rev. Gastroenterol. Hepatol.* **6**, 306–314.
- Ribeiro, S.C., Kennedy, S.E., Smith, Y.R., Stohler, C.S. & Zubieta, J.-K. (2005) Interface of physical and emotional stress regulation through the endogenous opioid system and μ -opioid receptors. *Prog. Neuro-Psychopharmacology Biol. Psychiatry* **29**, 1264–1280.
- Riedel, C.U., Schwartz, A. & Egert, M. (2014) The stomach and small and large intestinal microbiomes. *The Human Microbiota and Microbiome*, ed. Marchesi, J.R., 1–19 (CABI, Wallingford).
- Rivera-Chávez, F. & Bäumler, A.J. (2015) The pyromaniac inside you: *Salmonella* metabolism in the host gut. *Annu. Rev. Microbiol.* **69**, 31–48.
- Roberts, S.G.B., Wilson, R., Fedurek, P. & Dunbar, R.I.M. (2008) Individual differences and personal social network size and structure. *Pers. Individ. Dif.* **44**, 954–964.
- Rogers, G.B. *et al.* (2016) From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. *Mol. Psychiatry* **21**, 738–748.
- Rook, G.A.W. (2012) Hygiene hypothesis and autoimmune diseases. *Clin. Rev. Allergy Immunol.* **42**, 5–15.
- Rook, G.A.W. & Lowry, C.A. (2008) The hygiene hypothesis and psychiatric disorders. *Trends Immunol.* **29**, 150–158.
- Rooks, M.G. & Garrett, W.S. (2016) Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.* **16**, 341–352.
- Rosenbaum, M., Knight, R. & Leibel, R.L. (2015) The gut microbiota in human energy homeostasis and obesity. *Trends Endocrinol. Metab.* **26**, 493–501.
- Roshchina, V.V. (2010) Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. *Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health*, eds Lyte, M. & Freestone, P.E., 17–52 (Springer, New York).
- Rosshart, S.P. *et al.* (2017) Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell* **171**, 1015–1028.

- Roth, G. & Dicke, U. (2005) Evolution of the brain and intelligence. *Trends Cogn. Sci.* **9**, 250–257.
- Rothschild, D. *et al.* (2018) Environmental factors dominate over host genetics in shaping human gut microbiota composition. *Nature* **555**, 210–215.
- Round, J.L. & Mazmanian, S.K. (2009) The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323.
- Rousseaux, C. *et al.* (2007) *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat. Med.* **13**, 35–37.
- Rowland, I. *et al.* (2018) Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* **57**, 1–24.
- Ruzich, E. *et al.* (2015) Measuring autistic traits in the general population: a systematic review of the Autism-Spectrum Quotient (AQ) in a nonclinical population sample of 6,900 typical adult males and females. *Mol. Autism* **6**, 2.
- Sachs, J.L., Skophammer, R.G. & Regus, J.U. (2011) Evolutionary transitions in bacterial symbiosis. *Proc. Natl. Acad. Sci. USA* **108**, 10800–10807.
- Sampson, T.R. & Mazmanian, S.K. (2015) Control of brain development, function, and behavior by the microbiome. *Cell Host Microbe* **17**, 565–576.
- Sana, T.G., Lugo, K.A. & Monack, D.M. (2017) T6SS: the bacterial “fight club” in the host gut. *PLoS Pathog.* **13**, e1006325.
- Sansonetti, P.J. & di Santo, J.P. (2007) Debugging how bacteria manipulate the immune response. *Immunity* **26**, 149–161.
- Sapolsky, R.M. (2005) The influence of social hierarchy on primate health. *Science* **308**, 648–652.
- Sarkar, A. *et al.* (2016) Psychobiotics and the manipulation of bacteria–gut–brain signals. *Trends Neurosci.* **39**, 763–781.
- Savignac, H.M., Kiely, B., Dinan, T.G. & Cryan, J.F. (2014) *Bifidobacteria* exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterol. Motil.* **26**, 1615–1627.
- Schino, G. (2001) Grooming, competition and social rank among female primates: a meta-analysis. *Anim. Behav.* **62**, 265–271.
- Schirmer, M. *et al.* (2018) Dynamics of metatranscription in the inflammatory bowel disease gut microbiome. *Nat. Microbiol.* **3**, 337–346.
- Schloss, P.D., Iverson, K.D., Petrosino, J.F. & Schloss, S.J. (2014) The dynamics of a family's gut microbiota reveal variations on a theme. *Microbiome* **2**, 25.
- Schluter, J. & Foster, K.R. (2012) The evolution of mutualism in gut microbiota via host epithelial selection. *PLoS Biol.* **10**, e1001424.
- Schmidt, K. *et al.* (2015) Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology* **232**, 1793–1801.
- Schmittgen, T.D. & Livak, K.J. (2008) Analyzing real-time PCR data by the comparative C_T method. *Nat. Protoc.* **3**, 1101–1108.
- Schnorr, S.L. *et al.* (2014) Gut microbiome of the Hadza hunter-gatherers. *Nat. Commun.* **5**, 3654.
- Schorscher-Petcu, A. *et al.* (2010) Oxytocin-induced analgesia and scratching are mediated by the vasopressin-1A receptor in the mouse. *J. Neurosci.* **30**, 8274–8284.
- Schwab, C., Cristescu, B., Boyce, M.S., Stenhouse, G.B. & Gänzle, M. (2009) Bacterial populations and metabolites in the feces of free roaming and captive grizzly bears. *Can. J. Microbiol.* **55**, 1335–1346.

- Schwarz, E. *et al.* (2017) Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophr. Res.* **192**, 398–403.
- Scott, K.A. *et al.* (2017) Revisiting Metchnikoff: age-related alterations in microbiota–gut–brain axis in the mouse. *Brain Behav. Immun.* **65**, 20–32.
- Segata, N. (2015) Gut microbiome: westernization and the disappearance of intestinal diversity. *Curr. Biol.* **25**, R611–R613.
- Segerstrom, S.C. (2000) Personality and the immune system: models, methods, and mechanisms. *Ann. Behav. Med.* **22**, 180–190.
- Selhub, E.M., Logan, A.C. & Bested, A.C. (2014) Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry. *J. Physiol. Anthropol.* **33**, 2.
- Sender, R., Fuchs, S. & Milo, R. (2016) Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* **164**, 337–340.
- Shaaban, S.Y. *et al.* (2017) The role of probiotics in children with autism spectrum disorder: a prospective, open-label study. *Nutr. Neurosci.* **21**, 676–681.
- Shapira, M. (2016) Gut microbiotas and host evolution: scaling up symbiosis. *Trends Ecol. Evol.* **31**, 539–549.
- Sharon, G., Sampson, T.R., Geschwind, D.H. & Mazmanian, S.K. (2016) The central nervous system and the gut microbiome. *Cell* **167**, 915–932.
- Shemesh, Y. *et al.* (2014) High-order social interactions in groups of mice. *eLife* **3**, e03602.
- Shen, Y. *et al.* (2018) Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophr. Res.* **197**, 470–477.
- Sheth, R.U., Cabral, V., Chen, S.P. & Wang, H.H. (2016) Manipulating bacterial communities by *in situ* microbiome engineering. *Trends Genet.* **32**, 189–200.
- Shimizu, K. *et al.* (2012) Monkeys in the middle: parasite transmission through the social network of a wild primate. *PLoS One* **7**, 15–21.
- Shiro, Y., Arai, Y.-C., Ikemoto, T. & Hayashi, K. (2017) Stool consistency is significantly associated with pain perception. *PLoS One* **12**, e0182859.
- Shultz, S. & Dunbar, R.I.M. (2006) Both social and ecological factors predict ungulate brain size. *Proc. R. Soc. B Biol. Sci.* **273**, 207–215.
- Shultz, S. & Dunbar, R.I.M. (2010) Encephalization is not a universal macroevolutionary phenomenon in mammals but is associated with sociality. *Proc. Natl. Acad. Sci. USA* **107**, 21582–21586.
- Silk, J.B. (2007) The adaptive value of sociality in mammalian groups. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 539–559.
- Silverman, J.L., Yang, M., Lord, C. & Crawley, J.N. (2010) Behavioural phenotyping assays for mouse models of autism. *Nat. Rev. Neurosci.* **11**, 490–502.
- Slykerman, R.F. *et al.* (2017) Antibiotics in the first year of life and subsequent neurocognitive outcomes. *Acta Paediatr.* **106**, 87–94.
- Smith, K., McCoy, K.D. & Macpherson, A.J. (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* **19**, 59–69.
- Smith, P.M. *et al.* (2013) The microbial metabolites, short-chain fatty acids, regulate colonic T_{reg} cell homeostasis. *Science* **341**, 569–573.

- Snyder-Mackler, N. *et al.* (2016) Social status alters immune regulation and response to infection. *Science* **354**, 1041–1045.
- Sokol, H. *et al.* (2008) *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl. Acad. Sci. USA* **105**, 16731–16736.
- Sommer, F. & Bäckhed, F. (2013) The gut microbiota – masters of host development and physiology. *Nat. Rev. Microbiol.* **11**, 227–238.
- Sommer, F. *et al.* (2016) The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. *Cell Rep.* **14**, 1655–1661.
- Son, J.S. *et al.* (2015) Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the Simons Simplex Collection. *PLoS One* **10**, e0137725.
- Song, S.J. *et al.* (2013) Cohabiting family members share microbiota with one another and with their dogs. *eLife* **2**, e00458.
- Song, Y., Liu, C. & Finegold, S.M. (2004) Real-time PCR quantitation of Clostridia in feces of autistic children. *Appl. Environ. Microbiol.* **70**, 6459–6465.
- Sonnenburg, E.D. & Sonnenburg, J.L. (2014) Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab.* **20**, 779–786.
- Sonnenburg, E.D. *et al.* (2016) Diet-induced extinctions in the gut microbiota compound over generations. *Nature* **529**, 212–215.
- Sonnenburg, J.L., Xu, J. & Leip, D.D. (2005) Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* **307**, 1955–1959.
- Sonnenburg, J.L. & Bäckhed, F. (2016) Diet–microbiota interactions as moderators of human metabolism. *Nature* **535**, 56–64.
- Soto, M. *et al.* (2018) Gut microbiota modulate neurobehavior through changes in brain insulin sensitivity and metabolism. *Mol. Psychiatry* **23**, 2287–2301.
- Spielberger, C.D. Gorsuch, R.L., Lushene, R., Vagg, P.R. & Jacobs, G.A. (1983) *Manual for the State–Trait Anxiety Inventory* (Consulting Psychologists Press, California).
- Springer, A. *et al.* (2017) Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (*Propithecus verreauxi*). *Ecol. Evol.* **7**, 5732–5745.
- Sprockett, D., Fukami, T. & Relman, D.A. (2018) Role of priority effects in the early-life assembly of the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 197–205.
- Srinivasan, R. *et al.* (2015) Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS One* **10**, e0117617.
- Stadlbauer, U., Woods, S.C., Langhans, W. & Meyer, U. (2015) PYY_{3–36}: beyond food intake. *Front. Neuroendocrinol.* **38**, 1–11.
- Stein, R.R. *et al.* (2013) Ecological modeling from time-series inference: insight into dynamics and stability of intestinal microbiota. *PLoS Comput. Biol.* **9**, e1003388.
- Steinman, L. (2004) Elaborate interactions between the immune and nervous systems. *Nat. Immunol.* **5**, 575–581.
- Stevenson, T.J., Duddlestone, K.N. & Buck, C.L. (2014) Effects of season and host physiological state on the diversity, density, and activity of the Arctic ground squirrel cecal microbiota. *Appl. Environ. Microbiol.* **80**, 5611–5622.

- Stewart, C.J. *et al.* (2017) Cesarean or vaginal birth does not impact the longitudinal development of the gut microbiome in a cohort of exclusively preterm infants. *Front. Microbiol.* **8**, 1008.
- Stewart, I., Schluter, P.J. & Shaw, G.R. (2006) Cyanobacterial lipopolysaccharides and human health – a review. *Environ Health* **5**, 7.
- Stiemsma, L.T., Reynolds, L.A., Turvey, S.E. & Finlay, B.B. (2015) The hygiene hypothesis: current perspectives and future therapies. *ImmunoTargets Ther.* **4**, 143–157.
- Stilling, R.M., Bordenstein, S.R., Dinan, T.G. & Cryan, J.F. (2014) Friends with social benefits: host–microbe interactions as a driver of brain evolution and development? *Front. Cell. Infect. Microbiol.* **4**, 147.
- Stilling, R.M. *et al.* (2016a) The neuropharmacology of butyrate: the bread and butter of the microbiota–gut–brain axis? *Neurochem. Int.* **99**, 110–132.
- Stilling, R.M., Dinan, T.G. & Cryan, J.F. (2016b) The brain's Geppetto – microbes as puppeteers of neural function and behaviour? *J. Neurovirol.* **22**, 14–21.
- Stilling, R.M. *et al.* (2018) Social interaction-induced activation of RNA splicing in the amygdala of microbiome-deficient mice. *eLife* **7**, e33070.
- Stokes, J. & Pillsbury, D. (1930) The effect on the skin of emotional and nervous states: theoretical and practical consideration of a gastrointestinal mechanism. *Arch Dermatol Syphilol.* **22**, 962–993.
- Strachan, D.P. (1989) Hay fever, hygiene, and household size. *Br. Med. J.* **299**, 1259–1260.
- Strandwitz, P. (2018) Neurotransmitter modulation by the gut microbiota. *Brain Res.* **1693**, 128–133.
- Strandwitz, P. *et al.* (2018) GABA-modulating bacteria of the human gut microbiota. *Nat. Microbiol.* doi: 10.1038/s41564-018-0307-3.
- Strati, F. *et al.* (2017) New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* **5**, 24.
- Sudo, N. *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J. Physiol.* **558**, 263–275.
- Suez, J. & Elinav, E. (2017) The path towards microbiome-based metabolite treatment. *Nat. Microbiol.* **2**, 17075.
- Sun, B. *et al.* (2016) Marked variation between winter and spring gut microbiota in free-ranging Tibetan Macaques (*Macaca thibetana*). *Sci. Rep.* **6**, 26035.
- Suzuki, T.A. & Worobey, M. (2014) Geographical variation of human gut microbial composition. *Biol. Lett.* **10**, 20131037.
- Suzuki, T.A. & Nachman, M.W. (2016) Spatial heterogeneity of gut microbial composition along the gastrointestinal tract in natural populations of house mice. *PLoS One* **11**, e0163720.
- Suzuki, T.A. (2017) Links between natural variation in the microbiome and host fitness in wild mammals. *Integr. Comp. Biol.* **57**, 756–769.
- Sylvia, K.E., Jewell, C.P., Rendon, N.M., St John, E.A. & Demas, G.E. (2017) Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters. *Brain Behav. Immun.* **60**, 51–62.
- Szyszkowicz, J.K., Wong, A., Anisman, H., Merali, Z. & Audet, M.-C. (2017) Implications of the gut microbiota in vulnerability to the social avoidance effects of chronic social defeat in male mice. *Brain Behav. Immun.* **66**, 45–55.
- Takada, M. *et al.* (2016) Probiotic *Lactobacillus casei* strain Shirota relieves stress-associated symptoms by modulating the gut–brain interaction in human and animal models. *Neurogastroenterol. Motil.* **28**, 1027–1036.

- Tao, R. & Auerbach, S.B. (2002) Opioid receptor subtypes differentially modulate serotonin efflux in the rat central nervous system. *Pharmacology* **303**, 549–556.
- Taylor, A.M., Edwards, C.G., Khan, N.A. & Holscher, H.D. (2017) Gastrointestinal microbiota and cognitive function in adult females. *FASEB J.* **31** (Suppl. 1), abstract no. 965.34.
- Taylor, B., Jick, H. & MacLaughlin, D. (2013) Prevalence and incidence rates of autism in the UK: time trend from 2004–2010 in children aged 8 years. *BMJ Open* **3**, e003219.
- Terekhova, D., Standwitz, P., Liston, C., Lewis, K. & Dubin, M. (2018) Abundance of GABA-producing gut bacteria correlates with functional brain connectivity in depression. *Biol. Psychiatry* **83** (Suppl.), S176.
- The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214.
- de Theije, C.G.M. *et al.* (2014) Altered gut microbiota and activity in a murine model of autism spectrum disorders. *Brain Behav. Immun.* **37**, 197–206.
- Thion, M.S. *et al.* (2018) Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell* **172**, 500–516.e16.
- Thomas, F., Adamo, S.A. & Moore, J. (2005) Parasitic manipulation: where are we and where should we go? *Behav. Processes* **68**, 185–199.
- Thomas, L.V., Suzuki, K. & Zhao, J. (2015) Probiotics: a proactive approach to health. A symposium report. *Br. J. Nutr.* **114** (Suppl. 1), S1–S15.
- Thompson, J.N. (1982) *Interaction and Coevolution* (University of Chicago Press, New York).
- Tillisch, K. *et al.* (2013) Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* **144**, 1394–1401.
- Tillisch, K. *et al.* (2017) Brain structure and response to emotional stimuli as related to gut microbial profiles in healthy women. *Psychosom. Med.* **79**, 905–913.
- Togher, K. *et al.* (2017) Depression-associated alterations in the maternal microbiome during pregnancy: priming for adverse infant outcomes? *Gut* **66** (Suppl. 1), A15–A16.
- Tomova, A. *et al.* (2015) Gastrointestinal microbiota in children with autism in Slovakia. *Physiol. Behav.* **138**, 179–187.
- Tremaroli, V. (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* **489**, 242–249.
- Trezza, V., Damsteegt, R., Achterberg, E.J.M. & Vanderschuren, L.J.M.J. (2011) Nucleus accumbens μ -opioid receptors mediate social reward. *J. Neurosci.* **31**, 6362–6370.
- Trompette, A. *et al.* (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20**, 159–166.
- Trosvik, P. *et al.* (2018) Multilevel social structure and diet shape the gut microbiota of the gelada monkey, the only grazing primate. *Microbiome* **6**, 84.
- Tsuboi, M. *et al.* (2015) Comparative support for the expensive tissue hypothesis: big brains are correlated with smaller gut and greater parental investment in Lake Tanganyika cichlids. *Evolution* **69**, 190–200.
- Tung, J. & Barreiro, L. (2012) Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proc. Natl. Acad. Sci. USA* **109**, 6490–6495.
- Tung, J. *et al.* (2015) Social networks predict gut microbiome composition in wild baboons. *eLife* **4**, e05224.

- Turnbaugh, P.J. *et al.* (2005) Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. USA* **102**, 11070–11075.
- Turnbaugh, P.J. *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031.
- Turnbaugh, P.J. *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484.
- Turroni, S. *et al.* (2017) Temporal dynamics of the gut microbiota in people sharing a confined environment, a 520-day ground-based space simulation, MARS500. *Microbiome* **5**, 39.
- Uliaszek, A.A. *et al.* (2010) The role of neuroticism and extraversion in the stress–anxiety and stress–depression relationships. *Anxiety Stress Coping* **23**, 363–381.
- de Vadder, F. *et al.* (2014) Microbiota-generated metabolites promote metabolic benefits via gut–brain neural circuits. *Cell* **156**, 84–96.
- de Vadder, F. *et al.* (2018) Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc. Natl. Acad. Sci. USA* **115**, 6458–6463.
- Vandesompele, J. *et al.* (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* **3**, research0034.
- Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A. & Knight, R. (2013) EMPeror: a tool for visualizing high-throughput microbial community data. *Gigascience* **2**, 2–5.
- Vedhara, K. *et al.* (2015) Personality and gene expression: do individual differences exist in the leukocyte transcriptome? *Psychoneuroendocrinology* **52**, 72–82.
- Vera-Wolf, P. *et al.* (2018) Measures of reproducibility in sampling and laboratory processing methods in high-throughput microbiome analysis. *bioRxiv* doi: 10.1101/322677.
- Verdú, E.F. *et al.* (2006) Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* **55**, 182–190.
- Versalovic, J. (2016) The placenta harbors a unique microbiome. *Sci. Transl. Med.* **6**, 237ra65.
- Vickery, W.L. & Poulin, R. (2010) The evolution of host manipulation by parasites: a game theory analysis. *Evol. Ecol.* **24**, 773–788.
- Vogel, C. & Marcotte, E.M. (2012) Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **13**, 227–232.
- Vuong, H.E. & Hsiao, E.Y. (2017) Emerging roles for the gut microbiome in autism spectrum disorder. *Biol. Psychiatry* **81**, 411–423.
- Vuong, H.E., Yano, J.M., Fung, T.C. & Hsiao, E.Y. (2017) The microbiome and host behavior. *Annu. Rev. Neurosci.* **40**, 21–49.
- Vyas, A., Kim, S.-K., Giacomini, N., Boothroyd, J.C. & Sapolsky, R.M. (2007) Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. USA* **104**, 6442–6447.
- Waldor, M.K. *et al.* (2015) Where next for microbiome research? *PLOS Biol.* **13**, e1002050.
- Walker, A.W. *et al.* (2011) Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* **5**, 220–230.
- Wall, R. *et al.* (2012) Contrasting effects of *Bifidobacterium breve* NCIMB 702258 and *Bifidobacterium breve* DPC 6330 on the composition of murine brain fatty acids and gut microbiota. *Am. J. Clin. Nutr.* **95**, 1278–1287.

- Wall, R. *et al.* (2014) Bacterial neuroactive compounds produced by psychobiotics. *Microbial Endocrinology: The Microbiota–Gut–Brain Axis in Health and Disease*, eds Lyte, M. & Cryan, J.F., 221–239 (Springer, New York).
- Wallace, C.J.K. & Milev, R. (2017) The effects of probiotics on depressive symptoms in humans: a systematic review. *Ann. Gen. Psychiatry* **16**, 14.
- Wang, H., Lee, I.-S., Braun, C. & Enck, P. (2016) Effect of probiotics on central nervous system functions in animals and humans: a systematic review. *J. Neurogastroenterol. Motil.* **22**, 589–605.
- Wang, L. *et al.* (2011) Low relative abundances of the mucolytic bacterium *Akkermansia muciniphila* and *Bifidobacterium* spp. in feces of children with autism. *Appl. Environ. Microbiol.* **77**, 6718–6721.
- Wang, L. *et al.* (2013) Increased abundance of *Sutterella* spp. and *Ruminococcus* torques in feces of children with autism spectrum disorder. *Mol. Autism* **4**, 42.
- Wang, T. *et al.* (2015) *Lactobacillus fermentum* NS9 restores the antibiotic induced physiological and psychological abnormalities in rats. *Benef. Microbes* **6**, 707–717.
- Warren, D.L. & Iglesias, T.L. (2012) No evidence for the “expensive-tissue hypothesis” from an intraspecific study in a highly variable species. *J. Evol. Biol.* **25**, 1226–1231.
- Watson, K.K. & Platt, M.L. (2012) Of mice and monkeys: using non-human primate models to bridge mouse- and human-based investigations of autism spectrum disorders. *J. Neurodev. Disord.* **4**, 21.
- Weimer, P.J. (2015) Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. *Front. Microbiol.* **6**, 296.
- Weinersmith, K.L. & Earley, R.L. (2016) Better with your parasites? Lessons for behavioural ecology from evolved dependence and conditionally helpful parasites. *Anim. Behav.* **118**, 123–133.
- Weiss, S. *et al.* (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**, 27.
- Welch, M.G., Margolis, K.G., Li, Z. & Gershon, M.D. (2014) Oxytocin regulates gastrointestinal motility, inflammation, macromolecular permeability, and mucosal maintenance in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **307**, G848–G862.
- Wells, J.M. (2011) Immunomodulatory mechanisms of lactobacilli. *Microb. Cell Fact.* **10** (Suppl. 1), S17.
- Werling, D.M. & Geschwind, D.H. (2013) Sex differences in autism spectrum disorders. *Curr. Opin. Neurol.* **26**, 146–153.
- Wexler, A.G. *et al.* (2016) Human symbionts inject and neutralize antibacterial toxins to persist in the gut. *Proc. Natl. Acad. Sci. USA* **113**, 3639–3644.
- Wexler, H.M. (2007) Bacteroides: the good, the bad, and the nitty-gritty. *Clin. Microbiol. Rev.* **20**, 593–621.
- Whitman, W.B., Coleman, D.C. & Wiebe, W.J. (1998) Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. USA* **95**, 6578–6583.
- Wickham, M.E., Brown, N.F., Provias, J., Finlay, B.B. & Coombes, B.K. (2007) Oral infection of mice with *Salmonella enterica* serovar Typhimurium causes meningitis and infection of the brain. *BMC Infect. Dis.* **7**, 65.
- Wiles, T.J. *et al.* (2016) Host gut motility promotes competitive exclusion within a model intestinal microbiota. *PLoS Biol.* **14**, e1002517.
- Williams, B.L. *et al.* (2011) Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS One* **6**, e24585.

- Williams, B.L., Hornig, M., Parekh, T. & Lipkin, W.I. (2012) Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio* **3**, e00261-11.
- Williams, K.P. *et al.* (2010) Phylogeny of gammaproteobacteria. *J. Bacteriol.* **192**, 2305–2314.
- Wilson, E.O. (1975) *Sociobiology: The New Synthesis* (Harvard University Press, Cambridge, Massachusetts).
- Wing, L. (1988) The autistic continuum. *Aspects of Autism: Biological Research*, ed. Wing, L. (The Royal College of Psychiatrists, London).
- von Wintersdorff, C.J.H. *et al.* (2014) High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerg. Infect. Dis.* **20**, 649–657.
- Wohleb, E.S., Franklin, T., Iwata, M. & Duman, R.S. (2016) Integrating neuroimmune systems in the neurobiology of depression. *Nat. Rev. Neurosci.* **17**, 497–511.
- Wong, A.C.-N. *et al.* (2015) Behavioral microbiomics: a multi-dimensional approach to microbial influence on behavior. *Front. Microbiol.* **6**, 1359.
- Wong, J.M.W., de Souza, R., Kendall, C.W.C., Emam, A. & Jenkins, D.J.A. (2006) Colonic health: fermentation and short chain fatty acids. *J. Clin. Gastroenterol.* **40**, 235–243.
- Wong, M.-L. *et al.* (2016) Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol. Psychiatry* **21**, 797–805.
- Wostmann, B.S., Larkin, C., Moriarty, A. & Bruckner-Kardoss, E. (1983) Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats. *Lab. Anim. Sci.* **33**, 46–50.
- Wu, G.D. *et al.* (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108.
- Xavier, J.B., Kim, W. & Foster, K.R. (2011) A molecular mechanism that stabilizes cooperative secretions in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **79**, 166–179.
- Xia, Y. & Sun, J. (2017) Hypothesis testing and statistical analysis of microbiome. *Genes Dis.* **4**, 138–148.
- Xu, J. & Gordon, J.I. (2003) Honor thy symbionts. *Proc. Natl. Acad. Sci. USA* **100**, 10452–10459.
- Xu, J. *et al.* (2003) A genomic view of the human–*Bacteroides thetaiotaomicron* symbiosis. *Science* **299**, 2074–2076.
- Xu, L., Paterson, A.D., Turpin, W. & Xu, W. (2015) Assessment and selection of competing models for zero-inflated microbiome data. *PLoS One* **10**, e0129606.
- Yan, S. & Kentner, A.C. (2017) Mechanical allodynia corresponds to Oprm1 downregulation within the descending pain network of male and female rats exposed to neonatal immune challenge. *Brain Behav. Immun.* **63**, 148–159.
- Yang, C. *et al.* (2017) *Bifidobacterium* in the gut microbiota confer resilience to chronic social defeat stress in mice. *Sci. Rep.* **7**, 45942.
- Yang, H. *et al.* (2016a) Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. *Sci. Rep.* **6**, 27427.
- Yang, Y.C. *et al.* (2016b) Social relationships and physiological determinants of longevity across the human life span. *Proc. Natl. Acad. Sci. USA* **113**, 578–583.
- Yano, J.M. *et al.* (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276.

- Yasuda, K. *et al.* (2015) Biogeography of the intestinal mucosal and luminal microbiome in the rhesus macaque. *Cell Host Microbe* **17**, 385–391.
- Yatsunenکو, T. *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227.
- Yissachar, N. *et al.* (2017) An intestinal organ culture system uncovers a role for the nervous system in microbe–immune crosstalk. *Cell* **168**, 1135–1148.e12.
- Yoo, B.B. & Mazmanian, S.K. (2017) The enteric network: interactions between the immune and nervous systems of the gut. *Immunity* **46**, 910–926.
- Young, L.J., Lim, M.M., Gingrich, B. & Insel, T.R. (2001) Cellular mechanisms of social attachment. *Horm. Behav.* **40**, 133–138.
- Young, L.J. & Wang, Z. (2004) The neurobiology of pair bonding. *Nat. Neurosci.* **7**, 1048–1054.
- Yu, M. *et al.* (2017) Variations in gut microbiota and fecal metabolic phenotype associated with depression by 16S rRNA gene sequencing and LC/MS-based metabolomics. *J. Pharm. Biomed. Anal.* **138**, 231–239.
- Yunes, R.A. *et al.* (2016) Anaerobe GABA production and structure of gadB/gadC genes in *Lactobacillus* and *Bifidobacterium* strains from human microbiota. *Anaerobe* **42**, 197–204.
- Yuval, B. (2017) Symbiosis: gut bacteria manipulate host behaviour. *Curr. Biol.* **27**, R746–R747.
- Zarrinpar, A. *et al.* (2018) Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat. Commun.* **9**, 2872.
- Zhang, J. *et al.* (2017) Blockade of interleukin-6 receptor in the periphery promotes rapid and sustained antidepressant actions: a possible role of gut–microbiota–brain axis. *Transl. Psychiatry* **7**, e1138.
- Zheng, P. *et al.* (2016) Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* **21**, 786–796.
- Zhernakova, A. *et al.* (2016) Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352**, 565–569.
- Zhu, Y. *et al.* (2015) Meat, dairy and plant proteins alter bacterial composition of rat gut bacteria. *Sci. Rep.* **5**, 15220.
- Zmora, N., Zeevi, D., Korem, T., Segal, E. & Elinav, E. (2016) Taking it personally: personalized utilization of the human microbiome in health and disease. *Cell Host Microbe* **19**, 12–20.
- Zmora, N. *et al.* (2018) Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell* **174**, 1388–1405.e21.
- Zoetendal, E.G., Akkermans, A.D.L., Akkermans-van Vliet, W.M., de Visser, J.A.G.M. & de Vos, W.M. (2001) The host genotype affects the bacterial community in the human gastrointestinal tract. *Microb. Ecol. Health Dis.* **13**, 129–134.
- Zubieta, J.-K. *et al.* (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* **293**, 311–315.

Appendix

Chapter 2

Figures

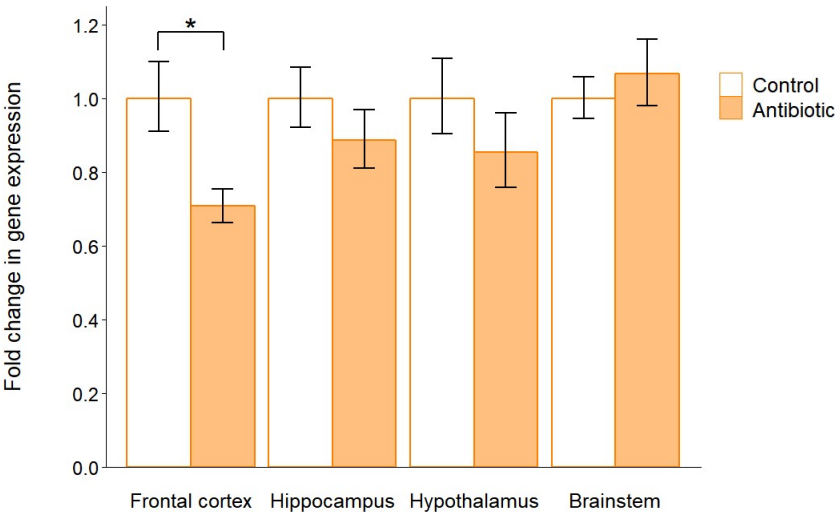


Figure A.1. Effects of post-weaning antibiotic treatment on the gene expression of *Bdnf*. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Bdnf* (encoding brain-derived neurotrophic growth factor) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

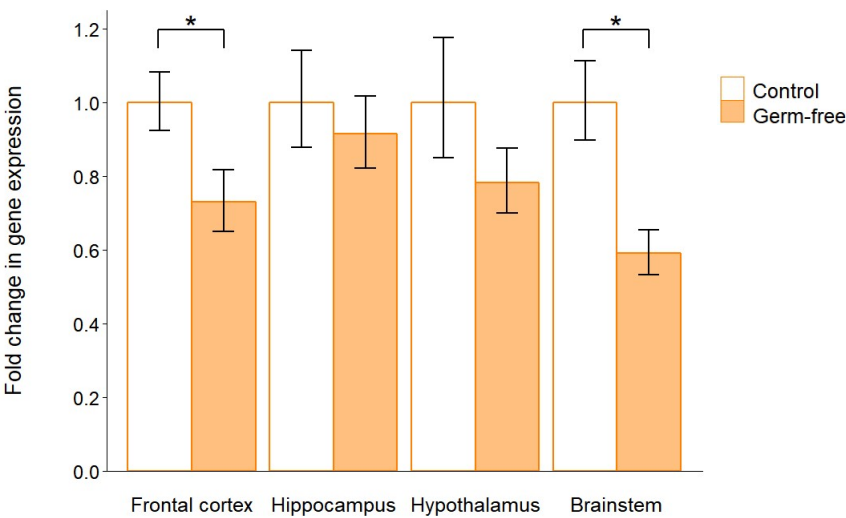


Figure A.2. Effects of germ-free status on the gene expression of *Bdnf*. Data are plotted as mean expression (relative to the control group) $\pm$ SEM for *Bdnf* (encoding brain-derived neurotrophic growth factor) and all comparisons are with $n = 8$ per group. Two-sample t-tests have 14 degrees of freedom (df) and asterisks denote $P < 0.05$.

Tables

Table A.1. Gene expression assays used for quantitative real-time PCR. All IDs refer to TaqMan assays (Applied Biosystems).

Gene	Protein encoded by gene	TaqMan gene expression assay ID
<i>Oprm1</i>	Opioid receptor μ 1	Mm01188089_m1
<i>Oxtr</i>	Oxytocin receptor	Mm01182684_m1
<i>Avpr1a</i>	Arginine vasopressin receptor 1A	Mm00444092_m1
<i>Pomc</i>	Pro-opiomelanocortin	Mm00435874_m1
<i>Oxt</i>	Oxytocin	Mm01329577_g1
<i>Avp</i>	Vasopressin	Mm00437761_g1
<i>MyD88</i>	Myeloid differentiation factor 88	Mm00440338_m1
<i>Bdnf</i>	Brain-derived neurotrophic factor	Mm00432069_m1
<i>Gapdh</i>	Glyceraldehyde 3-phosphate dehydrogenase	Mm99999915_g1
<i>B2m</i>	Beta-2-microglobulin	Mm00437762_m1
<i>Polr2a</i>	Polymerase (RNA) II subunit A	Mm00839502_m1

Chapter 3

Figures

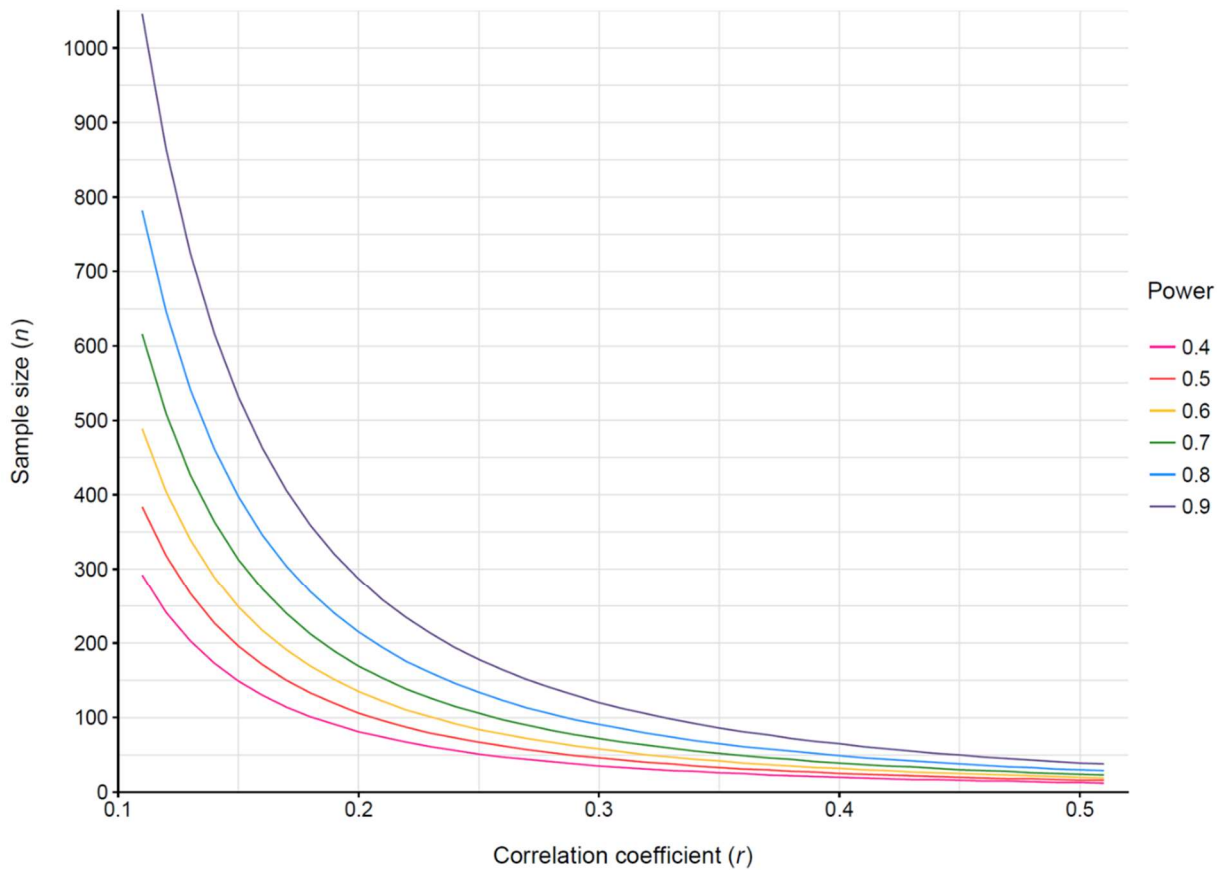


Figure A.3. Statistical power analysis for sample size estimation. Graph is plotted for a range of statistical powers, with a power of at least 0.8 considered acceptable.

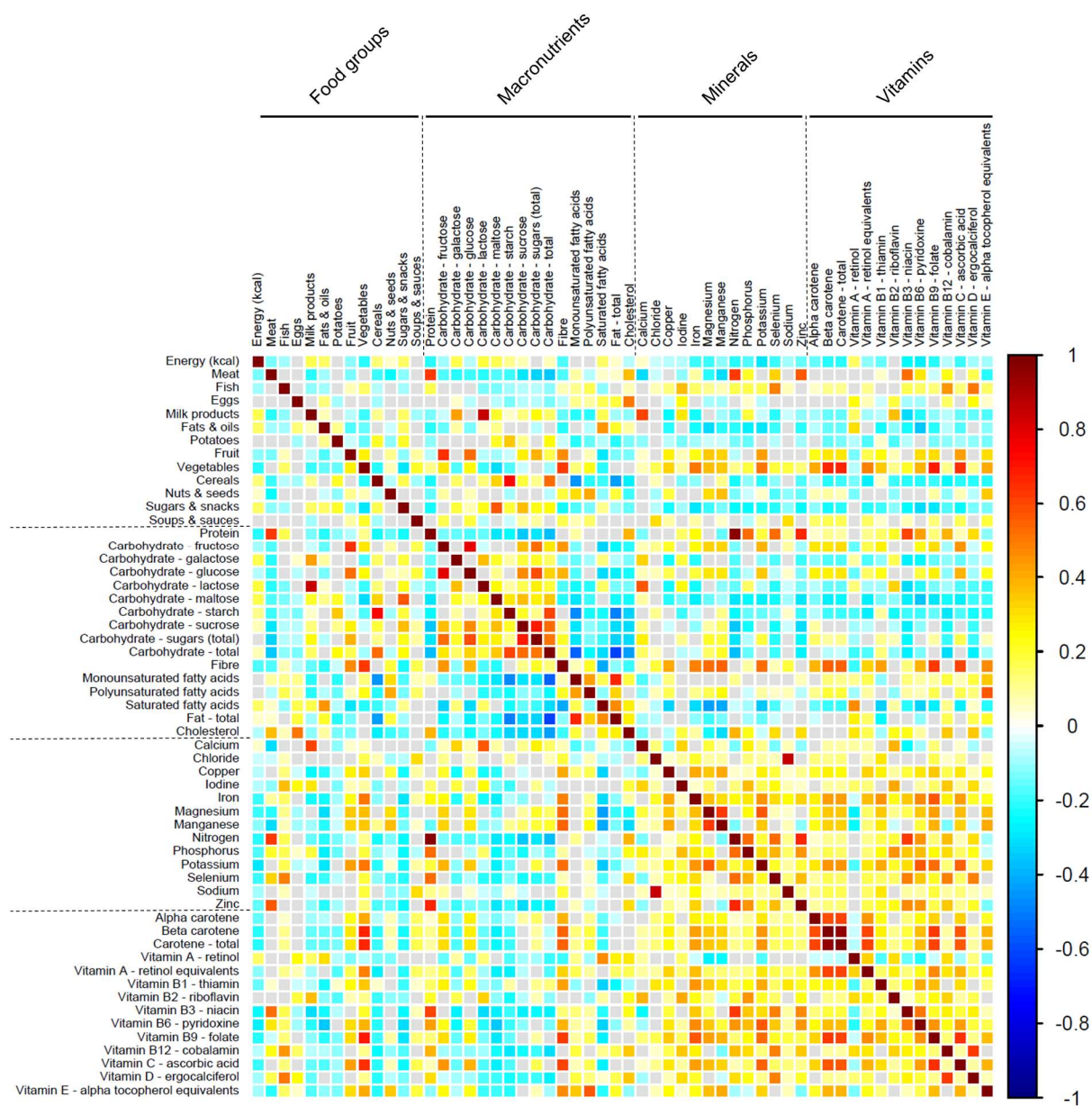


Figure A.4. Intercorrelation plot for the nutrient variables, divided into the four categories: food groups, macronutrients, minerals and vitamins. Plot depicts strength and direction of pairwise correlations, as measured by Kendall's Tau-b correlation coefficient. Grey squares denote associations that were not statistically significant at FDR < 0.1. Note that the quantity of each nutrient variable was calculated relative to estimated total calorie intake prior to the intercorrelation analysis.

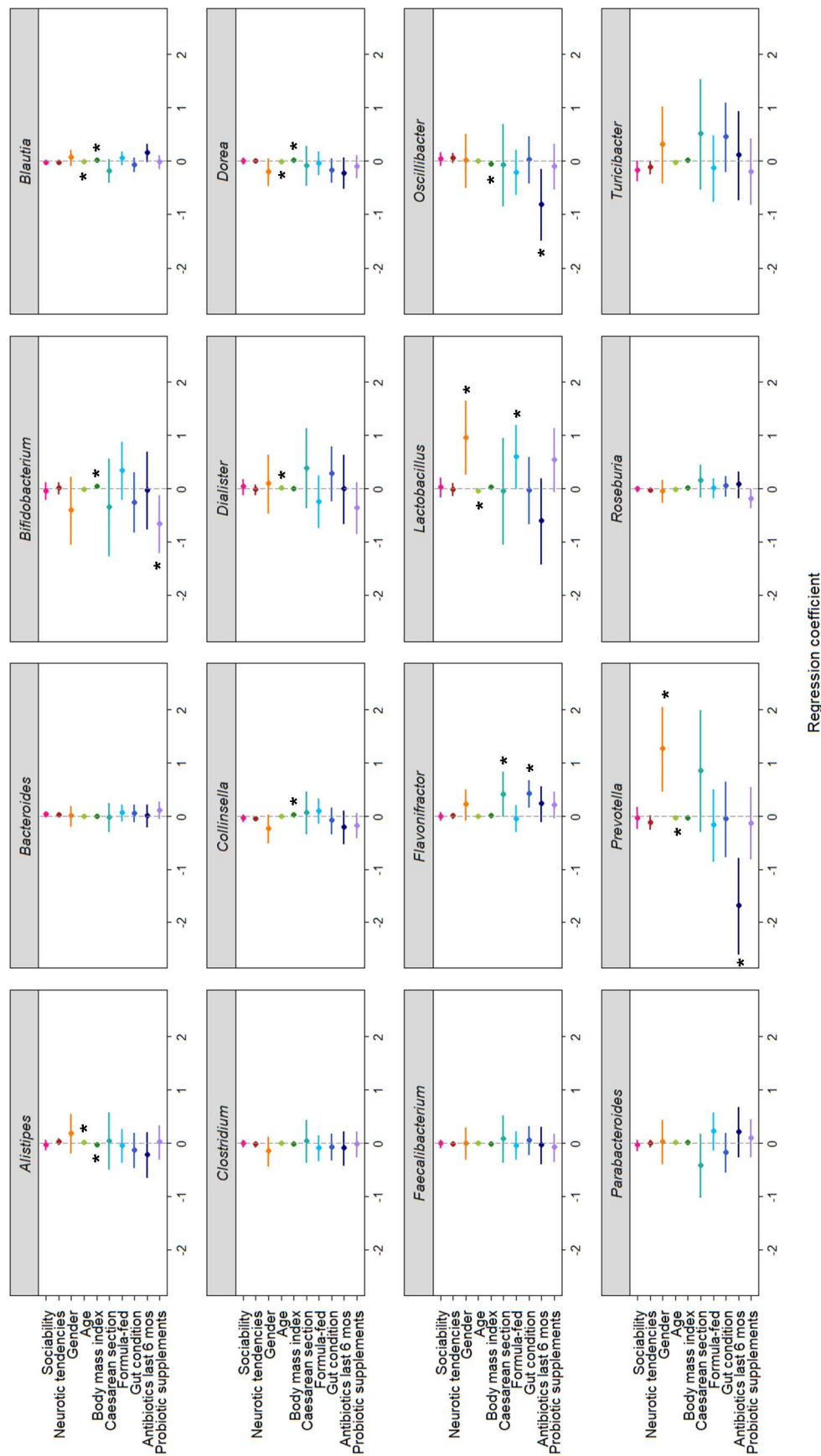


Figure A.5. Coefficient plots from regression models predicting abundances of genera in the human gut microbiome. Asterisks denote significant predictors of genus abundance where $P < 0.05$ and bars indicate 95% confidence intervals. The key variables of interest for this study were those relating to behavioural traits (sociability and neurotic tendencies), while other variables were also included to avoid potential confounding effects. A positive relationship with gender indicates a higher abundance of the genus in females. Plots displayed here depict genera whose abundances were not significantly related to differences in behavioural traits (for significant results see Figure 3.4).

Tables

Table A.2. Summary statistics for continuous variables. Mean, standard error and range for each study variable are given. For the questionnaires assessing personality phenotype, the maximum possible range of scores are 10 to 50 for the 50-item International Personality Item Pool Inventory, 20 to 80 for the State–Trait Anxiety Inventory and 0 to 20 for the Autism Spectrum Quotient (social skill and communication).

Category	Variable	Mean	SE	Minimum	Maximum
Behaviour	Extraversion	29.6	0.3	10	50
	Agreeableness	40.2	0.2	16	50
	Conscientiousness	37.2	0.3	13	50
	Neuroticism	30.5	0.3	10	50
	Openness	38.8	0.2	14	50
	Social network size	33.8	2.3	2	1005
	Social skill/commn	14.0	0.1	0	20
	Anxiety	42.4	0.5	20	77
	Stress	5.6	0.1	1	10
Diet	Adventurous eater	6.8	0.1	1	10
	Probiotic foods	8.0	0.2	0	35
	Prebiotic foods	19.8	0.3	0	43
	Total supplements	4.6	0.1	0	15
Health & lifestyle	General health	6.9	0.1	1	10
	Body mass index	24.5	0.2	12.4	54.9
	Days exercise	2.9	0.1	0	7
	Travel abroad/yr	0.7	0.0	0	6
	Different countries/yr	0.8	0.0	0	6
	Sleep quality	5.8	0.1	1	10
	Pain tolerance	6.5	0.1	1	10
	Stomach sickness freq	2.1	0.1	0	6
	Constipation freq	2.0	0.1	0	6
Sociodemographics	Age	41.9	0.5	18	84
	Siblings	1.7	0.1	0	10+

Table A.3. Data summary for binary variables. Values represent percentage of participants who answered 'yes' to each variable.

Category	Variable	Percent (%)
Diet	Gluten free	34
	Dairy free	22
	Probiotic supplements	53
	Prebiotic supplements	12
Health & lifestyle	Caesarean section	9
	Premature birth	9
	Breast-fed	53
	Formula-fed	47
	Allergy/asthma/eczema	67
	Mental illness	19
	Smoking history	14
	Alcohol/drug history	5
	Antibiotics last 6 mos	17
	Irritable bowel syndrome	24
	Inflammatory bowel disease	3
	Cancer	6
	Chronic infectious disease	6
	Immunodeficiency	13
Sociodemographics	Male	29
	Female	71
	Unemployed	13
	Dog owner	39
	Cat owner	29

Table A.4. Output from regression models predicting abundances of genera in the human gut microbiome. *P* values highlighted in green denote significant predictors of genus abundance where *P* < 0.05. Note that two *P* values are given for zero-inflated negative binomial regression models, with the first *P* value indicating whether the variable predicts genus abundance, and the second *P* value indicating whether the variable predicts the presence versus absence of the genus. CL represents 95% confidence limit for each regression coefficient. A positive regression coefficient for gender indicates a higher abundance of the genus in females.

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Akkermansia</i>	<i>P</i> value	0.038	0.497	0.897	0.052	0.851	0.384	0.375	0.113	0.001	0.161
	Coefficient	0.210	0.210	0.050	0.025	-0.005	-0.486	0.294	-0.552	-1.452	-0.467
	Lower CL	0.011	0.011	-0.706	0.000	-0.058	-1.580	-0.356	-1.234	-2.326	-1.120
	Upper CL	0.408	0.408	0.806	0.051	0.048	0.608	0.944	0.130	-0.577	0.186
<i>Alistipes</i>	<i>P</i> value	0.573	0.343	0.336	0.019	0.026	0.874	0.792	0.445	0.320	0.890
	Coefficient	-0.028	0.032	0.181	0.015	-0.030	0.043	-0.043	-0.130	-0.216	0.022
	Lower CL	-0.125	-0.034	-0.188	0.002	-0.056	-0.491	-0.360	-0.462	-0.643	-0.296
	Upper CL	0.069	0.098	0.550	0.027	-0.004	0.577	0.274	0.203	0.210	0.341
<i>Bacteroides</i>	<i>P</i> value	0.120	0.108	0.947	0.983	0.774	0.919	0.394	0.468	0.901	0.163
	Coefficient	0.039	0.028	0.006	0.000	0.002	-0.014	0.070	0.063	0.014	0.116
	Lower CL	-0.010	-0.006	-0.182	-0.006	-0.011	-0.287	-0.091	-0.107	-0.204	-0.047
	Upper CL	0.089	0.061	0.195	0.006	0.015	0.258	0.232	0.233	0.232	0.278
<i>Bifidobacterium</i>	<i>P</i> value	0.643	0.773	0.211	0.348	0.043	0.464	0.212	0.393	0.936	0.018
	Coefficient	-0.039	0.017	-0.402	-0.010	0.046	-0.340	0.345	-0.248	-0.030	-0.658
	Lower CL	-0.205	-0.096	-1.033	-0.031	0.002	-1.252	-0.196	-0.816	-0.759	-1.202
	Upper CL	0.126	0.129	0.228	0.011	0.091	0.571	0.887	0.321	0.699	-0.114
<i>Blautia</i>	<i>P</i> value	0.126	0.211	0.357	0.018	0.036	0.109	0.347	0.369	0.075	0.901
	Coefficient	-0.031	-0.017	0.072	-0.006	0.012	-0.181	0.063	-0.063	0.160	-0.008
	Lower CL	-0.071	-0.045	-0.081	-0.011	0.001	-0.401	-0.068	-0.201	-0.016	-0.140
	Upper CL	0.009	0.010	0.225	-0.001	0.022	0.040	0.194	0.075	0.337	0.123
<i>Clostridium</i>	<i>P</i> value	0.926	0.384	0.287	0.307	0.152	0.852	0.456	0.576	0.584	0.883
	Coefficient	-0.003	-0.022	-0.151	-0.005	-0.014	0.038	-0.091	-0.071	-0.090	-0.018
	Lower CL	-0.076	-0.072	-0.428	-0.014	-0.034	-0.363	-0.329	-0.322	-0.410	-0.258
	Upper CL	0.069	0.028	0.127	0.004	0.005	0.439	0.148	0.179	0.231	0.222

Table A.4. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
Collinsella	P value	0.484	0.101	0.101	0.553	0.015	0.739	0.380	0.551	0.215	0.168
	Coefficient	-0.026	-0.041	-0.231	-0.003	0.024	0.068	0.106	-0.076	-0.202	-0.167
	Lower CL	-0.098	-0.091	-0.506	-0.012	0.005	-0.331	-0.131	-0.324	-0.520	-0.405
	Upper CL	0.046	0.008	0.045	0.006	0.044	0.466	0.343	0.173	0.117	0.071
	P value	0.129	0.000	0.147	0.000	0.168	0.769	0.604	0.011	0.062	0.377
Corynebacterium	Coefficient	-0.165	-0.320	-0.599	-0.063	-0.040	-0.176	0.184	-0.943	-0.893	0.315
	Lower CL	-0.377	-0.465	-1.409	-0.090	-0.098	-1.347	-0.512	-1.674	-1.830	-0.384
	Upper CL	0.048	-0.175	0.210	-0.036	0.017	0.995	0.881	-0.213	0.044	1.014
	P value	0.019	0.981	0.964	0.601	0.792	0.602	0.760	0.807	0.091	0.953
	P value _{zero-infl}	0.317	0.909	0.972	0.016	0.137	0.701	0.038	0.042	0.429	0.371
Desulfovibrio	Coefficient	-0.201	0.001	0.015	0.006	0.007	-0.252	0.088	-0.074	-0.735	0.017
	Lower CL	-0.369	-0.115	-0.630	-0.016	-0.042	-1.200	-0.474	-0.671	-1.587	-0.544
	Upper CL	-0.033	0.118	0.659	0.027	0.056	0.696	0.650	0.522	0.117	0.578
	P value	0.608	0.762	0.729	0.035	0.713	0.301	0.353	0.275	0.991	0.157
	P value _{zero-infl}	0.243	0.464	0.748	0.000	0.091	0.443	0.981	0.287	0.966	0.517
Dialister	Coefficient	0.039	-0.016	0.096	0.022	0.007	0.393	-0.234	0.288	-0.004	-0.354
	Lower CL	-0.110	-0.116	-0.448	0.002	-0.031	-0.352	-0.726	-0.229	-0.650	-0.843
	Upper CL	0.188	0.085	0.641	0.043	0.046	1.137	0.259	0.805	0.643	0.136
	P value	0.800	0.683	0.134	0.047	0.010	0.662	0.764	0.143	0.134	0.394
	Coefficient	0.009	0.009	-0.193	-0.009	0.023	-0.082	-0.033	-0.170	-0.223	-0.095
Dorea	Lower CL	-0.058	-0.036	-0.445	-0.017	0.006	-0.447	-0.250	-0.398	-0.515	-0.313
	Upper CL	0.075	0.055	0.060	0.000	0.041	0.284	0.184	0.058	0.069	0.123
	P value	0.854	0.667	0.995	0.218	0.338	0.712	0.754	0.698	0.837	0.571
	Coefficient	-0.007	-0.012	-0.001	-0.006	-0.011	0.083	-0.042	0.054	-0.037	-0.076
	Lower CL	-0.087	-0.066	-0.305	-0.017	-0.032	-0.357	-0.303	-0.220	-0.389	-0.339
Faecalibacterium	Upper CL	0.072	0.042	0.303	0.004	0.011	0.523	0.220	0.329	0.315	0.187
	P value	0.881	0.648	0.130	0.254	0.362	0.048	0.769	0.001	0.160	0.083
	Coefficient	0.006	0.012	0.222	0.006	0.009	0.420	-0.037	0.430	0.238	0.219
	Lower CL	-0.070	-0.039	-0.065	-0.004	-0.011	0.004	-0.284	0.171	-0.094	-0.029
	Upper CL	0.081	0.063	0.510	0.015	0.030	0.836	0.210	0.689	0.571	0.468

Table A.4. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Lactobacillus</i>	P value	0.750	0.884	0.007	0.002	0.223	0.938	0.048	0.931	0.141	0.074
	Coefficient	0.030	-0.009	0.960	-0.037	0.030	-0.040	0.602	-0.028	-0.602	0.545
	Lower CL	-0.152	-0.133	0.266	-0.060	-0.019	-1.042	0.006	-0.653	-1.404	-0.053
	Upper CL	0.212	0.115	1.654	-0.014	0.079	0.963	1.198	0.598	0.200	1.144
<i>Lactococcus</i>	P value	0.002	0.401	0.613	0.159	0.843	0.064	0.207	0.093	0.115	0.972
	P value _{zero-infl}	0.237	0.623	0.612	0.814	0.319	0.990	0.845	0.920	0.992	0.806
	Coefficient	0.287	0.054	-0.183	0.017	0.005	0.817	0.386	0.542	0.646	0.011
	Lower CL	0.103	-0.072	-0.890	-0.007	-0.041	-0.046	-0.214	-0.090	-0.159	-0.591
<i>Oscillibacter</i>	Upper CL	0.472	0.180	0.525	0.042	0.051	1.680	0.986	1.173	1.451	0.613
	P value	0.468	0.143	0.953	0.867	0.005	0.856	0.342	0.888	0.019	0.662
	P value _{zero-infl}	0.502	0.566	0.877	0.023	0.752	0.919	0.612	0.044	0.004	0.662
	Coefficient	0.048	0.066	0.015	0.001	-0.052	-0.071	-0.205	0.032	-0.809	-0.095
<i>Oscillospira</i>	Lower CL	-0.082	-0.022	-0.491	-0.015	-0.089	-0.843	-0.629	-0.409	-1.484	-0.521
	Upper CL	0.177	0.154	0.521	0.018	-0.016	0.700	0.218	0.472	-0.135	0.331
	P value	0.000	0.783	0.290	0.000	0.539	0.742	0.209	0.004	0.366	0.003
	P value _{zero-infl}	0.643	0.075	0.937	0.052	0.128	0.481	0.363	0.347	0.037	0.730
<i>Parabacteroides</i>	Coefficient	0.179	0.010	0.207	0.026	-0.009	0.098	0.208	0.497	-0.220	0.496
	Lower CL	0.080	-0.059	-0.177	0.014	-0.037	-0.486	-0.117	0.156	-0.698	0.169
	Upper CL	0.277	0.078	0.591	0.039	0.019	0.682	0.533	0.839	0.257	0.822
	P value	0.506	0.984	0.915	0.174	0.780	0.167	0.210	0.369	0.381	0.603
<i>Prevotella</i>	Coefficient	-0.037	-0.001	0.022	0.010	0.004	-0.419	0.226	-0.170	0.212	0.094
	Lower CL	-0.144	-0.074	-0.388	-0.004	-0.025	-1.013	-0.127	-0.540	-0.263	-0.260
	Upper CL	0.071	0.073	0.433	0.023	0.033	0.175	0.579	0.201	0.687	0.449
	P value	0.792	0.129	0.002	0.010	0.408	0.142	0.642	0.891	0.000	0.722
<i>Roseburia</i>	Coefficient	-0.028	-0.109	1.262	-0.035	-0.023	0.854	-0.160	-0.050	-1.679	-0.123
	Lower CL	-0.235	-0.250	0.474	-0.061	-0.079	-0.285	-0.838	-0.760	-2.591	-0.803
	Upper CL	0.179	0.032	2.050	-0.008	0.032	1.992	0.517	0.660	-0.768	0.557
	P value	0.983	0.115	0.697	0.114	0.167	0.327	0.896	0.554	0.522	0.060
<i>Roseburia</i>	Coefficient	0.001	-0.031	-0.043	-0.006	0.011	0.155	0.012	0.058	0.081	-0.177
	Lower CL	-0.056	-0.069	-0.256	-0.013	-0.004	-0.155	-0.172	-0.135	-0.167	-0.362
	Upper CL	0.057	0.008	0.171	0.001	0.026	0.464	0.196	0.251	0.328	0.007

Table A.4. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Streptococcus</i>	P value	0.652	0.029	0.030	0.335	0.004	0.144	0.591	0.027	0.086	0.011
	Coefficient	-0.026	-0.085	0.472	-0.007	0.044	0.460	-0.101	0.436	0.432	-0.478
	Lower CL	-0.138	-0.162	0.045	-0.021	0.014	-0.158	-0.468	0.051	-0.062	-0.847
	Upper CL	0.086	-0.009	0.899	0.007	0.074	1.078	0.267	0.821	0.926	-0.109
<i>Sutterella</i>	P value	0.028	0.672	0.920	0.747	0.735	0.807	0.956	0.189	0.909	0.541
	Coefficient	-0.248	0.033	0.043	-0.005	0.010	-0.152	0.020	-0.509	0.056	-0.227
	Lower CL	-0.469	-0.118	-0.798	-0.033	-0.049	-1.368	-0.703	-1.267	-0.916	-0.953
	Upper CL	-0.027	0.183	0.884	0.024	0.070	1.065	0.743	0.250	1.029	0.500
<i>Turicibacter</i>	P value	0.071	0.101	0.394	0.105	0.409	0.329	0.677	0.167	0.792	0.542
	Coefficient	-0.173	-0.107	0.311	-0.020	0.021	0.516	-0.131	0.456	0.111	-0.192
	Lower CL	-0.361	-0.235	-0.405	-0.044	-0.029	-0.519	-0.747	-0.190	-0.717	-0.811
	Upper CL	0.015	0.021	1.027	0.004	0.072	1.551	0.485	1.101	0.939	0.426

Table A.5. Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and study variables. Highlighted cells denote significant associations where $P < 0.05$ and where $P_{adj} < 0.1$. Values in bold indicate $P_{adj} < 0.05$.

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P_{adj} value	Tau	P value	P_{adj} value	Tau	P value	P_{adj} value
Behaviour	Extraversion	-0.014	0.608	0.710	-0.018	0.490	0.594	-0.019	0.471	0.575
	Agreeableness	-0.063	0.020	0.048	-0.053	0.050	0.097	-0.069	0.012	0.034
	Conscientiousness	-0.050	0.062	0.115	-0.043	0.114	0.178	-0.078	0.004	0.014
	Neuroticism	-0.029	0.277	0.366	-0.038	0.154	0.220	0.006	0.819	0.855
	Openness	0.029	0.287	0.375	0.031	0.253	0.337	0.029	0.296	0.383
	Social network size	0.064	0.017	0.043	0.077	0.004	0.014	0.031	0.248	0.334
	Social skill/commn	-0.048	0.079	0.134	-0.045	0.104	0.163	-0.058	0.038	0.077
	Anxiety	-0.056	0.038	0.077	-0.063	0.019	0.047	-0.022	0.430	0.531
Diet	Stress	-0.066	0.018	0.045	-0.062	0.026	0.059	-0.039	0.165	0.235
	Adventurous eater	0.072	0.010	0.029	0.084	0.003	0.010	0.056	0.046	0.090
	Gluten free	-0.049	0.129	0.198	-0.054	0.092	0.151	-0.021	0.525	0.624
	Dairy free	-0.066	0.038	0.077	-0.061	0.058	0.109	-0.070	0.031	0.067
	Probiotic foods	0.090	0.001	0.005	0.087	0.001	0.006	0.067	0.015	0.039
	Prebiotic foods	0.118	0.000	0.000	0.128	0.000	0.000	0.051	0.063	0.115
	Probiotic supplements	-0.098	0.002	0.008	-0.098	0.002	0.009	-0.049	0.133	0.198
	Prebiotic supplements	-0.046	0.150	0.218	-0.048	0.134	0.198	-0.058	0.071	0.125
	Total supplements	-0.109	0.000	0.000	-0.129	0.000	0.000	-0.048	0.079	0.134
	Caesarean section	0.000	0.995	0.995	-0.008	0.821	0.855	0.004	0.914	0.921
Health & lifestyle	Premature birth	-0.004	0.890	0.910	-0.007	0.833	0.859	0.007	0.821	0.855
	Formula-fed	-0.086	0.011	0.033	-0.077	0.024	0.056	-0.043	0.212	0.298
	General health	0.132	0.000	0.000	0.136	0.000	0.000	0.107	0.000	0.001
	Body mass index	-0.027	0.310	0.397	-0.023	0.385	0.479	-0.010	0.705	0.788
	Days exercise	0.035	0.216	0.300	0.026	0.343	0.432	0.007	0.803	0.855
	Travel abroad/yr	0.104	0.001	0.003	0.096	0.002	0.006	0.114	0.000	0.001
	Different countries/yr	0.123	0.000	0.000	0.109	0.000	0.002	0.135	0.000	0.000
	Sleep quality	0.081	0.003	0.013	0.077	0.006	0.018	0.049	0.082	0.137
	Pain tolerance	0.007	0.799	0.855	0.011	0.698	0.788	0.010	0.734	0.814
	Allergy/asthma/eczema	-0.022	0.501	0.601	-0.014	0.662	0.760	-0.038	0.245	0.334
	Mental illness	-0.039	0.227	0.312	-0.054	0.092	0.151	-0.009	0.789	0.855
	Smoking history	-0.019	0.561	0.661	-0.007	0.823	0.855	-0.014	0.674	0.767
	Alcohol/drug history	-0.067	0.037	0.077	-0.056	0.077	0.134	-0.083	0.010	0.031

Table A.5. (cont.)

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P _{adj} value	Tau	P value	P _{adj} value	Tau	P value	P _{adj} value
Health & lifestyle (cont.)	Antibiotics last 6 mos	-0.163	0.000	0.000	-0.151	0.000	0.000	-0.158	0.000	0.000
	Stomach sickness freq	-0.128	0.000	0.000	-0.132	0.000	0.000	-0.087	0.002	0.009
	Constipation freq	0.043	0.130	0.198	0.014	0.632	0.732	0.096	0.001	0.005
	Gut condition	-0.140	0.000	0.000	-0.141	0.000	0.000	-0.103	0.001	0.006
	Cancer	-0.081	0.011	0.033	-0.114	0.000	0.002	-0.062	0.057	0.109
	Chronic infectious disease	-0.104	0.001	0.006	-0.103	0.001	0.006	-0.075	0.020	0.048
	Immunodeficiency	-0.146	0.000	0.000	-0.140	0.000	0.000	-0.121	0.000	0.001
Sociodemographics	Gender	-0.098	0.017	0.043	-0.091	0.027	0.059	-0.040	0.331	0.421
	Age	-0.048	0.067	0.119	-0.053	0.045	0.090	-0.049	0.067	0.119
	Unemployed	-0.092	0.004	0.014	-0.079	0.013	0.035	-0.135	0.000	0.000
	Siblings	-0.047	0.102	0.163	-0.042	0.144	0.212	-0.078	0.008	0.025
	Dog owner	-0.088	0.006	0.020	-0.080	0.013	0.035	-0.071	0.029	0.063
	Cat owner	0.008	0.802	0.855	-0.004	0.896	0.910	0.054	0.094	0.151

Table A.6. Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and nutrient variables. Highlighted cells denote significant associations where $P < 0.05$ and $P_{adj} < 0.1$. Values in bold indicate $P_{adj} < 0.05$.

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P_{adj} value	Tau	P value	P_{adj} value	Tau	P value	P_{adj} value
Food	Energy (kcal)	0.060	0.027	0.098	0.076	0.005	0.032	0.007	0.787	0.860
	Meat	-0.008	0.756	0.847	-0.006	0.835	0.893	0.009	0.752	0.847
	Fish	0.056	0.044	0.133	0.042	0.129	0.235	0.077	0.006	0.033
	Eggs	0.048	0.094	0.199	0.047	0.101	0.207	0.058	0.046	0.134
	Milk products	0.040	0.149	0.258	0.041	0.133	0.237	0.039	0.157	0.269
	Fats & oils	0.018	0.500	0.641	0.021	0.444	0.592	0.015	0.594	0.718
	Potatoes	0.053	0.058	0.151	0.061	0.029	0.100	0.034	0.224	0.361
	Fruit	0.083	0.002	0.018	0.094	0.001	0.009	-0.004	0.884	0.922
	Vegetables	0.048	0.078	0.174	0.040	0.136	0.240	0.008	0.765	0.848
	Cereals	0.064	0.019	0.072	0.095	0.001	0.009	0.015	0.585	0.712
	Nuts & seeds	0.049	0.077	0.174	0.052	0.061	0.156	-0.002	0.953	0.969
	Sugars & snacks	0.043	0.112	0.222	0.054	0.048	0.134	0.030	0.276	0.415
	Soups & sauces	-0.001	0.977	0.977	-0.010	0.705	0.813	-0.025	0.368	0.529
	Protein	0.033	0.219	0.357	0.044	0.103	0.207	0.011	0.689	0.804
Macronutrients	Carbohydrate - fructose	0.070	0.009	0.044	0.088	0.001	0.010	-0.016	0.559	0.696
	Carbohydrate - galactose	0.035	0.226	0.361	0.025	0.384	0.537	0.035	0.232	0.364
	Carbohydrate - glucose	0.074	0.007	0.035	0.089	0.001	0.010	-0.007	0.811	0.874
	Carbohydrate - lactose	0.041	0.133	0.237	0.042	0.124	0.232	0.032	0.236	0.364
	Carbohydrate - maltose	0.032	0.242	0.370	0.049	0.068	0.163	0.017	0.538	0.680
	Carbohydrate - starch	0.054	0.045	0.133	0.084	0.002	0.016	0.002	0.937	0.965
	Carbohydrate - sucrose	0.059	0.030	0.100	0.077	0.004	0.027	-0.010	0.707	0.813
	Carbohydrate - sugars (total)	0.072	0.008	0.039	0.089	0.001	0.010	-0.007	0.793	0.860
	Carbohydrate - total	0.066	0.014	0.059	0.093	0.001	0.009	-0.007	0.790	0.860
	Fibre	0.081	0.003	0.022	0.094	0.001	0.009	-0.008	0.768	0.848
	Monounsaturated fatty acids	0.041	0.129	0.235	0.042	0.121	0.232	0.014	0.607	0.719
	Polyunsaturated fatty acids	0.052	0.056	0.148	0.050	0.063	0.156	0.014	0.603	0.719
	Saturated fatty acids	0.030	0.266	0.403	0.036	0.187	0.317	0.024	0.389	0.537
	Fat - total	0.040	0.139	0.243	0.044	0.103	0.207	0.018	0.512	0.651
	Cholesterol	0.015	0.579	0.710	0.016	0.559	0.696	0.020	0.468	0.610

Table A.6. (cont.)

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P _{adj} value	Tau	P value	P _{adj} value	Tau	P value	P _{adj} value
Minerals	Calcium	0.059	0.029	0.100	0.069	0.011	0.048	0.021	0.442	0.592
	Chloride	0.033	0.229	0.364	0.054	0.047	0.134	-0.019	0.484	0.626
	Copper	0.091	0.001	0.009	0.102	0.000	0.006	0.024	0.377	0.532
	Iodine	0.035	0.199	0.335	0.034	0.212	0.349	0.024	0.390	0.537
	Iron	0.070	0.010	0.047	0.090	0.001	0.009	-0.009	0.737	0.837
	Magnesium	0.094	0.000	0.009	0.109	0.000	0.003	0.004	0.876	0.922
	Manganese	0.108	0.000	0.003	0.120	0.000	0.001	0.021	0.438	0.592
	Nitrogen	0.034	0.212	0.349	0.045	0.094	0.199	0.010	0.717	0.820
	Phosphorus	0.061	0.025	0.091	0.074	0.006	0.035	0.013	0.637	0.748
	Potassium	0.090	0.001	0.009	0.102	0.000	0.006	0.004	0.873	0.922
	Selenium	0.051	0.061	0.156	0.059	0.030	0.100	0.029	0.286	0.425
	Sodium	0.025	0.357	0.519	0.046	0.089	0.194	-0.025	0.358	0.519
	Zinc	0.024	0.374	0.532	0.042	0.124	0.232	-0.014	0.608	0.719
	Alpha carotene	0.063	0.020	0.075	0.067	0.013	0.057	0.032	0.236	0.364
Vitamins	Beta carotene	0.049	0.071	0.164	0.045	0.097	0.204	0.020	0.457	0.604
	Carotene - total	0.050	0.062	0.156	0.047	0.079	0.175	0.020	0.461	0.605
	Vitamin A - retinol	0.050	0.067	0.162	0.044	0.101	0.207	0.050	0.069	0.164
	Vitamin A - retinol equivalents	0.049	0.071	0.164	0.042	0.121	0.232	0.029	0.296	0.436
	Vitamin B1 - thiamin	0.078	0.004	0.027	0.097	0.000	0.009	-0.004	0.881	0.922
	Vitamin B2 - riboflavin	0.071	0.008	0.041	0.077	0.004	0.027	0.023	0.400	0.547
	Vitamin B3 - niacin	0.066	0.014	0.059	0.078	0.004	0.027	0.016	0.565	0.698
	Vitamin B6 - pyridoxine	0.075	0.006	0.033	0.091	0.001	0.009	0.001	0.973	0.977
	Vitamin B9 - folate	0.073	0.007	0.035	0.082	0.002	0.019	0.001	0.957	0.969
	Vitamin B12 - cobalamin	0.055	0.041	0.126	0.054	0.045	0.133	0.043	0.120	0.232
	Vitamin C - ascorbic acid	0.053	0.050	0.138	0.058	0.033	0.105	-0.004	0.889	0.922
	Vitamin D - ergocalciferol	0.068	0.012	0.055	0.064	0.018	0.071	0.053	0.053	0.145
	Vitamin E - alpha tocopherol equivalents	0.058	0.033	0.105	0.059	0.029	0.100	-0.002	0.942	0.965

Table A.7. Results of PERMANOVA to test for an association between gut microbiome composition and study variables. PERMANOVA with 1,000 permutations was conducted on both Bray–Curtis and Jaccard distance matrices. Highlighted cells denote significant associations where $P < 0.05$ and $P_{\text{adj}} < 0.1$. Values in bold indicate $P_{\text{adj}} < 0.05$.

Category	Variable	Bray–Curtis distance matrix						Jaccard distance matrix					
		R ²	P value	P_{adj} value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	P_{adj} value	Sum of sqs	Mean sqs	F-ratio
Behaviour	Extraversion	0.001	0.837	0.857	0.069	0.069	0.615	0.001	0.923	0.923	0.129	0.129	0.661
	Agreeableness	0.002	0.192	0.264	0.145	0.145	1.294	0.002	0.222	0.296	0.232	0.232	1.190
	Conscientiousness	0.003	0.083	0.118	0.183	0.183	1.629	0.002	0.143	0.196	0.252	0.252	1.290
	Neuroticism	0.002	0.229	0.305	0.139	0.139	1.239	0.002	0.138	0.196	0.258	0.258	1.320
	Openness	0.001	0.719	0.754	0.083	0.083	0.738	0.001	0.680	0.768	0.163	0.163	0.835
	Social network size	0.003	0.073	0.107	0.220	0.220	1.962	0.003	0.079	0.120	0.319	0.319	1.633
	Social skill/commn	0.002	0.361	0.450	0.120	0.120	1.074	0.002	0.365	0.458	0.204	0.204	1.047
	Anxiety	0.003	0.060	0.094	0.195	0.195	1.762	0.003	0.034	0.057	0.331	0.331	1.708
	Stress	0.003	0.027	0.052	0.221	0.221	1.991	0.003	0.026	0.050	0.329	0.329	1.701
	Adventurous eater	0.007	0.001	0.004	0.516	0.516	4.647	0.006	0.001	0.004	0.743	0.743	3.833
Diet	Gluten free	0.005	0.003	0.008	0.347	0.347	3.113	0.004	0.003	0.008	0.478	0.478	2.460
	Dairy free	0.004	0.008	0.020	0.282	0.282	2.523	0.003	0.010	0.022	0.392	0.392	2.013
	Probiotic foods	0.006	0.002	0.006	0.426	0.426	3.817	0.005	0.003	0.008	0.563	0.563	2.894
	Prebiotic foods	0.014	0.001	0.004	0.970	0.970	8.773	0.011	0.001	0.004	1.315	1.315	6.811
	Probiotic supplements	0.006	0.002	0.006	0.431	0.431	3.869	0.005	0.002	0.006	0.623	0.623	3.209
	Prebiotic supplements	0.003	0.020	0.042	0.239	0.239	2.143	0.003	0.015	0.030	0.357	0.357	1.836
	Total supplements	0.012	0.001	0.004	0.880	0.880	7.944	0.009	0.001	0.004	1.188	1.188	6.146
	Caesarean section	0.002	0.608	0.686	0.091	0.091	0.833	0.002	0.700	0.770	0.160	0.160	0.828
	Premature birth	0.001	0.684	0.753	0.086	0.086	0.763	0.001	0.729	0.783	0.158	0.158	0.807
	Formula-fed	0.003	0.073	0.107	0.194	0.194	1.727	0.003	0.086	0.126	0.286	0.286	1.467
Health & lifestyle	General health	0.012	0.001	0.004	0.859	0.859	7.765	0.009	0.001	0.004	1.111	1.111	5.747
	Body mass index	0.004	0.021	0.042	0.252	0.252	2.261	0.003	0.010	0.022	0.401	0.401	2.061
	Days exercise	0.003	0.043	0.073	0.204	0.204	1.828	0.002	0.043	0.070	0.302	0.302	1.553
	Travel abroad/yr	0.007	0.001	0.004	0.478	0.478	4.297	0.005	0.001	0.004	0.672	0.672	3.462
	Different countries/yr	0.007	0.001	0.004	0.544	0.544	4.881	0.006	0.001	0.004	0.752	0.752	3.868
	Sleep quality	0.005	0.001	0.004	0.365	0.365	3.269	0.004	0.001	0.004	0.482	0.482	2.480
	Pain tolerance	0.001	0.881	0.881	0.064	0.064	0.572	0.001	0.884	0.905	0.136	0.136	0.696
	Allergy/asthma/eczema	0.001	0.574	0.665	0.096	0.096	0.857	0.001	0.575	0.666	0.176	0.176	0.904

Table A.7. (cont.)

Category	Variable	Bray-Curtis distance matrix						Jaccard distance matrix					
		R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio
Health & lifestyle (cont.)	Mental illness	0.004	0.009	0.020	0.294	0.294	2.632	0.003	0.006	0.015	0.433	0.433	2.226
	Smoking history	0.002	0.379	0.450	0.115	0.115	1.028	0.002	0.403	0.479	0.196	0.196	1.005
	Alcohol/drug history	0.002	0.370	0.450	0.119	0.119	1.059	0.002	0.402	0.479	0.198	0.198	1.019
	Antibiotics last 6 mos	0.008	0.001	0.004	0.567	0.567	5.094	0.006	0.001	0.004	0.760	0.760	3.917
	Stomach sickness freq	0.008	0.001	0.004	0.581	0.581	5.229	0.006	0.001	0.004	0.716	0.716	3.691
	Constipation freq	0.009	0.001	0.004	0.620	0.620	5.576	0.007	0.001	0.004	0.860	0.860	4.438
	Gut condition	0.007	0.002	0.006	0.501	0.501	4.502	0.006	0.002	0.006	0.740	0.740	3.814
	Cancer	0.004	0.005	0.013	0.325	0.325	2.915	0.004	0.003	0.008	0.500	0.500	2.571
	Chronic infectious disease	0.003	0.038	0.067	0.228	0.228	2.038	0.003	0.029	0.051	0.352	0.352	1.809
	Immunodeficiency	0.005	0.001	0.004	0.394	0.394	3.531	0.005	0.001	0.004	0.588	0.588	3.023
	Gender	0.007	0.009	0.020	0.289	0.289	2.730	0.005	0.012	0.025	0.395	0.395	2.090
Sociodemographics	Age	0.007	0.001	0.004	0.538	0.538	4.838	0.006	0.001	0.004	0.733	0.733	3.779
	Unemployed	0.003	0.030	0.055	0.232	0.232	2.074	0.003	0.029	0.051	0.343	0.343	1.761
	Siblings	0.002	0.240	0.310	0.136	0.136	1.214	0.002	0.274	0.354	0.216	0.216	1.111
	Dog owner	0.003	0.053	0.086	0.195	0.195	1.742	0.002	0.063	0.099	0.292	0.292	1.498
	Cat owner	0.001	0.718	0.754	0.080	0.080	0.712	0.001	0.788	0.826	0.146	0.146	0.750

Table A.8. Results of PERMANOVA to test for an association between gut microbiome composition and nutrient variables. PERMANOVA with 1,000 permutations was conducted on both Bray–Curtis and Jaccard distance matrices. Highlighted cells denote significant associations where $P < 0.05$ and $P_{\text{adj}} < 0.1$. Values in bold indicate $P_{\text{adj}} < 0.05$.

Category	Variable	Bray–Curtis distance matrix						Jaccard distance matrix					
		R ²	P value	P_{adj} value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	P_{adj} value	Sum of sqs	Mean sqs	F-ratio
Food	Energy (kcal)	0.003	0.074	0.153	0.184	0.184	1.658	0.002	0.061	0.131	0.294	0.294	1.518
	Meat	0.002	0.387	0.550	0.117	0.117	1.050	0.002	0.515	0.641	0.183	0.183	0.943
	Fish	0.003	0.103	0.192	0.170	0.170	1.531	0.002	0.117	0.211	0.261	0.261	1.345
	Eggs	0.001	0.521	0.664	0.098	0.098	0.879	0.002	0.525	0.641	0.179	0.179	0.925
	Milk products	0.003	0.091	0.182	0.183	0.183	1.647	0.003	0.074	0.143	0.298	0.298	1.537
	Fats & oils	0.001	0.861	0.861	0.064	0.064	0.574	0.001	0.739	0.828	0.152	0.152	0.783
	Potatoes	0.003	0.054	0.121	0.195	0.195	1.750	0.003	0.053	0.124	0.298	0.298	1.536
	Fruit	0.008	0.001	0.005	0.560	0.560	5.062	0.007	0.001	0.004	0.779	0.779	4.035
	Vegetables	0.004	0.013	0.040	0.247	0.247	2.219	0.003	0.012	0.035	0.362	0.362	1.866
	Cereals	0.006	0.001	0.005	0.411	0.411	3.705	0.005	0.001	0.004	0.578	0.578	2.986
	Nuts & seeds	0.001	0.607	0.709	0.091	0.091	0.816	0.002	0.514	0.641	0.184	0.184	0.948
	Sugars & snacks	0.003	0.095	0.183	0.176	0.176	1.585	0.002	0.085	0.159	0.279	0.279	1.441
	Soups & sauces	0.002	0.196	0.332	0.147	0.147	1.322	0.002	0.190	0.322	0.241	0.241	1.242
	Protein	0.001	0.789	0.840	0.071	0.071	0.641	0.001	0.857	0.873	0.136	0.136	0.699
	Carbohydrate - fructose	0.009	0.001	0.005	0.621	0.621	5.624	0.007	0.001	0.004	0.831	0.831	4.303
Macronutrients	Carbohydrate - galactose	0.001	0.631	0.722	0.085	0.085	0.762	0.001	0.629	0.734	0.164	0.164	0.844
	Carbohydrate - glucose	0.008	0.001	0.005	0.556	0.556	5.025	0.006	0.001	0.004	0.746	0.746	3.860
	Carbohydrate - lactose	0.003	0.073	0.153	0.186	0.186	1.674	0.003	0.064	0.133	0.299	0.299	1.541
	Carbohydrate - maltose	0.004	0.012	0.039	0.275	0.275	2.474	0.003	0.008	0.026	0.409	0.409	2.111
	Carbohydrate - starch	0.006	0.001	0.005	0.382	0.382	3.444	0.005	0.001	0.004	0.550	0.550	2.843
	Carbohydrate - sucrose	0.006	0.002	0.008	0.399	0.399	3.601	0.005	0.002	0.008	0.558	0.558	2.885
	Carbohydrate - sugars (total)	0.007	0.001	0.005	0.497	0.497	4.492	0.006	0.001	0.004	0.677	0.677	3.501
	Carbohydrate - total	0.008	0.001	0.005	0.513	0.513	4.634	0.006	0.001	0.004	0.713	0.713	3.688
	Fibre	0.008	0.001	0.005	0.513	0.513	4.634	0.006	0.001	0.004	0.691	0.691	3.576
	Monounsaturated fatty acids	0.001	0.825	0.840	0.071	0.071	0.640	0.001	0.873	0.873	0.139	0.139	0.715
	Polyunsaturated fatty acids	0.001	0.802	0.840	0.074	0.074	0.660	0.001	0.836	0.873	0.142	0.142	0.732
	Saturated fatty acids	0.001	0.607	0.709	0.092	0.092	0.827	0.002	0.526	0.641	0.180	0.180	0.925
	Fat - total	0.001	0.795	0.840	0.074	0.074	0.661	0.001	0.811	0.873	0.145	0.145	0.745
	Cholesterol	0.002	0.293	0.431	0.127	0.127	1.138	0.002	0.285	0.443	0.213	0.213	1.097

Table A.8. (cont.)

Category	Variable	Bray–Curtis distance matrix						Jaccard distance matrix					
		R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio
Minerals	Calcium	0.003	0.044	0.109	0.215	0.215	1.933	0.003	0.037	0.094	0.330	0.330	1.700
	Chloride	0.002	0.281	0.425	0.129	0.129	1.162	0.002	0.281	0.443	0.218	0.218	1.124
	Copper	0.002	0.125	0.226	0.165	0.165	1.483	0.002	0.071	0.142	0.289	0.289	1.489
	Iodine	0.002	0.277	0.425	0.133	0.133	1.195	0.002	0.307	0.452	0.215	0.215	1.107
	Iron	0.004	0.007	0.026	0.294	0.294	2.647	0.004	0.007	0.026	0.422	0.422	2.178
	Magnesium	0.007	0.001	0.005	0.477	0.477	4.304	0.006	0.001	0.004	0.658	0.658	3.403
	Manganese	0.009	0.001	0.005	0.590	0.590	5.343	0.007	0.001	0.004	0.810	0.810	4.195
	Nitrogen	0.001	0.795	0.840	0.071	0.071	0.641	0.001	0.850	0.873	0.136	0.136	0.699
	Phosphorus	0.002	0.140	0.245	0.168	0.168	1.510	0.002	0.127	0.222	0.266	0.266	1.370
	Potassium	0.007	0.001	0.005	0.444	0.444	4.005	0.005	0.001	0.004	0.617	0.617	3.189
	Selenium	0.001	0.557	0.679	0.093	0.093	0.838	0.001	0.677	0.774	0.159	0.159	0.820
	Sodium	0.002	0.407	0.550	0.113	0.113	1.013	0.002	0.418	0.563	0.196	0.196	1.011
	Zinc	0.001	0.814	0.840	0.071	0.071	0.636	0.001	0.872	0.873	0.136	0.136	0.699
	Alpha carotene	0.002	0.221	0.364	0.142	0.142	1.276	0.002	0.199	0.327	0.240	0.240	1.235
Vitamins	Beta carotene	0.003	0.045	0.109	0.200	0.200	1.797	0.003	0.035	0.093	0.305	0.305	1.573
	Carotene - total	0.003	0.047	0.110	0.204	0.204	1.832	0.003	0.045	0.109	0.312	0.312	1.607
	Vitamin A - retinol	0.001	0.516	0.664	0.095	0.095	0.851	0.002	0.423	0.563	0.191	0.191	0.983
	Vitamin A - retinol equivalents	0.002	0.413	0.550	0.109	0.109	0.983	0.002	0.336	0.481	0.209	0.209	1.078
	Vitamin B1 - thiamin	0.006	0.002	0.008	0.404	0.404	3.648	0.005	0.001	0.004	0.579	0.579	2.993
	Vitamin B2 - riboflavin	0.003	0.036	0.101	0.227	0.227	2.040	0.003	0.027	0.076	0.343	0.343	1.771
	Vitamin B3 - niacin	0.001	0.543	0.676	0.097	0.097	0.872	0.001	0.595	0.709	0.172	0.172	0.884
	Vitamin B6 - pyridoxine	0.004	0.014	0.041	0.263	0.263	2.365	0.003	0.008	0.026	0.394	0.394	2.032
	Vitamin B9 - folate	0.004	0.009	0.031	0.297	0.297	2.672	0.004	0.012	0.035	0.423	0.423	2.182
	Vitamin B12 - cobalamin	0.002	0.408	0.550	0.114	0.114	1.021	0.002	0.344	0.481	0.207	0.207	1.069
	Vitamin C - ascorbic acid	0.005	0.002	0.008	0.316	0.316	2.848	0.004	0.001	0.004	0.461	0.461	2.379
	Vitamin D - ergocalciferol	0.002	0.256	0.409	0.134	0.134	1.200	0.002	0.306	0.452	0.213	0.213	1.098
	Vitamin E - alpha tocopherol equivalents	0.003	0.045	0.109	0.197	0.197	1.774	0.002	0.056	0.125	0.293	0.293	1.512

Table A.9. Output from regression models predicting abundances of genera in the gut microbiome, with sufferers of mental illness excluded from dataset. *P* values highlighted in green denote significant predictors of genus abundance where $P < 0.05$. Note that two *P* values are given for zero-inflated negative binomial regression models, with the first *P* value indicating whether the variable predicts genus abundance, and the second *P* value indicating whether the variable predicts the presence versus absence of the genus. CL represents 95% confidence limit for each regression coefficient.

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Akkermansia</i>	<i>P</i> value	0.186	0.532	0.652	0.047	0.563	0.412	0.491	0.196	0.005	0.458
	Coefficient	0.151	0.055	0.194	0.029	0.018	-0.502	-0.263	-0.514	-1.511	-0.284
	Lower CL	-0.073	-0.118	-0.649	0.000	-0.043	-1.702	-1.010	-1.292	-2.557	-1.033
	Upper CL	0.375	0.228	1.037	0.058	0.079	0.697	0.485	0.265	-0.465	0.466
<i>Alistipes</i>	<i>P</i> value	0.975	0.253	0.914	0.002	0.020	0.769	0.886	0.598	0.161	0.852
	Coefficient	-0.002	0.048	0.022	0.022	-0.034	0.085	-0.026	-0.099	-0.353	-0.034
	Lower CL	-0.107	-0.034	-0.376	0.008	-0.063	-0.481	-0.379	-0.466	-0.847	-0.387
	Upper CL	0.104	0.129	0.420	0.036	-0.005	0.651	0.327	0.268	0.140	0.320
<i>Bacteroides</i>	<i>P</i> value	0.259	0.555	0.774	0.564	0.817	0.455	0.100	0.217	0.909	0.318
	Coefficient	0.031	0.012	0.029	-0.002	0.002	-0.108	0.148	0.116	0.014	0.090
	Lower CL	-0.022	-0.029	-0.170	-0.009	-0.013	-0.392	-0.029	-0.068	-0.233	-0.087
	Upper CL	0.084	0.053	0.229	0.005	0.016	0.176	0.325	0.300	0.262	0.268
<i>Bifidobacterium</i>	<i>P</i> value	0.268	0.669	0.433	0.323	0.008	0.761	0.588	0.488	0.913	0.031
	Coefficient	-0.108	0.032	-0.288	-0.012	0.070	-0.159	0.176	-0.235	-0.049	-0.705
	Lower CL	-0.299	-0.115	-1.007	-0.037	0.018	-1.183	-0.462	-0.899	-0.942	-1.344
	Upper CL	0.083	0.179	0.431	0.012	0.122	0.864	0.814	0.429	0.843	-0.065
<i>Blautia</i>	<i>P</i> value	0.043	0.221	0.802	0.038	0.096	0.195	0.511	0.840	0.032	0.862
	Coefficient	-0.045	-0.021	0.021	-0.006	0.010	-0.154	0.049	-0.016	0.223	0.013
	Lower CL	-0.089	-0.055	-0.143	-0.012	-0.002	-0.388	-0.097	-0.167	0.019	-0.133
	Upper CL	-0.001	0.013	0.185	0.000	0.022	0.079	0.194	0.136	0.427	0.159
<i>Clostridium</i>	<i>P</i> value	0.865	0.261	0.181	0.716	0.080	0.980	0.689	0.127	0.265	0.714
	Coefficient	-0.007	0.034	-0.200	-0.002	-0.019	-0.005	-0.053	-0.210	-0.206	-0.049
	Lower CL	-0.084	-0.026	-0.492	-0.012	-0.040	-0.421	-0.312	-0.480	-0.569	-0.309
	Upper CL	0.071	0.094	0.093	0.008	0.002	0.411	0.206	0.060	0.157	0.211

Table A.9. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Collinsella</i>	P value	0.587	0.120	0.039	0.153	0.102	0.860	0.643	0.335	0.301	0.695
	Coefficient	-0.020	-0.044	-0.285	-0.007	0.016	0.035	0.057	-0.123	-0.178	-0.048
	Lower CL	-0.092	-0.100	-0.557	-0.016	-0.003	-0.352	-0.184	-0.374	-0.515	-0.290
	Upper CL	0.052	0.011	-0.014	0.003	0.036	0.421	0.298	0.127	0.159	0.193
<i>Corynebacterium</i>	P value	0.718	0.000	0.126	0.000	0.125	0.707	0.384	0.018	0.612	0.010
	Coefficient	-0.044	-0.341	-0.697	-0.073	-0.050	0.244	-0.352	-0.997	-0.287	1.038
	Lower CL	-0.281	-0.524	-1.589	-0.104	-0.115	-1.027	-1.144	-1.822	-1.395	0.243
	Upper CL	0.194	-0.158	0.196	-0.043	0.014	1.515	0.440	-0.172	0.822	1.833
<i>Desulfovibrio</i>	P value	0.002	0.447	0.713	0.561	0.985	0.839	0.665	0.375	0.022	0.400
	P value _{zero-infl}	0.499	0.735	0.663	0.175	0.233	0.758	0.171	0.022	0.304	0.132
	Coefficient	-0.306	0.058	-0.135	0.007	-0.001	-0.108	0.144	0.317	-1.245	0.280
	Lower CL	-0.499	-0.092	-0.853	-0.017	-0.058	-1.151	-0.510	-0.383	-2.310	-0.372
<i>Dialister</i>	Upper CL	-0.114	0.209	0.583	0.031	0.057	0.935	0.799	1.017	-0.179	0.933
	P value	0.406	0.138	0.474	0.194	0.103	0.287	0.006	0.422	0.150	0.934
	P value _{zero-infl}	0.761	0.681	0.623	0.007	0.078	0.369	0.729	0.755	0.140	0.704
	Coefficient	0.068	-0.093	0.209	0.014	0.033	0.419	-0.752	0.228	-0.491	-0.103
<i>Dorea</i>	Lower CL	-0.092	-0.216	-0.363	-0.007	-0.007	-0.353	-1.286	-0.329	-1.159	-0.634
	Upper CL	0.227	0.030	0.780	0.036	0.073	1.191	-0.218	0.786	0.178	0.428
	P value	0.397	0.319	0.067	0.118	0.038	0.355	0.961	0.174	0.196	0.878
	Coefficient	0.031	0.028	-0.249	-0.007	0.020	-0.179	-0.006	-0.170	-0.218	0.019
<i>Faecalibacterium</i>	Lower CL	-0.040	-0.027	-0.515	-0.016	0.001	-0.558	-0.242	-0.416	-0.548	-0.218
	Upper CL	0.101	0.082	0.018	0.002	0.039	0.200	0.230	0.075	0.112	0.255
	P value	0.813	0.852	0.814	0.330	0.516	0.973	0.817	0.650	0.893	0.521
	Coefficient	-0.011	-0.007	0.040	-0.006	-0.008	0.008	0.035	0.072	-0.029	-0.097
<i>Flavonifractor</i>	Lower CL	-0.100	-0.075	-0.294	-0.017	-0.032	-0.467	-0.261	-0.237	-0.443	-0.395
	Upper CL	0.078	0.062	0.374	0.006	0.016	0.484	0.331	0.380	0.386	0.200
	P value	0.453	0.179	0.419	0.295	0.743	0.007	0.021	0.010	0.870	0.107
	Coefficient	0.033	0.045	0.132	0.006	-0.004	0.622	-0.334	0.387	0.033	0.234
	Lower CL	-0.052	-0.021	-0.188	-0.005	-0.027	0.167	-0.617	0.092	-0.364	-0.050
	Upper CL	0.118	0.110	0.452	0.017	0.019	1.077	-0.050	0.682	0.430	0.518

Table A.9. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Lactobacillus</i>	P value	0.176	0.780	0.046	0.000	0.572	0.879	0.223	0.901	0.087	0.136
	Coefficient	0.139	-0.022	0.771	-0.049	-0.016	0.084	0.417	0.044	-0.819	0.512
	Lower CL	-0.062	-0.177	0.014	-0.074	-0.070	-0.993	-0.253	-0.654	-1.759	-0.161
	Upper CL	0.340	0.133	1.528	-0.023	0.039	1.160	1.088	0.743	0.120	1.185
<i>Lactococcus</i>	P value	0.000	0.747	0.387	0.047	0.288	0.047	0.681	0.047	0.329	0.060
	P value _{zero-infl}	0.124	0.337	0.189	0.085	0.820	0.993	0.327	0.591	0.245	0.534
	Coefficient	0.392	-0.028	0.337	0.029	0.030	1.033	-0.149	0.750	0.499	-0.683
	Lower CL	0.179	-0.194	-0.427	0.000	-0.025	0.013	-0.856	0.011	-0.502	-1.394
<i>Oscillibacter</i>	Upper CL	0.606	0.139	1.102	0.057	0.086	2.053	0.559	1.488	1.499	0.029
	P value	0.759	0.754	0.752	0.461	0.002	0.959	0.391	0.819	0.035	0.927
	P value _{zero-infl}	0.062	0.039	0.646	0.014	0.887	0.245	0.567	0.283	0.004	0.550
	Coefficient	0.022	0.018	0.089	-0.007	-0.064	0.022	-0.207	-0.057	-0.903	0.023
<i>Oscillospira</i>	Lower CL	-0.120	-0.094	-0.463	-0.025	-0.105	-0.827	-0.680	-0.546	-1.741	-0.458
	Upper CL	0.164	0.130	0.642	0.011	-0.023	0.871	0.266	0.432	-0.065	0.503
	P value	0.004	0.515	0.691	0.007	0.782	0.812	0.767	0.014	0.001	0.135
	P value _{zero-infl}	0.970	0.061	0.694	0.105	0.030	0.394	0.657	0.222	0.035	0.621
<i>Parabacteroides</i>	Coefficient	0.169	0.030	0.090	0.020	-0.005	0.081	0.059	0.509	-1.002	0.297
	Lower CL	0.054	-0.061	-0.353	0.005	-0.040	-0.589	-0.330	0.104	-1.619	-0.092
	Upper CL	0.284	0.121	0.532	0.035	0.030	0.751	0.447	0.915	-0.385	0.686
	P value	0.817	0.383	0.592	0.720	0.886	0.339	0.569	0.515	0.691	0.227
<i>Prevotella</i>	Coefficient	-0.014	-0.041	0.122	0.003	0.002	-0.309	0.115	-0.136	-0.112	0.243
	Lower CL	-0.132	-0.132	-0.323	-0.012	-0.030	-0.941	-0.279	-0.547	-0.663	-0.152
	Upper CL	0.104	0.051	0.566	0.018	0.034	0.324	0.509	0.274	0.440	0.639
	P value	0.316	0.002	0.012	0.002	0.296	0.301	0.229	0.356	0.018	0.283
<i>Roseburia</i>	Coefficient	-0.121	-0.291	1.147	-0.047	-0.034	0.670	0.486	0.389	-1.340	-0.435
	Lower CL	-0.359	-0.475	0.254	-0.078	-0.099	-0.600	-0.306	-0.436	-2.449	-1.229
	Upper CL	0.116	-0.108	2.041	-0.017	0.030	1.941	1.277	1.213	-0.231	0.359
	P value	0.934	0.359	0.982	0.520	0.113	0.240	0.656	0.786	0.110	0.321
<i>Roseburia</i>	Coefficient	0.003	-0.022	0.003	-0.003	0.014	0.198	0.047	0.030	0.235	-0.105
	Lower CL	-0.059	-0.070	-0.230	-0.011	-0.003	-0.133	-0.159	-0.185	-0.053	-0.311
	Upper CL	0.064	0.025	0.235	0.005	0.030	0.529	0.253	0.244	0.524	0.102

Table A.9. (cont.)

Genus	Model results	Sociability	Neurotic tendencies	Gender	Age	Body mass index	Caesarean section	Formula-fed	Gut condition	Antibiotics last 6 mos	Probiotic supplements
<i>Streptococcus</i>	P value	0.954	0.025	0.043	0.420	0.039	0.047	0.331	0.059	0.115	0.017
	Coefficient	-0.004	-0.114	0.503	-0.007	0.037	0.700	-0.214	0.432	0.485	-0.529
	Lower CL	-0.133	-0.213	0.017	-0.023	0.002	0.009	-0.644	-0.017	-0.118	-0.961
	Upper CL	0.125	-0.014	0.989	0.010	0.072	1.392	0.217	0.881	1.088	-0.097
<i>Sutterella</i>	P value	0.019	0.627	0.910	0.754	0.937	0.758	0.837	0.242	0.507	0.199
	Coefficient	-0.292	0.047	0.053	-0.005	-0.003	0.206	0.086	-0.508	-0.387	-0.537
	Lower CL	-0.537	-0.142	-0.867	-0.036	-0.069	-1.104	-0.730	-1.357	-1.529	-1.355
	Upper CL	-0.047	0.235	0.973	0.026	0.064	1.516	0.902	0.342	0.756	0.282
<i>Turicibacter</i>	P value	0.080	0.835	0.408	0.122	0.358	0.425	0.703	0.551	0.412	0.607
	Coefficient	-0.190	-0.017	0.338	-0.022	0.027	0.464	-0.138	0.225	0.416	-0.187
	Lower CL	-0.403	-0.182	-0.463	-0.049	-0.031	-0.676	-0.848	-0.515	-0.578	-0.900
	Upper CL	0.023	0.147	1.140	0.006	0.085	1.603	0.572	0.965	1.410	0.525

Table A.10. Results of Kendall's Tau-b correlation analysis between gut microbiome diversity and study variables, with sufferers of mental illness excluded from dataset. Highlighted cells denote significant associations where $P < 0.05$ and $P_{adj} < 0.1$. Values in bold indicate $P_{adj} < 0.05$.

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P_{adj} value	Tau	P value	P_{adj} value	Tau	P value	P_{adj} value
Behaviour	Extraversion	-0.032	0.282	0.379	-0.034	0.257	0.361	-0.044	0.147	0.261
	Agreeableness	-0.055	0.072	0.155	-0.046	0.127	0.242	-0.057	0.064	0.139
	Conscientiousness	-0.046	0.134	0.246	-0.044	0.146	0.261	-0.081	0.008	0.032
	Neuroticism	-0.043	0.157	0.271	-0.045	0.132	0.246	-0.008	0.786	0.838
	Openness	0.032	0.295	0.392	0.033	0.274	0.374	0.038	0.222	0.329
	Social network size	0.066	0.027	0.080	0.086	0.004	0.017	0.026	0.386	0.488
	Social skill/commn	-0.047	0.127	0.242	-0.042	0.176	0.285	-0.068	0.030	0.084
	Anxiety	-0.078	0.010	0.036	-0.078	0.010	0.036	-0.039	0.204	0.314
	Stress	-0.073	0.020	0.063	-0.062	0.047	0.115	-0.040	0.205	0.314
	Adventurous eater	0.092	0.003	0.015	0.103	0.001	0.005	0.059	0.063	0.139
Diet	Gluten free	-0.035	0.331	0.431	-0.045	0.207	0.314	0.009	0.813	0.854
	Dairy free	-0.047	0.187	0.298	-0.040	0.264	0.366	-0.043	0.237	0.340
	Probiotic foods	0.120	0.000	0.001	0.114	0.000	0.001	0.093	0.003	0.012
	Prebiotic foods	0.137	0.000	0.000	0.148	0.000	0.000	0.072	0.019	0.063
	Probiotic supplements	-0.078	0.030	0.084	-0.075	0.036	0.093	-0.043	0.233	0.338
	Prebiotic supplements	-0.056	0.118	0.232	-0.050	0.168	0.282	-0.054	0.135	0.246
	Total supplements	-0.115	0.000	0.001	-0.127	0.000	0.001	-0.058	0.060	0.135
	Caesarean section	-0.008	0.856	0.876	-0.024	0.554	0.668	0.017	0.677	0.772
	Premature birth	-0.011	0.765	0.836	-0.009	0.815	0.854	0.008	0.826	0.859
	Formula-fed	-0.073	0.055	0.130	-0.063	0.098	0.201	-0.016	0.672	0.772
Health & Lifestyle	General health	0.147	0.000	0.000	0.142	0.000	0.000	0.122	0.000	0.001
	Body mass index	-0.016	0.581	0.679	-0.010	0.746	0.829	0.005	0.880	0.894
	Days exercise	0.027	0.389	0.488	0.018	0.561	0.670	-0.010	0.761	0.836
	Travel abroad/yr	0.097	0.004	0.017	0.086	0.011	0.039	0.100	0.003	0.015
	Different countries/yr	0.117	0.001	0.003	0.100	0.003	0.014	0.118	0.001	0.003
	Sleep quality	0.071	0.023	0.071	0.063	0.043	0.109	0.040	0.204	0.314
	Pain tolerance	-0.026	0.405	0.502	-0.027	0.384	0.488	-0.013	0.682	0.772
	Allergy/asthma/eczema	-0.033	0.362	0.467	-0.020	0.584	0.679	-0.058	0.109	0.216
	Smoking history	-0.021	0.551	0.668	-0.010	0.771	0.836	-0.013	0.727	0.816
	Alcohol/drug history	-0.010	0.786	0.838	-0.004	0.922	0.929	-0.027	0.451	0.554

Table A.10. (cont.)

Category	Variable	Shannon diversity index			Inverse Simpson diversity index			Genus richness		
		Tau	P value	P _{adj} value	Tau	P value	P _{adj} value	Tau	P value	P _{adj} value
Health & lifestyle (cont.)	Antibiotics last 6 mos	-0.149	0.000	0.001	-0.138	0.000	0.001	-0.148	0.000	0.036
	Stomach sickness freq	-0.125	0.000	0.001	-0.129	0.000	0.001	-0.087	0.007	0.008
	Constipation freq	0.068	0.035	0.091	0.040	0.216	0.324	0.102	0.002	0.002
	Gut condition	-0.157	0.000	0.000	-0.152	0.000	0.000	-0.137	0.000	0.289
	Cancer	-0.071	0.047	0.115	-0.111	0.002	0.010	-0.050	0.168	0.075
	Chronic infectious disease	-0.076	0.035	0.091	-0.063	0.079	0.166	-0.079	0.031	0.007
Sociodemographics	Immunodeficiency	-0.148	0.000	0.001	-0.140	0.000	0.001	-0.123	0.001	0.550
	Gender	-0.077	0.095	0.198	-0.063	0.172	0.285	-0.026	0.575	0.270
	Age	-0.036	0.230	0.338	-0.034	0.257	0.361	-0.049	0.105	0.002
	Unemployed	-0.068	0.058	0.132	-0.051	0.155	0.271	-0.132	0.000	0.082
	Siblings	-0.036	0.275	0.374	-0.032	0.324	0.426	-0.073	0.027	0.082
	Dog owner	-0.084	0.019	0.063	-0.079	0.027	0.080	-0.070	0.056	0.299
	Cat owner	0.000	0.996	0.996	-0.007	0.855	0.876	0.049	0.175	0.221

Table A.11. (cont.)

Category	Variable	Bray–Curtis distance matrix						Jaccard distance matrix					
		R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	P _{adj} value	Sum of sqs	Mean sqs	F-ratio
Healthy & lifestyle (cont.)	Mental illness	0.002	0.286	0.372	0.132	0.132	1.180	0.002	0.329	0.354	0.211	0.224	1.150
	Smoking history	0.001	0.858	0.858	0.064	0.064	0.572	0.001	0.882	0.883	0.130	0.130	0.668
	Alcohol/drug history	0.008	0.002	0.008	0.463	0.463	4.161	0.006	0.002	0.004	0.620	0.620	3.200
	Antibiotics last 6 mos	0.009	0.001	0.005	0.513	0.513	4.617	0.006	0.001	0.004	0.627	0.607	3.133
	Stomach sickness freq	0.008	0.001	0.005	0.472	0.472	4.236	0.006	0.001	0.004	0.628	0.623	3.215
	Constipation freq	0.008	0.001	0.005	0.471	0.471	4.232	0.007	0.001	0.004	0.696	0.675	3.486
	Gut condition	0.006	0.004	0.012	0.327	0.327	2.925	0.005	0.003	0.007	0.517	0.530	2.731
	Cancer	0.003	0.158	0.242	0.162	0.162	1.450	0.002	0.174	0.198	0.246	0.268	1.380
	Chronic infectious disease	0.006	0.004	0.012	0.344	0.344	3.079	0.005	0.003	0.014	0.517	0.473	2.432
	Immunodeficiency	0.006	0.035	0.088	0.200	0.200	1.934	0.005	0.057	0.076	0.284	0.308	1.651
Sociodemographics	Gender	0.008	0.001	0.005	0.456	0.456	4.093	0.006	0.001	0.004	0.613	0.624	3.221
	Age	0.002	0.231	0.323	0.139	0.139	1.240	0.002	0.163	0.267	0.245	0.241	1.239
	Unemployed	0.003	0.116	0.192	0.167	0.167	1.488	0.003	0.121	0.225	0.259	0.255	1.312
	Siblings	0.003	0.094	0.184	0.172	0.172	1.536	0.003	0.105	0.176	0.262	0.286	1.472
	Dog owner	0.001	0.832	0.852	0.071	0.071	0.630	0.001	0.855	0.876	0.140	0.139	0.714
	Cat owner	0.002	0.286	0.372	0.132	0.132	1.180	0.002	0.329	0.354	0.211	0.136	0.696

Supplementary results

The data collected in Chapter 3 also provided the opportunity to assess whether the positive relationship between pain tolerance and social network size that I reported in a previous study (Johnson & Dunbar, 2016) was reproducible in a different dataset. Multiple regression analysis (Table A.12) revealed that pain tolerance also significantly predicted social network size in this dataset ($P = 0.005$). As in my previously published study, stress was also included in the regression model as a possible confounding factor. Three outliers were removed and social network size was log-transformed to ensure that the residuals were normally distributed (Shapiro–Wilk test, $P > 0.05$) and satisfied the assumption of homoscedasticity (non-constant variance test, $P > 0.05$). While the previous finding was based on a physical test of pain tolerance (where participants' fitness levels also needed to be taken into account), this result relies on self-reporting but has the advantage of a much larger sample size ($n = 629$). Since the same key result was reproduced using a different method, this provides further evidence in support of the positive relationship between pain tolerance and social network size, hypothesized to be mediated by the endogenous μ -opioid system (Johnson & Dunbar, 2016).

Table A.12. Regression statistics for predictors of social network size.

Predictor	Coefficient $\pm$ SE	<i>t</i> value	<i>P</i> value	ΔR^2
Pain tolerance	0.038 $\pm$ 0.014	2.795	0.005	0.012
Stress	-0.021 $\pm$ 0.015	-1.458	0.145	0.003

Total R^2 = 0.016

Chapter 4

Figures

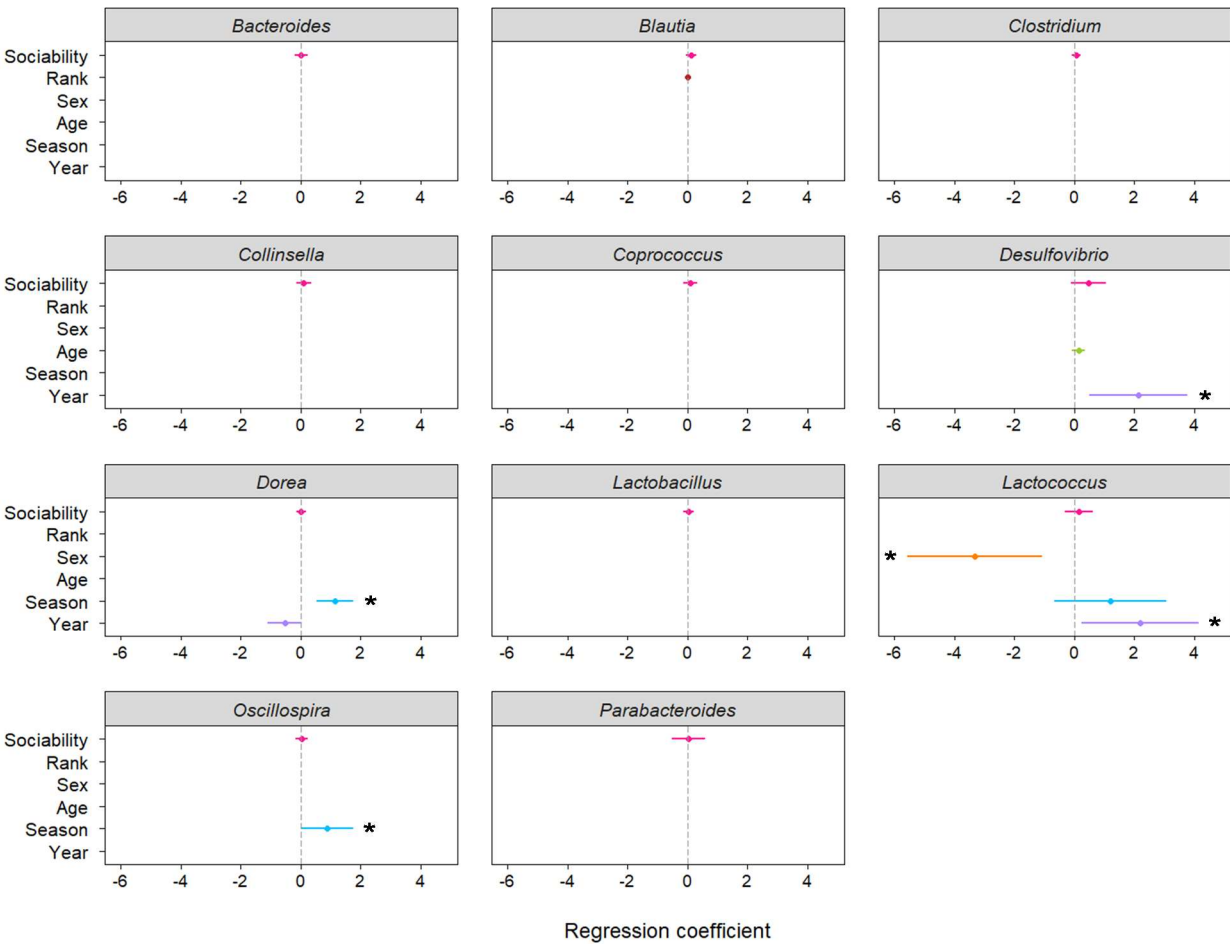


Figure A.6. Coefficient plots from regression models predicting abundances of genera in the macaque gut microbiome. Asterisks denote significant predictors of genus abundance where $P < 0.05$ and bars indicate 95% confidence intervals. The main variable of interest was the sociability index (as measured by the number of grooming partners and duration of grooming interactions), while other variables were also included where appropriate, as determined using Akaike information criterion. A positive relationship with sex indicates a higher abundance of the genus in females. Plots displayed here depict genera whose abundances were not significantly related to sociability (for significant results see Figure 4.3).

Tables

Table A.13. Summary of statistically significant associations between bacterial genera in the gut and sociability or autistic traits reported in the literature. Table lists findings from research in both animal models and human populations and includes all genera (16 in total) associated with autism or social behaviour in at least two independent studies. Adapted from Table 3.1, but including only bacterial genera that are linked to sociability or autistic traits and are also sufficiently prevalent in the macaque population to conduct regression analyses. This table additionally includes the genus *Coprococcus* that was identified in the gut microbiome of this macaque population but not in the human cohort (Chapter 3).

Genus	Change in abundance	Sociability/ autistic traits	Study subject	References
<i>Bacteroides</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
<i>Blautia</i>	↓	Autism	Infants	Inoue <i>et al.</i> , 2016
	↓	Autism	Children	Luna <i>et al.</i> , 2017
	↓	Autism	Mice	Golubeva <i>et al.</i> , 2017
<i>Clostridium</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2002; Song <i>et al.</i> , 2004; Parracho <i>et al.</i> , 2005; de Angelis <i>et al.</i> , 2013; Tomova <i>et al.</i> , 2015; Grimaldi <i>et al.</i> , 2017; Luna <i>et al.</i> , 2017; Strati <i>et al.</i> , 2017
	↓	Autism	Children	Finegold <i>et al.</i> , 2010
<i>Collinsella</i>	↑	Autism	Children	Strati <i>et al.</i> , 2017
	↓	Autism	Children	Finegold <i>et al.</i> , 2010
<i>Coprococcus</i>	↓	Autism	Children	de Angelis <i>et al.</i> , 2013; Kang <i>et al.</i> , 2013
<i>Desulfovibrio</i>	↑	Autism	Children	Finegold <i>et al.</i> , 2010; Tomova <i>et al.</i> , 2015
<i>Dialister</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; Strati <i>et al.</i> , 2017
	↑	Sociability	Infants	Christian <i>et al.</i> , 2015
<i>Dorea</i>	↑	Autism	Children	de Angelis <i>et al.</i> , 2013; Strati <i>et al.</i> , 2017
	↓	Autism	Children	Luna <i>et al.</i> , 2017
<i>Faecalibacterium</i>	↑	Autism	Infants	Inoue <i>et al.</i> , 2016
	↓	Autism	Children	de Angelis <i>et al.</i> , 2013; Kang <i>et al.</i> , 2018
<i>Lactobacillus</i>	↑	Autism	Children	Adams <i>et al.</i> , 2011; Kang <i>et al.</i> , 2013; Tomova <i>et al.</i> , 2015; Strati <i>et al.</i> , 2017; Pulikkan <i>et al.</i> , 2018
	↓	Autism	Children	de Angelis <i>et al.</i> , 2013
<i>Lactococcus</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
<i>Oscillospira</i>	↓	Autism	Children	de Angelis <i>et al.</i> , 2013
	↑	Sociability	Mice	Szyszkowicz <i>et al.</i> , 2017
<i>Parabacteroides</i>	↓	Autism	Children	Strati <i>et al.</i> , 2017
	↑	Autism	Children	Finegold <i>et al.</i> , 2010
	↑	Sociability	Infants	Christian <i>et al.</i> , 2015
<i>Prevotella</i>	↑	Autism	Children	de Angelis <i>et al.</i> , 2013
	↓	Autism	Children	Kang <i>et al.</i> , 2013
<i>Streptococcus</i>	↓	Autism	Children	Finegold <i>et al.</i> , 2010; de Angelis <i>et al.</i> , 2013
<i>Sutterella</i>	↑	Autism	Children	Wang <i>et al.</i> , 2011, 2013, Williams <i>et al.</i> , 2011, 2012
	↓	Autism	Children	Luna <i>et al.</i> , 2017

Table A.14. Output from regression models predicting abundances of genera in the macaque gut microbiome. *P* values highlighted in green denote significant predictors of genus abundance where $P < 0.05$. CL represents 95% confidence limit for each regression coefficient. A positive regression coefficient for sex indicates a higher abundance of the genus in females.

Genus	Model results	Sociability	Rank	Sex	Age	Season	Year
<i>Bacteroides</i>	<i>P</i> value	0.894	—	—	—	—	—
	Coefficient	0.000	—	—	—	—	—
	Lower CL	-0.198	—	—	—	—	—
	Upper CL	0.227	—	—	—	—	—
<i>Blautia</i>	<i>P</i> value	0.135	0.088	—	—	—	—
	Coefficient	0.128	0.015	—	—	—	—
	Lower CL	-0.040	-0.002	—	—	—	—
	Upper CL	0.295	0.031	—	—	—	—
<i>Clostridium</i>	<i>P</i> value	0.336	—	—	—	—	—
	Coefficient	0.070	—	—	—	—	—
	Lower CL	-0.073	—	—	—	—	—
	Upper CL	0.213	—	—	—	—	—
<i>Collinsella</i>	<i>P</i> value	0.400	—	—	—	—	—
	Coefficient	0.107	—	—	—	—	—
	Lower CL	-0.141	—	—	—	—	—
	Upper CL	0.354	—	—	—	—	—
<i>Coprococcus</i>	<i>P</i> value	0.397	—	—	—	—	—
	Coefficient	0.097	—	—	—	—	—
	Lower CL	-0.127	—	—	—	—	—
	Upper CL	0.320	—	—	—	—	—
<i>Desulfovibrio</i>	<i>P</i> value	0.120	—	—	0.181	—	0.010
	Coefficient	0.471	—	—	0.149	—	2.146
	Lower CL	-0.121	—	—	-0.069	—	0.507
	Upper CL	1.063	—	—	0.366	—	3.785
<i>Dialister</i>	<i>P</i> value	0.008	—	—	—	—	0.106
	Coefficient	0.367	—	—	—	—	0.628
	Lower CL	0.097	—	—	—	—	-0.133
	Upper CL	0.636	—	—	—	—	1.390
<i>Dorea</i>	<i>P</i> value	0.814	—	—	—	0.000	0.067
	Coefficient	0.019	—	—	—	1.136	-0.533
	Lower CL	-0.139	—	—	—	0.520	-1.103
	Upper CL	0.177	—	—	—	1.752	0.038
<i>Faecalibacterium</i>	<i>P</i> value	0.002	0.098	0.021	—	—	—
	Coefficient	0.494	0.020	-1.367	—	—	—
	Lower CL	0.185	-0.004	-2.528	—	—	—
	Upper CL	0.803	0.043	-0.206	—	—	—
<i>Lactobacillus</i>	<i>P</i> value	0.728	—	—	—	—	—
	Coefficient	0.032	—	—	—	—	—
	Lower CL	-0.146	—	—	—	—	—
	Upper CL	0.209	—	—	—	—	—
<i>Lactococcus</i>	<i>P</i> value	0.487	—	0.004	—	0.204	0.029
	Coefficient	0.166	—	-3.322	—	1.211	2.188
	Lower CL	-0.302	—	-5.571	—	-0.659	0.228
	Upper CL	0.635	—	-1.073	—	3.082	4.147
<i>Oscillospira</i>	<i>P</i> value	0.799	—	—	—	0.042	—
	Coefficient	0.027	—	—	—	0.887	—
	Lower CL	-0.182	—	—	—	0.032	—
	Upper CL	0.236	—	—	—	1.743	—

Table A.14. (cont.)

Genus	Model results	Sociability	Rank	Sex	Age	Season	Year
<i>Parabacteroides</i>	<i>P</i> value	0.873	–	–	–	–	–
	Coefficient	0.045	–	–	–	–	–
	Lower CL	-0.509	–	–	–	–	–
	Upper CL	0.599	–	–	–	–	–
<i>Prevotella</i>	<i>P</i> value	0.019	–	–	0.003	0.038	–
	Coefficient	0.374	–	–	-0.165	-1.353	–
	Lower CL	0.062	–	–	-0.275	-2.633	–
	Upper CL	0.686	–	–	-0.055	-0.073	–
<i>Streptococcus</i>	<i>P</i> value	0.001	–	–	0.004	–	0.003
	Coefficient	-0.341	–	–	-0.137	–	1.210
	Lower CL	-0.549	–	–	-0.231	–	0.403
	Upper CL	-0.133	–	–	-0.043	–	2.017
<i>Sutterella</i>	<i>P</i> value	0.006	–	–	–	–	–
	Coefficient	0.514	–	–	–	–	–
	Lower CL	0.148	–	–	–	–	–
	Upper CL	0.880	–	–	–	–	–

Table A.15. Results of linear mixed model to test the factors predicting gut microbiome diversity.

Diversity is estimated using the Chao1 index.

Fixed effects:

Predictor	Coefficient	SE	<i>t</i> value	<i>P</i> value
Grooming received	1.324	50.366	0.026	0.980
Grooming given	12.532	63.587	0.197	0.850
Rank	2.863	3.291	0.870	0.418
Sex	-37.915	123.324	-0.307	0.760
Age	4.790	11.724	0.409	0.697
Season	60.780	131.209	0.463	0.660
Year	88.908	108.633	0.818	0.444

Fixed effects $R^2 = 0.067$

Random effects:

Groups	Variance	SD
Individual ID	10668	103.284
Residual	91318	302.188

Total $R^2 = 0.165$

Table A.16. Results of PERMANOVA to test the factors affecting gut microbial community composition. PERMANOVA with 1,000 permutations was conducted on both weighted and unweighted UniFrac distance matrices. Highlighted cells denote significant associations where $P < 0.05$.

Variable	Weighted UniFrac distance					Unweighted UniFrac distance				
	R ²	P value	Sum of sqs	Mean sqs	F-ratio	R ²	P value	Sum of sqs	Mean sqs	F-ratio
Individual ID	0.690	0.327	3.088	0.086	1.150	0.738	0.130	8.220	0.228	1.079
Grooming received	0.050	0.022	0.224	0.224	3.000	0.023	0.103	0.255	0.255	1.203
Grooming given	0.014	0.468	0.065	0.065	0.867	0.020	0.252	0.226	0.226	1.069
Rank	0.010	0.776	0.043	0.043	0.571	0.018	0.574	0.202	0.202	0.953
Sex	0.012	0.616	0.055	0.055	0.737	0.019	0.362	0.216	0.216	1.023
Age	0.043	0.039	0.194	0.194	2.600	0.021	0.232	0.231	0.231	1.091
Season	0.070	0.007	0.311	0.311	4.174	0.029	0.015	0.324	0.324	1.533
Year	0.011	0.658	0.050	0.050	0.666	0.017	0.687	0.194	0.194	0.916

Chapter 5

Tables

Table A.17. List of the 60 mammalian species (and their taxonomic classification) for which data on gut microbiome composition were available. Abbreviations used for provenance: Wild, W; Zoo, Z.

Common name	Species name	Family	Suborder	Order	Provenance
Springbok	<i>Antidorcas marsupialis</i>	Bovidae	Ruminantia	Artiodactyla	W & Z
Banteng	<i>Bos javanicus</i>	Bovidae	Ruminantia	Artiodactyla	Z
Dairy cow (Holstein)	<i>Bos taurus</i>	Bovidae	Ruminantia	Artiodactyla	W
Takin	<i>Budorcas taxicolor</i>	Bovidae	Ruminantia	Artiodactyla	Z
Speke's gazelle	<i>Gazella spekei</i>	Bovidae	Ruminantia	Artiodactyla	Z
Argali sheep	<i>Ovis ammon</i>	Bovidae	Ruminantia	Artiodactyla	W
Bighorn sheep	<i>Ovis canadensis</i>	Bovidae	Ruminantia	Artiodactyla	W & Z
Transcaspian urial sheep	<i>Ovis vignei</i>	Bovidae	Ruminantia	Artiodactyla	Z
Reticulated giraffe	<i>Giraffa camelopardalis reticulata</i>	Giraffidae	Ruminantia	Artiodactyla	Z
Okapi	<i>Okapia johnstoni</i>	Giraffidae	Ruminantia	Artiodactyla	Z
Babirusa	<i>Babirusa babyrussa</i>	Suidae	Suiformes	Artiodactyla	Z
Red river hog	<i>Potamochoerus porcus</i>	Suidae	Suiformes	Artiodactyla	Z
Visayan warty pig	<i>Sus cebifrons</i>	Suidae	Suiformes	Artiodactyla	Z
Cheetah	<i>Acinonyx jubatus</i>	Felidae	Feliformia	Carnivora	Z
Spotted hyena	<i>Crocuta crocuta</i>	Hyaenidae	Feliformia	Carnivora	Z
Lion	<i>Panthera leo</i>	Pantherinae	Feliformia	Carnivora	Z
Bush dog	<i>Speothos venaticus</i>	Canidae	Caniformia	Carnivora	Z
Red panda	<i>Ailurus fulgens</i>	Ailuridae	Caniformia	Carnivora	Z
Giant panda	<i>Ailuropoda melanoleuca</i>	Ursidae	Caniformia	Carnivora	Z
Spectacled bear	<i>Tremarctos ornatus</i>	Ursidae	Caniformia	Carnivora	Z
North American black bear	<i>Ursus americanus</i>	Ursidae	Caniformia	Carnivora	Z
Polar bear	<i>Ursus maritimus</i>	Ursidae	Caniformia	Carnivora	Z
Seba's short-tailed bat	<i>Carollia perspicillata</i>	Phyllostomidae	Microchiroptera	Chiroptera	Z
Indian flying fox	<i>Pteropus giganteus</i>	Pteropodidae	Megachiroptera	Chiroptera	Z
Southern three-banded armadillo	<i>Tolypeutes matacus</i>	Dasyopodidae	–	Cingulata	Z
Red kangaroo	<i>Macropus rufus</i>	Macropodidae	Macropodiformes	Diprotodontia	Z
Rock hyrax	<i>Procavia capensis</i>	Procaviidae	–	Hyracoidea	Z
Hedgehog	<i>Atelerix albiventris</i>	Erinaceidae	–	Insectivora	Z
European rabbit	<i>Oryctolagus cuniculus</i>	Leporidae	–	Lagomorpha	Z
Short-beaked echidna	<i>Tachyglossus aculeatus</i>	Tachyglossidae	–	Monotremata	Z

Table A.17. (cont.)

Common name	Species name	Family	Suborder	Order	Provenance
Somali wild ass	<i>Equus asinus</i>	Equidae	Hippomorpha	Perissodactyla	Z
Horse	<i>Equus equus</i>	Equidae	Hippomorpha	Perissodactyla	W
Grévy's zebra	<i>Equus grevyi</i>	Equidae	Hippomorpha	Perissodactyla	Z
Hartmann's mountain zebra	<i>Equus hartmannae</i>	Equidae	Hippomorpha	Perissodactyla	W
Black rhinoceros	<i>Diceros bicornis</i>	Rhinocerotidae	Ceratomorpha	Perissodactyla	Z
Indian rhinoceros	<i>Rhinoceros unicornis</i>	Rhinocerotidae	Ceratomorpha	Perissodactyla	Z
Black-handed spider monkey	<i>Ateles geoffroyi</i>	Atelidae	Anthropoidea	Primates	Z
Geoffroy's marmoset	<i>Callithrix geoffroyi</i>	Callitrichidae	Anthropoidea	Primates	Z
Goeldi's marmoset	<i>Callimico goeldii</i>	Cebidae	Anthropoidea	Primates	Z
Eastern Angolan colobus	<i>Colobus angolensis</i>	Cercopithecidae	Anthropoidea	Primates	Z
Eastern black-and-white colobus	<i>Colobus guereza</i>	Cercopithecidae	Anthropoidea	Primates	Z
Hamadryas baboon	<i>Papio hamadryas</i>	Cercopithecidae	Anthropoidea	Primates	W & Z
François' langur	<i>Presbytis francoisi</i>	Cercopithecidae	Anthropoidea	Primates	Z
Douc langur	<i>Pygathrix nemaeus</i>	Cercopithecidae	Anthropoidea	Primates	Z
Western lowland gorilla	<i>Gorilla gorilla</i>	Hominidae	Anthropoidea	Primates	Z
Bwindi gorilla	<i>Gorilla beringei</i>	Hominidae	Anthropoidea	Primates	W
Bonobo	<i>Pan paniscus</i>	Hominidae	Anthropoidea	Primates	Z
Chimpanzee	<i>Pan troglodytes</i>	Hominidae	Anthropoidea	Primates	Z
Sumatran orangutan	<i>Pongo pygmaeus abelii</i>	Hominidae	Anthropoidea	Primates	Z
Human	<i>Homo sapiens</i>	Hominidae	Anthropoidea	Primates	–
Black lemur	<i>Eulemur macaco</i>	Lemuridae	Strepsirrhini	Primates	Z
Mongoose lemur	<i>Eulemur mongoz</i>	Lemuridae	Strepsirrhini	Primates	Z
Ring-tailed lemur	<i>Lemur catta</i>	Lemuridae	Strepsirrhini	Primates	Z
White-faced saki	<i>Pithecia pithecia</i>	Lemuridae	Strepsirrhini	Primates	Z
Asian elephant	<i>Elephas maximus</i>	Elephantidae	–	Proboscidea	Z
African elephant	<i>Loxodonta africana</i>	Elephantidae	–	Proboscidea	W
Naked mole-rat	<i>Heterocephalus glaber</i>	Bathyergidae	Hystricomorpha	Rodentia	Z
Capybara	<i>Hydrochaeris hydrochaeris</i>	Caviidae	Hystricomorpha	Rodentia	Z
Norway rat (Wistar)	<i>Rattus norvegicus</i>	Muridae	Myomorpha	Rodentia	W
Prevost's squirrel	<i>Callosciurus prevosti</i>	Sciuridae	Sciromorpha	Rodentia	Z

Table A.18. Data on mammalian encephalization, gut microbiome composition and fibre intake used for linear regression analyses. Data are summarized at suborder level since encephalization slopes were calculated over evolutionary time within suborders.

Suborder	Order	Fibre index	Relative abundance of Bacteroidetes	Relative abundance of Firmicutes	Bacteroidetes: Firmicutes ratio	Encephalization slope
Ruminantia	Artiodactyla	1485.3	0.126	0.728	0.173	0.0023
Feliformia	Carnivora	39.3	0.014	0.785	0.018	0.0010
Caniformia	Carnivora	525.7	0.038	0.723	0.053	0.0043
Hippomorpha	Perissodactyla	2232.5	0.144	0.450	0.320	0.0086
Ceratomorpha	Perissodactyla	1907.0	0.245	0.729	0.336	0.0069
Anthropoidea	Primates	195.1	0.268	0.620	0.432	0.0160
Strepsirrhini	Primates	225.6	0.199	0.718	0.277	0.0090

Table A.19. Data on fibre intake and gut microbiome diversity for the 30 mammalian species. Abbreviations used for provenance: Wild, W; Zoo, Z.

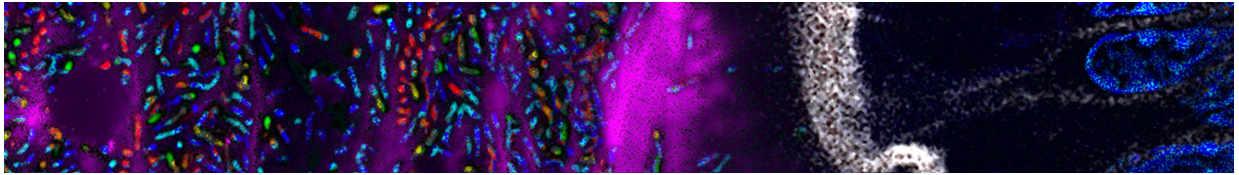
Common name	Species name	Family	Suborder	Order	Provenance	Fibre index	Shannon diversity index
Springbok	<i>Antidorcas marsupialis</i>	Bovidae	Ruminantia	Artiodactyla	W & Z	1493.8	7.69
Speke's gazelle	<i>Gazella spekei</i>	Bovidae	Ruminantia	Artiodactyla	Z	1238.4	7.99
Bighorn sheep	<i>Ovis canadensis</i>	Bovidae	Ruminantia	Artiodactyla	W & Z	1447.3	6.44
Transcaspien urial sheep	<i>Ovis vignei</i>	Bovidae	Ruminantia	Artiodactyla	Z	1258.3	8.24
Reticulated giraffe	<i>Giraffa camelopardalis reticulata</i>	Giraffidae	Ruminantia	Artiodactyla	Z	1494.9	7.53
Okapi	<i>Okapia johnstoni</i>	Giraffidae	Ruminantia	Artiodactyla	Z	1176.0	8.04
Visayan warty pig	<i>Sus cebifrons</i>	Suidae	Suiformes	Artiodactyla	Z	1416.7	7.18
Spotted hyena	<i>Crocuta crocuta</i>	Hyenidae	Feliformia	Carnivora	Z	33.0	6.13
Lion	<i>Panthera leo</i>	Pantherinae	Feliformia	Carnivora	Z	42.8	3.84
Bush dog	<i>Speothos venaticus</i>	Canidae	Caniformia	Carnivora	Z	17.0	4.22
Spectacled bear	<i>Tremarctos ornatus</i>	Ursidae	Caniformia	Carnivora	Z	292.5	2.01
North American black bear	<i>Ursus americanus</i>	Ursidae	Caniformia	Carnivora	Z	14.0	0.93
Polar bear	<i>Ursus maritimus</i>	Ursidae	Caniformia	Carnivora	Z	18.3	3.23
Southern three-banded armadillo	<i>Tolypeutes matacus</i>	Dasypodidae	–	Cingulata	Z	73.0	6.06
Red kangaroo	<i>Macropus rufus</i>	Macropodidae	Macropodiformes	Diprotodontia	Z	1012.7	7.93
Rock hyrax	<i>Procavia capensis</i>	Procaviidae	–	Hyracoidea	Z	529.3	6.61
European rabbit	<i>Oryctolagus cuniculus</i>	Leporidae	–	Lagomorpha	Z	8.5	5.49
Short-beaked echidna	<i>Tachyglossus aculeatus</i>	Tachyglossidae	–	Monotremata	Z	3.4	5.45
Grévy's zebra	<i>Equus grevyi</i>	Equidae	Hippomorpha	Perissodactyla	Z	2562.0	7.95
Black rhinoceros	<i>Diceros bicornis</i>	Rhinocerotidae	Ceratomorpha	Perissodactyla	Z	1813.0	7.19
Goeldi's marmoset	<i>Callimico goeldii</i>	Cebidae	Anthropoidea	Primates	Z	2.6	3.56
Eastern Angolan colobus	<i>Colobus angolensis</i>	Cercopithecidae	Anthropoidea	Primates	Z	276.5	7.24
Hamadryas baboon	<i>Papio hamadryas</i>	Cercopithecidae	Anthropoidea	Primates	W & Z	175.5	5.96
Western lowland gorilla	<i>Gorilla gorilla</i>	Hominidae	Anthropoidea	Primates	Z	317.9	7.06
Chimpanzee	<i>Pan troglodytes</i>	Hominidae	Anthropoidea	Primates	Z	281.4	7.14
Sumatran orangutan	<i>Pongo pygmaeus abelii</i>	Hominidae	Anthropoidea	Primates	Z	232.8	7.12
Ring-tailed lemur	<i>Lemur catta</i>	Lemuridae	Strepsirrhini	Primates	Z	319.6	7.19
White-faced saki	<i>Pithecia pithecia</i>	Lemuridae	Strepsirrhini	Primates	Z	24.3	5.96
Capybara	<i>Hydrochaeris hydrochaeris</i>	Caviidae	Hystricomorpha	Rodentia	Z	1197.0	7.49
Prevost's squirrel	<i>Callosciurus prevosti</i>	Sciuridae	Sciuromorpha	Rodentia	Z	8.0	2.39

Table A.20. Results of linear regression analyses on the relationships between mammalian encephalization, gut microbiome composition and fibre intake. *P* values highlighted in green denote significant predictors where *P* < 0.05. CL represents 95% confidence limit for each regression coefficient.

Response	Predictor	Coefficient	SE	Lower CL	Upper CL	<i>t</i> value	<i>P</i> value	R ²
Encephalization slope	Bacteroidetes: Firmicutes ratio	0.029	0.007	0.010	0.047	3.994	0.010	0.761
Encephalization slope	Relative abundance of Bacteroidetes	0.042	0.014	0.006	0.077	3.015	0.030	0.645
Encephalization slope	Relative abundance of Firmicutes	-0.024	0.017	-0.068	0.020	-1.407	0.218	0.284
Bacteroidetes: Firmicutes ratio	Fibre index	5.41E-05	7.17E-05	-1.30E-04	2.39E-04	0.755	0.484	0.102
	Fibre index	2.37E-05	4.68E-05	-9.66E-05	1.44E-04	0.505	0.635	0.049
	Fibre index	-6.43E-05	4.72E-05	-1.86E-04	5.69E-05	-1.365	0.231	0.271
Encephalization slope	Fibre index	-4.44E-07	2.48E-06	-6.81E-06	5.92E-06	-0.179	0.865	0.006

Table A.21. Output from non-linear regression model predicting gut microbiome diversity from fibre intake for the 30 mammalian species. *P* values are highlighted in green where *P* < 0.05. CL represents 95% confidence limit for each parameter.

Parameter	Estimate	SE	Lower CL	Upper CL	<i>t</i> value	<i>P</i> value
a	7.078	0.382	6.294	7.861	18.509	<2.20E-16
b	9.267	3.517	2.063	16.471	2.635	0.014



Appendix Chapter

Applying evolutionary theory to the microbiome–gut–brain axis

A.1 Abstract

Growing evidence indicates that the mammalian microbiome can affect behaviour, and various symbionts even produce neurotransmitters. One common explanation for these observations is that symbionts have evolved to manipulate host behaviour for their benefit. Here, we evaluate the manipulation hypothesis by applying evolutionary theory to recent work on the microbiome–gut–brain axis. Although the theory predicts manipulation by symbionts under certain conditions, these appear rarely satisfied by the genetically diverse mammalian microbiome. Specifically, any symbiont investing its resources to manipulate host behaviour is expected to be outcompeted within the microbiome by strains that do not manipulate but instead redirect their resources into growth and survival. Moreover, current data provide no clear evidence for manipulation. Instead, we show how behavioural effects can readily arise as a by-product of natural selection on microorganisms to grow within the host and natural selection on hosts to depend upon their symbionts. We argue that understanding why the microbiome influences behaviour requires a focus on microbial ecology and local effects within the host.

A.2 Introduction

The link between the gut and brain has been discussed for centuries, with multiple proposed mechanisms underlying this relationship (Mayer, 2011). These include communication through

the vagus nerve (Forsythe *et al.*, 2014), the immune (Fung *et al.*, 2017) and endocrine systems (Neuman *et al.*, 2015) and microorganism-derived neuroactive chemicals (Lyte, 2013). The relative importance of these routes and how they might interact are unclear, but studies are increasingly documenting effects of gut microorganisms on the brain and behaviour (Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013; Sarkar *et al.*, 2016; Sharon *et al.*, 2016). For example, faecal microbiota transplantation in mice can cause the behavioural traits of the recipient to become more like those of the donor (Bercik *et al.*, 2011a). Behavioural effects have also been traced to specific subsets of the microbiota. Evidence suggests that *Lactobacillus* and *Bifidobacterium* species can alleviate anxiety and depressive-like symptoms (Desbonnet *et al.*, 2010; Bercik *et al.*, 2011b; Bravo *et al.*, 2011; Messaoudi *et al.*, 2011; Savignac *et al.*, 2014; Davis *et al.*, 2016b), including in humans (Pinto-Sanchez *et al.*, 2017; Wallace & Milev, 2017). Particular *Lactobacillus* species can also improve social interactions in stressed mice (Bharwani *et al.*, 2017) and restore impaired oxytocin production and social deficits driven by a high-fat maternal diet (Buffington *et al.*, 2016). In addition, *Bacteroides* species have been shown to ameliorate repetitive and anxiety-like behaviours and communicative impairments in mice, seemingly through restoration of a specific bacterial metabolite (Hsiao *et al.*, 2013).

Such microbiota-driven alterations of host behaviour have led to the hypothesis that some symbionts manipulate the host for their own ends (Alcock *et al.*, 2014; Wong *et al.*, 2015; Stilling *et al.*, 2016b; Yuval, 2017). For example, microorganisms might make us more sociable (Stilling *et al.*, 2014), and even altruistic (Lewin-Epstein *et al.*, 2017), in order to increase host contacts and enhance their transmission. The general idea of behavioural manipulation — whereby a microorganism evolves to change host behaviour because this increases microbial fitness, for example by promoting its own transmission (Brown, 2005) — has its roots in parasitology (Thomas *et al.*, 2005). Numerous parasites affect the host nervous system and drive atypical behaviour (Figure A.7), often by interfering with neurotransmitter or neuropeptide signalling (Adamo, 2002; Perrot-Minnot & Cézilly, 2013). These effects are commonly attributed to manipulation by the parasite (Thomas *et al.*, 2005). Many examples come from invertebrate hosts, and one particularly striking example is the fungus *Ophiocordyceps unilateralis*, which

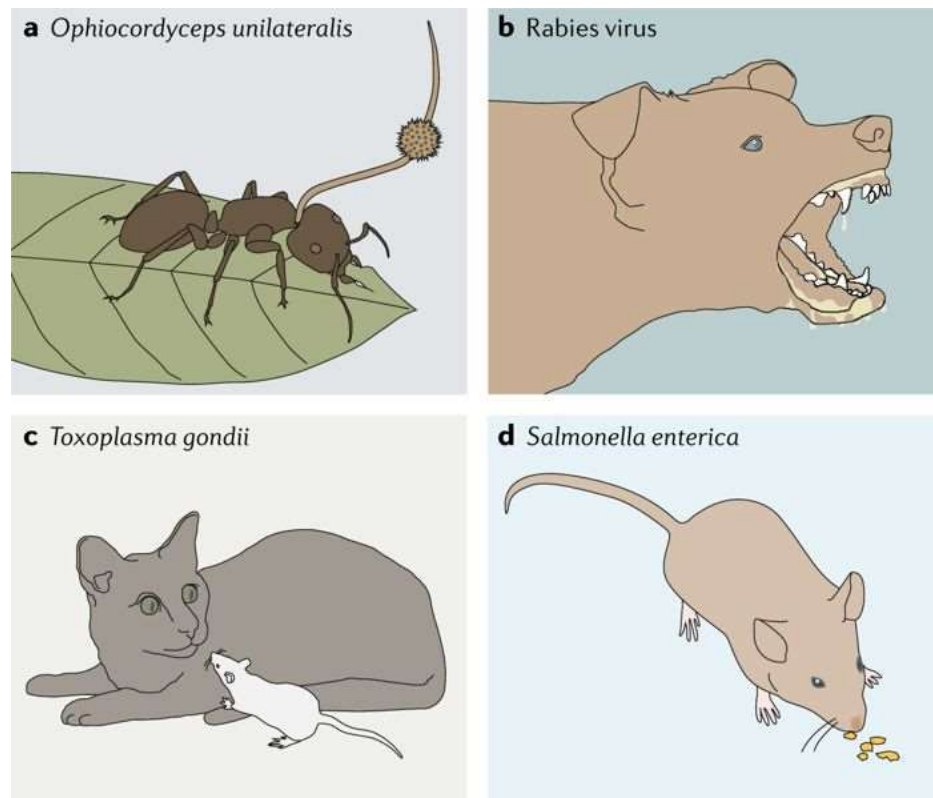


Figure A.7. Examples of parasites affecting host behaviour. (a) The fungal parasite *Ophiocordyceps unilateralis* induces ants to reach a certain elevation in the canopy, where they then bite on vegetation with the so-called death grip (Andersen *et al.*, 2009), thus securing a position in the canopy that is favourable for fungal growth. The fungus then emerges from the base of the ant's head to sporulate (Andersen *et al.*, 2009). (b) In vertebrates, parasite infections can change the social behaviour of hosts in ways that may promote parasite transmission (Klein, 2003). For example, rabies virus infects mammals, including dogs and humans. The virus causes inflammation of the central nervous system and increased host aggression, which leads to biting and transmission (Klein, 2003). (c) The protozoan parasite *Toxoplasma gondii* infects birds and mammals and has been shown to reduce the aversion of rodents to cat urine (Berdoy *et al.*, 2000; Vyas *et al.*, 2007). This may put the rodent at greater risk of predation and increase the chance of parasite transmission to feline hosts, which is necessary for the parasite to reproduce sexually (Berdoy *et al.*, 2000). (d) Infection can cause sickness behaviour in hosts, including behaviours such as appetite loss (Murray & Murray, 1979). The evolutionary basis for sickness behaviour is not always clear, but loss of appetite may have evolved to decrease nutrient supply to intestinal pathogens. Interestingly, there is evidence that *Salmonella enterica* subsp. *enterica* serovar Typhimurium suppresses this appetite loss, which may represent manipulation of host feeding behaviour (Rao *et al.*, 2017).

infects insects, including ants. In this example, two key pieces of evidence support evolved parasite manipulation. First, infection with the fungus induces ant hosts to adopt a certain elevation in the canopy and then bite on vegetation, anchoring the ant before fungal sporulation (Andersen *et al.*, 2009; Hughes *et al.*, 2011). Second, and critically, there is evidence that this

provides fitness benefits to the parasite, as the particular elevation (and likely humidity) that the ant adopts appears important for fungal development (Andersen *et al.*, 2009). In mammals, examples of parasites that affect the behaviour of their hosts are rare but include rabies virus and *Toxoplasma gondii* (Berdoy *et al.*, 2000; Klein, 2003; Vyas *et al.*, 2007). However, although evidence indicates that parasites can influence host behaviour, the second step — that the parasite gains a fitness benefit from its effects — is difficult to demonstrate. This difficulty is because it is typically challenging to show that the change in host behaviour resulting from parasite infection increases the fitness of the parasite in its natural environment. Thus, even in the parasite field, it is unclear whether some long-discussed examples of parasite manipulation are genuine (Poulin, 1995).

The parasite literature, therefore, teaches us that demonstrating evolved manipulation is experimentally challenging. This literature also makes evolutionary predictions about when one should expect host manipulation. Here, we apply the evolutionary theory of parasite manipulation (Brown, 1999; Vickery & Poulin, 2010) and host–symbiont interactions (Schluter & Foster, 2012; Box A.1) to the mammalian microbiome (Figure A.8). We consider the possible routes by which natural selection may have led to host manipulation by gut microorganisms and conclude that manipulation of host behaviour is often unlikely. We explore other evolutionary explanations for the behavioural effects of mammalian symbionts and propose that they modulate behaviour as a side effect of natural selection on other functions. In particular, host-affecting compounds can arise as a by-product of natural selection on microorganisms to compete within or control the local environment. Finally, hosts may evolve to depend on microbial metabolites for normal physiological function; consequently, if the microorganism that produces these metabolites is lacking, then behavioural dysfunction can result.

A.3 Manipulation of host behaviour

The potential benefits to a symbiont from manipulating host behaviour, which we define here as global manipulation (Figure A.8), are clear; how a host behaves can strongly affect the growth and survival of a symbiont and its transmission to other hosts. Despite this, the conditions that

Box A.1. The semantics of host–microbiota systems. Diverse definitions abound in the study of host–microbiota systems, but the fields of ecology and evolution have a set of mostly agreed-upon definitions that can be applied consistently to avoid confusion. This box outlines these definitions along with those of the microbiota and microbiome.

Co-evolution Reciprocal evolutionary adaptations in different species in response to one another. If species A changes, then species B changes in response; critically, this feeds back, and then species A changes again (Thompson, 1982).

Commensalism Interaction between species in which individuals on one side receive net fitness benefits, whereas the other species are unaffected.

Commensal Party in commensalism that receives benefit but has no net fitness effect on the other party.

Competition Interaction between species in which individuals on both sides suffer net fitness costs.

Manipulation A manipulating symbiont alters the host phenotype in such a way as to improve the fitness of the symbiont. For example, symbiont fitness may be increased by increased transmission to new hosts or increased access to resources.

Microbiome The community of microorganisms plus the environment. In host-associated microorganisms, this translates to the microbiota plus the host environment. This follows the proposed definition (Marchesi & Ravel, 2015) and logically stems from the meaning of ‘biome’ as a major type of ecological community. Others limit the definition of the microbiome to the genomic material of the microbiota.

Microbiota A community of microorganisms associated with a particular environment.

Mutualism Interaction between species in which individuals receive net fitness benefits from the interaction.

Parasitism Interaction between species in which individuals on one side receive net fitness benefits, whereas the other species experience net fitness costs. Parasites can be members of the microbiota with ecologies similar to those of commensal and mutualistic microorganisms.

Symbiosis Close ecological interaction between organisms (from Greek, meaning ‘living with’). Examples of symbiosis include mutualism, parasitism, commensalism and more.

Symbiont Member of a symbiosis that lives in or on the other member.

These definitions highlight that members of the mammalian microbiota are best described as symbionts rather than the commonly used commensal, because the former term is silent on the potentially varied effects on the host. Indeed, one limitation of definitions based on fitness benefits is that a single symbiont may switch, for example, from mutualist to parasite under certain conditions (Méthot & Alizon, 2014), making the classification of symbionts challenging without full knowledge of their effects (May & Nelson, 2014).

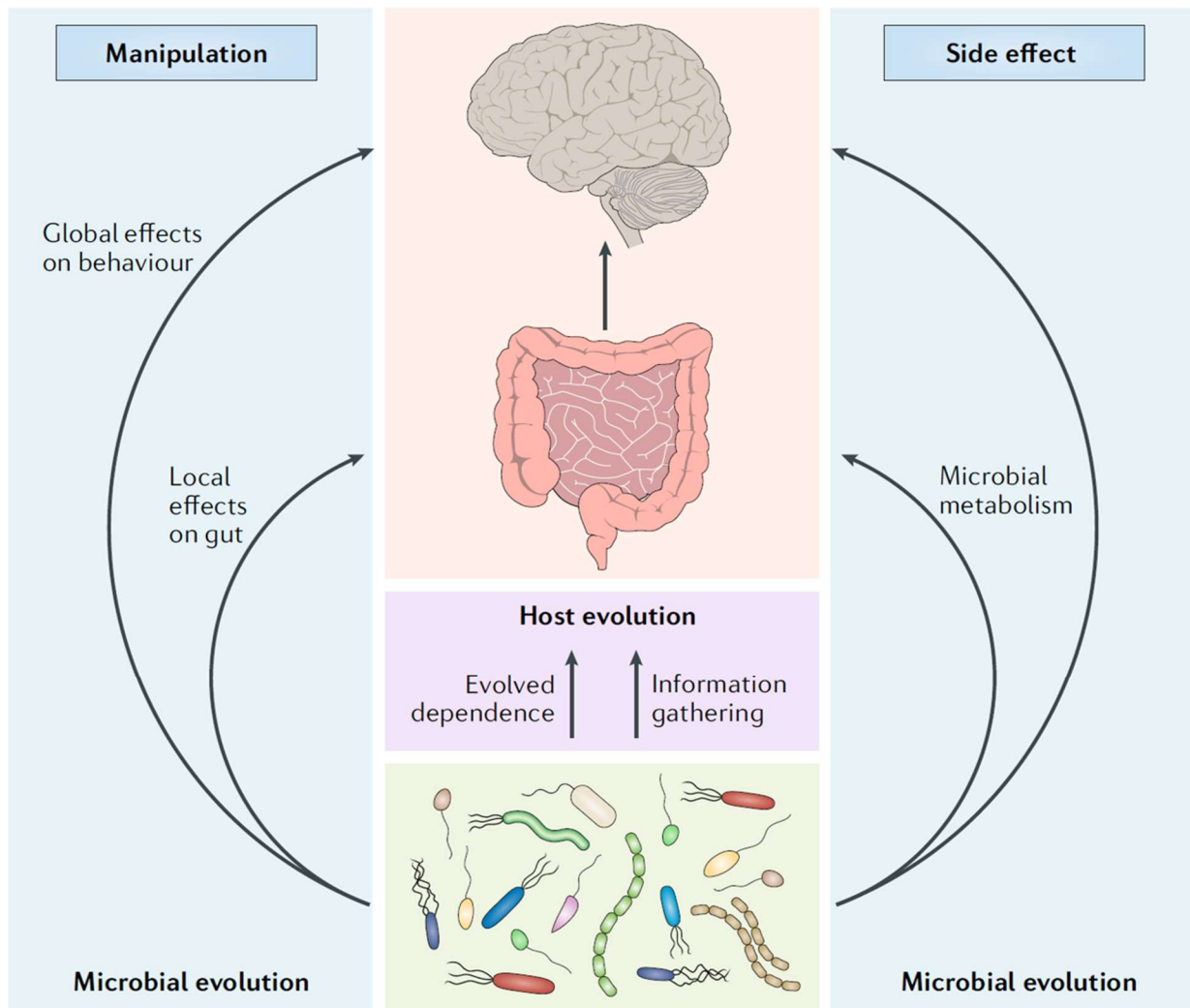


Figure A.8. Evolution of microbial effects on the brain. Arrows denote the potential routes by which microorganisms may influence host behaviour. Effects driven by natural selection on members of the microbiota are shown in blue. The left-hand side captures microbial manipulation, in which case the effects on the host increase microbial fitness. Here, the microbiota–gut–brain axis arises as an evolutionary adaptation of microorganisms to influence either the gut environment (local manipulation of host physiology) or host behaviour (global manipulation of the host). The right-hand side depicts the evolution of microbial traits that affect the brain without the evolution of manipulation. For example, the evolution of the metabolism used by the microbiota to survive and divide in the gut may generate compounds, such as metabolic waste products, that affect host behaviour as a side effect. In this case, the compounds are not adapted to influence the host, and host effects are a by-product. Effects driven by natural selection on the host are shown in purple. The host may evolve to depend on the microbiota for particular functions, including nutrient provision or immune system maturation, such that a missing microbial species leads to strong physiological effects and, potentially, behavioural effects. In addition, natural selection is expected to favour hosts that use the microbiota to provide information on nutrition and health in a manner that influences feeding, foraging and sickness behaviour. In all cases, the effects of the microbiota may be due to multiple mechanisms, including the production of neuroactive chemicals (or their precursors) that then trigger the vagus nerve or travel to the brain through the blood or lymphatic system, or through effects on the immune system.

favour the evolution of a manipulative trait (Table A.22) are quite restrictive (Brown, 1999; Vickery & Poulin, 2010). Consider a bacterial strain that uses a dedicated set of enzymes to generate a compound that affects host behaviour. Moreover, let us assume that this compound influences host behaviour in a way that benefits the bacterium. Hypothetically, it could immediately make the host more sociable and increase the potential routes of transmission to new hosts, or it might make a host consume resources that the bacterium needs. When will the production of this compound be favoured by natural selection? If the host is colonized only by this single strain, then production of the compound is predicted to evolve, so long as any fitness cost of production is outweighed by the benefits of increased nutrients or transmission.

Table A.22. Conditions favouring manipulation of a host by a symbiont. Evolutionary theory predicts specific conditions that favour the persistence of a manipulating symbiont (Brown, 1999; Vickery & Poulin, 2010; Schluter & Foster, 2012). Not all conditions are necessary for manipulation to evolve, for example, a symbiont that experiences little competition (low genetic diversity) might evolve an energetically costly manipulation trait. Critically, however, theory predicts that either limited within-host evolution or low genetic diversity is necessary for the evolution of manipulation (as they prevent a non-manipulating strain from outcompeting a slower-growing manipulating strain). The best candidate examples of host manipulation come from a few types of parasite or pathogen, and we use these as illustrations. However, many parasites and pathogens do not appear to manipulate host behaviour. Indeed, some are members of the microbiota with ecologies very similar to those of commensal and mutualistic microorganisms, making them subject to the same constraints on the evolution of manipulation.

Evolutionary parameter	Prediction	Parasite or pathogen example	Hypothetical microbiota example	Likelihood for mammalian gut symbionts
High benefit	Host behaviour affects symbiont abundance within the host and/or transmission.	The fungus <i>Ophiocordyceps unilateralis</i> needs ants to move to specific elevation to develop (Andersen <i>et al.</i> , 2009).	Changes in host social interactions promote microbial transmission.	High
Low cost	Manipulation has limited negative effect on symbiont growth rate and survival, or manipulation is transient.	Nematomorph hairworms disperse by inducing their locust or grasshopper host to jump into water; this involves only transient manipulation (Biron <i>et al.</i> , 2005).	Microbial waste product or signalling molecule happens to strongly affect host neurophysiology. Microorganism evolves manipulation by upregulating this pathway under specific conditions.	High

Table A.22. (cont.)

Evolutionary parameter	Prediction	Parasite or pathogen example	Hypothetical microbiota example	Likelihood for mammalian gut symbionts
High within-host abundance	Abundant symbionts may benefit most if they can generate large amounts of manipulating compounds.	Many manipulative parasites reach high biomass within the host, for example, <i>O. unilateralis</i> .	Highly abundant strain influences host behaviour. Bacteroidales strains reach high frequencies in the gut, although each strain is typically only a few percent of total microbial cell number (Chatzidaki-Livanis <i>et al.</i> , 2016).	Low
Limited within-host evolution	Symbiont undergoes few cell divisions within the host, either owing to transient colonization or occupying a slow-growing ecological niche.	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium, which promotes host appetite, only transiently infects the host (Rao <i>et al.</i> , 2017).	Microorganism is specialist on a low-abundance nutrient in the gut.	Low
Low genetic diversity	Few other genotypes (mutants, strains or species) within the niche of the symbiont, which prevents a slow-growing manipulating strain being outcompeted.	<i>Wolbachia</i> strains have a diverse range of manipulative effects on insects and are intracellular, so there is little competition from other genotypes (Pannebakker <i>et al.</i> , 2007).	Microorganism is in a discrete compartment within the host, limiting competition.	Low

If the bacterium must compete for resources and space with other strains and species, however, the prediction is very different. Whereas the metabolic cost of the enzymes falls on the producing bacterium, the benefits are now shared by multiple members of the microbiota. Indeed, in the case of a transmission effect, it is likely that much of the microbiota benefits. If a bacterial strain manipulates host food preference, then only strains in a niche similar to that of the producing strain may benefit, but these are also the main competitors of this strain. In either of these cases of hypothetical manipulation, therefore, the prediction is that a strain in the same niche that lacks the enzymes will outcompete the producing strain because it receives the benefit without paying the cost. This competitor could be a loss-of-function mutant of the producing strain or another species that inhabits the same niche. Ultimately, this is predicted to lead to the

loss of the manipulating compound (Brown, 1999; Vickery & Poulin, 2010). For high costs of production, this loss is expected to happen rapidly, in a few microbial generations. For low costs, the loss is predicted to take longer. Low costs are possible because natural selection is expected to minimize the costs associated with a given trait for a given level of benefit (Xavier *et al.*, 2011). Consistent with this concept, some parasites of invertebrates are thought to act by increasing synthesis of neuromodulators, such as serotonin, by the host, which may be less costly than producing them themselves (Adamo, 2002, 2013). A low cost may also be facilitated when a microorganism can use pre-existing metabolic pathways to drive host effects. However, even for low metabolic costs, the prediction is that a manipulative trait will eventually be lost in the face of prolonged competition from a non-producing strain within a host.

Evolutionary theory then predicts that the evolution of manipulation will critically depend on the diversity within the microbiota and, more specifically, how much competition a given strain experiences with other genotypes in its niche. If a strain is largely free from such competition, then manipulation is predicted to evolve if affecting host behaviour can increase resources or transmission. However, when a strain faces competition from other genotypes, the evolution of costly mechanisms of manipulation is disfavoured, as these will undermine the ability of a strain to persist in the microbiota. The question then is which of these two scenarios best represents a given host microbiome. The human gut is an ecologically complex community, estimated to contain hundreds to thousands of interacting species and strains (Bäckhed *et al.*, 2005; Qin *et al.*, 2010). Moreover, there is growing evidence for the importance of direct competition between strains of the same species and between species. This evidence derives from ecological modelling (Coyte *et al.*, 2015), empirical estimates of species interactions in the mouse gut (Stein *et al.*, 2013; Marino *et al.*, 2014) and from studies revealing the key role of bacteriocins and type VI secretion systems for ecological success in the gut (Kommineni *et al.*, 2015; Chatzidaki-Livanis *et al.*, 2016; Wexler *et al.*, 2016). These competitive conditions are predicted to lead to natural selection against symbionts — whether they are mutualists, parasites or commensals — that manipulate host behaviour (Table A.22; Box A.2).

Box A.2. Social evolution, relatedness and host manipulation. Application of social evolution theory to the mammalian microbiota reveals that natural selection likely operates against symbionts that manipulate host behaviour.

Our prediction that the microbiota rarely manipulates mammalian hosts originates from the field of social evolution (Hamilton, 1964a, 1964b; Wilson, 1975; Bourke, 2011). Social evolutionists seek to understand the origin of traits in one organism that affect the survival and reproduction of other individuals. A classic example is the sterile, and sometimes suicidal, workers of insect societies. Such phenotypes, which harm the reproduction of the individual but benefit others, are known as altruism in evolutionary biology. Altruistic traits can evolve when there is genetic similarity between the carrier and the benefiting recipients (Hamilton, 1964a, 1964b; Bourke, 2011), because this means that an actor can increase the propagation of its alleles through the copies in a recipient. More specifically, the key determinant in social evolution is that of relatedness, which captures the genetic similarity between individuals at the locus that drives the altruistic trait, relative to the population average. The main way to create relatedness is family life; the evolution of sterility in workers is explained by the fact that the queen in the colony is typically the mother of the workers. This means that the workers are raising siblings and are therefore able to pass on their genetic information, even though they do not themselves reproduce.

In microorganisms such as bacteria, relatedness emerges easily by binary fission, which can create a large group of a single genotype. At the scale of such groups, cooperative phenotypes in which several bacterial cells work together are extremely common, including the production of signalling molecules, enzymes to break down complex molecules and siderophores that scavenge iron (Nadell *et al.*, 2016). However, beyond the scale of a clonal group, competition between genotypes (through both nutrient acquisition and the many toxins used by strains to kill others) is commonly predicted and observed (Mitri & Foster, 2013). The challenge to host manipulation then is that multiple competing strains can benefit, whereas only the strain that actually invests in manipulation will experience the cost, putting it at a disadvantage.

The problem of competition for manipulation was realized over 15 years ago in a seminal social evolution paper that predicted a positive relationship between relatedness within a group of parasites in a host and potential investment in host manipulation (Brown, 1999). Although caution is required when applying relatedness measures to microbial communities, in which many strains and species may compete and share genes (Mitri & Foster, 2013), this prediction from the parasitology literature (Brown, 1999) remains relevant for the mammalian microbiota. Two sources of competition threaten to undermine a manipulating strain, one being strain diversity within its niche. If many different competing microorganisms exist, then genetic relatedness will be very low, which disfavours any trait that costs a manipulating strain but benefits all others at the scale of the host (Schluter & Foster, 2012; Foster *et al.*, 2017). If a manipulating strain can prevent immigration of other strains into its niche, then the prospects for manipulation are improved. Such colonization resistance is seen in the microbiota, and some species, such as *Bacteroides fragilis*, often seem to occur as a single strain within a host (Sana *et al.*, 2017). However, even for such cases, a manipulating strain may be outcompeted by a second source of competitors: a mutant in the genetic background of the strain that lacks the manipulative trait. Low costs to manipulation and genetic constraints on the emergence of loss-of-function mutants may slow this process (Mitri & Foster, 2016). Nevertheless, the expectation is that a manipulative trait will be lost under long-term competition in the mammalian gut as any small growth cost associated with manipulation can drive the loss of a strain given the many microbial generations that commonly occur within the lifetime of a host (Schluter & Foster, 2012).

How do our predictions relate to current discussions of host manipulation by the microbiota? In contrast to our predictions, a recent theory paper proposed that social (specifically altruistic) behaviour in animals can be explained by the evolution of microbial manipulation (Lewin-Epstein *et al.*, 2017). However, unlike the models of parasite manipulation (Brown, 1999; Vickery & Poulin, 2010), this study simply assumed there was no microbial competition within hosts to disfavour a manipulating strain. It does not, therefore, challenge our predictions. If the microbial competition that occurs within the gut were accounted for in this microorganism-induced altruism model (Lewin-Epstein *et al.*, 2017), then the expectation is still natural selection against manipulation (Brown, 1999; Vickery & Poulin, 2010). Nevertheless, there is empirical evidence consistent with host manipulation by *Salmonella enterica* subsp. *enterica* serovar Typhimurium, mediated by the vagus nerve (Rao *et al.*, 2017). Bacterial infections can trigger sickness-induced behaviour in hosts (Murray & Murray, 1979; Wickham *et al.*, 2007). It is challenging to demonstrate that sickness behaviour is an adaptation to combat infection rather than a by-product of compromised physiology. However, one feature — loss of appetite — may function to decrease nutrient supply to intestinal pathogens (Murray & Murray, 1979). *S. Typhimurium* seems to suppress this appetite loss, which may represent manipulation of host feeding behaviour in order to counteract a potential reduction in nutrient supply (Rao *et al.*, 2017). Curiously, in this case, this effect seemed to improve host fitness as well as microbial fitness, though estimates of host fitness in a laboratory system may not capture fitness effects seen in the natural environment. In any case, the data appear consistent with the evolution of microbial manipulation of behaviour.

Does this *S. Typhimurium* example contradict our predictions? Consideration of *S. Typhimurium* biology suggests not; rather, this example is consistent with the predictions of when true manipulation can evolve. The evolutionary success of *S. Typhimurium* is based on its ability to transiently outcompete other species and become dominant in the gut (David *et al.*, 2014a; Rivera-Chávez & Bäumlér, 2015). This competitive dominance means that *S. Typhimurium* is not expected to be outcompeted by other species that do not invest in manipulation (Table A.22). There remains the potential for a non-manipulating strain of *S. Typhimurium* to outcompete a

manipulating one. However, this outcome would require simultaneous co-infection with multiple strains to be common, yet multiple-strain infections are rare for bacterial pathogens (Balmer & Tanner, 2011). Importantly, the ecology of *S. Typhimurium* contrasts with the typical ecology expected in the gut microbiota. Many species exist at relatively low frequency and face competition from other strains and species over long periods, spanning many symbiont generations. These conditions are well captured by the theory (Brown, 1999; Vickery & Poulin, 2010), with the expectation that manipulation will often be disfavoured. Many species in the microbiota are likely to experience long-term competition, including members of the genera *Bifidobacterium* and *Lactobacillus*, which are most associated with effects on host behaviour (Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013; Sarkar *et al.*, 2016; Sharon *et al.*, 2016).

A.4 Local manipulation in the host

We have discussed how the manipulation of global host phenotypes, such as behaviour, is expected only under specific conditions. However, growing evidence indicates that the microbiota affects host behaviour. What then are the alternative explanations for these effects of microbial symbionts on host behaviour? One explanation is that symbionts are naturally selected to manipulate the local gut environment, and this then influences host behaviour as a side effect. Local manipulation is predicted to be more likely than behavioural manipulation because there is a greater chance that any benefits fall preferentially on the strain that invests in manipulation (Box A.2). Potential benefits of such manipulation include increased nutrient supply and decreased inhibition by attenuating host immune responses. However, to explain effects on behaviour, any manipulation must also have side effects on the central nervous system of the host. One potential route is through local changes to the enteric neurobiology of the host, which may then influence host behaviour through communication between the enteric and central nervous systems (Rao & Gershon, 2016). Gut bacteria can modulate intestinal motility (Quigley, 2011) through metabolites, including short-chain fatty acids and bile acids, that affect serotonin synthesis in the host (Fukumoto *et al.*, 2003; Dey *et al.*, 2015; Reigstad *et al.*, 2015; Yano *et al.*, 2015), and gut motility can in turn influence the competitive ability of certain

species (Wiles *et al.*, 2016). Moreover, a recent study found that the *Vibrio cholerae* type VI secretion system increases the strength of gut contractions in larval zebrafish and that this can displace a symbiont bacterial species (Logan *et al.*, 2018). This is consistent with local manipulation that provides a competitive benefit, although the example involves an acute pathogen rather than a symbiont. Whether or not such effects also have impacts on behaviour is unknown. However, effects on the enteric nervous system have the potential to modulate the mood and behaviour of the host through the gut–brain connection.

Symbiotic microorganisms may also have local effects on immune responses of the host (Sansonetti & di Santo, 2007; Ayres, 2016), including reducing the inflammatory response (Neish *et al.*, 2000; Kelly *et al.*, 2004). However, it is not clear whether these effects represent local manipulation by the microorganisms or arise purely as a function of natural selection on the host to discriminate between different microbial phenotypes (Hooper, 2009). Nevertheless, the immune and nervous systems are extensively connected (Steinman, 2004; Baganz & Blakely, 2013; Wohleb *et al.*, 2016), not only mechanistically but also anatomically (Louveau *et al.*, 2015), such that any microbial effects on the immune system may elicit behavioural changes as a side effect (without any natural selection on symbionts to manipulate behaviour). Therefore, the possibility exists that many of the effects described in studies of the microbiome–gut–brain axis may actually reflect an immune response. Consistent with this, recent findings show that colonizing mice with the faecal microbiota from patients with irritable bowel syndrome can drive anxiety-like behaviour, but only when the mice also exhibit immune activation (de Palma *et al.*, 2017).

The evolutionary basis for local manipulation by symbionts is on more solid ground than the global manipulation of host phenotypes. As such, some effects of the microbiota on host behaviour may be a side effect of local manipulation. However, even at the local scale, it is challenging to demonstrate the evolution of symbiont manipulation of the host and distinguish it from other explanations, such as the evolution of host adaptations that serve to detect and respond to particular strains and species in the gut (Foster *et al.*, 2017).

A.5 By-products of microbial metabolism

If the microbial compounds that affect host behaviour do not arise for manipulation, then why are they produced? The simplest explanation is that they are generated as part of the metabolism that helps the microorganism to grow and divide, as occurs with metabolic waste products. Short-chain fatty acids (for example, butyrate, propionate and acetate) are key waste products made by gut bacteria that can influence gut motility (Fukumoto *et al.*, 2003; Reigstad *et al.*, 2015; Yano *et al.*, 2015), modulate host immune responses (Rooks & Garrett, 2016) and have substantial neuromodulatory effects, possibly because they function as histone deacetylase inhibitors (Koh *et al.*, 2016). Polysaccharide A, a component of the bacterial capsule, can also affect gut motility (Mao *et al.*, 2013) and host immune responses (Mazmanian & Kasper, 2006). In addition, microbial compounds may affect the brain. Butyrate helps maintain the integrity of the blood–brain barrier (Braniste *et al.*, 2014), which typically functions to separate the neuroactive agents of the brain and periphery (Abbott *et al.*, 2006). Furthermore, acetate produced in the colon can cross the blood–brain barrier and directly enter the brain (Frost *et al.*, 2014).

Compounds that function as host neurotransmitters (Figure A.9) are particularly relevant. *Lactobacillus* and *Bifidobacterium* species from the human intestine are prolific producers of GABA in culture (Barrett *et al.*, 2012). Moreover, expressing a *Bifidobacterium dentium* gene for GABA production in the mouse gut using transformed *Bifidobacterium breve* can modulate indicators of visceral pain (Pokusaeva *et al.*, 2017). A GABA uptake system has also been reported in *Pseudomonas fluorescens*, a plant-associated bacterium (Guthrie & Nicholson-Guthrie, 1989), and more recently, a bacterial species from the human gut microbiome, *Eutepia gabavorous*, has been shown to require GABA as a growth factor (Strandwitz *et al.*, 2018). Uptake of a neurotransmitter therefore provides another potential route to influence host behaviour. In addition, bacteria seem to have an important role in activating precursors of dopamine and noradrenaline in the gut (Asano *et al.*, 2012), and some species also synthesize serotonin, acetylcholine and histamine (Wall *et al.*, 2014). The production of these molecules

raises the possibility that microorganism-derived neurotransmitters can bind directly to host receptors (Wall *et al.*, 2014).

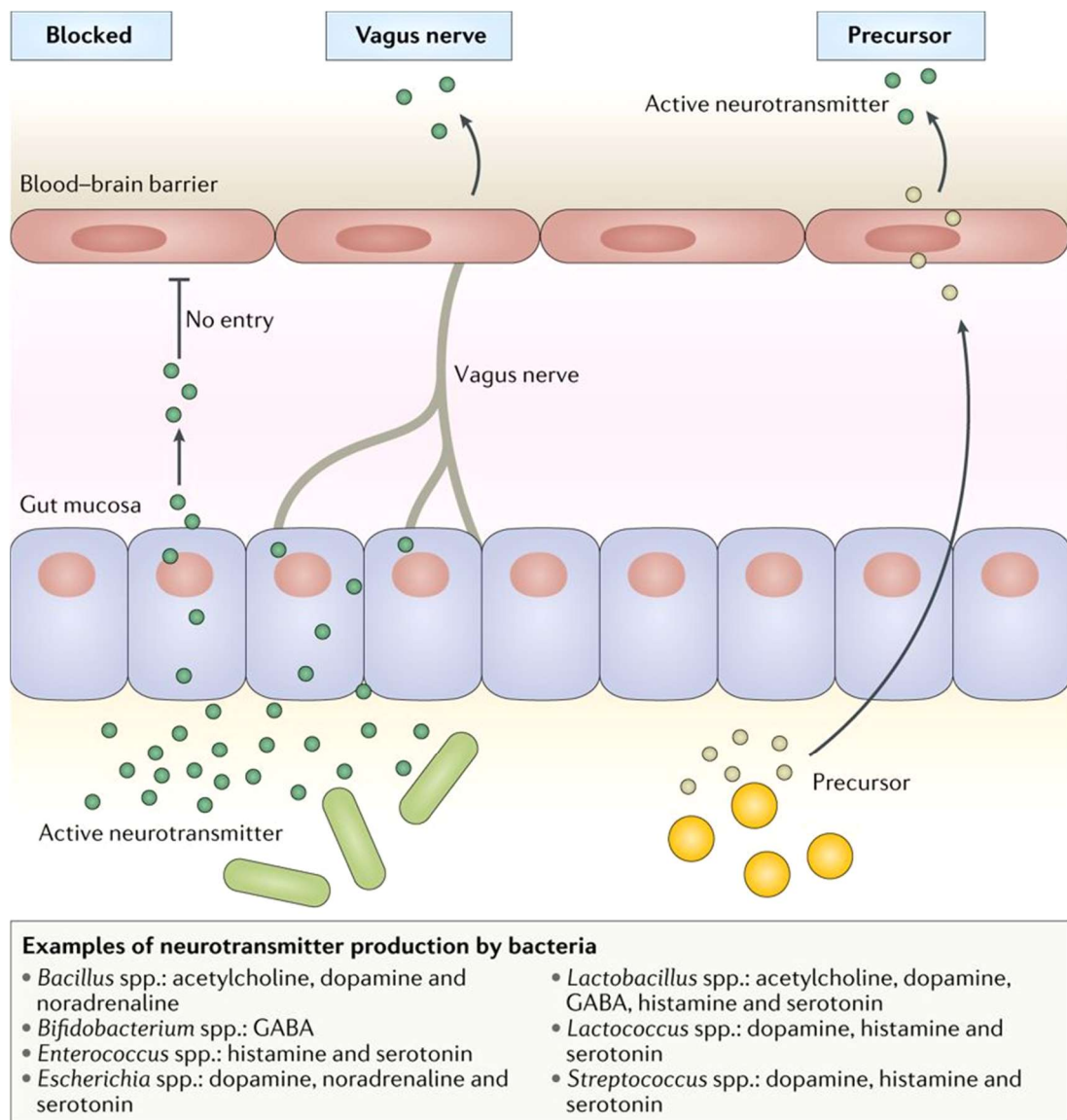


Figure A.9. How neurotransmitters in the gut lumen might influence the central nervous system.

Various neurotransmitters have been isolated from microbial species known to occur in the human gut, as listed in the diagram (Wall *et al.*, 2014). The microbial production of neurotransmitters represents a potential mechanism to directly influence the brain and behaviour. In reality, this route is limited because most neurotransmitters, including serotonin, dopamine and GABA, cannot typically breach the protective blood–brain barrier (Lattera *et al.*, 1999; Abbott *et al.*, 2006). Alternative modes of action include the possibility that microorganism-derived neurotransmitters affect the brain through the vagus nerve and its afferent neurones (Forsythe & Kunze, 2013). Another option is that precursors of neurotransmitters cross the blood–brain barrier (Fernstrom, 1983; Banks, 2009) and are then converted into active neurotransmitters. For example, gut bacteria can influence the metabolism and availability of the serotonin precursor tryptophan (O’Mahony *et al.*, 2015). This may affect serotonergic signalling in the central nervous system as tryptophan concentration in the blood plasma correlates with brain serotonin levels (Fernstrom & Wurtman, 1971).

Does the production of neurotransmitters identify symbionts that have evolved to manipulate the mammalian brain? This is far from clear. First, neurotransmitters may not be produced at meaningful levels by bacteria in the gut, as studies describing bacterial neurotransmitter production are largely performed *in vitro* (Sampson & Mazmanian, 2015). Second, it remains unknown whether lumen-produced neurotransmitters (or their precursors) can strongly influence the brain (Figure A.9). Moreover, even if microorganism-derived neurotransmitters can affect the brain, their production may well be explained by another bacterial function rather than host manipulation. Although these compounds are known as neurotransmitters in animals, they are produced not only by bacteria but also by fungi and plants (Roshchina, 2010). Indeed, their use in multicellular species may even be explained by horizontal gene transfer from bacteria (Iyer *et al.*, 2004). Most importantly, bacteria isolated from the environment can also produce neurotransmitters (Mountfort & Pybus, 1992; Roshchina, 2010), which suggests that these compounds have a role in bacterial biology outside of the host. Determining the functions of these compounds in free-living bacteria, therefore, is an important open question, as is whether these functions translate to symbiotic species. Initial work in this area suggests functions in both core metabolism and signalling between cells (Roshchina, 2010).

A.6 The evolution of host dependence

Our focus has been on the microbiota and how microbial evolution can lead to effects on the host. However, considering how host evolution can influence the microbiota–gut–brain axis is also important. Here, there are several non-mutually exclusive routes for natural selection on hosts to affect or forge links from the microbiome to behaviour. The simplest stems from the possibility that a host behaves differently when certain microorganisms are lacking simply because the physiology of the host is compromised. Such effects can have multiple causes, but evolutionarily, they are expected from what is often called ‘evolved dependence’ (de Mazancourt *et al.*, 2005; Weinersmith & Earley, 2016). When a host evolves alongside a symbiont, even a harmful one, there is the potential for it to come to rely on that symbiont for certain functions. For example, the wasp *Asobara tabida* has evolved to depend on the bacterial endosymbiont

Wolbachia for normal oocyte development, even though this bacterium is commonly a parasite of insects (Pannebakker *et al.*, 2007).

Evolved dependence may affect the nervous system, such that removing a particular microorganism creates a maladaptive physiological state that translates to behavioural effects. This can then lead to specific microorganisms having specific effects on host behaviour without any natural selection on the microorganisms to influence host physiology. Given the apparent functional redundancy of the gut microbiota (Moya & Ferrer, 2016), multiple phylogenetically diverse symbionts may complement any host dependence. Therefore, it may be the loss of the microbial trait, rather than specific microbial species, that leads to an impairment in host behaviour. More generally, evolved dependence may explain why an altered gut microbiome composition (such as in the case of germ-free or antibiotic-treated animals) is associated with behavioural changes (Diaz Heijtz *et al.*, 2011; Desbonnet *et al.*, 2014, 2015; Hoban *et al.*, 2016a). If we have evolved to depend on members of the microbiota to modulate our own neurochemistry, then we might expect their absence to influence brain function.

There is also the potential for evolved dependence through the evolution of the immune system. The long evolutionary association with the symbiotic microbiota has provided many opportunities for immune regulation to evolve dependencies on bacterial phenotypes. Broadly consistent with this, the gut microbiota affects various aspects of the host immune response (Mazmanian & Kasper, 2006; Round & Mazmanian, 2009; Hill & Artis, 2010; Rooks & Garrett, 2016). For example, microbial metabolites influence the differentiation and functioning of immune cells (Furusawa *et al.*, 2013; Smith *et al.*, 2013) and can have anti-inflammatory effects (Brestoff & Artis, 2013). Evolved dependence has also been linked to the hygiene hypothesis, which posits a causal association between improved hygiene and the rise in autoimmune conditions (Strachan, 1989; Weinersmith & Earley, 2016). This is based on the idea that an absence of symbiotic microorganisms or parasites leads to immune dysregulation. Most relevant here is the suggestion that the hygiene hypothesis is linked to mental health through neuroimmune connections (Rook & Lowry, 2008). Thus, immune processes may underpin many of the effects of the microbiome on the brain. Indeed, the bacterial genera *Lactobacillus* and *Bifidobacterium*,

which are commonly associated with behavioural changes, are also known for their immunomodulatory properties (Wells, 2011; Fanning *et al.*, 2012). Although the study of the microbiota–gut–brain axis has not explicitly considered evolved dependence, we believe it may prove fundamental to how the microbiome affects the brain.

Evolved dependence rests upon the idea that host physiology may come to depend upon symbionts for normal functioning. Host evolution can also generate new functions through the microbiota, and these may again affect behaviour. The gut microbiota contains much information of value to a host. When the microbiota informs on nutritional state, natural selection on the host may link the state of the microbiota to host appetite, feeding and foraging behaviour (Wong *et al.*, 2015; Fetissov, 2016). For example, short-chain fatty acids produced by microbial fermentation are implicated in satiety regulation (Rosenbaum *et al.*, 2015). Another evolved response may be sickness behaviour resulting from the spread of a pathogen within the gut (Figure A.7d). Furthermore, when particular symbionts provide valuable information or perform a useful function, even if just through a by-product of microbial metabolism, a host may evolve mechanisms to favour these bacterial species and thereby reinforce their effects. There are many potential routes to such host control of the microbiota by compounds secreted from the host epithelium, including both specific nutrients and antimicrobials (Foster *et al.*, 2017). Thus, hosts are expected to evolve to depend upon, monitor and regulate their microbiota. This evolution may readily forge and modulate links between the microbiota and host behaviour.

A.7 Outlook

There is growing evidence that the mammalian gut microbiome affects the brain and behaviour, raising the hypothesis that our microbiome has evolved to manipulate us (Alcock *et al.*, 2014; Stilling *et al.*, 2014, 2016b; Wong *et al.*, 2015; Lewin-Epstein *et al.*, 2017; Yuval, 2017). However, taking stock of both data and evolutionary theory casts serious doubt on this hypothesis. The theory predicts that manipulation is most likely when the manipulative trait has low cost and high benefit for the manipulating bacteria and, critically, when there is limited competition from non-manipulating strains (Table A.22). This last condition does not seem

easily satisfied in the diverse microbial ecosystem of the gut. We should not then assume that our microorganisms are our ‘puppeteers’. Instead, the behavioural effects of the microbiota might be better explained as a side effect of either local manipulation of the host environment or the microbial metabolism needed to grow and survive in the gut. Moreover, it is clear that hosts can evolve to depend upon the microbiota and use it to respond to nutritional and disease states, thereby cementing a link from symbionts to host physiology.

Our perspective has implications for both understanding and manipulating how the microbiota affects behaviour. We predict that microbial compounds that influence host physiology, such as neurotransmitters, typically evolve either because of their local impacts on host physiology (local manipulation) or as a by-product of natural selection on microorganisms to grow and compete within the microbiota (Figure A.8). Local effects on mucus production (Schluter & Foster, 2012; McLoughlin *et al.*, 2016), the inflammatory response (Rivera-Chávez & Bäumlér, 2015) and gut motility (Wiles *et al.*, 2016; Logan *et al.*, 2018) all have the potential to influence microbial strains differently in ways that are important for evolutionary success. However, the clearest evidence for local manipulation currently comes from acute pathogens, such as *Salmonella* strains and *V. cholerae*, rather than from beneficial or commensal symbionts. The demonstration of local manipulation by symbionts requires more than simply showing effects of a microorganism on host physiology. Critically, local manipulation is also predicted to increase the competitive ability of the manipulating strain in the gut, in contrast to behavioural manipulation, which is not expected to improve local competitive ability (Table A.22). For symbionts that spend a long time in the host, this predicts that a locally manipulating strain will outcompete an otherwise isogenic strain lacking the manipulative trait. Moreover, this competitive benefit must arise from effects on host physiology.

Arguably, the simplest explanation for microbial traits that influence host behaviour is that they are a by-product of the way that microorganisms grow and compete in the gut (Figure A.8). Similar to local manipulation, this scenario predicts that the production of a host-affecting compound will provide a competitive advantage to the strain that carries it. However, in contrast to local manipulation, the advantage will occur independently of the effects on host physiology.

This implies that any advantage can also be observed experimentally *ex vivo* (to the extent that the experiment can capture the growth conditions in the host). What kinds of molecule help a microorganism to compete but might also affect host physiology as a side effect? Many candidates exist. We have focused our discussion on metabolic products such as short-chain fatty acids, which are known to strongly influence the physiology of host cells (Fukumoto *et al.*, 2003; Reigstad *et al.*, 2015; Yano *et al.*, 2015; Rooks & Garrett, 2016). However, bacteria produce vast numbers of compounds whose effects on gut physiology are currently unknown (Franzosa *et al.*, 2015).

Metabolic waste products are just one source of compounds released by bacteria. To compete in any community, microorganisms produce a wide variety of compounds that influence the survival and division of other cells. These include enzymes that break down complex molecules, biosurfactants, siderophores that scavenge iron, diverse toxins that inhibit other microorganisms, extracellular polymeric substances (including carbohydrates and DNA), molecules that function as electron acceptors and molecules that function in cell–cell communication, for example quorum sensing (Mitri & Foster, 2013; Nadell *et al.*, 2016). Such compounds are potential candidates for influencing host physiology because they can be released in large quantities and, moreover, have often evolved because of their physiological effects on other cells. For example, iron is a key currency for both host and microbial cells (Markel *et al.*, 2007), and bacterial siderophores that scavenge iron are known to affect epithelial cell physiology (Choi *et al.*, 2004).

A focus on microbial ecology also has implications for the goal of engineering the microbiota (Foster *et al.*, 2017). It has been suggested that probiotic strains can be used to improve mental health outcomes (Wallace & Milev, 2017). A major challenge with many probiotics is getting a strain to establish itself in a new community (Weimer, 2015; Foster *et al.*, 2017; Zmora *et al.*, 2018). However, a focus on naturally occurring human symbionts should circumvent this challenge. If we are correct that host-affecting traits are accompanied by a competitive advantage, then probiotic strategies should be viable in the sense that the strains can compete and establish themselves in communities. Another limitation of probiotic strategies is the

tendency to seek a single strain to provide a given benefit. In reality, the benefits that the microbiota provides to the host, such as protection against pathogens, can arise from the interactions of multiple species within a community (Caballero *et al.*, 2017). If this is also true for behavioural effects, then we will need to embrace the full ecological complexity of the microbiota in order to understand the microbiome–gut–brain axis.

Embrace your other half



Image courtesy of Steven Lee, Broad Institute