THE ROLE OF GUT-BRAIN SIGNALLING IN CHRONIC SOCIAL STRESS

THE ROLE OF GUT-BRAIN SIGNALLING IN FUNCTIONAL RESPONSES TO CHRONIC SOCIAL STRESS

By:

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy

DOCTOR OF PHILOSOPHY (2019) McMaster University

(Medical Sciences) Hamilton, Ontario, Canada

TITLE: The role of gut-brain signalling in functional responses to chronic social stress

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NO. OF PAGES: xiv, 224

LAY ABSTRACT

Stress, which is a leading risk factor for mental illnesses such as depression, drastically affects the microbiota—the community of intestinal bacteria. However, this influence is bidirectional as gut bacteria can also influence the brain. Thus, we sought to understand the role of the microbiota in the negative effects of stress and how these microorganisms interact with the brain. We observed that behavioural changes in mice after chronic stress were associated with inflammation and community-wide changes in the microbiota. Treatment with a bacterial strain, *Lactobacillus rhamnosus* (JB-1), attenuated changes in behaviour and inflammation, but had no effect on the microbiota composition. We observed that the brain rapidly responded to JB-1 via the vagus nerve, and that chronic treatment caused long-term changes in brain regions. This work will allow us to discover novel pathways that can be targeted with greater specificity in clinical settings, providing an innovative approach to treatment of psychiatric conditions.

ABSTRACT

Chronic stress has a cumulative physiological impact, causing dysregulation of multiple systems due to allostatic overload. There is growing evidence that one such system, the microbiota, is engaged in persistent bidirectional interplay with the brain—a phenomenon that influences neural function and behaviour. However, the functional role of the microbiota in stress-associated changes and the underlying pathways of communication are unknown. Using a murine model of depression, we demonstrate that chronic stress has top-down effects on the structure of the microbiota community, reducing its richness and diversity, altering its profile, and causing differential abundance of various bacterial genera. These structural changes have functional consequences, including in metabolic pathways responsible for the synthesis of short chain fatty acids, tryptophan, and tyrosine. Using a physiologically active bacteria, *Lactobacillus rhamnosus* (JB-1), we probed for bottom-up signalling in chronic stress. JB-1 attenuated deficits in anxiety-like and social behaviours, and induced systemic immunoregulatory effects, independent of affecting stress-induced changes in the microbiota. In examining possible mechanisms of gut-brain brain signalling, we observed that in unstressed mice, a single dose of JB-1 causes rapid expression of c-Fos—a marker of neuronal activation—in distributed areas of the brain within 165 minutes, absent behavioural changes. No such effects were observed with heat-killed JB-1, despite that both live and heat-killed preparations facilitated vagal activity. Sub-diaphragmatic vagotomy prevented neuronal activation in most but not all brain regions, suggesting that vagal signalling is critical but indicating the presence of additional independent pathways. Finally, only chronic JB-1 treatment increased ΔFosB

expression in the brain, which is indicative of long-term neuronal adaptations, in association with behavioural changes. These studies demonstrate a role for bidirectional gut-brain signalling in chronic stress, and highlight the signalling pathways and brain regions through which gut bacteria exert their influence on host behaviour.

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisors, Dr. Paul Forsythe and Dr. John Bienenstock for their immense support, guidance, and mentorship throughout my graduate school career; for advocating a wide-eyed, yet responsible pursuit of insatiable enquiry; and for encouraging a curious student to run with the ideas that eventually engendered parts of this dissertation.

I am ever-grateful and deeply indebted to members of my supervisors committee for their mentorship and advice: to Dr. Jane Foster, who has been extremely generous with her expertise and time whilst training me on the nuances of behavioural neuroscience, and for always advocating for me as I navigated the world of academia; to Dr. Michael Surette, without whom I could not have possibly embarked on this interdisciplinary journey that attempts to bridge the neurosciences with microbiology.

I would like to thank Dr. Karen-Anne McVey Neufeld, whose enthusiasm and passion as a teacher inspired me to pursue my dissertation in the field of neuroimmunology, and for her time and assistance in carrying out some of the work described herein.

I would like to thank Dr. Firoz Mian and Dr. Andrew Stanisz, both an ever-sturdy presence in the lab, who eased my transition into the basic sciences and who have been extremely helpful throughout my time in graduate school.

Finally, to my parents Fernaz and Firoz Bharwani, who sacrificed stability, embraced precarity, and uprooted their lives to move across the world to a new country, in the hopes that their children could pursue a brighter future. Your tireless efforts and the burdens you shouldered did not go unnoticed, and without which, this body of work would not exist. This dissertation is dedicated to you.

“For my part, I know nothing with any certainty, but the sight of the stars makes me dream” (V. van Gogh, The Complete Letters of Vincent van Gogh, New York Graphic Society, 1958)

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapter 3

Chapter 4

Chapter 5

LIST OF ABBREVIATIONS AND SYMBOLS

5-HT	5-hydroxytryptamine (serotonin)
BDNF	Brain-derived neurotrophic factor
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
CA1, CA3	Cornu Ammonis area 1, 3
CeA	Central amydala
CFU	Colony-forming units
CNS	Central nervous system
CSD	Chronic social defeat
CRF/CRH	Corticoptropin-releasing factor/hormone
DA	Dopamine
DCs	Dendritic cells
DG	Dentate gyrus
dHpc	Dorsal hippocampus
DRN	Dorsal raphe nucleus
ENS	Enteric nervous system
GABA	γ-aminobutyric acid
GF	Germ free
GR	Glucocorticoid receptor
HDAC	Histone deacetylase
HPA	Hypothalamus-pituitary-adrenal axis
IBS	Irritable bowel syndrome
IPAN	Intrinsic primary afferent neuron
JB-1	*Lactobacillus rhamnosus* (JB-1)
LC	Locus coeruleus
LD	Light-dark
LPS	Lipopolysaccharide
MCPP	Monte Carlo permutation procedure
MDD	Major Depression Disorder
MHC	Major Histocompatibility Complex
MIP-2	Macrophage inflammatory protein 2
NA	Noradrenaline
NAc	Nucleus accumbens
NTS	Nucleus tractus solitarius
PAG	Periaqueductal grey
PBS	Phosphate buffer saline
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PTSD	Post-traumatic stress disorder
PVH	Paraventricular nucleus of the hypothalamus
PVT	Paraventricular nucleus of the thalamus
OFT	Open field test
OTU	Operational taxonomic unit
SCFA	Short chain fatty acid

Tph1	Tryptophan hydroxylase 1
Tregs	Regulatory T cells
TST	Tail suspension test
vHpc	Ventral hippocampus
VNS	Vagal nerve stimulation
Vx	Vagotomy

DECLARATION OF ACADEMIC ACHIEVEMENT

The author gratefully acknowledges the following individuals for their contributions towards this project: Dr. Firoz Mian for his assistance in sample preparation and flow cytometry analysis in Chapters 3 and 4; Dr. Jane Foster for providing training and expertise on behavioural experiments conducted in Chapters 3 and 4; Dr. Michael Surette for performing sequencing of the 16S rRNA data and providing expertise on the associated analysis in Chapters 3 and 4; Christine West for the mesenteric nerve recordings in Chapter 5; Kevin Champagne-Jorgensen and Dr. Karen-Anne McVey Neufeld for the tail suspension test data in Chapter 5; and Joseph Ruberto for the tryptophan hydroxylase 2 staining data in Chapter 5. The author's contributions associated with each study are presented sequentially. Chapter 3: chronic social defeat stress procedures, behavioural experiments, tissue and stool collection, data analysis and interpretation, preparation and writing of the associated manuscript. Chapter 4: chronic social defeat stress procedures, animal treatments, behavioural experiments, tissue and stool collection, data analysis and interpretation, preparation and writing of the associated manuscript. Chapter 5: animal treatment, cardiac perfusions, brain tissue collection and sectioning, immunohistochemistry, imaging, data analysis and interpretation, preparation and writing of the associated manuscript.

CHAPTER 1. INTRODUCTION

1.1. The intestinal microbiota is a rich source of peripheral signals.

The central nervous system (CNS) incessantly communicates with the periphery, interacting with signals in an effort to integrate sensory information and maintain homeostasis. The gastrointestinal tract harbours a vast community of microorganisms, collectively termed the 'microbiota', that is one such rich source of peripheral signals. Current estimates suggest that this community comprises nearly 1000 different bacterial species, $4x10^{13}$ bacterial cells that rival the number of cells in the human body at a ratio of 1.3:1, and 3.3 million non-redundant genes, which is nearly 150x more than the human gene complement (Qin et al., 2010; Sender, Fuchs, & Milo, 2016). Following its colonization of the intestinal tract at birth, the microbiota develops alongside the host under regulation of host genetics whilst sensitive to a variety of environmental factors such as diet and stress, eventually forming a core profile that exhibits the complex structural characteristics of an adult community by 1 year of age (Palmer, Bik, DiGiulio, Relman, & Brown, 2007). During both, early development and later in life, the microbiota plays a critical role in the organism's developmental trajectory and in the maintenance of homeostasis by influencing various aspects of physiology, thus engendering a mutualistic relationship with the host.

The microbiota initially garnered attention for its role in host immune function. Intestinal bacteria contribute to innate immunity by occupying colonization niches and outcompeting pathogenic microorganisms for nutrients and other resources. Much of the early research also focused on its role in the appropriate development of the mucosal and systemic immune systems. Using germ-free (GF) models, in which

animals are bred and raised with no exposure to bacteria, scientists observed the role of gut bacteria in establishing the mucosal architecture. In the mucosa, GF mice exhibit hypoplastic Peyer's patchers and significantly smaller populations of IgA-secreting plasma cells and $CD4^+$ lamina propria T cells (Macpherson & Harris, 2004). Similarly, in the serum, these mice exhibit fewer circulating lymphocytes and immunoglobulin levels, along with an underdeveloped spleen and lymph nodes. Elegantly highlighting the role of gut bacteria in immune development is the observation that introduction and colonization of the gastrointestinal tract with a 'normal' consortium of bacteria, and often even a single species, is sufficient to correct such deficits. For instance, inoculation of GF mice with faecal microorganisms restores the expression of major histocompatibility complex class II molecules on intestinal epithelial cells and expands the population of intraepithelial lymphocytes (Umesaki, Okada, Matsumoto, Imaoka, & Setoyama, 1995). Similar effects were observed following the introduction of only a single indigenous species, a population of segmented filamentous bacteria.

Intestinal bacteria also play an important role in influencing the development, physiology, and function of the enteric nervous system (ENS)—a vast network of 500 million neurons and glial cells divided into myenteric and submucosal plexuses (Furness, Callaghan, Rivera, & Cho, 2014). GF animals show significant abnormalities relative to conventionally housed animals and recolonized, ex-GF mice. Specifically, these animals exhibit less frequent migrating myoelectric complexes, which are waves of activity that propagate through the intestine at regular intervals during fasting, and slower intestinal transit—deficits that are restored following recolonization with conventional microflora (Caenepeel, Janssens, Vantrappen, Eyssen, & Coremans,

1989; Husebye, Hellström, & Midtvedt, 1994). GF animals also exhibit decreased basal excitability of intrinsic primary afferent neurons (IPANs) due to prolonged slow afterhyperpolarization, which decreases the discharge of these neurons in response to sensory stimuli (McVey Neufeld, Mao, Bienenstock, Foster, & Kunze, 2013). These effects were similarly reversed following exposure of adult mice to intestinal bacteria over four weeks. Beyond the developmental period, ENS function can also be influenced by single species. In conventional animals possessing a normal microbiota community, exposure to a specific *Lactobacillus* strain enhances the excitability of colonic AH neurons by reducing slow afterhyperpolarization and influences colonic and jejunal motility (Kunze et al., 2009; B. Wang et al., 2010). Thus, these data highlight the interactions between intestinal bacteria and the enteric nervous system across the host's lifespan.

Given its close proximity to the immune system and the complex nexus of enteric neurons that form anatomical synapses with ascending vagal nerve endings (Perez-Burgos, Mao, Bienenstock, & Kunze, 2014), it is then hardly surprising that the microbiota drew attention for its potential role in gut-brain signalling. An oft-referenced example of gut-brain communication involves the regulation of feeding behavior. Sensors at dendritic terminals in the gut respond to signals that influence satiety and ingestive behaviour through afferent projections to the paraventricular nucleus of the hypothalamus (PVH) from the gastrointestinal tract. In humans and rodent models, the fermentation of dietary non-digestible carbohydrate compounds by intestinal bacteria induces the secretion of appetite-regulating peptides, leading to increased satiety and lowered energy intake (Cani et al., 2009). However, the influence of microbiota on the

brain extends far beyond feeding. Akin to its impact on the immune and enteric nervous systems, the presence of intestinal bacteria in early life is critical to the appropriate development of the brain. The physiology of the CNS is significantly different in mice that lack intestinal bacteria, notably in the hippocampus, amygdala, and striatum. For instance, GF mice exhibit differential turnover rates of dopamine, serotonin, and noradrenaline, along with altered expression of brain-derived neurotrophic factor (BDNF), synaptophysin, and 5-HT_{1A} receptor, with the specific direction of the change depending on the sex and strain of the animal (Clarke et al., 2012; Crumeyrolle-Arias et al., 2014; Heijtz et al., 2011; Neufeld, Kang, Bienenstock, & Foster, 2011; Nishino et al., 2013) . These animals also exhibit altered microglia development and global deficits in the associated innate immune response (Erny et al., 2015). Consequently, these physiological differences are associated with altered behavioural development in adulthood, including changes in anxiety-like, exploratory, and locomotive behaviours. GF mice also exhibit baseline impairments in working and reference memory systems, suggesting aberrant hippocampal development (Gareau et al., 2011). Similarly, antibiotic treatment with low dose-penicillin in late pregnancy and the postnatal period has lasting effects on the microbiota, inducing deficits in anxiety-like and social behaviours, which are attenuated upon concurrent treatment with an exogenous *Lactobacillus* strain (Leclercq et al., 2017). While certain physiological changes can be reversed by bacteria colonization during adulthood, behavioural deficits only appear to be restored by colonization in early life, implicating the critical role of the microbiota during a narrow developmental window in neurodevelopment (Clarke et al., 2012; Erny et al., 2015; Heijtz et al., 2011).

Recent work has also probed the influence of the microbiota on CNS development in models of neurodevelopmental disorders. In mouse models of maternal immune activation and maternal high fat diet, both of which exhibit certain core behavioural features of autism spectrum disorders, offspring show changes in exploratory and social behaviours, deficits in sensorimotor gating, repetitive and stereotyped behaviour, and changes in communication (Buffington et al., 2016; Hsiao et al., 2013; Malkova, Collin, Hsiao, Moore, & Patterson, 2012). These animals also exhibit significant deficits in intestinal barrier integrity—a phenomenon also observed in subsets of patient populations—along with dysbiosis of the intestinal bacterial community. Treatment with specific bacteria however, can correct these peripheral changes and reverse the associated behavioural deficits, with certain strains even exerting their effects independently of the microbiota (Buffington et al., 2016; Hsiao et al., 2013; Sgritta et al., 2018).

1.2. The role of the microbiota in the stress response and mood-related behaviours

The hypothalamus-pituitary-adrenal axis (HPAA) is the neuroendocrine network that is responsible for coordinating the response to stress exposure. A common physiological manifestation in major depression (MDD) and other mood-related disorders is impaired functioning of the HPA axis. Patients with depression exhibit higher baseline cortisol levels in the plasma and corticotrophin-releasing-hormone (CRH) levels in the cerebrospinal fluid, blunted HPA axis reactivity following stressor onset, and impaired recovery of the stress response (Burke, Davis, Otte, & Mohr, 2005). There is extensive evidence demonstrating that the development of the HPA axis during

early-life is highly sensitive to various stimuli, including stress exposure and changes to the gut microbiota. GF rodents have significantly higher circulating levels of plasma corticosterone and exhibit a more exaggerated release of adrenocorticotrophic hormone and corticosterone in response to an acute stressor, likely due to reduced expression of glucocorticoid receptors in the brain (Crumeyrolle-Arias et al., 2014; Neufeld et al., 2011; Sudo et al., 2004). Recolonization with the complete microbiota or monoassociation with a single bacterial species reversed these changes and increased sensitivity to negative feedback regulation of the HPA axis. However, this was contingent upon reconstitution occurring in early life, highlighting the importance of the intestinal bacteria in the development of the stress response.

Beyond its role in development, the intestinal microbiota also plays a role in the stress response later in life. Notably, this influence is bidirectional—both top-down, i.e. brain-to-gut, and bottom-up, i.e. gut-to-brain. In top-down signalling, stress exposure throughout life induces downstream changes in the structure and composition of the microbiota community (Bailey et al., 2011; Galley et al., 2015; O'Mahony et al., 2009b). Intestinal bacteria also respond to stress-associated release of catecholamines via Qsec sensory kinase—a quorum-sensing receptor that detects host adrenaline and noradrenaline levels—which consequently induces the expression of various virulence genes (Clarke, Hughes, Zhu, Boedeker, & Sperandio, 2006). Cross-kingdom interactions between bacteria and host have also been observed in humans. For instance, exposure to maternal prenatal stress is associated with an altered microbiota composition in infants, along with differential abundance of specific taxonomic groups (Zijlmans, Korpela, Riksen-Walraven, de Vos, & de Weerth, 2015). Recent studies have

revealed that such stress-induced changes in the microbiota can, in turn, influence the host and drive stress-related changes in physiology and behaviour. Using GF mice, De Palma et al. demonstrate that the presence of intestinal bacteria is necessary for the development of stress-induced changes in anxiety-like behaviour and behavioural despair—GF mice do not exhibit behavioural changes exhibited by wild-type mice following stress exposure; however colonization of stress-exposed GF mice with microbiota from unstressed mice is sufficient to restore these behavioural deficits, demonstrating the interaction between host and microbiota factors in stress (De Palma et al., 2015). Similarly, transplantation of the microbiota from vulnerable, stress-exposed rodents into naïve animals is sufficient to recapture various changes associated with stress, including behavioural alterations and central inflammation (Chunyu et al., 2019). These effects of the microbiota on the host can also be transmitted across progeny: prenatal stress produced widespread changes in the maternal vaginal microbiota and altered the composition of the community that colonizes the neonatal gut. Exposure of offspring with stress-naïve mothers to this stress-associated colonizing community was sufficient to significantly change the response of the PVH to chronic stress later in life (Jašarević et al., 2018).

Using models of immune stress, early research observed evidence of bacteria-driven gut-to-brain signalling and its influence on behaviour. In models of infection-induced colonic inflammation and chronic colitis, mice exhibited anxiety-like behaviours and decreased expression of BNDF in the brain—changes that were reversed by treatment with a specific exogenous bacterial strain, *Bifidobacterium longum*, independently of normalizing systemic inflammation (Bercik et al., 2011; Bercik et al.,

2010). Similarly, increased anxiety-like behaviour and deficits in memory were observed in models of infection with *C. rodentium* and subclinical infection with *C. jejunum* (Lyte, Li, Opitz, Gaykema, & Goehler, 2006; Lyte, Varcoe, & Bailey, 1998). Given that this was observed in the early stages of infection prior to the immune response, or in the absence of any overt inflammation in the subclinical infection model, suggests gut-brain signalling driven by bacteria-associated signals rather than those associated with sickness behaviour.

Further evidence that bacteria can influence stress-related behaviours has also been observed using treatment with exogeneous bacterial strains. In a maternal separation model of early life stress, offspring exhibited systemic inflammation and alterations in neurochemistry, along with depressive-like behaviours, as exhibited by changes in swim behaviour and immobility on the forced swim test (Desbonnet et al., 2010). Chronic oral treatment with *Bifidobacterium infantis* however, reversed these behavioural deficits, imparting effects similar to citalopram treatment. Moreover, these effects appear to be limited to specific bacteria strains. Only treatment with *Bifidobacterium longum*, but not *Lactobacillus salivarius* attenuated the response of the HPA axis and autonomic nervous system to water avoidance stress, in addition to restoring deficits in gut permeability and hippocampal neurogenesis (Ait-Belgnaoui et al., 2014). Host genetics and environmental factors also play a role in influencing the host response to physiologically active bacteria, as was determined by a study in which the behavioural response of mice to *Lactobacillus helveticus* appeared to differ based on diet and genotype (Ohland et al., 2013). Such effects of bacterial treatment on the stress response may not be limited to oral treatment. In a model of chronic psychosocial

stress, repeated immunization of mice with an immunoregulatory agent, a heat-killed preparation of *Mycobacterium vaccae*, engendered proactive social coping behaviour and decreased submissive and avoidance behaviours towards a dominant male animal, suggesting increased resilience to stress-induced behavioural changes (Reber et al., 2016).

Beyond stress models, bacteria-driven signalling can also influence facets of mood-related behaviours in unstressed mice. Through oral administration of nonabsorbable antimicrobials—neomycin, bacitracin, pimaricin—it was demonstrated that transient shifts in the gut microbiota composition of developed mice can induce behavioural changes: treated mice exhibited anxiolytic behaviour, along with altered BDNF expression in the hippocampus and amygdala (Bercik et al., 2011). These changes were not a direct effect of the antimicrobials, as oral treatment of GF mice and parenteral administration of mice with a normal microbiota failed to induce such changes, suggesting that shifts in the microbiota composition were necessary for behavioural alterations. This was further demonstrated through adoptive transfer of the microbiota between Balb/c and NIH swiss mice, which exhibit contrasting behavioural phenotypes. Colonization of Balb/c mice with microflora from NIH swiss mice induced lower levels of anxiety-like behaviour while conversely, colonization of NIH swiss mice with Balb/c microflora induced greater levels of anxiety-like behaviours, with recipients exhibiting the behavioural phenotype associated with the donor in each case. Similar effects have also been demonstrated with exposure to specific bacterial strains such as *Lactobacillus rhamnosus* (JB-1). Long-term JB-1 treatment reduced anxiety- and depressive-like behaviours along with specific neurochemical changes, including the

levels of glutamate/glutamine and GABA, none of which were observed until at least two weeks of treatment (Bravo et al., 2011; Janik, Thomason, Stanisz, Forsythe, Stanisz, et al., 2016). These observations parallel the delayed therapeutic effects observed with antidepressant treatment in humans (Andrews, Bharwani, Lee, Fox, & Thomson, 2015; Frazer & Benmansour, 2002). In a double blind, randomized, placebo-controlled trial, administration of a mixed bacterial formulation to a small group of healthy volunteers for 30 days reduced scores on various measures of distress, including depression, and anxiety, along with 24-hour urinary cortisol levels (Messaoudi et al., 2011). In another study using functional magnetic resonance imaging, healthy women were randomly assigned to a group that consumed a fermented milk product with added bacterial strains for four weeks, or a placebo group that consumed a non-fermented milk product adjusted for taste and texture (Tillisch et al., 2013). Consumption of the fermented product produced changes in resting-state connectivity of a network of brain regions that process affective and sensory stimuli. Together, these data demonstrate the bidirectional nature of microbiota-brain interactions in stress and mood-related behaviours.

1.3. Anatomical and functional connections of the gut-brain axis.

1.3.1. Vagus nerve

The vagus, or the tenth cranial nerve, maintains homeostasis as part of the parasympathetic arm of the autonomic nervous system. As a mixed nerve, it carries both afferent and efferent signals between viscera and the brain, thus enabling rapid tonic communication between the CNS and the periphery. The length of the gastrointestinal tract is innervated by the vagus at three distinct locations, each with

characteristic afferent terminals: in the external muscle layers, in the myenteric plexus, and in the mucosal lamina propria (Berthoud & Neuhuber, 2000; Fülling, Dinan, & Cryan, 2019). In the musculature, most vagal afferents detect shear forces and respond to stretch and tension, whilst others form synapses with neurons of the ENS, which provides an additional, indirect route of gut-brain signalling. Vagal afferents in the mucosal lamina propria rapidly respond to chemical mediators such as cytokines, peptides, and hormones that are released by epithelial cells or those that traverse the epithelial lining. Bacteria of certain genera can also synthesize neurotransmitters such as serotonin, GABA, dopamine, and noradrenaline, although whether these bacteria-derived neurochemicals can influence gut-brain signalling is unclear (Forsythe, Kunze, & Bienenstock, 2016; Lyte, 2013). In the small intestine, the vagal chemosensory response is mediated by two different types of mucosal vagal endings: vagal crypt afferents, which encircle the luminal end of intestinal crypts, and vagal villi, which innervate the apical villus near the epithelial layer. Recent studies have also demonstrated that a subset of enteroendocrine cells in the intestine possess genes encoding pre- and post-synaptic proteins necessary for synaptic adhesion (Bohórquez & Shahid, 2015). These enteroendocrine cells synapse with vagal afferent terminals in the villus, forming a monosynapse between the gut and the brain that uses glutamate-mediated signalling for rapid sensory responses (Kaelberer et al., 2018).

80% of vagal fibres carry afferent signals to cell bodies in the nodose ganglia, which in turn innervate the nucleus tractus solitarius (NTS), organized topographically such that only gut terminals innervate the ventromedial NTS (Foley & DuBois, 1937; Han et al., 2018; Nemeroff et al., 2006). Direct and indirect projections from the NTS

innervate various brain regions, including the locus coeruleus (LC), dorsal raphe nucleus (DRN), periaqueductal grey (PAG), ventral hippocampus (vHpc), and the paraventricular nucleus of the thalamus (PVT) (Castle, Comoli, & Loewy, 2005; Groves & Brown, 2005; Han et al., 2018). While the vagus has been traditionally regarded for its role in satiety and feeding behaviour, growing evidence points to its role in reward- and mood-related behaviours (Fülling et al., 2019; Han et al., 2018). Indeed, vagus nerve stimulation has been successfully employed in humans for the treatment of refractory MDD (Aaronson, Sears, Ruvuna, Ph, et al., 2017). Recent work has also demonstrated that the vagus can be recruited by bacteria-driven gut-brain signalling. Exposure of the intestinal lumen to JB-1 increases the firing frequency of afferent vagal fibres, driven by an increase in the firing of individual fibres rather than an increase in the number of active fibres (Perez-Burgos et al., 2013). These effects are observed in the absence of bacterial translocation across the epithelium, implicating the presence of soluble signalling mediators. The mechanism for bacteria-induced vagal activity involves intrinsic primary afferent neurons (IPANs) of the ENS, which provide input for vagal afferents via an intramural nicotinic sensory synapse (Perez-Burgos et al., 2014). IPANs can also be activated by other bacterial strains, *Bacteroides fragilis*, and an isolated component of its cellular membrane (Mao et al., 2013). Similar effects are observed following exposure of the intestinal lumen to JB-1 microvescicles, which are shed by the parent bacteria and contain a variety of molecules, including DNA and RNA (Al-Nedawi et al., 2014; Dorward, Garon, & Judd, 1989). In this instance, signalling is mediated via the intestinal epithelium, given that this was observed when microvesicles were placed on the apical surface of the epithelium, but not when in direct contact with myenteric

plexus neurons. In a model of subclinical infection with *C. jejuni*, anxiogenic behaviour in mice with associated with an early response that involved bilateral activation of the brainstem and the NTS, as indicated by elevated c-Fos expression (Goehler et al., 2005).

Vagus-mediated gut-brain signalling is critical for the behavioural and neural effects of specific bacterial strains. Unstressed mice that were orally administered *L. rhamnosus* (JB-1) for four weeks showed reduced anxiety- and depressive-like behaviours, along with differential expression of GABA receptor levels in the prelimbic region, amygdala, Hpc, and LC (Bravo et al., 2011). Severing the vagus below the diaphragm however, prevented these behavioural and central changes, suggesting that vagal signalling was necessary. Similarly, in a model of colitis, *B. longum* treatment normalized changes in anxiety-like behaviour absent attenuating inflammation levels, but failed to exert any effects in mice that underwent a subdiaphragmatic vagotomy (Bercik, Park, et al., 2011). Another *Lactobacillus* strain, *L. reuteri*, also utilizes the vagus nerve to exert its effects on the brain and behaviour. In multiple models of autism spectrum disorder, treatment with this bacterial strain restored synaptic potentiation of dopaminergic neurons in the ventral tegmental areas and consequently, reversed deficits in social behaviour (Sgritta et al., 2018). These effects were mediated by the vagus nerve, via oxytocin-dependent signalling between the PVT and ventral tegmental area, demonstrating the critical role of the vagus in bacteria-driven gut-brain signalling.

1.3.2. Immune system

Given that non-inflammatory states are characterized by an intact intestinal epithelial barrier that prevents microbial translocation, the immune system presents an

important interface for signalling between the microbiota and the host. Through recognition of microbe-associated molecular patterns by pattern recognition receptors that are ubiquitously distributed on epithelial cells and cells of the mucosal immune system, the immune system can detect and respond to the lumen-confined bacterial population (Murphy & Weaver, 2016). Dendritic cells in the lamina propria can detect commensal microorganisms and microbial antigens through extending their processes into the lumen, or indeed via the transport of commensals and their antigens across the epithelial layer by enterocytes and specialized M cells in Peyer's patches. Intestinal dendritic cells can also retain and carry a small number of live commensals for several days (Macpherson & Uhr, 2004). Through such interactions, gut bacteria can induce downstream immune responses, such as cytokine release, activation of immune effector cells, and immunoglobulin responses. IgA is the most abundantly secreted immunoglobulin at mucosal membranes, contributing to regulation of intestinal bacteria and immunity against invading pathogens (Tsuji, Suzuki, Kinoshita, & Fagarasan, 2008). Conversely, altering the composition of the microbiota community affects the release of secretory IgA (He et al., 2007). Such bacterial-immune interactions are also observed following administration of specific strains. *Lactobacillus acidophilus* and *casei* increase the production and release of IgA and IgA^+ cells in Peyers patches and in the gut (de LeBlanc et al., 2008; Torii et al., 2007). Oral treatment with *B. infantis* in early life stress models prevents systemic inflammation and reverses the associated changes in neurochemistry and depressive-like behaviours (Desbonnet et al., 2010). In human subjects diagnosed with irritable bowel syndrome—which is highly comorbid with depressive and anxiety symptoms and is hypothesized to be a consequence of altered

gut-brain signalling (Henningsen, Zimmermann, & Sattel, 2003; Wu, 2012)—treatment with *B. infantis* reduced symptoms of pain, distention, and difficult bowel movements, and normalized the pro-inflammatory state characterized by an abnormal IL-10/IL-12 ratio (O'Mahony, Mccarthy, et al., 2005). Systemic immunoregulatory effects can also be induced by specific components of bacteria. Oral treatment with polysaccharide A, an isolated component of *B. fragilis*, provides protection from colitis through suppression of IL-17, a proinflammatory cytokine, from intestinal immune cells—a phenomenon that requires the presence of IL-10-secreting $CD4^+$ T cells (Mazmanian, Round, & Kasper, 2008). Similarly, microvesicles isolated from JB-1 replicate the immunomodulatory effects of the parent bacteria, increasing IL-10 and intracellular HO-1 in dendritic cells (Al-Nedawi et al., 2014).

There is extensive research describing the influence of cytokines and other immune signals on the CNS to bring about changes in the brain and behaviour (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). The adaptive immune system also plays a critical role in learning behaviour and cognition (Brynskikh, Warren, Zhu, & Kipnis, 2008). Paralleling such data, recent studies have demonstrated the role of the immune system in mediating gut-brain signalling. Ischemic and traumatic brain injury produce shifts in the composition of the microbiota and are associated with a robust response from intestinal T cells, which drives 1L-17-producing γδ T cells to the brain where they accumulate and induce inflammation, exacerbating injury and tissue damage (Benakis et al., 2016; Houlden et al., 2016). Disruption of the microbiota with antibiotics, which reduces the complexity and diversity of the community, altered mucosal immunity and intestinal dendritic cell function, leading to a larger population of

regulatory T cells, and impaired differentiation and trafficking of IL-17^{+} γδ T cells. This phenomenon, which was transmissible by faecal transplants, reduced ischaemic brain injury and offered neuroprotection following stroke.

As described in section 1.1., the intestinal microbiota influences the levels of BDNF in various brain regions along with associated behaviours (Clarke et al., 2012; Heijtz et al., 2011; Neufeld et al., 2011). In line with this data, recent work has shed light on putative immune mechanisms through which this effect might be mediated into adulthood. Toll-like receptors are a family of highly conserved pattern recognition receptors that play a role in the development and function of the innate immune system (Murphy & Weaver, 2016). These receptors are also found on adult neural progenitor cells, regulating neuronal differentiation and neurogenesis in the hippocampus (Rolls et al., 2007). In mice, antibiotic treatment reduced neurogenesis in the hippocampus during adulthood, leading to deficits on cognitive tasks (Möhle et al., 2016). This effect was a direct result of fewer Ly6C^{hi} monocytes in the bone marrow, blood, and consequently, in the brain, suggesting that this population of immune cells provides a signalling pathway between the gut and the brain. These antibiotic-induced deficits in Ly6C^{hi} monocyte levels were restored by treatment with a mixture of bacterial strains, which consequently reversed the deficits in neurogenesis and normalized performance on cognitive tasks. Together, these data demonstrate that interactions between the intestinal bacteria and the immune system at the gut mucosal surface can influence host physiology on a systemic scale, and that the immune system serves as an important signalling mediator along the gut-brain axis.

1.3.3. Other signalling pathways

In addition to the vagus nerve and the immune system, there exist other putative signalling pathways along the gut-brain axis that warrant mentioning. Spinal afferent fibres play a particularly important role in modulating the perception of visceral pain. In rodents, administration of specific bacterial strains can inhibit visceral pain induced by colorectal distention. TRPV1 is a non-selective cation channel that serves a variety of functions. In the gut, TRPV1 plays an important role in pain and is found on the terminals of spinal and vagal afferent fibres (Holzer, 2011). Through blocking TRPV1 ion channels on spinal sensory neurons, *L. reuteri* reduces firing of spinal fibres and confers anti-nociceptive effects (Perez-Burgos et al., 2015). JB-1 treatment on the other hand, accomplishes anti-nociceptive activity through the inhibition of dorsal root ganglion activity in response to colorectal distention independent of TRPV1 antagonism, suggesting strain-specific mechanisms (Kamiya et al., 2006; Perez-Burgos et al., 2015).

While it is evident that bacteria can influence vagal and ENS activity without translocating across the epithelium, the identity of mediators that enable interactions between bacteria, epithelial cells, and sensory neurons is unknown. Putative signalling molecules include humoural mediators that are released by enteroendocrine and epithelial cells, including serotonin, GABA, substance P, somatostatin, and other hormones (Forsythe et al., 2016). Furthermore, in addition to synthesizing various neuroactive compounds (Lyte, 2011), gut bacteria can also regulate host levels of neurotransmitters such as serotonin. GF mice exhibit prolonged gut transit time, which is reversed following recolonization by increasing tissue Tph1 and 5-HT levels (Reigstad et al., 2014). As described previously, these effects are mediated by the release of

SCFA and other metabolites from specific spore-forming bacteria, which induce Tph1 expression in colonic enterochromaffin cells, thus increasing 5-HT synthesis and accelerating gut motility and faecal output (Yano et al., 2015). Regulation of peripheral 5-HT levels by gut bacteria also influences other aspects of physiology, including platelet function. Whether this bacterial influence on peripheral humoural factors can influence CNS physiology and gut-brain signalling remains yet to be fully explored, however recent evidence suggests this may indeed be the case. In murine seizure models, changes in the gut microbiota mediate the anti-seizure effects of the ketogenic diet, which reduces the diversity of the community and increases levels of *A. mucinphilia* and *Parabacteroides* (Olson et al., 2018). The corollary of this is a reduction in the levels of gamma-glutamyl amino acids in the gut lumen and serum, along with an increase in the ratio of GABA: glutamate in the hippocampus, which provides neuroprotection and reduces seizure susceptibility. Through the release of SCFAs, gut bacteria also influence microglia development and function (Erny et al., 2015). In the absence of a microbiota and signalling at SCFA receptors, microglia exhibit an immature phenotype and an impaired innate immune response. Thus, these studies highlight the distinct pathways along the gut-brain axis that can be recruited by bacteria to influence the host.

1.4. Chronic social defeat stress as a model of depression.

Chronic social defeat (CSD) stress is one of the best described animal models of depression. Briefly, test mice are subjected to repeated daily bouts of social defeat by aggressive CD-1 mice over 10 days, with each individual defeat episode lasting for a duration of 5 minutes (Golden, Covington, Berton, & Russo, 2011). Following cessation

of physical interactions, the test mouse is placed in a shared home cage with the aggressor for 24 hours, separated only by a clear, perforated divider, thus enabling incessant sensory interactions and engendering a period of psychological distress. The CSD paradigm satisfies important requirements of validity for animal models of psychiatric disorders, laid out by McKinney and Bunney (McKinney & Bunney, 1969): the model should possess a similar etiology; it should induce an array of symptoms analogous to those observed in depressed patients; and that these changes should be sensitive to treatment modalities that are effective in the treatment of depression in humans. Per these criteria, CSD maintains ethological or construct validity by employing chronic social subordination as a means to reproduce the depressive-like state in the animals (Malatynska & Knapp, 2005); face validity, through reproducing the symptoms encompassed by stress-related disorders, including anhedonia, weight loss, anxiety-like behaviours, and social avoidance (Avgustinovich, Kovalenko, & Kudryavtseva, 2005; Krishnan et al., 2007); and predictive validity, since the associated behavioural and neural changes are reversed only by chronic, not acute, antidepressant treatment (Bagot et al., 2016; Berton, McClung, et al., 2006).

Despite the use of genetically inbred mice, the CSD paradigm engenders two distinct phenotypes: susceptible animals, which exhibit pronounced social avoidance of a novel CD-1 aggressors—a phenomenon only reversed by chronic but not acute treatment with antidepressants (Berton, McClung, et al., 2006)—and constellation of depression-like behaviours such as anhedonia; and resilient animals, which fail to develop such behaviours (Krishnan et al., 2007). By capturing phenotypic variability in stress vulnerability, this model allows for the investigation of biological mechanisms that

drive stress-induced changes in neural function and behaviour. Environmental factors have traditionally been attributed as sources of variability in genetically identical animals, including the prenatal environment, differences in postnatal development and care, and social dominance hierarchies (Krishnan et al., 2007; Wong, Gottesman, & Petronis, 2005). However, growing evidence has also highlighted epigenetic factors as a source of naturally occurring variation that, along with environmental differences, may underlie differences in stressor susceptibility (Gärtner, 1990). Finally, and notably for this dissertation, CSD also models changes in peripheral systems, such as aspects of immune dysregulation that are observed in clinical settings and in patients with MDD (Hodes et al., 2014; Menard et al., 2017).

1.5. Fos family of transcription factors.

Proto-oncogenes are a normally occurring class of genes that are progenitors of "oncogenes", defined for their role in the induction of tumours. Proto-oncoproteins serve a critical role in intra- and inter-cellular signal transduction and are found in various forms that range from extracellular polypeptide messengers and cell surface proteins to nuclear transcription factors (Morgan & Curran, 1991). The *fos* proto-oncogene is one such family of nuclear transcription factors that are readily induced in response to environmental signals, and which act as mediators that couple extra-cellular signals to changes in cellular expression of genes. This family includes c-Fos, FosB, Fra1, and Fra2. Fos proteins act as transcriptional regulators in concert with the Jun family of proteins, heterodimerizing to form the activator protein-1 (AP-1) complex that binds AP-1 sites (consensus sequence: TGAC/GTCA) in gene promoters (Curran & Franza Jr, 1988). In most cases, cellular Fos levels are highly regulated and only transiently

increase following stimuli. Thus, long-term alterations in cellular phenotype only arise following incessant or dysregulated expression, the latter being the source of cancerous cellular transformations. This tight expression is partly a consequence of negative regulation by Fos proteins, acting to suppress its own promoter (König et al., 1989). Furthermore, in some cases there exists a refractory period for Fos re-expression that lasts for several hours following exposure to the inducing stimulus.

1.5.1. c-Fos as a marker of acute neuronal activity.

c-Fos, part of the aforementioned family of Fos genes, is an immediate early gene that is expressed constitutively at low basal levels due to the instability of the transcribed mRNA, auto-negative regulation by Fos proteins, and the presence of active promoter repression (Chung, 2015; Lucibello, Lowag, Neuberg, & Müller, 1989; Morgan & Curran, 1991). Following stimulus exposure, intracellular calcium influx links neuronal activity to *c-Fos* induction through the recruitment of mitogen-activated protein kinase pathways, following which *c-Fos* undergoes transcriptional activation within 5 minutes and continues over a period of 20 minutes (Greenberg, Greene, & Ziff, 1985; Greenberg & Ziff, 1984). mRNA expression is transient, peaking between 30-45 minutes post-stimulation and degrading relatively quickly, with a half-life of 12 minutes (Müller, Bravo, Burckhardt, & Curran, 1984). c-Fos is a 55 kDa nuclear protein encoded by the *c-fos* gene that is post-transcriptionally modified to a 62 kDa product. c-Fos expression is closely linked temporally with *c-Fos* transcription (Sharp, Sagar, Hicks, Lowenstein, & Hisanaga, 1991). It appears 30 minutes following stimulus exposure, is maximal at 1-2 hours following a stimulus, and degrades within 6 hours (Hoffman, Smith, & Verbalis, 1993; Jung et al., 2014; Sharp et al., 1991). Following expression, c-Fos can rapidly

upregulate or downregulate gene transcription, via heterodimerization with either c-Jun or Jun B dimers respectively (Chiu, Angel, & Karin, 1989; Schütte et al., 1989). These properties of rapid induction and tight regulation make c-Fos an excellent marker of acute neuronal activity and thus a useful tool to investigate the rapid CNS response to acute bacteria administration.

1.5.2. ΔFosB reflects long-term molecular adaptations in the brain.

ΔFosB is encoded by the *fosB* gene and is a truncated splice variant of FosB—a group of proteins that peak at 6 hours following a stimulus and are part of the Fos family of transcription factors (Chen, Kelz, Hope, Nakabeppu, & Nestler, 1997; Hope et al., 1994; Morgan & Curran, 1991). Unlike all other members of the Fos protein family, which gradually exhibit desensitization to repeated exposure to a stimulus, the 35-37 kDa ΔFosB isoforms show no such habituation. Rather, this protein accumulates and can persist in the brain for extended periods, often lasting anywhere from weeks to months (Mcclung et al., 2004; Perrotti et al., 2004; Vialou et al., 2010). The accumulating response of ΔFosB to chronic stimulation is due to unique properties that render it highly stable, thus conferring a long half-life. One underlying mechanism for this stability is the phosphorylation of serine residue sites of ΔFosB at its N-terminus by protein kinases (Ulery, Rudenko, & Nestler, 2006). Furthermore, this particular splice variant of FosB also lacks a C-terminus, which is targeted for degradation and destabilizes the full-length protein (Carle et al., 2007). Given these properties, ΔFosB is uniquely positioned to regulate long-term neuronal adaptations and has thus been studied in several brain regions, notably the nucleus accumbens and dorsal striatum, for its role in behavioural changes in chronic stress models, chronic drug use, and repeated

natural rewards (Nestler, 2015). For instance, recent studies have observed that chronic administration of selective serotonin reuptake inhibitors such as fluoxetine increase expression of ΔFosB in various brain regions (Vialou et al., 2015). ΔFosB induction following exposure to chronic social defeat stress actively opposes changes induced by stress exposure and is thus critical for the expression of stress resilience (Vialou et al., 2010). Although the precise mechanisms underlying the effects of ΔFosB are unclear, it heterodimerizes with JunD to form long-lasting AP-1 complexes, or can homodimerize, which confers unique physico-chemical properties (Chen et al., 1997; Jorissen et al., 2007). Among its many targets, ΔFosB regulates the expression of AMPA and NMDA glutamate receptor subunits, thus influencing neuronal excitability (Mcclung et al., 2004; Vialou et al., 2010). Other targets include NF-κB (Ang et al., 2001), dynorphin (Zachariou et al., 2006), and Ca^{2+}/calmodulin-dependent protein kinase II (Robison et al., 2013). Given that the accumulating levels of ΔFosB reflect the presence of long-term adaptations, determining the brain regions that exhibit such a response to chronic bacterial treatment may reveal which regions are critical for the effects gut-brain signalling on behaviour, and studying the targets of this transcription factor may reveal the molecular underpinnings of such changes.

CHAPTER 2. HYPOTHESIS AND AIMS

Central hypothesis: Bidirectional gut-brain signalling influences the peripheral, neural, and behavioural changes induced by chronic stress exposure. Specifically, I **hypothesise** that 1) chronic stress alters the structure and function of the gut bacterial community; 2) That chronic treatment with a physiologically active bacterial strain will attenuate stress-induced changes in behaviour and the gut microbiota; 3) That acute versus chronic bacterial treatment induces differential expression of Fos gene products in distributed brain regions, and that this response is mediated by vagus-dependent pathways. These hypotheses are addressed by the following three aims.

Aims:

1. Determine the impact of chronic social stress exposure on the structure and function of the gut microbiota community and peripheral immune signals.
2. Determine whether chronic treatment with a physiologically active bacterial strain modulates the gut microbiota and influences chronic stress-induced behavioural changes.
3. Identify brain regions that respond to acute versus chronic bacterial treatment, and whether the vagus nerve is critical in mediating the Fos response of the central nervous system.

CHAPTER 3.

Structural and functional consequences of chronic psychosocial stress on the microbiome and host

(Published in Psychoneuroendocrinology 63: 217-227, 2016)

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Abstract

Introduction

Given the lasting impact of psychological distress on behaviour, along with the role of the microbiome in neurobehavioural development, we sought to examine the relationship between the microbiota and stress-induced behavioural deficits.

Methods

Male C57BL/6 mice exposed to chronic social defeat were subjected to behavioural analysis and profiling of the intestinal microbiome. Mice were also analyzed for phenotypic and functional immune changes. A computational approach on 16S rRNA marker gene sequences was used to predict functional changes in the metagenome as a consequence of structural shifts in the microbiota.

Results

Chronic social defeat induced behavioural changes that were associated with reduced richness and diversity of the gut microbial community, along with distinct shifts at the level of operational taxonomic units (OTU) across phyla. The degree of deficits in social, but not exploratory behaviour was correlated with group differences between the microbial community profile. *In silico* analysis predicted a shift in the functional profile of the microbiome: defeated mice exhibited reduced functional diversity and a lower prevalence of pathways involved in the synthesis and metabolism of neurotransmitter precursors and short-chain fatty acids. Defeated mice also exhibited sustained alterations in dendritic cell activation, and transiently elevated levels of IL-10+ T regulatory cells that were suppressed over time.

Conclusions

This study indicates that stress-induced disruptions in neurologic function are associated with altered immunoregulatory responses and complex OTU-level shifts in the microbiota. It is thus suggested that a dysbiotic state, along with specific changes in microbial markers, may predict the onset of adverse neurocognitive deficits commonly observed following exposure to severe stressors. The data also predict novel pathways that might underlie microbiota-mediated effects on brain and behaviour, thus presenting targets for investigations into mechanisms and potential therapy.

Keywords: Psychosocial stress, gut microbiota, behaviour, immune regulation, functional metagenome, chronic social defeat

3.1. Introduction

There is abundant evidence demonstrating the adverse impact of stress on physiology and neurocognitive correlates during development and adulthood: trauma or abuse during early life increase the risk of psychiatric conditions and can impair the development of the stress response (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008), while inadequate coping behaviour contributes to the etiology of diseases such as gastrointestinal disorders and increased risk of depression (Dinan, 2005; Mayer, 2000). Amidst the efforts to elucidate the mechanisms underpinning this association, there has been a growing recognition of the importance of the microbiota to normal development and function of several physiological processes, including metabolism, immunity, and behaviour. Indeed, so integral are these symbionts to host function that it has been suggested that almost all animals, including humans, should be viewed as multi-species organisms or "holobionts" (S. F. Gilbert, Sapp, & Tauber, 2012).

The body of work demonstrating the systemic role of the microbiome, especially in neural development and function, is extensive. Disruption or absence of the microbiome impairs behaviour and its development, leading to increased exploration, decreased apprehension, and impaired social behaviour (Bercik, Denou, et al., 2011; Desbonnet, Clarke, Shanahan, Dinan, & Cryan, 2014). Conversely, chronic administration of *Lactobacillus rhamnosus* (JB-1) alters GABAR expression in the brain, and reduces anxiety-like and depressive behaviour (Bravo et al., 2011). Particularly, there is compelling evidence of bidirectional interaction between stress and the microbiome. Exposure to stress alters the structural composition of the intestinal microbiota (Bailey et al., 2011; O'Mahony et al., 2009a), while germ-free (GF) status

and intestinal colonization alter the developmental trajectory of the stress response (Neufeld et al., 2011; Sudo et al., 2004). Within the context of the holobiont paradigm, the influence exerted by these microorganisms on brain development and behaviour is a consequence of the evolution of a multi-species organism. To provide clearer insight into the implications of the concept of the collective "self" for health and disease, and understand the functional relationship between the microbiota and stress-induced alterations, we need greater insight into the mechanisms, pathways, and consequences of communication along the microbiota-gut-brain axis.

Here, an anthropomorphic model is used to examine the impact of psychosocial stress on host-microbiota interactions, and the relationship between the microbiome and stress-induced behavioural deficits. We profile the community structure and species-level shifts in the intestinal microbiota, including in the relative abundance of *Akkermancia* and Coriobacteriaceae. These specific taxa have been previously reported in the literature to be associated with healthy and stress-exposed microbiome communities respectively (Bendtsen et al., 2012; Everard et al., 2013). Accordingly, we examined whether such alterations are retained, amidst broader shifts in the microbiota, across experimental studies in an effort to investigate the use of specific microbial community markers to predict adverse consequences on the host. We also examine the nature of the stress-induced dysbiosis—specifically, the *Firmicutes/Bacteroidetes* ratio, which signals the status of the human gut microbiota in models of obesity and antibiotic-induced dysbiosis (Mariat et al., 2009; Sanderson, Boardman, Ciofi, & Gibson, 2006; Thompson, Oliveira, Djukovic, Ubeda, & Xavier, 2015).

Given evidence of immune-mediated signalling along the microbiota-gut-brain axis in the literature (Desbonnet et al., 2010; Forsythe, Sudo, Dinan, Taylor, & Bienenstock, 2010), we profile the immunoregulatory and innate immune phenotype, as well as the function of the peripheral immune system in order to unravel gut-brain communication. Moreover, using a computational approach, we address the biological pathways that may be driving the effects of the microbiome on brain and behaviour by profiling the predicted functional implications of structural shifts in the microbiome.

3.2. Methods

3.2.1. Animals. Male C57BL/6 mice, eight-weeks old, and male CD-1 mice, retired breeders, were acquired from Charles River (Montreal, QC, Canada). All animals were allowed to acclimatize to the housing facility for seven days prior to beginning the experiment. Animals were housed in standard conditions (12-h light-dark (LD) cycle, lights on at 7 am) with *ad libitum* access to standard rodent chow and water. All experiments followed the guidelines of the Canadian Council on Animal Care and were approved by the McMaster Animal Research Ethics Board.

3.2.2. Social Defeat. Chronic Social Defeat (CSD) procedures were conducted as previously described (Berton, Mcclung, et al., 2006). Defeats were conducted over the course of 10 consecutive days. During each defeat session, intruder C57BL/6 mice were allowed to interact for 5-10 minutes with a novel resident CD-1 mouse. Intruder mice were carefully observed to ensure the demonstration of subordinate posturing. For 24 hours after each defeat session, mice were house in the same cage across a perforated Plexiglas divider to enable the transmission of visual and olfactory cues.

Control mice were housed two per cage on either side of a Plexiglas divider while preventing any physical contact.

3.2.3. Behavioural Testing

Open Field Test (OFT). Testing was carried out in the dark phase of the LD cycle under dim-light conditions, one day after exposure to the final defeat session (see Fig. S1 for experimental timeline). After a one-hour habituation period in the testing room, mice were singly placed into an 18 x 38 cm clear Plexiglas enclosure for a period of 30 minutes. Total distance traveled, rearing count, and time spent in the center of the field were recorded via photo beam sensors outfitted around the arena (Motor Monitor; Kinder Scientific). The equipment was cleaned between each test.

Three-Chambered Sociability Test. All tests were conducted 2 days after the final defeat session, during the light phase of the LD cycle following a 30-minute habituation period in the testing room. The testing apparatus was a three-chambered Plexiglas box, with each chamber possessing the dimensions 24.5 cm L x 44 cm W x 30 cm H. The dividing walls of the chambers possessed small openings that allowed mice access to each chamber. During the habituation phase of the test, a single test mouse was placed in the center chamber—with access to side chambers obstructed—and allowed to freely explore for five minutes. Following this, an unfamiliar sex- and strain-matched conspecific (stranger) was placed within a round, wire cup in one of the side chambers. An identical inverted wire cup was placed in the other side chamber. During the sociability phase, the test mouse was placed in the center chamber and allowed to freely explore all three chambers for a period of ten minutes. During each phase, distanced moved, time spent in each chamber, and time spent in within-chamber zones

were recorded by a video camera positioned directly over the testing apparatus (EthoVision XT; Noldus). Sociability scores were calculated using (time spent in mouse-chamber interaction zone/time spent in empty-chamber interaction zone). The equipment was cleaned and wiped down between test mice.

Aggressor Interaction Test. Immediately following completion of the three-chambered sociability test, mice were placed for 10 minutes in a 24.5 cm x 44 cm arena. A novel aggressor CD-1 was placed under a round, wired cage at one end of the arena. Time spent in the aggressor interaction zone and non-interaction zone was calculated for each mouse (EthoVision XT; Noldus).

Light-Dark (LD) Test. In a separate cohort of mice, on day 3 following the final defeat session, testing was carried out in the dark phase of the LD cycle under dim-light conditions. After a one-hour habituation period in the testing room, mice were singly placed into an 18 x 38 cm clear Plexiglas enclosure containing a black insert at one end for a period of 10 minutes. Kinderscientific Motor Monitor software was used to record time spent in the light zone and number of entries into the light zone. The equipment was cleaned and wiped down between test mice.

3.2.4. Tissue Collection and Splenocyte Isolation. 5 days after the final defeat session, following completion of all behavioural tests, or 17 days after the final defeat session —due to an opportunity afforded by the use of these mice in a separate, independent experiment—mice were euthanized and trunk blood was collected for serum analysis. Spleens were harvested 5 or 17 days after the final defeat session and dispersed using a cell strainer in cold, sterile PBS. Cell suspensions were centrifuged at 1500 rpm for 10 minutes at 4°C, then re-suspended in RBC lysis buffer for 1-2 minutes.

The resulting solution was centrifuged before cell pellets were washed with 5ml of complete RPMI 1640 medium: 10% fetal bovine serum, penicillin/streptomycin antibiotics, 2mM L-glutamine, and 0.01% β -mercaptoethanol. Viable cell numbers were assessed by Trypan Blue exclusion and diluted in RPMI to a concentration of 10^7 cells/ml.

3.2.5. Flow Cytometry. Splenocytes (10^6) were stained for markers of dendritic cell (DC) maturation and function—CD11c-APC-Cy7, MHCII-PE-Cy7, CD80-PerCP-Cy5, CD86-APC—or regulatory T cells—CD3-APC, CD4-FITC, CD25-PE-Cy7, intracellular Foxp3- PerCP-Cy5, intracellular IL-10-PE (BD Pharmingen, San Diego, CA, USA; eBiosciences, San Diego, CA, USA). Following surface staining, cells were fixed and permeabilized with BD Cytofix/cytosperm before staining for intracellular markers. Data were acquired with FACSCanto (Becton Dickinson, Oakville, ON, Canada) and analyzed using FlowJo (TreeStar, Ashland, OR, USA).

3.2.6. In vitro Splenocyte Stimulation. For anti-CD3/CD28 stimulation, splenocytes ($1x10^6$) were incubated in a 96-well cell culture plate coated with anti-CD3 antibody (2μg/ml) and in the presence of anti-CD28 antibody (2μg/ml). For stimulation with lipopolysaccharide (LPS), isolated splenocytes were incubated for 48 hours at 37°C with 5% CO2, following which they were stimulated with 20μL of 10μg/ml of LPS. After incubation for a total of 72 hours, all samples were centrifuged at 1500rpm for 10 minutes at 4°C. Supernatants were isolated and stored at -20°C for cytokine analyses (eBiosciences, San Diego, CA, USA).

3.2.7. 16s rRNA Analysis of Bacterial Composition. One day before the first defeat session and one day following the final defeat session, faecal pellets were collected and

stored at -80^{o}C for molecular analysis of microbiota**.** DNA extraction was carried out using a previously described protocol that enhances DNA recovery from microbial communities (Sibley et al., 2011) with modifications (Whelan et al., 2014) to increase quantitative recovery of bacteria across different taxa. Quantitative PCT (qPCR) was used to measure total eubacterial load using the universal bacterial primers, 8fM and Bact515R (Nadkarni, Martin, Jacques, & Hunter, 2002). Bacterial community profiling of 16S rRNA gene was carried out using a modified bar coded Illumina sequencing method (Bartram, Lynch, Stearns, Moreno-Hagelsieb, & Neufeld, 2011). Paired end reads of the V3 region were performed using the 341F and 518R primers (Muyzer, De Waal, & Uitterlinden, 1993). 250 nt paired-end sequencing was carried out on a MiSeq Illumina sequencer as per manufacturer's instructions. This approach provided overlapping sequence reads of the V3 region, which could be used for correcting poor quality base calls and increasing sequencing accuracy. Sequencing was carried out on a MiSeq Illumina sequencer in the McMaster Genome Center (McMaster University).

The MiSeq data was processed by an in-house bioinformatics pipeline (Whelan et al., 2014) that incorporates quality filtering. Sequencing results produced 7458 operational taxonomic units (OTUs), and a minimum, maximum, and median of 5800, 282124, and 206300.5 reads/sample respectively. Using QIIME (Caporaso et al., 2010), singletons were excluded and OTU tables underwent ten repeated rarefactions at multiple sequencing depths to enable equal reads across samples. For alpha diversity analysis, Chao1 and Phylogenetic Diversity metrics were recruited using the alpha diversity workflow script. Each metric was implemented using the same number of sequences as the most indigent sample (5800). Mann Whitney *U* tests were used to

assess statistical significance of measures derived from alpha diversity metrics. For beta-diversity analyses, Jackknife resampling at a sequencing depth equal to 80% of the most indigent sample was used to generate weighted and unweighted unifrac distance matrices. Between-group (dis)similarity was assessed using the Monte Carlo Permutation Procedure (MCPP) (999 permutations) and the analysis of similarities (ANOSIM) test. The Mann-Whitney U test was used to examine the abundance of *Akkermancia* and Coriobacteriaceae—groups predicted *a priori* to be altered following chronic stress. The G-test of goodness-of-fit and the Benjamini Hochberg correction for multiple comparisons (False Discovery Rate < 0.1) was used to analyze differential abundance of OTUs in either group using data first rarefied to a sequencing depth of 5800 and then filtered to eliminate OTUs observed fewer than 10 instances. To analyze the association between microbiome and behaviour, Procrustes analysis—which compares and transforms distance matrices through rotation, scaling, and translation to minimize the distance between corresponding points (Gower, 1975)—and the mantel test were performed on distance matrices for unweighted unifrac and behavioural data that distinguished between control and defeated groups: aggressor interaction and rearing behaviour.

3.2.8. In silico Metagenomics. Predictions regarding the functional composition of the microbiome were made on 16S rRNA-derived OTUs using a computation approach: phylogenetic investigation of communities using reconstruction of unobserved states (PICRUSt) (Langille et al., 2013). OTU abundances were normalized to known or predicted 16S copy number abundances. Using KEGG pathway metadata, KEGG orthologs (KO) were categorized by their function to level 3 of the pathway hierarchy.

Group differences in functional diversity were calculated using the Shannon Index and analyzed using the Mann Whitney U test. PCoA plots were created using Bray-Curtis distances with the QIIME v1.8.0 software suite (Caporaso et al., 2010), and differences between the functional profiles of control and defeated groups were analyzed with the ANOSIM test. Differential frequency of functionally categorized gene counts was analyzed using the G-test of goodness-of-fit and the Benjamini Hochberg correction for multiple comparisons (FDR < 0.05).

3.2.9. Statistical Analysis. Behavioural and immunological data were analyzed in GraphPad Prism 5 using two-tailed students *t* test, Mann-Whitney U test, or ANOVA, with post-hoc tests that utilized Bonferroni corrections. Effect size is reported for p-values near statistical significance using probability of superiority. Results in figures are expressed as mean ± SEM. Statistical significance is denoted as * (P < 0.05), ** (P < 0.01), and *** (P < 0.001).

3.3. Results

3.3.1. Exposure to chronic social defeat induces deficits in social and exploratory behaviour

In order to characterize changes in the behavioural phenotype following exposure to chronic stress, animals were subjected to measures that evaluate social, exploratory, and anxiety-like behaviours. Socially defeated mice exhibited pronounced avoidance of a novel CD1 aggressor mouse during the aggressor interaction test, opting to spend the majority of the time in the non-interaction zone of the field (Fig. 1A; Welch-corrected t= 15.74, df= 27, P <0.0001). Defeated mice also exhibited marked deficits in

social preference during the three-chambered test. Unlike unstressed control mice, which spent more time exploring the mouse chamber, defeated mice spent significantly more time exploring the empty chamber than the chamber containing the sex-, and strain-matched conspecific mouse (Fig. 1B; Group x chamber interaction, $F_{2, 66}$= 27.98, P < 0.0001; Bonferroni-corrected post hoc test, empty versus mouse chamber for defeated group, t= 4.36, P < 0.001). Although the defeated group also exhibited lower activity levels during the habituation phase (distance traveled, t= 3.045, df=33, P= 0.0046), this is unlikely to have contributed to differences in sociability, given that sociability scores—a measure of relative time spent interacting with the mouse or object in the respective chambers—were also lower in the defeated group (Fig. 1C*; t*= 3.60, df= 33, P= 0.001), demonstrating that defeated mice spent less time interacting with the novel conspecific.

Chronic exposure to social defeat also altered behaviour during the OFT. Defeated mice exhibited reduced locomotion (t= 2.46, df= 33, P= 0.019) and consistently lower levels of exploratory behaviour, as evidenced by fewer rearing counts across all time intervals (Fig. 1D; main effect of Group, $F_{1, 165}$= 83.14, P < 0.0001; Group x time interaction, $F_{5, 165}$= 3.59, P= 0.0042; Bonferroni-corrected post hoc test, control versus defeated group, P < 0.001 for all six time intervals). Anxiety-like behaviour on the OFT remained unaffected, as demonstrated by the lack of difference in time spent in the center of the field (Fig. 1E*; P*=0.5550). However, given previous evidence of the effect of chronic stress on anxiety (Kinsey, Bailey, Sheridan, Padgett, & Avitsur, 2007), anxiety-like behaviour was further evaluated in a separate cohort of mice using the LD test. Similar to previously, defeated mice exhibited reduced rearing

behaviour (t= 5.309, df= 15, P < 0.0001), but not time spent in the center (t= 2.021, df= 15, P= 0.0615, probability of superiority= 0.757) on the OFT. On the LD test, there was no statistical difference in time spent in the light zone (Fig. S2A; t= 0.1209, df= 14, P= 0.9055); however, defeated mice made fewer entries into the light zone (Fig. S2B; t= 2.545, df= 14, P= 0.0234), suggesting increased anxiety-like behaviour.

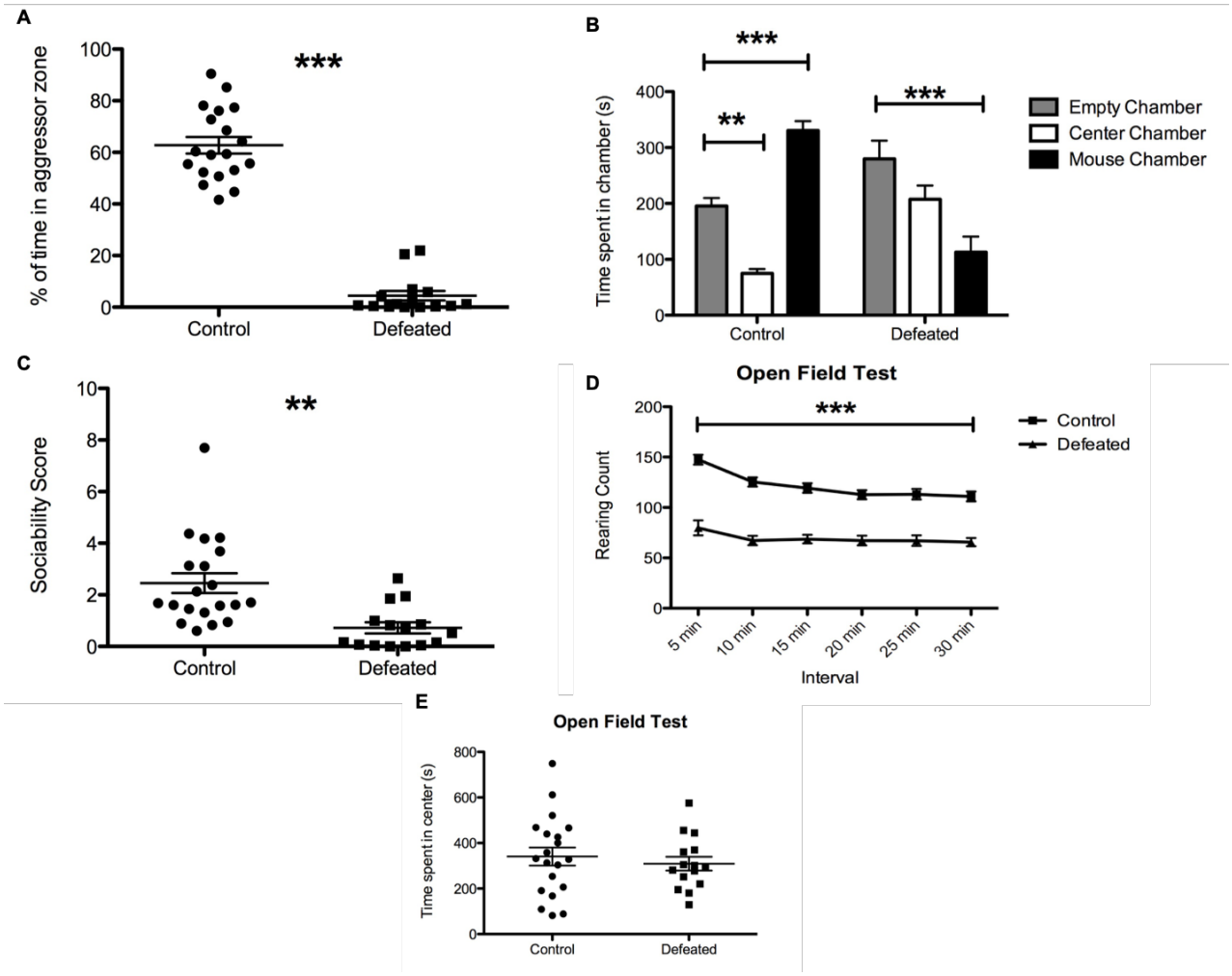

Figure 1. Effect of chronic social defeat on behaviour. (A) Percentage of time spent in the aggressor interaction zone by defeated (n= 15) and control mice (n= 19) during the aggressor interaction test. **(B)** and **(C)** Social preference and sociability scores of mice from control (n= 20) and defeated groups (n= 15) during the three-chambered sociability test. **(D)** Rearing behaviour exhibited by defeated mice (n= 15) versus the control group (n= 20) across all time intervals on the OFT. **(E)** Time spent in the center of the open field for control (n= 20) and defeated groups (n= 15). * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

3.3.2. Exposure to chronic social defeat alters the microbiome structure

To explore the functional relationship between stressor-induced changes in the microbiome and behaviour, we first sought to profile the structural composition of the

microbiota in mice exposed to prolonged social stress. Alpha-diversity analysis of faecal samples revealed differences within the composition of the microbial community of either group. As visualized in the rarefaction curves (Fig. 2A), there was a pronounced decrease in the overall diversity of the microbiome following exposure to stress. In contrast, the microbial community in the control group remained relatively stable at both time points and boasted greater species diversity than that of socially defeated mice (Faith's PD, *Mann-Whitney U*= 17, *P*= 0.04). Chronic stress also reduced the richness of the community, resulting in a lower estimate of rare species or OTU classes (Fig. 2B; Chao1, *Mann-Whitney U*= 15, *P*= 0.0244).

Examination of the microbiome profile revealed clustering of the gut microbiota by stress exposure group (Fig. 2C). Analysis of unweighted Unifrac PCoA plots using the MCPP revealed shorter distances between intra-group samples than samples between groups (999 permutations, non-parametric *P*= 0.018). These results were confirmed using the ANOSIM test on unweighted Unifrac distances, which revealed group differences in the microbial communities (999 permutations, *P*= 0.001). That there was no clustering of samples using weighted Unifrac distances (MCPP, 999 permutations, non-parametric *P*= 0.256) suggests that profile differences are driven by changes at the OTU-level of the community rather than shifts in the relative abundance

of the taxa.

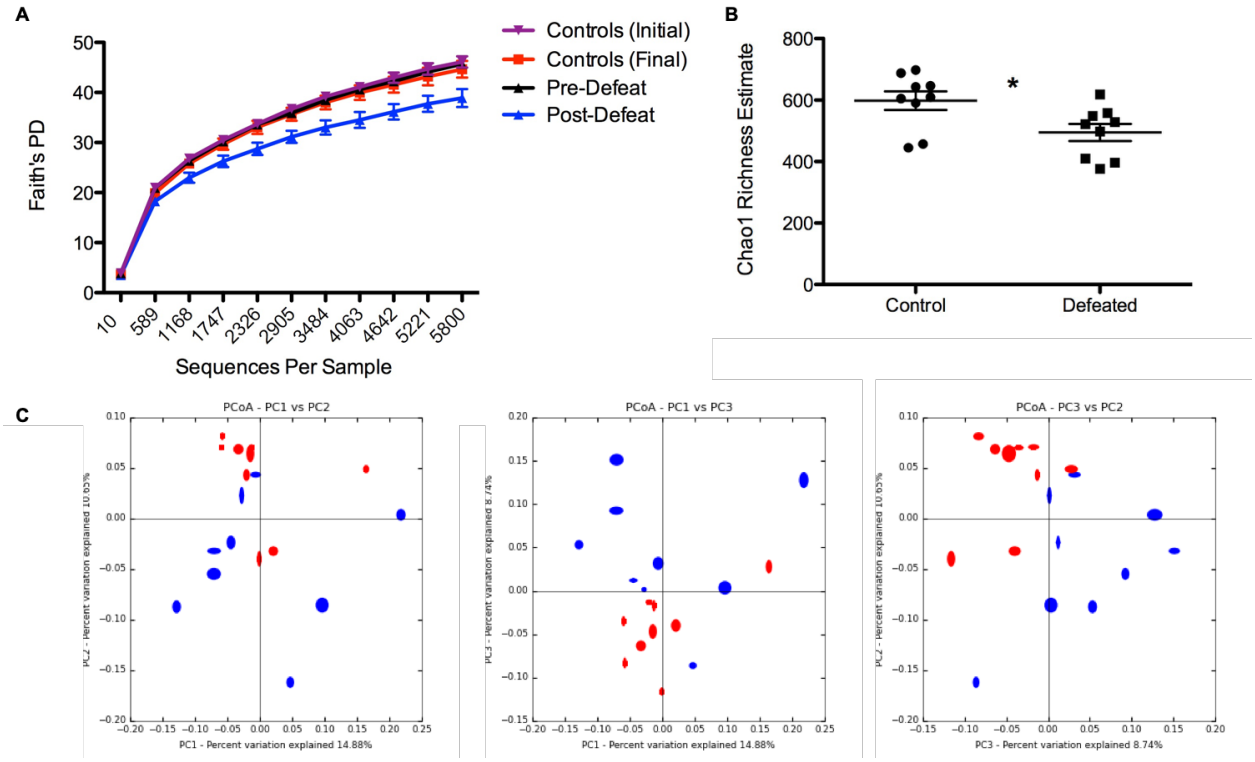

Figure 2. Microbial community diversity and profile differences between groups according to stress exposure. (A) Rarefaction curves of alpha diversity measure (Faith's PD) according to stress exposure (control versus defeated) and time point (before and after exposure to CSD). **(B)** Chao1 richness estimates for microbiome community in the control (n=9) and defeated groups (n= 9). **(C)** PCoA plots of unweighted Unifrac distances of samples from the control (red) and defeated groups (blue). * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

To examine the sensitivity of certain microbial groups to stress exposure and further explore changes in the community, we examined the abundance of species predicted, *a priori,* to be altered in the defeated group. Defeated mice exhibited a trend towards lowered faecal levels of *Akkermansia* (species: unclassified) (*Mann Whitney U*= 20, *P*= 0.0642, probability of superiority= 0.803) and significantly lower levels of Coriobacteriaceae (genus: other) than the control group (0.0635% versus 0.142%, *Mann Whitney U*= 12, *P*= 0.0418).

Previous studies have demonstrated dysbiotic states to be associated with a shift in dominant taxa at the phylum level (Mariat et al., 2009; Sanderson et al., 2006; Thompson et al., 2015). Thus, we examined the phylogenetic distribution of OTUs in the control and defeated microbial communities. The relative abundances of the major phyla were relatively similar between the two groups (Fig. 3A; Firmicutes, control versus defeated groups: 36.96% versus 39.16%; Bacteroidetes, control versus defeated groups: 60.32% versus 58.26%), revealing the absence of a straightforward shift in the structure of the community. Initial group differences however, were revealed upon analysis of distribution at the class level (Fig. 3A; Bacilli, control versus defeated groups: 12.06% versus 20.26%; Clostridia, control versus defeated groups: 24.4% versus 18.28%). The G-test of goodness-of-fit was implemented in QIIME (Caporaso et al., 2010) to investigate differential abundance of individual OTUs between the two communities (FDR <0.1). At this criterion, 25 OTUs were differentially represented between the two groups (Fig. S3); 23 of these OTUs belonged to either of the two major phyla (Fig. 3B). In the microbial community of defeated mice, the abundance of distinct OTUs belonging to Firmicutes and Bacteroidetes were either increased (8 members of

Firmicutes) or decreased (6 members of Firmicutes, 9 members of Bacteroidetes), demonstrating complex structural alterations following chronic exposure to social defeat.

To examine the relationship between changes in the microbiome and behavioural deficits, Procrustes analysis was recruited to compare the unweighted Unifrac matrix to a distance matrix of behaviour on the aggressor interaction test (Fig. 3C). The data exhibited a statistically significant fit between the two matrices, based on 10 000 Monte Carlo iterations (M^2= 0.786, *P*= 0.0052). This relationship was further evidenced by a statistically significant correlation between the two variables (Mantel *r*= 0.167, *P*= 0.029, 999 permutations) and appeared to be specific to social avoidance behaviour, as no such association was observed between the unweighted Unifrac data and rearing behaviour (M^2= 0.848, *P*= 0.056).

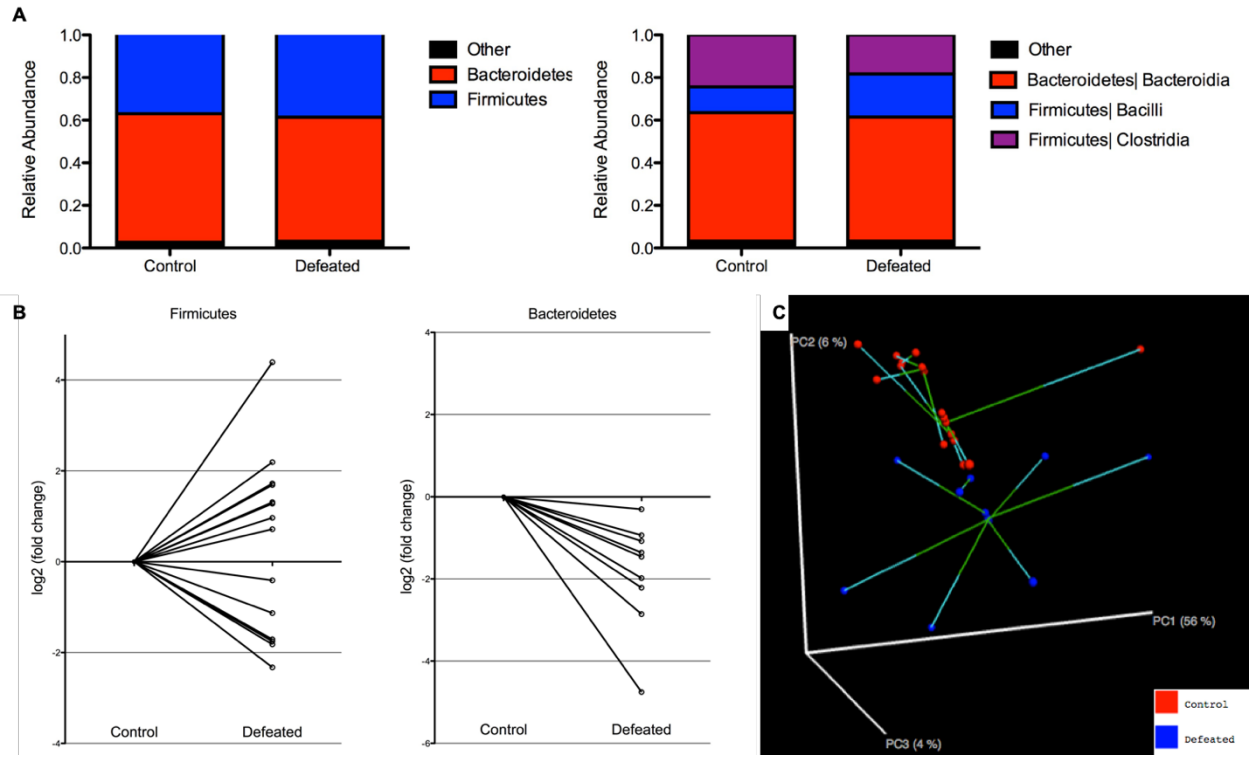

Figure 3. Effect of chronic social defeat on taxonomic changes in the microbiota and association with behavioural deficits. (A). Taxonomic distribution at the phylum and class level of faecal samples derived from the control and defeated groups. **(B).** Statistically significant changes in OTUs belonging to either the Firmicutes or Bacteroidetes phyla, displayed as fold change in defeated mice relative to the control group. **(C).** Procrustes plot comparing aggressor interaction behavioural data and the microbiome profile (unweighted unifrac distances) of each mouse from control and defeated groups. Lines connect the behavioural and phylogenetic data of a specific sample.

3.3.3. Chronic social defeat induces lasting changes in immunoregulatory responses

Given previous evidence of immune-mediated communication along the microbiota-gut-brain axis (Desbonnet et al., 2010; Forsythe et al., 2010), we investigated changes in immune phenotype and function as a consequence of exposure to social defeat.

Chronic stress altered immune function, with defeated mice exhibiting elevated serum IL-6 levels, five days after the final defeat session (Fig. 4A; t= 3.837, df= 15, P= 0.0016; Control group= 0.0838 ± 0.0838 pg/ml, Defeated group= 44.52 ± 14.04 pg/ml). No significant differences were observed in serum levels of IL-10 (P=0.327; Control group= 2.610 ± 0.2891 pg/ml, Defeated group= 3.188 ± 0.5522 pg/ml), IL-17 (P=0.0652; Control group= 1.691 ± 0.811 pg/ml, Defeated group= 5.585 ± 2.099 pg/ml, probability of superiority= 0.703), IL-4 (P=0.1120; Control group= 0.5000 ± 0.0099 pg/ml, Defeated group= 0.7663 ± 0.1216 pg/ml), or macrophage inflammatory protein (MIP)-2 (P=0.9151; Control group= 21.53 ± 2.880 pg/ml, Defeated group= 21.01 ± 4.046 pg/ml). Changes in IL-6 were transient, as serum IL-6 levels were no longer distinguishable between the two groups seventeen days following defeat (P=0.8020; Control group= 4.200 ± 0.3544 pg/ml, Defeated group= 3.967 ± 0.8442 pg/ml).

To investigate whether such changes were due to immunoregulatory alterations, we characterized the phenotype of regulatory T cells and dendritic cells. There were no significant differences in the number of splenocytes isolated from either group (P= 0.2705; Control group= 101.0 ± 9.648 $x10^6$ cells, Defeated group= 128.1 ± 23.82 $x10^6$ cells). Social defeat did result in sustained changes in phenotype of T cells and dendritic cells. While there were no differences in the population of Foxp3-expressing CD4+ CD25+ T cells at 5 (P= 0.6409) or 17 days (P= 0.0724) after stress exposure, there were distinct differences in the IL-10-expressing CD4+ CD25+ population over time. Chronic social defeated increased IL-10+ CD4+ CD25+ T cells in the spleens of defeated animals 5 days post-stressor (Fig. 4B; t= 2.748, df= 8, P= 0.0252), but led to a decrease in levels of this cell population 17 days after stress exposure (Fig. 4C; t=

3.109, df= 16, *P*= 0.0067). Given this, the supernatant from splenocytes isolated 5 or 17 days after the final defeat session was analyzed to examine whether the functional changes paralleled phenotypic alterations. At five days following the final stressor, there were no differences in the release of IL-10 from anti-CD3/CD28 antibody-stimulated splenocytes (*P*= 0.5007; Control group= 2019 ± 332.1 pg/ml, Defeated group= 2489 ± 578.0 pg/ml). At seventeen days following chronic social defeat, there was a trend towards reduced IL-10 release from splenocytes of defeated mice following anti-CD3/CD28 antibody stimulation (Fig. 4D; *t*= 2.020, df= 16, *P*= 0.0605, probability of superiority= 0.753), but not LPS stimulation (*P*= 0.353; Control group= 1577 ± 225.1 pg/ml, Defeated group= 1999 ± 406.7 pg/ml). In contrast, there was no difference in the release of TNF by splenocytes stimulated with either anti-CD3/CD28 antibodies (*P*= 0.2430; Control group= 1904 ± 411.0 pg/ml, Defeated group= 1283 ± 247.7 pg/ml) or LPS (*P*= 0.8144; Control group= 9727 ± 476.6 pg/ml, Defeated group= 9450 ± 1157 pg/ml) at seventeen days following CSD.

To investigate whether immunoregulatory alterations were associated with phenotypic changes in innate immune activation, dendritic cells were examined for markers of maturation and activation. Analysis revealed that following chronic stress exposure, levels of CD86 and MHCII expression on CD11c+ DCs at both 5 and 17 days post-stressor remain unchanged (CD86: 5 days post-stressor, *P*= 0.1609; 17 days post-stressor, *P*= 0.9750; MHCII: 5 days post-stressor, *P*= 0.7181; 17 days post-stressor, *P*= *0.*405). The levels of the co-stimulatory molecule CD80 however, were enhanced at both 5 days (Fig. 4E; *t*= 2.956 df= 8, *P*= 0.0183) and 17 days post-stressor (Fig. 4F; *t*= 3.268 df= 16, *P*= 0.0048), suggesting sustained DC activation.

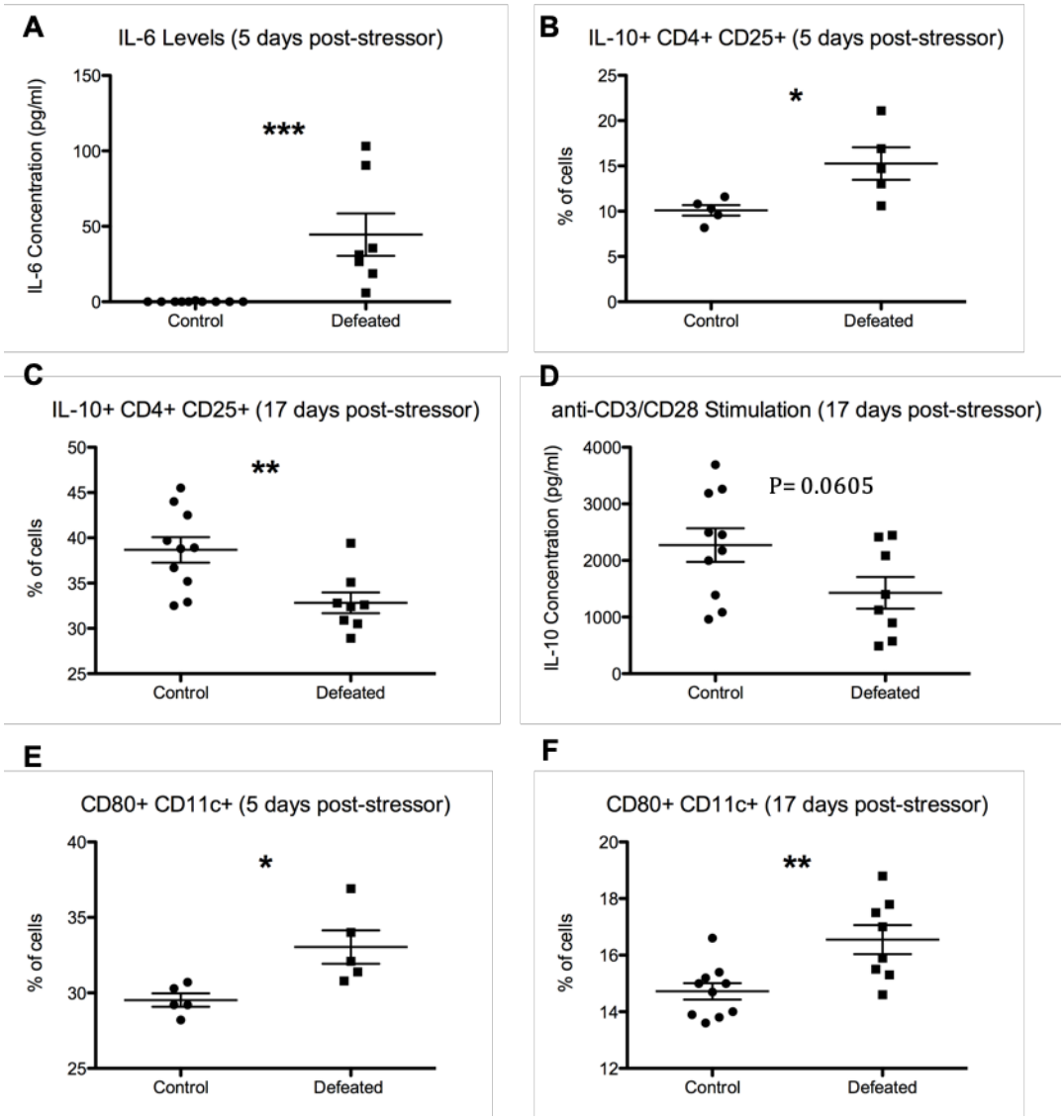

Figure 4. Effect of chronic social defeat on splenocyte phenotype and immune function. (A) IL-6 levels in the serum of defeated (n= 9) and unstressed control mice (n= 10). **(B)** IL-10+ CD4+ CD25+ splenocyte levels from control (n= 10) or defeated groups (n= 8) 5 days following exposure to CSD. **(C)** IL-10+ CD4+ CD25+ expression in the spleens of control (n= 10) and defeated mice (n=8) 17 days following exposure to CSD. **(D)** In vitro IL-10 release following stimulation of splenocytes with anti-CD3/CD28 antibodies; defeated group (n= 8), control group (n= 10). **(E)** Levels of CD80+ CD11c+ splenocytes in control (n=10) and defeated mice (n=7) 5 days following exposure to CSD. **(F)** Levels of CD80+ CD11c+ splenocytes in control (n=10) and defeated mice (n=7) 17 days following exposure to CSD. * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

3.3.4. Predictive analysis reveals altered functional microbiota profile following social defeat

To investigate the implications of structural changes in the microbiome, functional predictions were made on 16S rRNA-derived OTUs using PICRUSt, a computational method (Langille et al., 2013). Analysis using the Shannon Index revealed reduced functional diversity of KEGG pathways in the microbial community of defeated mice (Fig. 5A; t= 2.218, df= 16, P= 0.0413). PCoA plots of functional KEGG pathways generated using Bray-Curtis distances exhibited clustering of samples according to exposure to social defeat (Fig. 5B), which was confirmed following statistical analysis (ANOSIM R= 0.1918, P= 0.027, 999 permutations). Upon further investigation, a total of 145 KEGG pathways were found to be differentially represented between control and defeated groups (FDR < 0.05), of which a subset are displayed in Table 1. Predictive analysis indicated in the defeated group a lower frequency of pathways involved in fatty acid metabolism and biosynthesis, including the metabolism of propanoate and butanoate—conjugate bases of the short chain fatty acids (SCFAs), propionic and butyric acid. Defeated mice also exhibited reduced abundance of pathways involved in the biosynthesis and metabolism of tyrosine and tryptophan: molecules that serve as precursors for the synthesis of dopamine (DA), norepinephrine, serotonin (5-HT), and melatonin respectively.

Table 1. Functional pathways with reduced predicted representation in the microbiota of mice following chronic social defeat

Function	Log2 Fold Change in Frequency
Fatty acid biosynthesis	-0.218
Fatty acid metabolism	-0.214
Butanoate metabolism	-0.219
Propanoate metabolism	-0.221
Tryptophan biosynthesis	-0.222
Tyrosine biosynthesis	-0.222
Tryptophan metabolism	-0.217
Tyrosine metabolism	-0.218
Bacterial chemotaxis	-0.227
Bacterial motility proteins	-0.227
Bacterial secretion system	-0.223
Function	**Log2 Fold Change in Frequency**
Fatty acid biosynthesis	-0.218
Fatty acid metabolism	-0.214
Butanoate metabolism	-0.219
Propanoate metabolism	-0.221
Tryptophan biosynthesis	-0.222
Tyrosine biosynthesis	-0.222
Tryptophan metabolism	-0.217
Tyrosine metabolism	-0.218
Bacterial chemotaxis	-0.227
Bacterial motility proteins	-0.227
Bacterial secretion system	-0.223

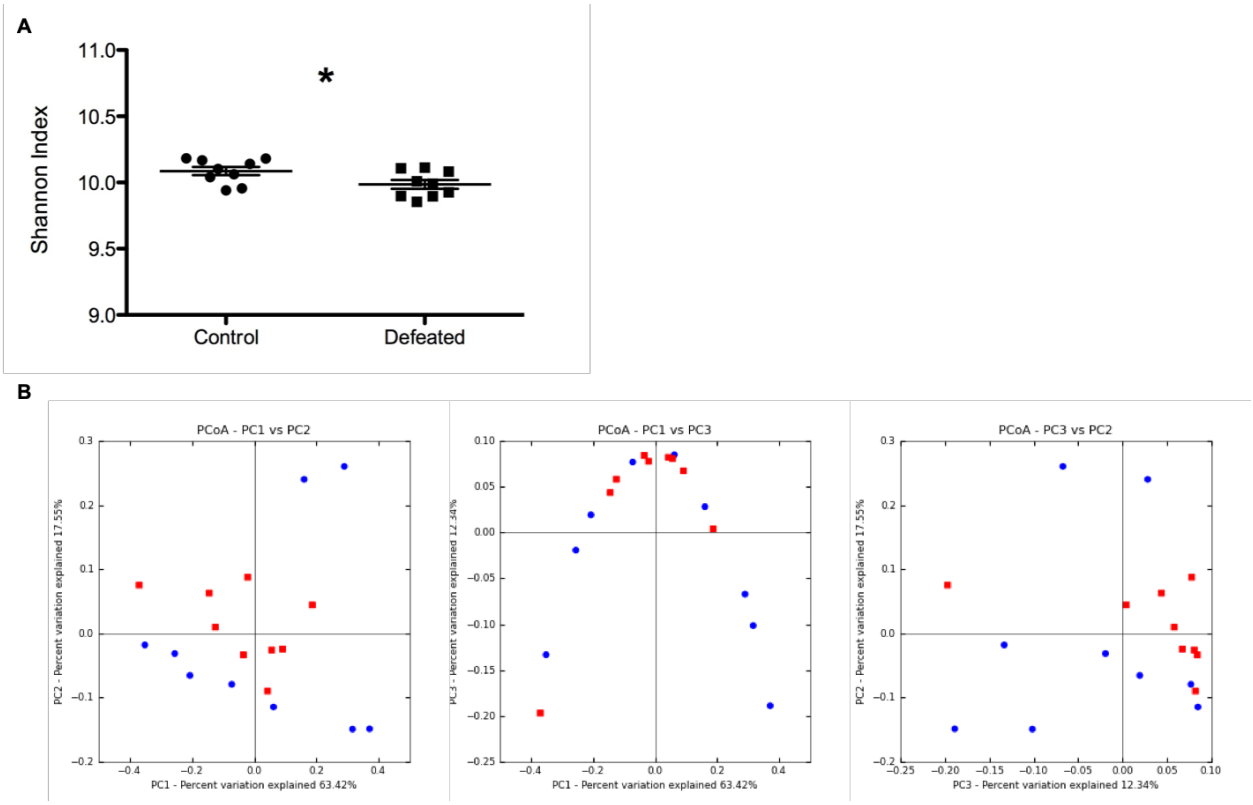

Figure 5. Effect of chronic social defeat on the predicted functional metagenome profile following CSD. **(A)** Predicted functional diversity using the Shannon index of the microbial communities in control (n=9) and defeated mice (n= 9). **(B)** PCoA plots of Bray-Curtis distances of the predicted functional profile of KEGG pathways in control (red) and defeated groups (blue). * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

3.4. Discussion

In this study, we offer a structural and functional analysis of the consequences of chronic social defeat stress on the murine microbiome and the host. We demonstrate that psychosocial stress induces complex and nuanced OTU-level shifts in the structural composition of the intestinal microbiota, including changes in the relative abundance of microbial groups that are sensitive to the state of the host (Bendtsen et al., 2012; Everard et al., 2013); these shifts are associated with the anthropomorphic neurocognitive and immunoregulatory disruptions commonly observed in both animal

models and patients with affective conditions (American Psychiatric Association, 2013; Maes, 1995). We also frame these structural changes in the microbiome within the context of the associated functional consequences on the metagenome in order to predict the biological pathways that may underlie the effects of the microbiota on brain and behaviour: social defeat reduces the prevalence of synthesis and metabolism pathways for short-chain fatty acids and neurotransmitter precursors.

Following chronic social stress exposure, defeated mice exhibited a distinct intestinal microbiota profile from that of the control group. Previous studies have also demonstrated an effect of stress on the microbiome profile, including a decrease in *Bacteroides* and an increase in the *Clostridium* genus (Bailey et al., 2011; O'Mahony et al., 2009a) However, little is known about the nature of these structural alterations at the species membership level, the functional implications associated with the resulting dysbiosis, and its relationship with stress-induced behavioural deficits. Studies investigating obesity and environmental stress-induced dysbiosis of the gut microbiome, such as that produced by antibiotic administration, have demonstrated a binary shift at the phylum level in the *Firmicutes/Bacteroidetes* ratio—a measure indicative of the normal healthy status of the human intestinal microbiome (Mariat et al., 2009; Sanderson et al., 2006; Thompson et al., 2015) Here however, in addition to decreasing the overall richness and diversity of the community, chronic stress engendered a more complex form of dysbiosis. Rather than a phylum-level binary shift in the community, we observed both increases and decreases in the frequency of distinct OTUs in the major phyla that comprise the microbial population. While not reaching statistical significance, defeated mice also exhibited lowered (relative abundance of *Akkermansia*—a taxa

whose relative abundance in the microbiota is associated with healthier states (Everard et al., 2013) . Furthermore, in contrast to a previous study demonstrating higher levels of Coriobacteriaceae following exposure to a grid floor model of stress in Balb/c mice (Bendtsen et al., 2012), social defeat led to reduced levels in stressed C57BL/6 mice. These observations demonstrate complex structural alterations and indicate the sensitivity of certain bacterial groups to stress exposure, although the precise magnitude and direction of the shift may vary between different mouse strains, models of stress. While our efforts here were limited to evaluating the microbiota community in faecal material, a recent study using the restraint stress model demonstrated the differential impact of stress on the composition of the luminal versus mucosa-associated colonic community (Galley et al., 2015). Only the diversity of the mucosa-associated community was impacted by the stressor. Furthermore, restraint stress induced compartment-specific shifts in the community-wide profile of the colonic microbiotas, as well as in the relative abundance of specific taxa such as *Lactobacillus*. These results emphasize the need for future studies to take into account compartment-specific niche differences in order to understand how such alterations might impact host health.

Exploring the role of these distinct microbiota alterations in stress-induced behavioural changes revealed an association between the microbiome profile and deficits in social behaviour on the aggressor interaction test. That the association was limited to this particular test, where defeated mice avoided a novel stimulus resembling the original traumatic episodes, lends weight to the specific nature of this nexus. No such association was observed with deficits in exploratory behaviour on the OFT. The relationship between the microbiota and social behaviour has been demonstrated

previously in GF mice, which display impairments in social cognition development, and in offspring from the maternal immune activation model of autism, which exhibit dysbiosis and deficits in social and communicative behaviour,(Desbonnet et al., 2014; Hsiao et al., 2013). Indeed, some of these deficits are normalized through post-weaning colonization or treatment with *B. fragilis*. From a clinical perspective, aggressor avoidance following social defeat bears remarkable similarity to phobic avoidance behaviour of trauma-related stimuli in individuals with post-traumatic stress disorder (American Psychiatric Association, 2013). These findings also parallel the comorbidity between stress-related psychiatric conditions and functional bowel disorders (Dinan et al., 2006; Mayer, 2000). Individuals exposed to severe wartime stress have a greater risk of irritable bowel syndrome (IBS), which is associated with alterations in the intestinal microbiota (Collins & Bercik, 2009; Klooker et al., 2009). Given the evidence provided here of the specific and nuanced nature of microbiota alterations, the sensitivity of certain microbial groups to stress exposure, and its association with stress-induced behavioural deficits, we propose that investigation into the use of microbial community markers to predict adverse neurocognitive consequences following exposure to severe stress is warranted.

Paralleling the suite of immunological changes observed in psychiatric conditions (Maes, 1995), exposure to social defeat induced alterations in immune profile. In addition to a transient increase in serum IL-6 there were long-term systemic changes at the cell population level. Spleens from defeated mice exhibited a greater population of activated dendritic cells at both 5- and 17-days following completion of the stress procedure, indicating sustained changes in innate immune activation. Defeated mice

also exhibited alterations in the IL-10+ CD4+CD25+ population, which is indicative of Tr1 regulatory T cells: a class of adaptive Tregs that produces high levels of the anti-inflammatory/regulatory cytokine, IL-10, and has been previously demonstrated to prevent the development of experimental colitis (Groux et al., 1997). The increased population of IL-10+ CD4+CD25+ T cells at 5 days post-defeat may be reflective of a homeostatic response to counteract stress-induced inflammation (Boer, Joosten, & Ottenhoff, 2015). In contrast, at 17 days following cessation of the social defeat procedure, the defeated group exhibited reduced levels of this Treg population, indicating long-term alterations in the immunoregulatory profile—consistent with the adverse consequences of chronic stress. Furthermore, while differences did not reach statistical significance, stimulation of spleen-derived T cells from defeated animals at this time-point resulted in a trend towards reduced IL-10 release. The immune alterations also exhibited a distinct temporal profile: unlike phenotypic changes in DCs and the Tr1 cell population, which were evident at least seventeen days following the cessation of chronic stress, group differences in serum IL-6 levels were no longer evident by this time point. Overall, the immune data suggests that chronic social defeat stress induces long-term changes in immune regulation and priming of the innate immune response. Furthermore, given that we only evaluated acute changes in the microbiome and behaviour (36 hours and days 1-2 following the final defeat session respectively), future studies are needed to provide insight into whether the observed changes are long lasting in order to investigate the extent of the impact of chronic stress on the adult microbiota and the associated behavioural deficits.

Although we cannot yet determine the direction of causality linking these immune alterations to behavioural and microbiome changes, our findings align with the critical role of the microbiota in priming the immune system (Clarke et al., 2010) and evidence implicating immune signalling in communication along the microbiota-gut-brain axis: the maternal separation stress-induced inflammatory profile induced and its associated behavioural deficits are both normalized upon administration of *B. infantis* (Desbonnet et al., 2010). Similarly, administration of *B. infantis* to human patients diagnosed with IBS induces immunomodulatory shifts in the cytokine levels of peripheral blood mononuclear cells (O'Mahony, McCarthy, et al., 2005).

While the role of immune and vagal signalling routes have been demonstrated in the gut-brain communications infrastructure (Bravo et al., 2011; Perez-Burgos et al., 2014), there remains much to be investigated regarding the functional alterations following structural changes in the microbiome. In defeated mice, *in silico* metagenomic analysis predicted a lower frequency of functional pathways for the synthesis and metabolism of tyrosine and tryptophan—precursors to DA and 5-HT respectively. The role of the microbiota in regulating host 5-HT levels has been highlighted extensively: more than 90% of the total 5-HT content is synthesized in the gut (Erspamer, 1966), while GF mice exhibit altered levels of 5-HT in the plasma and hippocampus (Clarke et al., 2012; Wikoff et al., 2009). These data indicate the presence of peripheral mechanisms through which indigenous species might influence the neurochemistry of the central nervous system (CNS). A recent study highlighted one such mechanism, which involved increased 5-HT synthesis by enterochromaffin cells through metabolites produced by colonic spore-forming bacteria (Yano et al., 2015). Interestingly, this

population of microbes is predominantly comprised of Clostridia, which potently induce IL-10 expression in Tregs (Atarashi et al., 2013). Both observations are consistent with our results: defeated mice exhibited a lower abundance of Clostridial species (Fig. 3A) as well as reduced IL-10+ Tr1 Tregs (Fig. 4C), thus indicating a high degree of logical agreement between the predicted functional profile, our measured observations, and previous evidence in the literature. Given the vast array of receptors acted upon by 5-HT, including those expressed on peripheral neurons and immune cells (Baganz & Blakely, 2012), further research is needed to explore the implications of microbiota-dependent deficits in 5-HT signalling on stress-induced changes in behaviour.

Our analyses also predicted reduced frequency of fatty acid biosynthesis and metabolism pathways, including that of propanoate and butanoate—the byproducts of dietary carbohydrate fermentation by intestinal microorganisms. Previous studies have extensively investigated the indirect neuroactive properties of these molecules. Butyrate is a potent histone deacetylase (HDAC) inhibitor that exerts antidepressant-like effects by increasing histone acetylation in the frontal cortex and hippocampus, and consequentially, raising *Bdnf* transcript levels (Schroeder, Lin, Crusio, & Akbarian, 2007). Through the SCFA receptor FFAR2, the microbiota also regulate microglia homeostasis in the CNS (Erny et al., 2015). Deficits in microglia maturation and function in GF mice were reversed by chronic administration of a SCFA mix comprising of butyrate, propionate, and acetate. Similarly, acetate influences appetitive and feeding behaviour by inducing the expression of regulatory neuropeptides, via effects exerted through central mechanisms in the hypothalamus (Frost et al., 2014). Although it was previously unclear whether the systemic levels of these metabolites achieved *in vivo*

were sufficient to produce behavioural changes, progress has been made by discovering their presence in the cerebrospinal fluid and the brain, and demonstrating that colon-derived SCFAs cross the blood-brain barrier and preferentially accumulate in the hypothalamus, where they can affect CNS activity (Frost et al., 2014; Wyss, Magistretti, Buck, & Weber, 2011). Such observations implicate functional changes in the microbiome as mediators of stress-induced changes in behaviour. It bears emphasizing that the *in silico* results are purely predictive in nature, and while useful in directing future functional studies, there exist obvious limitations in such techniques. Furthermore, although studies have shown a high degree of correlation (0.8-0.9) between PICRUSt predictions and measured observations of gene content through metagenomic sequencing (Langille et al., 2013), quantification of stress-induced microbiota activity is necessary in order to validate the aforementioned results and determine whether the predicted changes discovered in this particular study accurately reflect functional alterations in the microbiome in response to chronic stress. Thus, necessary caution should be undertaken whilst interpreting these data in the current context. However, that the inferred changes align with previous evidence in the literature lends weight to these results and paves the way for future studies by enabling targeted investigation of the biological pathways that may drive the effects of the microbiota on the host.

Although the literature is replete with evidence of microbiota-gut-brain communication, there exists a large gap in our understanding of the functional relationship between the microbiome and stress-induced changes in brain and behaviour. The challenge lies in unraveling the mechanisms underlying the symbiotic

microbiota-host relationships, and understanding how these relationships are altered upon exposure to chronic stress. In association with neurobehavioural disruptions that are analogous to those observed in psychiatric conditions, we demonstrate pronounced alterations in immune regulation, along with complex and nuanced shifts in the microbial community following chronic exposure to psychosocial stress. Using a computational approach, we address a critical gap in the literature by providing functional context to structural microbiota changes, thus enabling future research into the molecular mechanisms underlying signalling pathways along the microbiota-gut-brain axis. Furthermore, these findings emphasize the potential use of microbiota-based biomarkers and even novel therapeutic targets in and against the adverse psychiatric consequences of chronic stress.

Conflict of interest

The authors declare no conflicts of interest.

Contributors

P.F, J.B., &. A.B conceptualized the study. P.F, J.B., A.B. & J.A.F designed experiments. A.B. performed animal experiments. A.B. and M.F.M. prepared samples and carried out FACS analysis. M.G.S performed 16S rRNA DNA sequencing. A.B. acquired and analyzed the data and wrote the initial draft of the manuscript. A.B., M.F.M., J.A.F., M.G.S., J.B., & P.F. contributed to data interpretation and revised the manuscript. All authors approved the final version of this article.

Acknowledgments

This project is funded by a grant from the Office of Naval Research (#N00014-14-1-0787). A.B. gratefully acknowledges funding support from the Canadian Institutes of Health Research (GSM-136180). P.F. is supported by a Career Award from the Department of Medicine, McMaster University. Equipment support was provided by funds from the Canadian Foundation for Innovation to J.A.F., M.G.S is supported as a Canada Research Chair.

3.5. Supplementary Figures

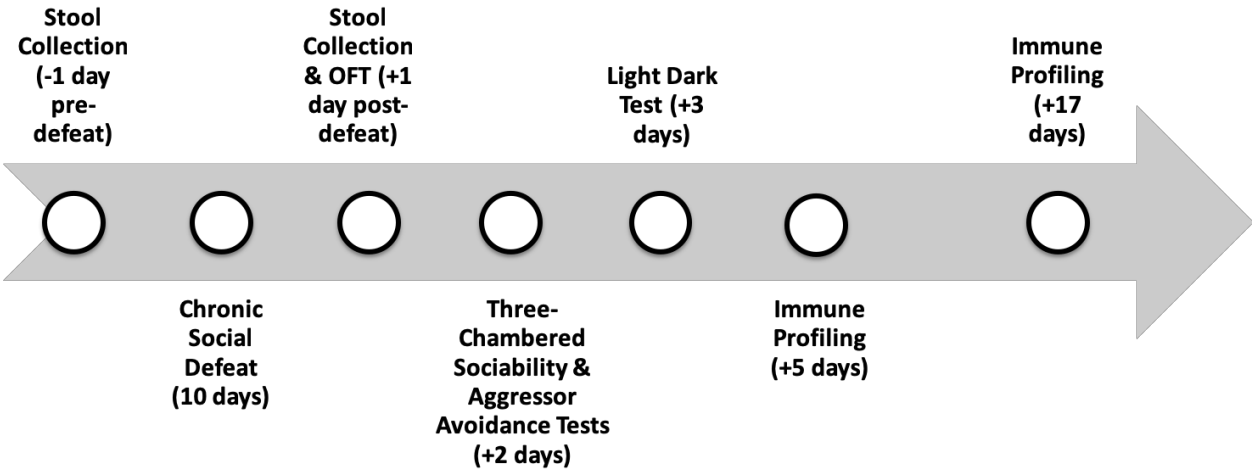

Figure S1. Timeline depicting the experimental design. Pre-defeat stools were collected one day before the initial defeat, while post-defeat stools were collected one day after the final defeat session. Open field tests were conducted on day 1 after the final defeat session. This was followed by the three-chambered sociability and aggressor avoidance tests on day 2. Light dark tests were conducted on day 3. Mice were euthanized on day 5 and day 17 for immune analysis.

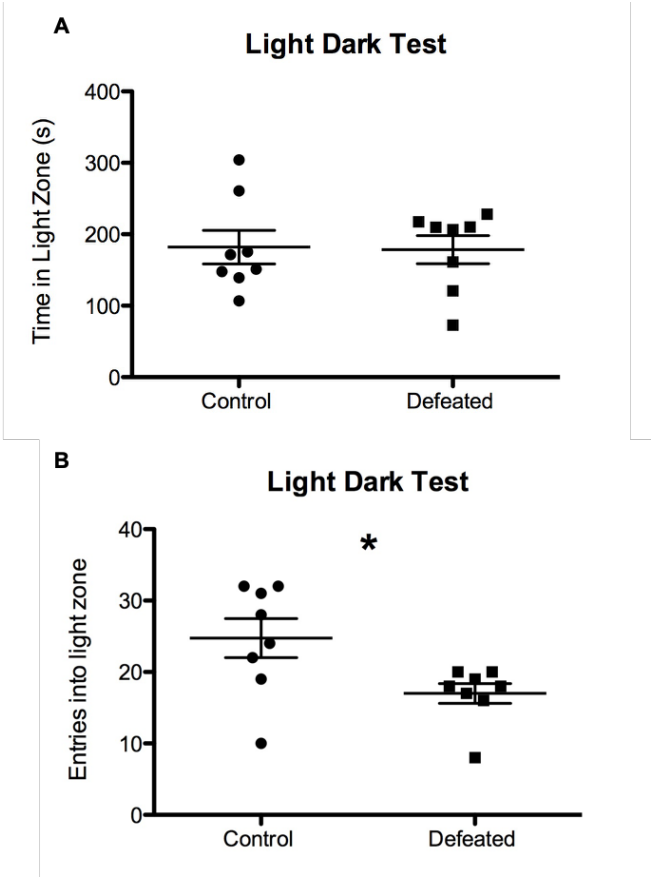

Figure S2. Effect of chronic social defeat on anxiety-like behaviour. (A) Amount of time spent in the light zone by defeated (n= 8) and control mice (n= 8) on the light-dark test. (B) Number of entries into the light zone by defeated (n= 8) and control mice (n= 8) on the light-dark test. * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

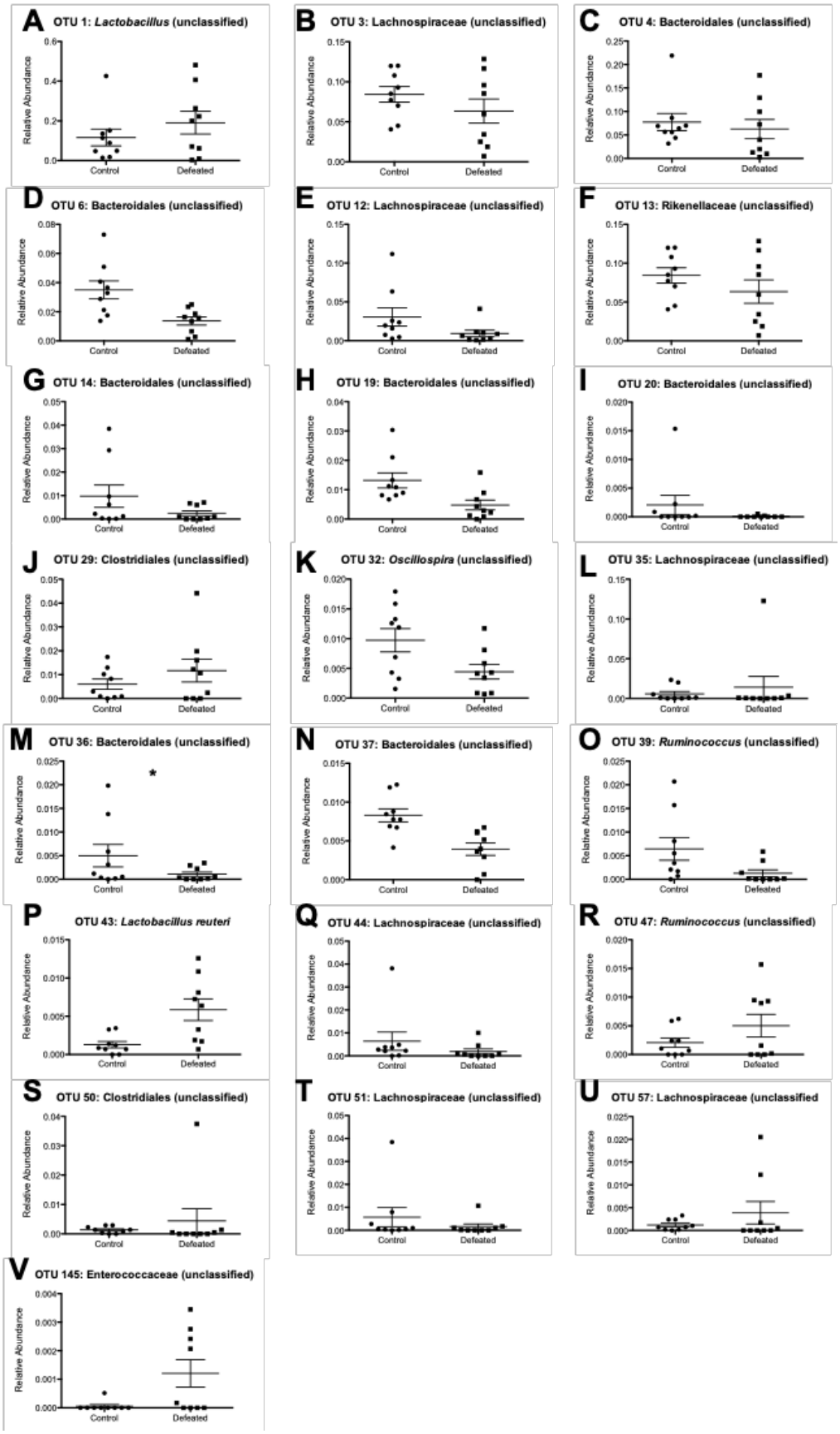
A OTU 1: Lactobacillus (unclassified)
B OTU 3: Lachnospiraceae (unclassified)
C OTU 4: Bacteroidales (unclassified)
D OTU 6: Bacteroidales (unclassified)
E OTU 12: Lachnospiraceae (unclassified)
F OTU 13: Rikenellaceae (unclassified)
G OTU 14: Bacteroidales (unclassified)
H OTU 19: Bacteroidales (unclassified)
I OTU 20: Bacteroidales (unclassified)
J OTU 29: Clostridiales (unclassified)
K OTU 32: Oscillospira (unclassified)
L OTU 35: Lachnospiraceae (unclassified)
M OTU 36: Bacteroidales (unclassified)
N OTU 37: Bacteroidales (unclassified)
O OTU 39: Ruminococcus (unclassified)
P OTU 43: Lactobacillus reuteri
Q OTU 44: Lachnospiraceae (unclassified)
R OTU 47: Ruminococcus (unclassified)
S OTU 50: Clostridiales (unclassified)
T OTU 51: Lachnospiraceae (unclassified)
U OTU 57: Lachnospiraceae (unclassified
V OTU 145: Enterococcaceae (unclassified)
Relative Abundance
Control
Defeated

Figure S3. Effect of chronic social defeat on the relative abundance of individual OTUs, related to Figure 3. (A-V) Relative abundance of individual OTUs that were significantly differentially represented between control (n=9) and defeated (n=9) mice (FDR < 0.1).

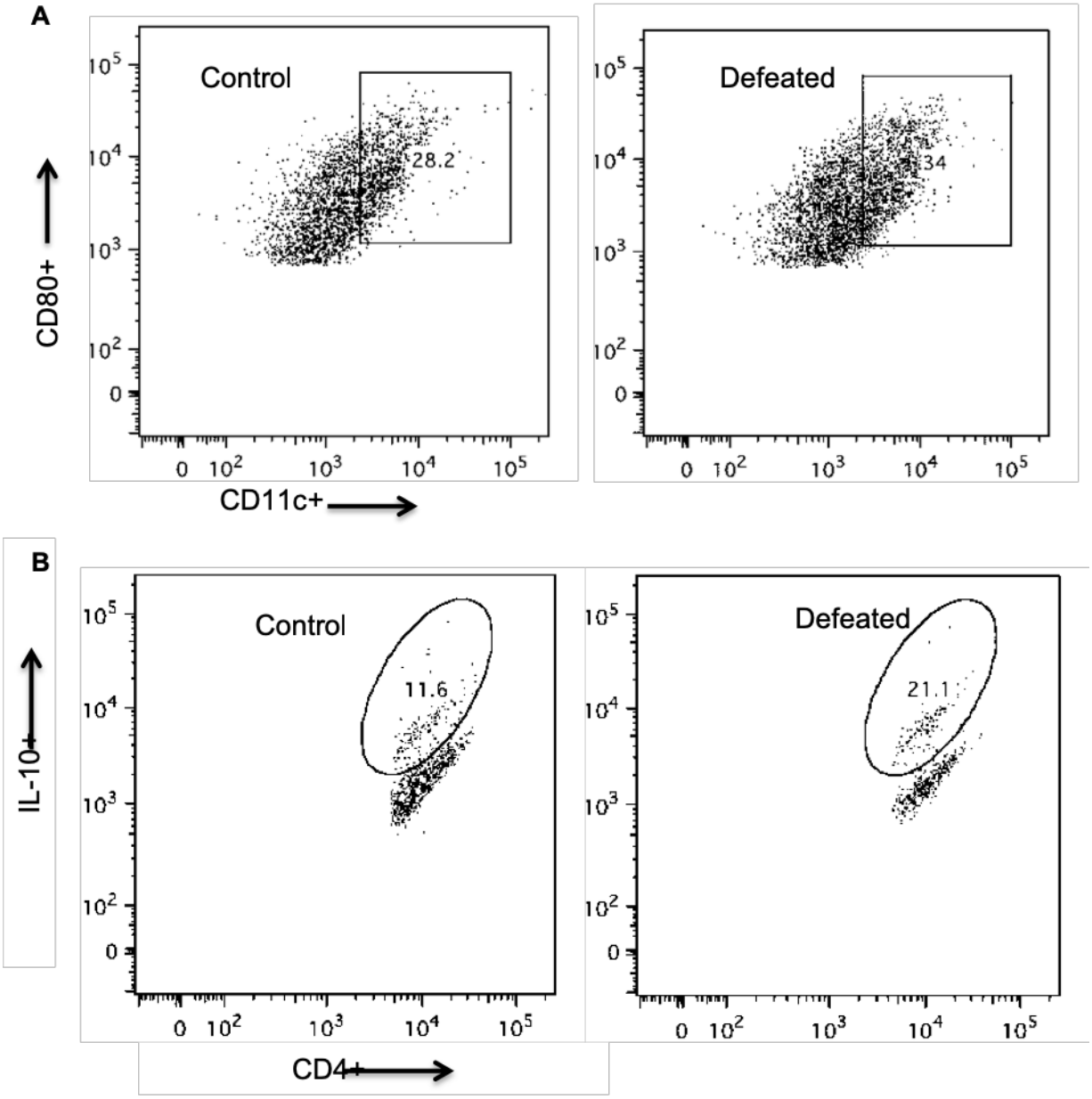

Figure S4. Effect of chronic social defeat on splenocyte phenotype. (A) FACSCanto data showing MHCII+ CD11c+ splenocytes from representative samples in each group. (B) FACSCanto data showing CD80+ CD11c+ splenocytes from representative samples in each group.

3.6. References

Aaronson, S. T., Sears, P., Ruvuna, F., Bunker, M., Conway, C. R., Dougherty, D. D., … Zajecka, J. M. (2017). A 5-year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: comparison of response, remission, and suicidality. *American Journal of Psychiatry*, *174*(7), 640–648.

Aaronson, S. T., Sears, P., Ruvuna, F., Ph, D., Bunker, M., Pharm, D., & Conway, C. R. (2017). A 5-Year Observational Study of Patients With Treatment-Resistant Depression Treated With Vagus Nerve Stimulation or Treatment as Usual : Comparison of Response , Remission , and Suicidality, (July). https://doi.org/10.1176/appi.ajp.2017.16010034

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., … Tompkins, T. (2014). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology and Motility*, *26*(4), 510–520. https://doi.org/10.1111/nmo.12295

Al-Nedawi, K., Mian, M. F., Hossain, N., Karimi, K., Mao, Y.-K., Forsythe, P., … Bienenstock, J. (2014). Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 1–12. https://doi.org/10.1096/fj.14-259721

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association.

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., … Hen, R. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, 1. https://doi.org/10.1038/s41586-018-0262-4

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neuroscience and Biobehavioral Reviews*, *51*, 164–188. https://doi.org/10.1016/j.neubiorev.2015.01.018

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E., & Nestler, E. J. (2001). Induction of nuclear factor-κB in nucleus accumbens by chronic cocaine administration. *Journal of Neurochemistry*, *79*(1), 221–224.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., … Hase, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461), 232–236.

Avgustinovich, D. F., Kovalenko, I. L., & Kudryavtseva, N. N. (2005). A model of anxious depression: Persistence of behavioral pathology. *Neuroscience and Behavioral Physiology*, *35*(9), 917–924. https://doi.org/10.1007/s11055-005-0146-6

Baganz, N. L., & Blakely, R. D. (2012). A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chemical Neuroscience*, *4*(1), 48–

63.

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., … Schöpf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486–496.

Bagot, R. C., Cates, H. M., Purushothaman, I., Vialou, V., Heller, E. A., Yieh, L., … Nestler, E. J. (2016). Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. *Biological Psychiatry*, *81*(4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012

Bagot, R. C., Parise, E. M., Pen, C. J., Zhang, H., Maze, I., Chaudhury, D., … Nestler, E. J. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms8062

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., & Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology*, *77*(11), 3846–3852.

Beas, B. S., Wright, B. J., Skirzewski, M., Leng, Y., Hyun, J. H., Koita, O., … Penzo, M. A. (2018). The locus coeruleus drives disinhibition in the midline thalamus via a dopaminergic mechanism. *Nature Neuroscience*, *21*(7), 963. https://doi.org/10.1038/s41593-018-0167-4

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., … Pamer, E. G. (2016). Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nature Medicine*, *22*(5), 516.

Bendtsen, K. M. B., Krych, L., Sørensen, D. B., Pang, W., Nielsen, D. S., Josefsen, K., … Hansen, A. K. (2012). Gut Microbiota Composition Is Correlated to Grid Floor Induced Stress and Behavior in the BALB/c Mouse. *PLoS ONE*, *7*(10), e46231. https://doi.org/10.1371/journal.pone.0046231

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., … Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609. https://doi.org/10.1053/j.gastro.2011.04.052

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., … Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and Motility*, *23*(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Bercik, P., Verdu, E. F., Foster, J. a, Macri, J., Potter, M., Huang, X., … Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and

alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102-2112.e1. https://doi.org/10.1053/j.gastro.2010.06.063

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience: Basic and Clinical*, *85*(1–3), 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0

Berton, O., Mcclung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress, (February), 864–869.

Berton, O., McClung, C. a, Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science (New York, N.Y.)*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural and functional consequences of chronic psychosocial stress on the microbiome and host. *Psychoneuroendocrinology*, *63*(2016), 217–227. https://doi.org/10.1016/j.psyneuen.2015.10.001

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 Medicine*, *15*(1), 7. https://doi.org/10.1186/s12916-016-0771-7

Blackshaw, L. A., Brookes, S. J. H., Grundy, D., & Schemann, M. (2007). Sensory transmission in the gastrointestinal tract. *Neurogastroenterology & Motility*, *19*, 1–19.

Boer, M. C., Joosten, S. A., & Ottenhoff, T. H. M. (2015). Regulatory T-cells at the interface between human host and pathogens in infectious diseases and vaccination. *Frontiers in Immunology*, *6*.

Bohórquez, DV, & Shahid, R. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *The Journal of …*, 1–5. https://doi.org/10.1172/JCI78361DS1

Bohórquez, Diego V., Samsa, L. A., Roholt, A., Medicetty, S., Chandra, R., & Liddle, R. A. (2014). An enteroendocrine cell - Enteric glia connection revealed by 3D electron microscopy. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0089881

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E., Taylor, C. M., Welsh, D. A., & Berthoud, H.-R. R. (2014). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry*, *77*(7), 607–615. https://doi.org/10.1016/j.biopsych.2014.07.012

Brynskikh, A., Warren, T., Zhu, J., & Kipnis, J. (2008). Adaptive immunity affects

learning behavior in mice. *Brain, Behavior, and Immunity*, *22*(6), 861–869. https://doi.org/10.1016/j.bbi.2007.12.008

Buffington, S. A., Viana, G., Prisco, D., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., … Petrosino, J. F. (2016). Microbial Reconstitution Reverses Maternal Diet- Induced Social and Synaptic Deficits in Offspring Article Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell*, *165*(7), 1762–1775. https://doi.org/10.1016/j.cell.2016.06.001

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856.

Caenepeel, P. H., Janssens, J., Vantrappen, G., Eyssen, H., & Coremans, G. (1989). Interdigestive myoelectric complex in germ-free rats. *Digestive Diseases and Sciences*, *34*(8), 1180–1184.

Cámara, R. J. A., Gander, M.-L., Begré, S., Von Känel, R., & Group, S. I. B. D. C. S. (2011). Post-traumatic stress in Crohn's disease and its association with disease activity. *Frontline Gastroenterology*, *2*(1), 2–9.

Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012). The tail suspension test. *JoVE (Journal of Visualized Experiments)*, (59), e3769.

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., … Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal 1 – 3, 1236–1243. https://doi.org/10.3945/ajcn.2009.28095.The

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335

Carle, T. L., Ohnishi, Y. N., Ohnishi, Y. H., Alibhai, I. N., Wilkinson, M. B., Kumar, A., & Nestler, E. J. (2007). Absence of conserved C-terminal degron domain contributes to ΔFosB's unique stability. *Eur J Neurosci*, *25*, 3009–3019.

Castelli, M. P., Ferraro, L., Mocci, I., Carta, F., Carai, M. A. M., Antonelli, T., … Gessa, G. L. (2003). Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of γ-hydroxybutyric acid. *Journal of Neurochemistry*, *87*(3), 722–732.

Castle, M., Comoli, E., & Loewy, A. D. (2005). Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience*, *134*(2), 657–669. https://doi.org/10.1016/j.neuroscience.2005.04.031

Chae, J., Nahas, Z., Lomarev, M., Denslow, S., Lorberbaum, J. P., Bohning, D. E., & George, M. S. (2003). A review of functional neuroimaging studies of vagus nerve stimulation (VNS), *37*, 443–455. https://doi.org/10.1016/S0022-3956(03)00074-8

Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., & Nestler, E. J. (1997). Chronic Fos-

related antigens: stable variants of ΔFosB induced in brain by chronic treatments. *Journal of Neuroscience*, *17*(13), 4933–4941.

Chiu, R., Angel, P., & Karin, M. (1989). Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. *Cell*, *59*(6), 979–986. https://doi.org/10.1016/0092-8674(89)90754-X

Chung, L. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. *Development & Reproduction*, *19*(2), 61–67. https://doi.org/10.12717/DR.2015.19.2.061

Chunyu, J. P., Kyle, Z., Darrell, B., Dayanim, G., Bhatnagar, S., Luz, S., & Vigderman, A. S. (2019). The gut microbiome regulates the increases in depressive-type behaviors and in in fl ammatory processes in the ventral hippocampus of stress vulnerable rats. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0380-x

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., … Cryan, J. F. (2012). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences*, *103*(27), 10420–10425.

Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, *16*(2), 228–231.

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The Impact of the Gut Microbiota on Human Health: An Integrative View - 1-s2.0-S0092867412001043-main.pdf. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*(6), 2003–2014.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews. Microbiology*, *10*(11), 735–742. https://doi.org/10.1038/nrmicro2876

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science*, *326*(5960), 1694–1697.

Crawley, J. N. (1985). Exploratory behavior models of anxiety in mice. *Neuroscience & Biobehavioral Reviews*, *9*(1), 37–44.

Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., … Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207–217. https://doi.org/10.1016/j.psyneuen.2014.01.014

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–

712.

Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009

Cunningham, J. T., Mifflin, S. W., Gould, G. G., & Frazer, A. (2008). Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. *Neuropsychopharmacology*, *33*(8), 1884–1895. https://doi.org/10.1038/sj.npp.1301570

Curran, T., & Franza Jr, B. R. (1988). Fos and Jun: the AP-1 connection. *Cell*, *55*(3), 395–397.

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. *Nature Reviews Neuroscience*, *9*(1), 46–56.

David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrotta, A., … Alm, E. J. (2014). Host lifestyle affects human microbiota on daily timescales. *Genome Biol*, *15*(7), R89.

de LeBlanc, A. de M., Dogi, C. A., Galdeano, C. M., Carmuega, E., Weill, R., & Perdigón, G. (2008). Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunology*, *9*(1), 27.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, a. J., Green, W., … Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*(August), 7735. https://doi.org/10.1038/ncomms8735

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Dinan, T G. (2005). Stress: the shared common component in major mental illnesses. *European Psychiatry*, *20*, S326–S328.

Dinan, Timothy G, Quigley, E. M. M., Ahmed, S. M. M., Scully, P., O'Brien, S., O'Mahony, L., … Keeling, P. W. N. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*, *130*(2), 304–311.

Dorward, D. W., Garon, C. F., & Judd, R. C. (1989). Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. *Journal of Bacteriology*, *171*(5), 2499–2505.

Duffy, L. C., Zielezny, M. A., Marshall, J. R., Byers, T. E., Weiser, M. M., Phillips, J. F., … Graham, S. (1991). Relevance of major stress events as an indicator of disease activity prevalence in inflammatory bowel disease. *Behavioral Medicine*, *17*(3), 101–110.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.

Eloe-fadrosh, E. A., Brady, A., Crabtree, J., Drabek, E. F., Ma, B., Mahurkar, A., … Fraser, M. (2015). Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption. *MBio*, *6*(2), e00231-15. https://doi.org/10.1128/mBio.00231-15.Editor

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, (October 2014). https://doi.org/10.1038/nn.4030

Erspamer, V. (1966). Occurrence of indolealkylamines in nature. In *5-Hydroxytryptamine and Related Indolealkylamines* (pp. 132–181). Springer.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., … Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, *110*(22), 9066–9071. https://doi.org/10.1073/pnas.1219451110

Foley, J. O., & DuBois, F. S. (1937). Quantitative studies of the vagus nerve in the cat. I. The ratio of sensory to motor fibers. *Journal of Comparative Neurology*, *67*(1), 49–67.

Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. *Immunological Investigations*, *39*(4–5), 429–448. https://doi.org/10.3109/08820131003667978

Forsythe, P., & Kunze, W. a. (2013). Voices from within: gut microbes and the CNS. *Cellular and Molecular Life Sciences : CMLS*, *70*(1), 55–69. https://doi.org/10.1007/s00018-012-1028-z

Forsythe, P., Kunze, W., & Bienenstock, J. (2016). Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? *BMC Medicine*, *14*(1), 58. https://doi.org/10.1186/s12916-016-0604-8

Forsythe, P., Sudo, N., Dinan, T., Taylor, V. H., & Bienenstock, J. (2010). Mood and gut feelings. *Brain, Behavior, and Immunity*, *24*(1), 9–16. https://doi.org/10.1016/j.bbi.2009.05.058

Forsythe, P., Wang, B., Khambati, I., & Kunze, W. a. (2012). Systemic effects of ingested lactobacillus rhamnosus: Inhibition of mast cell membrane potassium (IKCA) current and degranulation. *PLoS ONE*, *7*(7), 1–8. https://doi.org/10.1371/journal.pone.0041234

Frazer, A., & Benmansour, S. (2002). Delayed pharmacological effects of antidepressants. *Molecular Psychiatry*, *7*, S23–S28.

https://doi.org/10.1038/sj.mp.4001015

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., … Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, *5*, 3611. https://doi.org/10.1038/ncomms4611

Fuchs, D., Möller, A. A., Reibnegger, G., Stöckle, E., Werner, E. R., & Wachter, H. (1990). Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *3*(9), 873–876.

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling : What Happens in Vagus... *Neuron*, *101*, 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Furet, J.-P., Quénée, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology*, *97*(2), 197–207.

Furmaga, H., Sadhu, M., & Frazer, A. (2012). Comparison of FosB Immunoreactivity Induced by Vagal Nerve Stimulation with That Caused by Pharmacologically Diverse Antidepressants. *Journal of Pharmacology and Experimental Therapeutics*, *341*(2), 317–325. https://doi.org/10.1124/jpet.111.188953

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. In *Microbial endocrinology: The microbiota-gut-brain axis in health and disease* (pp. 39–71). Springer.

Furness, J. B., Kunze, W. A. A., & Clerc, N. (1999). II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *277*(5), G922–G928.

Galley, J. D., Yu, Z., Kumar, P., Dowd, S. E., Lyte, M., & Bailey, M. T. (2015). The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut Microbes*, *5*(6), 748–760. https://doi.org/10.4161/19490976.2014.972241

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., … Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515

Gärtner, K. (1990). A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Laboratory Animals*, *24*(1), 71–77.

Gębicki, J., Sysa-Jędrzejowska, A., Adamus, J., Woźniacka, A., Rybak, M., & Zielonka, J. (2003). 1-Methylnicotinamide: a potent anti-inflammatory agent of vitamin origin. *Pol. J. Pharmacol*, *55*, 109–112.

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., … Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*(7610), 94–103.

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: we have never been individuals. *The Quarterly Review of Biology*, *87*(4), 325–341.

Gobaille, S., Schleef, C., Hechler, V., Viry, S., Aunis, D., & Maitre, M. (2002). Gamma-hydroxybutyrate increases tryptophan availability and potentiates serotonin turnover in rat brain. *Life Sciences*, *70*(18), 2101–2112.

Goehler, L. E., Gaykema, R. P. A., Opitz, N., Reddaway, R., Badr, N., & Lyte, M. (2005). Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with Campylobacter jejuni. *Brain, Behavior, and Immunity*, *19*(4), 334–344. https://doi.org/10.1016/j.bbi.2004.09.002

Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols*, *6*(8), 1183–1191. https://doi.org/10.1038/nprot.2011.361

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 33–51.

Greenberg, M. E., Greene, L. A., & Ziff, E. B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *Journal of Biological Chemistry*, *260*(26), 14101–14110.

Greenberg, M. E., & Ziff, E. B. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature*, *311*(5985), 433.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., & Roncarolo, M. G. (1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*, *389*(6652), 737–742.

Groves, D. A., & Brown, V. J. (2005). Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neuroscience & Biobehavioral Reviews*, *29*(3), 493–500. https://doi.org/10.1016/j.neubiorev.2005.01.004

Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., … de Araujo, I. E. (2018). A Neural Circuit for Gut-Induced Reward. *Cell*, 1–14. https://doi.org/10.1016/j.cell.2018.08.049

He, B., Xu, W., Santini, P. A., Polydorides, A. D., Chiu, A., Estrella, J., … Plebani, A. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*, *26*(6), 812–826.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., … Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences*, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710.

Heitler, W. J. (2007). DataView: A Tutorial Tool for Data Analysis. Template-based Spike Sorting and Frequency Analysis. *Journal of Undergraduate Neuroscience Education*, *6*(1), A1.

Henningsen, P., Zimmermann, T., & Sattel, H. (2003). Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. *Psychosomatic Medicine*, *65*(4), 528–533.

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., … Warren, B. L. (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proceedings of the National Academy of Sciences*, *111*(52), 18799–18799. https://doi.org/10.1073/pnas.1423575112

Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Frontiers in Neuroendocrinology*, *14*(3), 173–213. https://doi.org/10.1006/frne.1993.1006

Holzer, P. (2011). Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacology & Therapeutics*, *131*(1), 142–170.

Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., … Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron*, *13*(5), 1235–1244.

Houlden, A., Goldrick, M., Brough, D., Vizi, E. S., Lénárt, N., Martinecz, B., … Denes, A. (2016). Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain, Behavior, and Immunity*, *57*, 10–20.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., … Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female Syrian hamsters. *Hormones and Behavior*, *44*(3), 293–299.

Husebye, E., Hellström, P. M., & Midtvedt, T. (1994). Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. *Digestive Diseases and Sciences*, *39*(5), 946–956.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuroimage*, *125*, 988–995.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Stanisz, G. J., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *NeuroImage*, *125*(2015), 988–995. https://doi.org/10.1016/j.neuroimage.2015.11.018

Jašarević, E., Howard, C. D., Misic, A. M., Beiting, D. P., & Bale, T. L. (2017). Stress

during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. *Scientific Reports*, *7*, 44182.

Jašarević, E., Howard, C. D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., … Bale, T. L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nature Neuroscience*. https://doi.org/10.1038/s41593-018-0182-5

Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., … Kheirbek, M. A. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron*, *0*(0), 1–14. https://doi.org/10.1016/j.neuron.2018.01.016

Jorissen, H. J. M. M., Ulery, P. G., Henry, L., Gourneni, S., Nestler, E. J., & Rudenko, G. (2007). Dimerization and DNA-binding properties of the transcription factor ΔFosB. *Biochemistry*, *46*(28), 8360–8372.

Jung, H. Y., Kim, W., Yoo, D. Y., Nam, S. M., Kim, J. W., Choi, J. H., … Hwang, I. K. (2014). Intragastric gavage with denatonium benzoate acutely induces neuronal activation in the solitary tract nucleus via the vagal afferent pathway. *Journal of Veterinary Science*, *15*(4), 459–464. https://doi.org/10.4142/jvs.2014.15.4.459

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B., Montoya, M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, *In Press*. https://doi.org/10.1126/science.aat5236

Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., … Bienenstock, J. (2006). Inhibitory effects of Lactobocillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, *55*(2), 191–196. https://doi.org/10.1136/gut.2005.070987

Karimi, K., Inman, M. D., Bienenstock, J., & Forsythe, P. (2009). Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *American Journal of Respiratory and Critical Care Medicine*, *179*(3), 186–193. https://doi.org/10.1164/rccm.200806-951OC

Karimi, K., Kandiah, N., Chau, J., Bienenstock, J., & Forsythe, P. (2012). A Lactobacillus rhamnosus Strain Induces a Heme Oxygenase Dependent Increase in Foxp3+ Regulatory T Cells. *PLoS ONE*, *7*(10), 1–12. https://doi.org/10.1371/journal.pone.0047556

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., … Cryan, J. F. (2016). Lost in Translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.11.018

Kidd, M., Modlin, I. M., Gustafsson, B. I., Drozdov, I., Hauso, O., & Pfragner, R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *295*(2), G260–G272.

Kinsey, S. G., Bailey, M. T., Sheridan, J. F., Padgett, D. A., & Avitsur, R. (2007). Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. *Brain, Behavior, and Immunity*,

21(4), 458–466.

Klooker, T. K., Braak, B., Painter, R. C., de Rooij, S. R., van Elburg, R. M., van den Wijngaard, R. M., … Boeckxstaens, G. E. (2009). Exposure to severe wartime conditions in early life is associated with an increased risk of irritable bowel syndrome: a population-based cohort study. *The American Journal of Gastroenterology*, *104*(9), 2250–2256.

König, H., Ponta, H., Rahmsdorf, U., Büscher, M., Schönthal, A., Rahmsdorf, H. J., & Herrlich, P. (1989). Autoregulation of fos: the dyad symmetry element as the major target of repression. *The EMBO Journal*, *8*(9), 2559–2566.

Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, *7*.

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Kunze, W. A., Mao, Y., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., & Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and Molecular Medicine*, *13*(8b), 2261–2270.

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. a, … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676

Lay, C., Rigottier-Gois, L., Holmstrøm, K., Rajilic, M., Vaughan, E. E., de Vos, W. M., … Blaut, M. (2005). Colonic microbiota signatures across five northern European countries. *Applied and Environmental Microbiology*, *71*(7), 4153–4155.

Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-amram, H., … Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. https://doi.org/10.1038/ncomms15062

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, *124*(4), 837–848.

Lindqvist, D., Wolkowitz, O. M., Mellon, S., Yehuda, R., Flory, J. D., Henn-Haase, C., … Neylan, T. C. (2014). Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. *Brain, Behavior, and Immunity*, *42*, 81–88.

Liu, Z.-H., & Smith, C. B. (2009). Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. *Neuroscience Letters*, *454*(1), 62–66.

Lucibello, F. C., Lowag, C., Neuberg, M., & Müller, R. (1989). Trans-repression of the mouse c-fos promoter: A novel mechanism of fos-mediated trans-regulation. *Cell*, *59*(6), 999–1007. https://doi.org/10.1016/0092-8674(89)90756-3

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, *33*(8), 574–581.

Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathogens*, *9*(11).

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. *Physiology & Behavior*, *89*(3), 350–357. https://doi.org/10.1016/j.physbeh.2006.06.019

Lyte, M., Varcoe, J. J., & Bailey, M. T. (1998). Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiology and Behavior*, *65*(1), 63–68. https://doi.org/10.1016/S0031-9384(98)00145-0

Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system, *4*(June), 1626–1632.

Macpherson, A. J., & Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*, *303*(5664), 1662–1665.

Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *19*(1), 11–38.

Maitre, M., Humbert, J.-P., Kemmel, V., Aunis, D., & Andriamampandry, C. (2005). [A mechanism for gamma-hydroxybutyrate (GHB) as a drug and a substance of abuse]. *Medecine Sciences: M/S*, *21*(3), 284–289.

Malatynska, E., & Knapp, R. J. (2005). Dominant–submissive behavior as models of mania and depression. *Neuroscience & Biobehavioral Reviews*, *29*(4–5), 715–737.

Malkova, N. V, Collin, Z. Y., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, Behavior, and Immunity*, *26*(4), 607–616.

Mao, Y.-K., Kasper, D. L., Wang, B., Forsythe, P., Bienenstock, J., & Kunze, W. a. (2013). Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.*, *4*, 1465. https://doi.org/10.1038/ncomms2478

Mariat, D., Firmesse, O., Levenez, F., Guimarães, V., Sokol, H., Doré, J., … Furet, J.-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, *9*, 123. https://doi.org/10.1186/1471-2180-9-123

Mayer, E. A. (2000). The neurobiology of stress and gastrointestinal disease. *Gut*, *47*(6), 861–869.

Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195), 620.

Mccall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., Mcelligott, Z. A., & Stuber, G. D. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections

promote anxiety-like behavior, 1–23. https://doi.org/10.7554/eLife.18247

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain. *Molecular Brain Research*, *132*(January), 146–154. https://doi.org/10.1016/j.molbrainres.2004.05.014

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2005). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain, (May 2018). https://doi.org/10.1016/j.molbrainres.2004.05.014

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine*, *338*(3), 171–179.

McHenry, J. A., Robison, C. L., Bell, G. A., Vialou, V. V, Bolaños-Guzmán, C. A., Nestler, E. J., & Hull, E. M. (2016). The role of ΔfosB in the medial preoptic area: Differential effects of mating and cocaine history. *Behavioral Neuroscience*, *130*(5), 469.

McKinney, W. T., & Bunney, W. E. (1969). Animal model of depression: I. Review of evidence: implications for research. *Archives of General Psychiatry*, *21*(2), 240–248.

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility*, *25*(2), 183-e88.

Menard, C., Pfau, M. L., Hodes, G. E., Kana, V., Wang, V. X., Bouchard, S., … Russo, S. J. (2017). Social stress induces neurovascular pathology promoting depression. *Nature Neuroscience*, *20*(12), 1752–1760. https://doi.org/10.1038/s41593-017-0010-3

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … Cazaubiel, M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition*, *105*(5), 755–764.

Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., … Wolf, S. A. (2016). Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports*, *15*(9), 1945–1956. https://doi.org/10.1016/j.celrep.2016.04.074

Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience*, *14*(1), 421–451.

Müller, R., Bravo, R., Burckhardt, J., & Curran, T. (1984). Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature*, *312*(5996), 716.

Mundorf, M. L., Hochstetler, S. E., & Wightman, R. M. (1999). Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *Journal of Neurochemistry*, *73*(6), 2397–2405.

Murphy, K., & Weaver, C. (2016). *Janeway's immunobiology*. Garland Science.

Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*(3), 695–700.

Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, *148*(1), 257–266.

Naritoku, D. K., Terry, W. J., & Helfert, R. H. (1995). Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Research*, *22*(1), 53–62. https://doi.org/10.1016/0920-1211(95)00035-9

Nemeroff, C. B., Mayberg, H. S., Krahl, S. E., McNamara, J., Frazer, A., Henry, T. R., … Brannan, S. K. (2006). VNS therapy in treatment-resistant depression: Clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology*, *31*(7), 1345–1355. https://doi.org/10.1038/sj.npp.1301082

Nestler, E. J. (2015). Δ FosB : A transcriptional regulator of stress and antidepressant responses. *European Journal of Pharmacology*, *753*, 66–72. https://doi.org/10.1016/j.ejphar.2014.10.034

Nestler, E. J., Barrot, M., & Self, D. W. (2001). ΔFosB: a sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences*, *98*(20), 11042–11046.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–265. https://doi.org/10.1111/j.1365-2982.2010.01620.x

Nishino, R., Mikami, K., Takahashi, H., Tomonaga, S., Furuse, M., Hiramoto, T., … Sudo, N. (2013). Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. *Neurogastroenterology & Motility*, *25*(6), 521-e371.

O'Garra, A., Vieira, P. L., Vieira, P., & Goldfeld, A. E. (2004). IL-10–producing and naturally occurring CD4+ Tregs: limiting collateral damage. *The Journal of Clinical Investigation*, *114*(10), 1372–1378.

O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. (2005). Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, L., Mccarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. M. (2005). Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., … Dinan, T. G. (2009a). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric

illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., … Dinan, T. G. (2009b). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., & Madsen, K. L. (2013). Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology*, *38*(9), 1738–1747.

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., & Hsiao, E. Y. (2018). The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell*, *173*(7), 1728-1741.e13. https://doi.org/10.1016/j.cell.2018.04.027

Padilla-coreano, N., Bolkan, S. S., Pierce, G. M., Spellman, T. J., Gordon, J. A., Padilla-coreano, N., … Hardin, W. D. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Article Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron*, *89*, 1–10. https://doi.org/10.1016/j.neuron.2016.01.011

Padmanabhan, P., Grosse, J., Asad, A. B. M. A., Radda, G. K., & Golay, X. (2013). Gastrointestinal transit measurements in mice with99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Research*, *3*(1), 1–8. https://doi.org/10.1186/2191-219X-3-60

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, *5*(7), e177.

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society*, *73*(04), 477–489.

Paxinos, G., & Franklin, K. B. J. (2004). *The mouse brain in stereotaxic coordinates*. Gulf professional publishing.

Perez-Burgos, A., Mao, Y.-K., Bienenstock, J., & Kunze, W. a. (2014). The gut-brain axis rewired: adding a functional vagal nicotinic "sensory synapse". *The FASEB Journal*, *28*(7), 3064–3074. https://doi.org/10.1096/fj.13-245282

Perez-Burgos, A., Wang, B., Mao, Y.-K., Mistry, B., McVey Neufeld, K.-A., Bienenstock, J., & Kunze, W. (2013). Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *304*(2), G211-20. https://doi.org/10.1152/ajpgi.00128.2012

Perez-Burgos, A., Wang, L., McVey Neufeld, K., Mao, Y., Ahmadzai, M., Janssen, L. J., … Kunze, W. A. (2015). The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of Physiology*, *593*(17), 3943–3957.

Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., & Nestler, E. J. (2004). Induction of ΔFosB in reward-related brain structures after chronic stress. *Journal of Neuroscience*, *24*(47), 10594–10602.

Peyron, C., Luppi, P. H., Fort, P., Rampon, C., & Jouvet, M. (1996). Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *Journal of Comparative Neurology*, *364*(3), 402–413. https://doi.org/10.1002/(SICI)1096-9861(19960115)364:3<402::AID-CNE2>3.0.CO;2-8

Phillips, J. G. P. (1910). The Treatment of Melancholia by the Lactic Acid Bacillus. *The British Journal of Psychiatry*, *56*(234), 422-NP. https://doi.org/10.1192/bjp.56.234.422

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Yamada, T. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59.

Ramos, A., & Mormède, P. (1997). Stress and emotionality: a multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, *22*(1), 33–57.

Reber, S. O., Siebler, P. H., Donner, N. C., Morton, J. T., Smith, D. G., Kopelman, J. M., … Lowry, C. A. (2016). Immunization with a heat-killed preparation of the environmental bacterium *Mycobacterium vaccae* promotes stress resilience in mice. *Proceedings of the National Academy of Sciences*, 201600324. https://doi.org/10.1073/pnas.1600324113

Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., … Kashyap, P. C. (2014). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, *29*(4), 1395–1403.

Robison, A. J., Vialou, V., Mazei-Robison, M., Feng, J., Kourrich, S., Collins, M., … Neve, R. (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving ΔFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *Journal of Neuroscience*, *33*(10), 4295–4307.

Rolls, A., Shechter, R., London, A., Ziv, Y., Ronen, A., Levy, R., & Schwartz, M. (2007). Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biology*, *9*(9), 1081.

Rong, W., Hillsley, K., Davis, J. B., Hicks, G., Winchester, W. J., & Grundy, D. (2004). Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *The Journal of Physiology*, *560*(3), 867–881.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1), 529.

Sanderson, S., Boardman, W., Ciofi, C., & Gibson, R. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/nature4441021a

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., …

Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676.

Schönfeld, C.-L., & Trendelenburg, U. (1989). The release of3H-noradrenaline by p-and m-tyramines and-octopamines, and the effect of deuterium substitution in α-position. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *339*(4), 433–440.

Schroeder, F. a, Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry*, *62*(1), 55–64. https://doi.org/10.1016/j.biopsych.2006.06.036

Schütte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J., & Minna, J. (1989). jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, *59*(6), 987–997. https://doi.org/10.1016/0092-8674(89)90755-1

Schwarz, J., Burguet, J., Rampin, O., Fromentin, G., Andrey, P., Tomé, D., … Darcel, N. (2010). Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. *PLoS ONE*, *5*(2), 13–15. https://doi.org/10.1371/journal.pone.0008974

Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, *164*(3), 337–340.

Serretti, A., Calati, R., Goracci, A., Di Simplicio, M., Castrogiovanni, P., & De Ronchi, D. (2010). Antidepressants in healthy subjects: what are the psychotropic/psychological effects? *European Neuropsychopharmacology*, *20*(7), 433–453.

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2018). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron*, 1–14. https://doi.org/10.1016/j.neuron.2018.11.018

Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P. C., & Mazmanian, S. K. (2014). Perspective Specialized Metabolites from the Microbiome in Health and Disease. *Cell Metabolism*, *20*(5), 719–730. https://doi.org/10.1016/j.cmet.2014.10.016

Sharp, F. R., Sagar, S. M., Hicks, K., Lowenstein, D., & Hisanaga, K. (1991). c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. *Journal of Neuroscience*, *11*(8), 2321–2331.

Sibley, C. D., Grinwis, M. E., Field, T. R., Eshaghurshan, C. S., Faria, M. M., Dowd, S. E., … Surette, M. G. (2011). Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PloS One*, *6*(7), e22702.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.-M., … Kolassa, I.-T. (2009). Substantial reduction of naive and regulatory T cells following traumatic stress. *Brain, Behavior, and Immunity*, *23*(8), 1117–1124.

Stam, R., Akkermans, L. M., & Wiegant, V. M. (1997). Trauma and the gut: interactions between stressful experience and intestinal function. *Gut*, *40*(6), 704.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., … Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal

system for stress response in mice. *The Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′Sialyllactose and 6′Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain, Behavior, and Immunity*, *50*, 166–177. https://doi.org/10.1016/j.bbi.2015.06.025

Thompson, J. A., Oliveira, R. A., Djukovic, A., Ubeda, C., & Xavier, K. B. (2015). Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota. *Cell Reports*, 1–11. https://doi.org/10.1016/j.celrep.2015.02.049

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … Mayer, E. a. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401, 1401.e1-4. https://doi.org/10.1053/j.gastro.2013.02.043

Torii, A., Torii, S., Fujiwara, S., Tanaka, H., Inagaki, N., & Nagai, H. (2007). Lactobacillus acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergology International*, *56*(3), 293–301.

Tsuji, M., Suzuki, K., Kinoshita, K., & Fagarasan, S. (2008). Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. In *Seminars in immunology* (Vol. 20, pp. 59–66). Elsevier.

Ulery, P. G., Rudenko, G., & Nestler, E. J. (2006). Regulation of ΔFosB stability by phosphorylation. *Journal of Neuroscience*, *26*(19), 5131–5142.

Umesaki, Y., Okada, Y., Matsumoto, S., Imaoka, A., & Setoyama, H. (1995). Segmented Filamentous Bacteria Are Indigenous Intestinal Bacteria That Activate Intraepithelial Lymphocytes and Induce MHC Class II Molecules and Fucosyl Asialo GM1 Glycolipids on the Small Intestinal Epithelial Cells in the Ex-Germ-Free Mouse. *Microbiology and Immunology*, *39*(8), 555–562.

van der Kleij, H., O'Mahony, C., Shanahan, F., O'Mahony, L., & Bienenstock, J. (2008). Protective effects of Lactobacillus reuteri and Bifidobacterium infantis in murine models for colitis do not involve the vagus nerve. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(4), R1131–R1137.

Vialou, V., Robison, A. J., Laplant, Q. C., Covington, H. E., Dietz, D. M., Ohnishi, Y. N., … Nestler, E. J. (2010). fosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neuroscience*, *13*(6), 745–752. https://doi.org/10.1038/nn.2551

Vialou, V., Thibault, M., Kaska, S., Cooper, S., Gajewski, P., Eagle, A., … Robison, A. J. (2015). Differential induction of FosB isoforms throughout the brain by fluoxetine and chronic stress. *Neuropharmacology*, *99*, 28–37. https://doi.org/10.1016/j.neuropharm.2015.07.005

Wang, B., Mao, Y.-K. K., Diorio, C., Pasyk, M., Wu, R. Y., Bienenstock, J., & Kunze, W. A. (2010). Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *The FASEB Journal*, *24*(10), 4078–4088.

https://doi.org/10.1096/fj.09-153841

Wang, F. Bin, & Powley, T. L. (2000). Topographic inventories of vagal afferents in gastrointestinal muscle. *The Journal of Comparative Neurology*, *421*(3), 302–324. https://doi.org/10.1002/(SICI)1096-9861(20000605)421:3<302::AID-CNE2>3.0.CO;2-N [pii]

Wehner, S., Koscielny, A., Vilz, T. O., Stoffels, B., Engel, D. R., Kurts, C., & Kalff, J. (2014). Measurement of gastrointestinal and colonic transit in mice, 1–9. https://doi.org/10.1038/protex.2011.219

Werner-Felmayer, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G., & Wachter, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells in vitro. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1012*(2), 140–147.

Whelan, F. J., Verschoor, C. P., Stearns, J. C., Rossi, L., Luinstra, K., Loeb, M., … Bowdish, D. M. E. (2014). The loss of topography in the microbial communities of the upper respiratory tract in the elderly. *Annals of the American Thoracic Society*, *11*(4), 513–521.

Wikoff, W. R., Anfora, A. T., Liu, J., Schultz, P. G., Lesley, S. A., Peters, E. C., & Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences*, *106*(10), 3698–3703.

Wohleb, E. S., McKim, D. B., Sheridan, J. F., & Godbout, J. P. (2015). Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Frontiers in Neuroscience*, *8*(January), 1–17. https://doi.org/10.3389/fnins.2014.00447

Wohleb, E. S., Powell, N. D., Godbout, J. P., & Sheridan, J. F. (2013). Stress-Induced Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(34), 13820–13833. https://doi.org/10.1523/JNEUROSCI.1671-13.2013

Wong, A. H. C., Gottesman, I. I., & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*, *14*(suppl_1), R11–R18.

Wu, J. C. Y. (2012). Psychological co-morbidity in functional gastrointestinal disorders: epidemiology, mechanisms and management. *Journal of Neurogastroenterology and Motility*, *18*(1), 13.

Wyss, M. T., Magistretti, P. J., Buck, A., & Weber, B. (2011). Labeled acetate as a marker of astrocytic metabolism. *Journal of Cerebral Blood Flow & Metabolism*, *31*(8), 1668–1674.

Yano, J. M. M., Yu, K., Donaldson, G. P. P., Shastri, G. G. G., Ann, P., Ma, L., … Hsiao, E. Y. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. https://doi.org/10.1016/j.cell.2015.02.047

Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B.,

… Dileone, R. J. (2006). An essential role for ΔFosB in the nucleus accumbens in morphine action. *Nature Neuroscience*, *9*(2), 205.

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T., … Vieira-Silva, S. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, *352*(6285), 565–569.

Zijlmans, M. a. C., Korpela, K., Riksen-Walraven, J. M., de Vos, W. M., & de Weerth, C. (2015). Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, *53*, 233–245. https://doi.org/10.1016/j.psyneuen.2015.01.006

Zucchi, R., Chiellini, G., Scanlan, T. S., & Grandy, D. K. (2006). Trace amine-associated receptors and their ligands. *British Journal of Pharmacology*, *149*(8), 967–978.

CHAPTER 4.

Oral treatment with neuroactive bacteria attenuates behavioral deficits and immune changes in chronic social stress

(Published in BMC Medicine 15(1): 7, 2017)

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Abstract

Background: Stress-related disorders involve systemic alterations, including disruption of the intestinal microbial community. Given the putative connections between the microbiota, immunity, neural function, and behaviour, we investigated the potential for microbe-induced gut-to-brain signalling to modulate the impact of stress on host behaviour and immunoregulation.

Methods: Male C57BL/6 mice treated orally over 28 days with either *Lactobacillus rhamnosus* (JB-1) ™ or vehicle were subjected to chronic social defeat and assessed for alterations in behaviour and immune cell phenotype. 16S rRNA sequencing and mass spectrometry were employed to analyse the faecal microbial community and metabolite profile.

Results: Treatment with JB-1 decreased stress-induced anxiety-like behaviour and prevented deficits in social interaction with con-specifics. However, JB-1 did not alter development of aggressor avoidance following social defeat.

Microbial treatment attenuated stress-related activation of dendritic cells while increasing IL-10+ regulatory T cells. Furthermore, JB-1 modulated the effect of stress on faecal metabolites with neuroactive and immunomodulatory properties. Exposure to social defeat altered faecal microbial community composition and reduced species richness and diversity, none of which were prevented by JB-1.

Stress-related microbiota disruptions persisted in vehicle-treated mice for three weeks following stressor cessation.

Conclusions: These data demonstrate that despite the complexity of the gut microbiota, exposure to a single microbial strain can protect against certain stress-induced behaviours and systemic immune alterations without preventing dysbiosis. This work supports microbe-based interventions for stress related disorders.

4.1. Introduction

Stress-related disorders have their roots in nuanced interactions between genetic and environmental risk factors, resulting in complex and multifactorial etiologies. The cumulative physiological effect of stressors (McEwen, 1998) causes the dysregulation of multiple host systems due to allostatic overload. The last decade has witnessed a growing interest in the potential contribution of gut-brain signalling to psychiatric disorders. Chronic severe stress is associated with inflammation and increased susceptibility to functional gastrointestinal conditions and there is strong evidence for co-morbidity between gastrointestinal symptoms and psychiatric disorders (Duffy et al., 1991; Klooker et al., 2009; Stam, Akkermans, & Wiegant, 1997). Although precise biological mechanisms remain unclear, it is possible that such bidirectional associations in stress are at least partly a consequence of alterations in gut-brain signalling pathways due to a disrupted gut microbial community. The latter is complex and dynamic, harbouring ~10^{13} bacterial cells that represent 3.3 million non-redundant genes, rivalling our human genome by at least two orders of magnitude (Ley, Peterson, & Gordon, 2006).The critical role of the gut microbial community in the regulation of diverse physiological functions, including immunity, is well established, and there is growing evidence of its influence on the central nervous system (Collins, Surette, & Bercik,

2012; Cryan & Dinan, 2012; Forsythe & Kunze, 2013). For instance, administration of specific bacterial strains decreases anxiety- and depressive-like behaviours (Bercik, Denou, et al., 2011; Bravo et al., 2011), while changes in the microbial community modulate stress-induced inflammation (Bailey et al., 2011; Reber et al., 2016). The emergent corollary demonstrates the inextricable relationship between the microbiota, immune, and nervous systems, and their roles in regulating behaviour and neural function. Indeed, along with other groups, we have demonstrated the top-down effect of psychological stress on the structure and function of the microbiota, resulting in reduced species diversity and richness, an altered community profile, and shifts in functional pathways (Bailey et al., 2011; Bharwani et al., 2016; O'Mahony et al., 2009a). Given microbial regulation of host signalling at the mucosal interface between microbiota and host, disruptions in this community may lead to systemic changes in peripheral signals (Forsythe & Bienenstock, 2010; Sharon et al., 2014). For instance, immune dysregulation has been implicated in psychological stressors and psychiatric disorders (Bailey et al., 2011; Sommershof et al., 2009). However, much is still unknown regarding how bottom-up signalling along the gut-brain axis might be utilized to modulate stress-related changes in behaviour and neural function.

The aim of the present study was to investigate the role of microbe-induced gut-to-brain signalling on the central and systemic disruptions induced by chronic exposure to a psychosocial stressor. Using a validated model of chronic stress and depression (Berton, McClung, et al., 2006; Krishnan et al., 2007), we determined whether oral administration of a bacteria with neuroactive and immunomodulatory properties could modulate stress-induced behavioural deficits, immune changes, and gut dysbiosis. We

selected *L. rhamnosus* JB-1™ (JB-1) as our test organism as oral treatment with this strain was previously demonstrated to lead to changes in neurotransmitters levels in the brains of mice (Janik, Thomason, Stanisz, Forsythe, Bienenstock, et al., 2016) and to have anxiolytic and anti-depressant-like activity on baseline behaviours—effects that were dependent on an intact vagus nerve (Bravo et al., 2011). Feeding the JB-1 strain also modulates enteric nervous system function (Kunze et al., 2009), increases the frequency of vagal afferent firing (Perez-Burgos et al., 2013), and has well described anti-inflammatory and immunoregulatory effects (Forsythe, Wang, Khambati, & Kunze, 2012; Karimi, Inman, Bienenstock, & Forsythe, 2009; Karimi, Kandiah, Chau, Bienenstock, & Forsythe, 2012). To elucidate metabolites that may drive effects of bacteria on the brain, we investigated candidate functional pathways using metabolomics profiling. Furthermore, we examined the duration of stress-induced disruptions in the microbiota, and whether administration of a single bacteria strain during stress exposure can facilitate recovery of the dysbiotic community.

4.2. Methods

4.2.1. Animals. Male C57BL/6 mice, eight-weeks old, and CD-1 retired breeders were acquired from Charles River (Montreal, Canada). Animals were acclimatized for seven days in standard conditions (12-h light-dark cycle) with *ad libitum* access to standard chow and water. All experiments followed Canadian Council on Animal Care guidelines and were approved by the McMaster Animal Research Ethics Board.

4.2.2. Preparation and treatment with Lactobacillus rhamnosus (JB-1). *Lactobacillus rhamnosus* (JB-1)™ bacteria were prepared as described previously

(Bravo et al., 2011). Briefly, bacteria from stock were suspended in tubes filled with Man-Rogosa-Sharpe (MRS) medium for 48-h under anaerobic conditions. Following this, bacteria were harvested, washed with sterile phosphate buffered saline (PBS) to the desired concentration, re-suspended in MRS broth, and stored at -80°C in 1ml aliquots at 10^{10} colony-forming units (CFUs)/ml.

4.2.3. Treatment and Social Defeat. Animals were gavaged with 200µl ($1.67x10^9$ CFU) of *Lactobacillus rhamnosus* (JB-1) ™—a gift from Alimentary Health Inc., Ireland—or equivalent volume of phosphate buffered saline. Treatment was administered over 28 days, Monday to Friday, between 1-3 pm. During the final 10 days of treatment (Fig. 1), chronic social defeat (CSD) was initiated as previously described (supplementary methods) (Berton, McClung, et al., 2006). For 24 hours after each defeat, mice were housed in the same cage across a perforated Plexiglas divider from their aggressors.

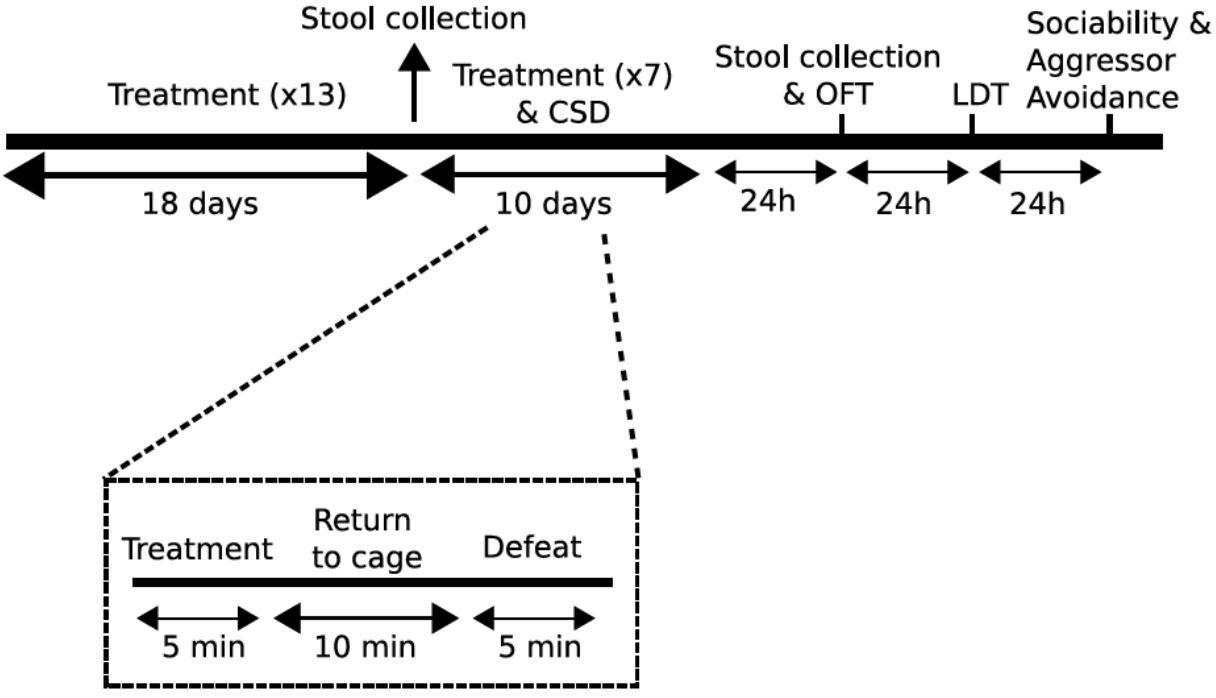


Figure 1. Schematic diagram of experimental approach. Mice were treated with JB-1

on 20 instances over a period of 28 days, including 7 instances over the final 10 days, during which mice were exposed to chronic social defeat (CSD) stress every day. OFT, open field test; LDT, light-dark box test.

4.2.4. Behavioural Testing. Details are provided in the supplementary. Behavioural testing began 24h after the final defeat session, and was recorded/analyzed using Motor Monitor (Kinder Scientific) and EthoVision XT (Noldus). Anxiety-like and exploratory behaviours were assessed using the light-dark box (LD) and open field tests (OFT). Sociability and susceptibility were assessed using the three-chamber sociability and aggressor approach-avoidance tests.

4.2.5. Tissue Analysis. Mice were euthanized 5 days after the final defeat session. Spleens were harvested and dispersed using a cell strainer in cold, sterile PBS. Cell suspensions were centrifuged at 1500 rpm for 10 minutes at 4°C, then re-suspended in RBC lysis buffer for 1-2 minutes. The resulting solution was centrifuged before cell pellets were washed with 5ml of complete RPMI 1640 medium: 10% foetal bovine serum, penicillin/streptomycin antibiotics, 2mM L-glutamine, and 0.01% β -mercaptoethanol. Viable cell numbers were assessed by Trypan Blue exclusion and diluted in RPMI to a concentration of 10^7 cells/ml. Splenocytes (10^6) were stained for markers of dendritic cell (DC) maturation and function—CD11c- PerCP-Cy5, MHCII-FITC, CD80-PE, CD86-APC—or regulatory T cells—CD3-APC, CD4-FITC, CD25-PE-Cy7, intracellular IL-10-PE (BD Pharmingen, San Diego, CA, USA; eBiosciences, San Diego, CA, USA). Following surface staining, cells were fixed and permeabilized with BD Cytofix/cytosperm before staining for intracellular markers. Data were acquired with FACSCanto (Becton Dickinson, Oakville, ON, Canada) and analyzed using FlowJo (TreeStar, Ashland, OR, USA).

4.2.6. RNA extraction and RT-qPCR analyses. Following rapid decapitation, the frontal cortex, and hippocampus were macrodissected using their stereotaxic coordinates according to the Mouse Brain Atlas and placed into RNA*later*® solution (Ambion, Life Technologies, CA, USA). Tissues were incubated overnight at 4°C, then transferred to -20°C to await further processing. RNA extraction was carried out using TRIzol® Reagent (Ambion, Life technologies) following manual homogenization. RNA quality was assessed using NanoDrop® Spectrophotometer ND-1000. 1 µg RNA was then converted into cDNA by using SuperscriptIII™ First-Strand Synthesis Supermix (Invitrogen, CA, USA). Diluted or non-diluted cDNA was used as template for qPCR reaction using PowerUp™ SYBR®Green Master Mix (Applied Biosystem, Life Techologies, TX, USA) containing ROX™ dye Passive Reference. The qPCR reactions were performed in the fast mode (UDG activation 50°C, 2min; Dual-Lock™DNA polymerase 95°C, 2min; Denaturation: 95°C, 1sec; Annealing/Extension 60°C, 30sec; number of cycles: 40) by using QuanStudio3™ (Applied Biosystem). Primers were designed with Primer Express™ Software and used at a concentration of 300 nM. Primers sequences are listed in Table S6. Transcripts were normalized to endogenous GAPDH and quantified using the $\Delta\Delta$Ct method, with related fold change expressed as $2^{(-\Delta\Delta Ct)}$.

4.2.7. 16s rRNA Analysis & Metabolomics. Faecal pellets were stored at -80°C**.** DNA extraction was carried out as previously described (Bharwani et al., 2016). Bacterial community profiling of 16S rRNA was carried out on a MiSeq Illumina sequencer in the McMaster Genome Center (McMaster University). Using rarified data in QIIME (Caporaso et al., 2010), Chao1 and Phylogenetic Diversity metrics were implemented, and Jackknife resampling was used to generate Bray-Curtis distances. (Dis)similarity

between the groups was calculated using the Monte Carlo Permutation Procedure (MCPP) (999 permutations) and *a priori* Bonferroni-corrected non-parametric t-tests. Kruskal-Wallis one-way ANOVA or Mann Whitney U test, followed by the Benjamini-Hochberg correction for multiple comparisons (False Discovery Rate < 0.05) was used to analyze differential abundance of OTUs in groups.

Metabolite profiling was performed by Metabolon, Inc. using the automated MicroLab STAR® system from Hamilton Company and analyzed using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) platforms. Samples were lyophilized and an identical mass equivalent was extracted and processed for the platform. Proteins were precipitated with methanol under vigorous shaking for 2 minutes (Glen Mills GenoGrinder 2000) followed by centrifugation. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis, when the extract was dried then reconstituted in compatible acidic and basic solvents. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution.

4.2.8. Detection of faecal L. rhamnosus levels. Faecal content in JB1 was estimated using qPCR of the 16S rRNA gene for *L. rhamnosus*. DNA was extracted from feces using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the

manufacturer's instructions, and including a bead-beating step. Real-time polymerase chain reaction (PCR) was performed with a StepOnePlus Real-Time PCR System and software (Applied Biosystems, Den Ijssel, The Netherlands) using SYBR Green (Applied Biosystems) for detection. Primers sequences were as followed: Lr1 GTGCTTGCATCTTGATTTAATTTT and Lr2 TGCGGTTCTTGGATCTATGCG as reported by (Furet, Quénée, & Tailliez, 2004) (annealing temperature of 50°C). All samples were run in duplicate in a single 96-well reaction plate. The purity of the amplified product was verified by analyzing the melting curve performed at the end of amplification. Quantification was achieved through a standard curve generated using DNA isolated from a culture-quantified aliquot of JB1 following the same extraction procedure.

4.2.9. Statistical Analysis. Data were analyzed in IBM's SPSS (version 22, Chicago) and GraphPad Prism 6 using two-tailed students *t*-test, Mann-Whitney U test, or ANOVAs, with Bonferroni-corrected post-hoc tests. Two-way ANOVAs with contrasts followed by Benjamini-Hochberg correction (FDR < 0.1) were used to analyze metabolomics data. No statistical methods were used to predetermine sample sizes; however, *n* values used herein are consistent with previous work. During the course of social defeat and testing, some animals were removed due to excessive wounding (open wounds exceeding 1 cm, as per the Animal Utilization Protocol approved by McMaster's Animal Research Ethics Board). Results in figures are expressed as mean ± SEM, where applicable. Statistical significance is denoted as * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$).

4.3. Results

4.3.1 Microbial treatment modulates specific stress-induced behavioural deficits

Chronic social defeat (CSD) reveals distinct phenotypes—susceptible and resilient—based on behaviour in the aggressor approach-avoidance test (Bagot et al., 2015; Berton, McClung, et al., 2006; Krishnan et al., 2007). CSD induced expression of both phenotypes in either treatment group, with no difference in the proportion of resilient mice: 18.1% (6/33) of vehicle-treated defeated mice, 15.6% (5/32) of defeated mice treated with JB-1 until CSD cessation. Only the susceptible group was used for all experiments.

We have previously demonstrated that mice subjected to CSD exhibit sociability deficits (Bharwani et al., 2016). Vehicle-treated defeated mice (DEF/VEH) exhibited pronounced avoidance of the social chamber (Group x chamber interaction [$F_{1, 23}$= 5.438, p = 0.029, post-hoc, $p < 0.05$]) (Fig. 2B). However, defeated mice administered JB-1 (DEF/JB-1) demonstrated no preference between the social and non-social chambers (post-hoc, $p > 0.05$) (Fig. 2B), and relative to DEF/VEH, exhibited a greater social: non-social ratio ($F_{1, 39}$= 9.660, p = 0.004, post-hoc, $p < 0.05$) (Fig. 2C), indicating a partial correction of stress-induced deficits in social behaviour. Notably, treatment did not alter baseline behaviour (Fig. 2A).

Susceptible mice markedly avoid interactions with a novel aggressor (Berton, McClung, et al., 2006). Thus, we investigated whether the positive effects of JB-1 extended to behaviour on the aggressor approach-avoidance test (Fig. 2D). DEF/JB-1 mice continued to exhibit pronounced avoidance of the zone surrounding the aggressor

('interaction zone'), exclusively during the presence of the aggressor ($F_{1, 23}$= 130.8, p < 0.0001) (Fig. 2E).

Chronic social stress also induces anxiety-like behaviour and deficits in exploration (Bharwani et al., 2016; Kinsey et al., 2007). On the OFT, stress decreased rearing behaviour ($F_{1, 81}$= 131.2, p < 0.0001), indicating reduced exploration. Simple effects analysis of defeated groups revealed JB-1 significantly attenuated deficits in rearing ($F_{1, 14}$= 6.888, p = 0.02) (Fig. 2F). Overall, there was no main effect of treatment on rearing or locomotion. Neither stress exposure nor treatment influenced time spent in the center of the open field (Fig. S1B). On the LD test, both defeated groups exhibited fewer transitions into the light compartment ($F_{1, 57}$= 36.34, p < 0.0001), which is a more salient measure of anxiety-like behaviour (Crawley, 1985) (Fig. 2G). However, DEF/JB-1 mice ventured into the light compartment more frequently than DEF/VEH mice, indicating an anxiolytic-like effect of JB-1 administration (stress exposure x treatment interaction [$F_{1, 57}$= 5.171, p = 0.027, post-hoc, p < 0.05]). Neither stress nor treatment affected time spent in the light compartment (Fig. S1C).

Given the paucity of literature regarding the long-term ramifications on behaviour following cessation of interventions, we re-tested a subset of mice 3 weeks following CSD exposure and treatment cessation. Entries into the light compartment 24 hours following the final defeat were significantly different between CON/VEH and DEF/VEH groups, but not between CON/VEH and DEF/JB-1 or DEF/VEH and DEF/JB-1 groups ($F_{1, 26}$= 6.738, p = 0.004, post-hoc, CON/VEH vs DEF/VEH at 24h, p < 0.01), further corroborating the anxiolytic-like effects of JB-1 (Fig. 2H). 3 weeks post-stressor, there were no significant differences between any of the three groups, indicating a recovery of

stress-induced anxiety-like behaviour. Neither JB-1 nor time influenced aggressor avoidance behaviour 3 weeks post-stressor (Fig. 2I).

To investigate the neural mechanisms underlying the effect of microbial treatment on the expression of stress-related behaviours, we examined changes in expression of genes related to the stress circuitry in the frontal cortex and hippocampus. Neither stress nor treatment altered the expression of corticotropin-releasing factor receptor type 1 or type 2 in the frontal cortex or the hippocampus, or the glucocorticoid receptor in the frontal cortex (Fig. S2A-S2E). Stress decreased the expression of glucocorticoid receptors in the hippocampus ($F_{1, 16}$= 10.67, p = 0.005); an effect that was not influenced by JB-1 treatment (Fig. S2F). Given that we have previously demonstrated the effects of JB-1 administration on central GABA receptors (Bravo et al., 2011), we examined whether similar changes might underlie the effects of the bacteria in a chronic stress model. Stress reduced the expression of $GABA_{A\alpha2}$ ($F_{1, 30}$= 6.126, p = 0.019) and $GABA_{B1b}$ mRNA ($F_{1, 30}$= 5.961, p = 0.021) in the frontal cortex, in the absence of a treatment effect (Fig. S2G; Fig. S2H). There were no effects of either stress or treatment on $GABA_{A\alpha2}$ or $GABA_{A\alpha2}$ mRNA levels in the hippocampus (Fig. S2I; Fig. S2J).

These data demonstrate that microbial treatment partially corrects the adverse effects of stress on social preference, exploration, and anxiety-like behaviours.

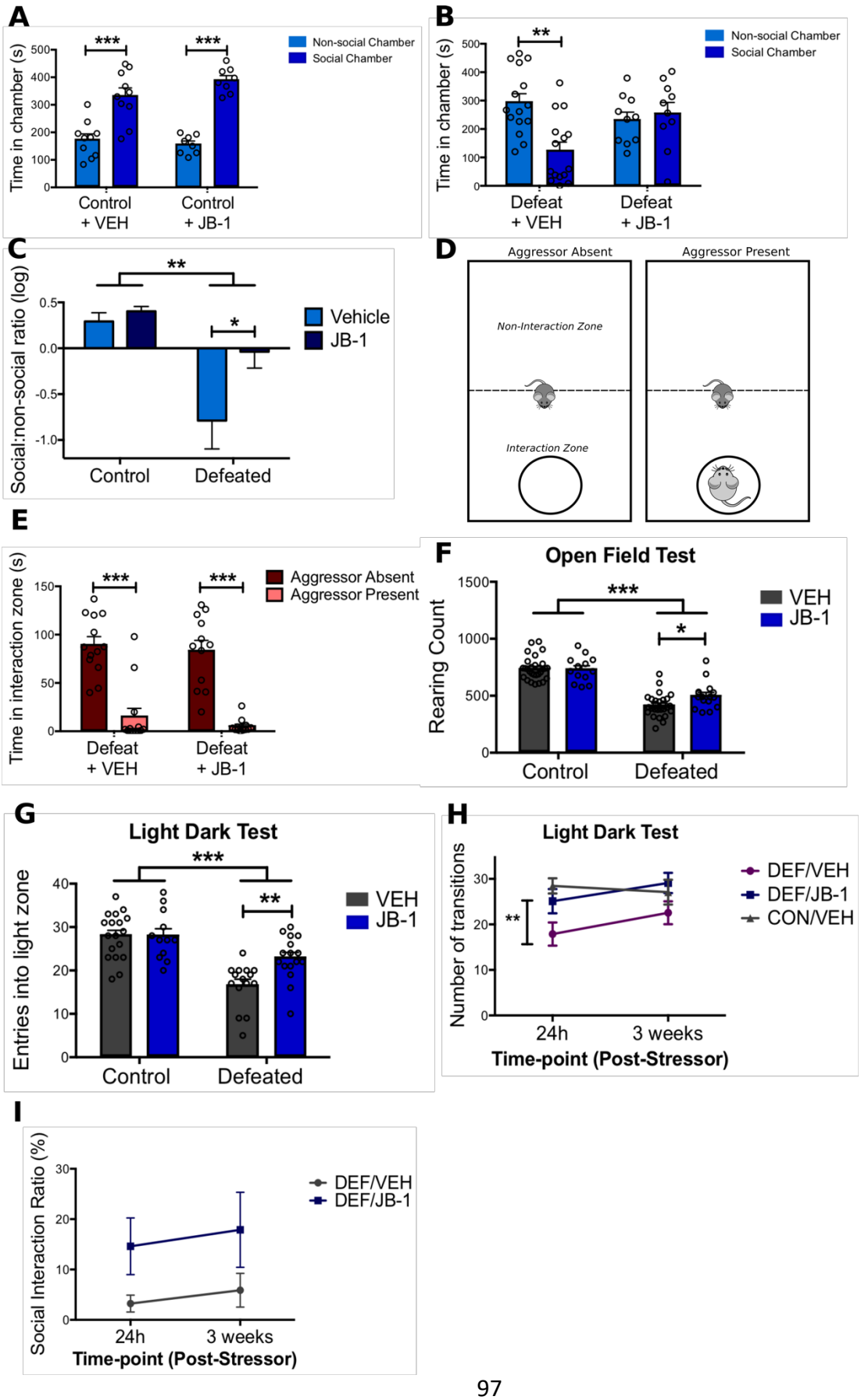

A
Time in chamber (s)
Non-social Chamber
Social Chamber
Control + VEH
Control + JB-1
B
Time in chamber (s)
Non-social Chamber
Social Chamber
Defeat + VEH
Defeat + JB-1
C
Social:non-social ratio (log)
Vehicle
JB-1
Control
Defeated
D
Aggressor Absent
Aggressor Present
Non-Interaction Zone
Interaction Zone
E
Time in interaction zone (s)
Aggressor Absent
Aggressor Present
Defeat + VEH
Defeat + JB-1
F
Open Field Test
Rearing Count
VEH
JB-1
Control
Defeated
G
Light Dark Test
Entries into light zone
VEH
JB-1
Control
Defeated
H
Light Dark Test
Number of transitions
DEF/VEH
DEF/JB-1
CON/VEH
24h
3 weeks
Time-point (Post-Stressor)
I
Social Interaction Ratio (%)
DEF/VEH
DEF/JB-1
24h
3 weeks
Time-point (Post-Stressor)

Figure 2. Chronic stress induces deficits in social and anxiety-like behaviours in mice that are partially corrected by microbial treatment. (A). Microbial treatment does not alter baseline sociability, measured by time spent in the social and non-social chambers, in vehicle-treated (n= 10) versus JB-1 treated (n= 8) unstressed mice. **(B).** Vehicle-treated defeated mice (n=15) exhibit avoidance of the social chamber—deficits that are corrected in defeated mice treated with JB-1 (n= 10). **(C).** Data demonstrating the time spent in the social and non-social chambers as a log ratio across all four groups. **(D).** Aggressor approach-avoidance test paradigm. **(E).** Socially defeated mice exhibit avoidance of a novel aggressor, independent of treatment. **(F).** Chronic stress reduced rearing behaviour on the OFT, but was partially rescued by JB-1 treatment (n, CON/VEH= 29, CON/JB-1= 13, DEF/VEH= 27, DEF/JB-1= 16). **(G).** Chronic stress reduced the number of entries into the light zone on the LD test, but was partially rescued by JB-1 treatment (n, CON/VEH= 18, CON/JB-1= 12, DEF/VEH= 15, DEF/JB-1= 16). **(H).** Anxiety-like behaviour across time, as measured by the number of entries into the light zone, at 24 hours and 3 weeks following cessation of CSD treatment (n, CON/VEH= 11, DEF/VEH= 9, DEF/JB-1= 9). **(I)** Avoidance behaviour on the aggressor approach-avoidance test, at 24 hours and 3 weeks following cessation of CSD treatment (n, DEF/VEH= 9, DEF/JB-1= 10).
* ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Data are represented as mean ± SEM.

4.3.2. Microbial treatment regulates stress-induced alterations in the immune phenotype

The immune system represents an important interface for bacteria-host signalling and has been hypothesized as a potential effector of gut-brain communication (Collins et al., 2012; Forsythe & Bienenstock, 2010). CSD increased the population of IL-10+ CD4+ CD25+ T cells (CD3+) ($F_{1, 16}$= 6.114, p = 0.025) (Fig. 3A). Microbial treatment alone similarly increased the population of these spleen-derived IL-10-expressing Tregs ($F_{1, 16}$= 5.621, p = 0.031). The immunomodulatory effects of JB-1 were not limited to adaptive immunity, as treatment also prevented the stress-induced increase in spleen-derived dendritic cells (MHCII+ CD11c+) expressing markers of activation, CD80 (stress exposure x treatment interaction [$F_{1, 15}$= 8.224, p = 0.012, post-hoc, $p < 0.05$]) and CD86, though the latter did not reach statistical significance (Fig. 3B; Fig. 3C).

This suggests that administration of JB-1 promoted systemic changes in the immunoregulatory phenotype and influenced the effects of chronic stress on host immunity.

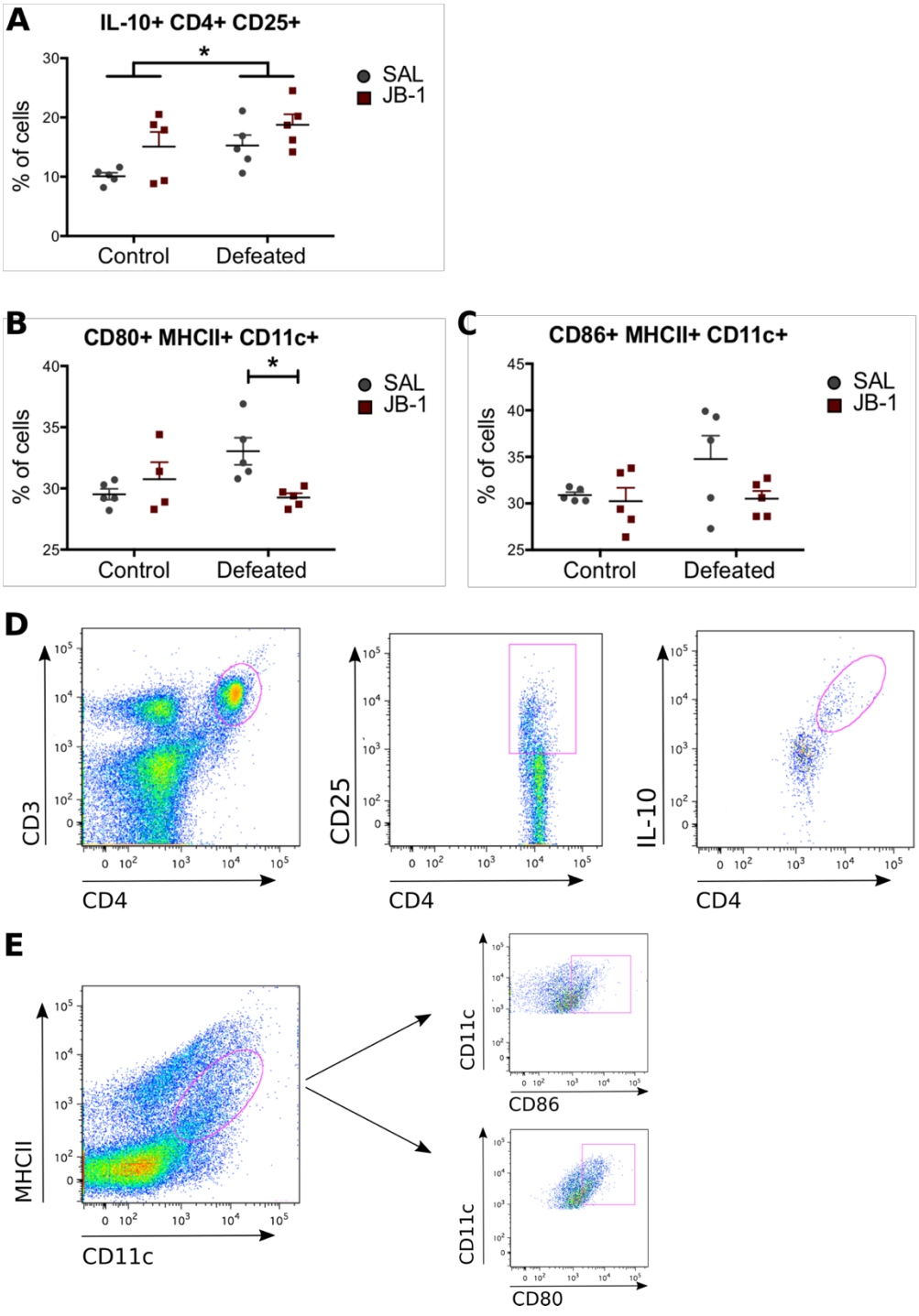


Figure 3. Effect of chronic social defeat stress and JB-1 treatment on splenocyte phenotype (n=5/group). **(A).** IL-10+ CD4+ CD25+ T cells in mice following exposure to chronic social defeat and JB-1 treatment. **(B).** JB-1 treatment prevents the stress-induced increase in CD80+ MHCII+ CD11c+ splenocyte levels in defeated mice **(C).** CD86+ MHCII+ CD11c+ splenocytes in mice following exposure to chronic social defeat and JB-1 treatment. **(D).** Fluorescence-activated cell sorting (FACS) gating strategy for IL-10+ CD4+ CD25+ T cells (CD3+). **(E).** FACS gating strategy for CD80+ and CD86+ on MHCII+ DCs (CD11c+).
* ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Data are represented as mean ± SEM.

4.3.3. Microbial treatment does not prevent stress-induced dysbiosis of the microbiota

Multiple groups have confirmed that stress induces dysbiosis (Bailey et al., 2011; Bendtsen et al., 2012; Bharwani et al., 2016), correction of which can impart positive effects on the host (Hsiao et al., 2013; Tarr et al., 2015). Thus, we investigated whether JB-1 exerted its neurobehavioural effects on the stressed host by restoring the microbiota.

Prior to social defeat, although significantly greater levels of *L. rhamnosus* cells per gram of faeces were detected in mice administered JB-1 (Fig. S1D), JB-1 did not significantly alter the overall profile of the microbial community (Fig. S1E) or post-defeat body weight across groups. As previously described (Bharwani et al., 2016), exposure to CSD reduced the diversity ($F_{1, 68}$= 13.21, p = 0.0005) and richness ($F_{1, 68}$= 12.50, p = 0.0007) of the microbiota (Fig. 4A). These alterations were not ameliorated in DEF/JB-1 mice (post-hoc, $p > 0.05$). Assessment of community richness did reveal a significant stress exposure x treatment interaction: CON/JB-1 mice had a richer gut microbiota relative to CON/VEH mice ($F_{1, 68}$= 5.616, p = 0.021, post-hoc, $p < 0.05$). However, there was no effect of treatment in the defeated groups (post-hoc, $p > 0.05$).

To compare group differences in the overall microbiota profile, Bray-Curtis distances (Fig. 4B) were analyzed using *a priori* planned comparisons. Stress altered the microbiota profile: distances within non-defeated mice were smaller than distances between defeated and non-defeated mice (Fig. 4C; Table S1; Bonferroni-corrected non-parametric $p = 0.013$). JB-1 treatment did not prevent stress-induced changes in the microbiota: profiles within a group (within-DEF/VEH and within-DEF/JB-1) were not significantly closer than to profiles from the opposing group (DEF/VEH vs DEF/JB-1), indicating a lack of clustering due to treatment (Bonferroni-corrected non-parametric $p > 0.05$). Moreover, JB-1- and vehicle-treated non-defeated mice formed a separate cluster from DEF/JB-1 (Bonferroni-corrected non-parametric $p = 0.013$).

We investigated whether microbial treatment restored the relative abundance of specific OTUs that discriminated defeated mice from the non-defeated controls. 18 OTUs (11 Bacteroidetes, 6 Firmicutes, 1 Proteobacteria) were altered by stress exposure ($q < 0.05$) (Table S2), none of which were restored by JB-1 treatment.

Alterations in the major microbial phyla—Firmicutes and Bacteroidetes—are associated with dysbiosis and disease (Clemente, Ursell, Parfrey, & Knight, 2012; Mariat et al., 2009; Sanderson et al., 2006; Thompson et al., 2015). However, there was no effect of treatment on the Bacteroidetes/Firmicutes ratio (Fig. 4D). Together, these data suggest that JB-1 treatment failed to prevent stress-induced alterations to the microbiota community.

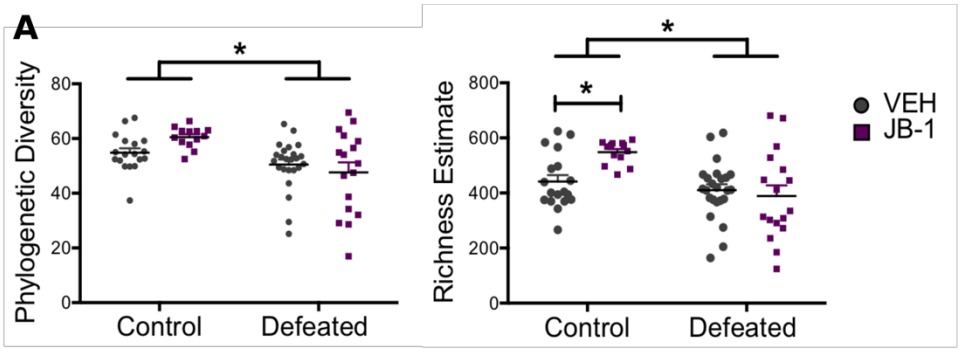
4.3.4. Stress induced dysbiosis persists for at least 3 weeks

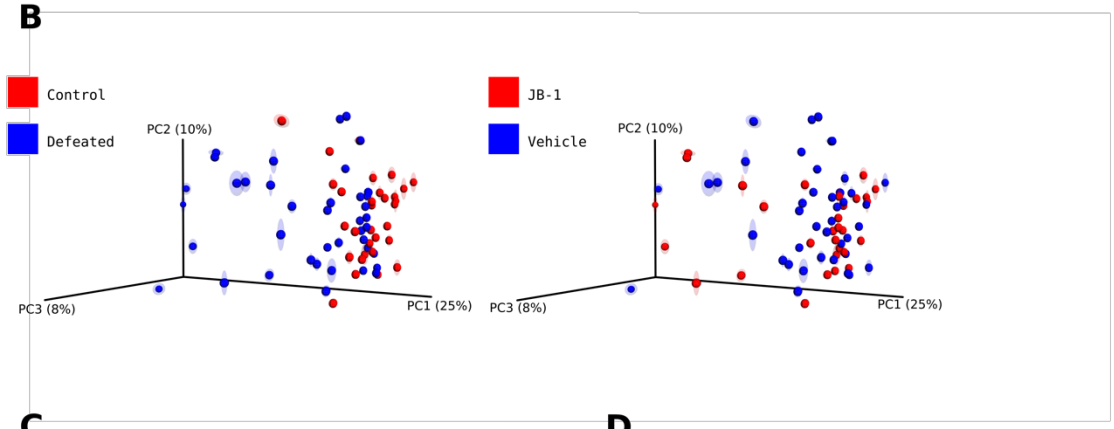
There is growing evidence from human reports indicating comorbidity between psychiatric conditions such as depression and post-traumatic stress disorder (PTSD)

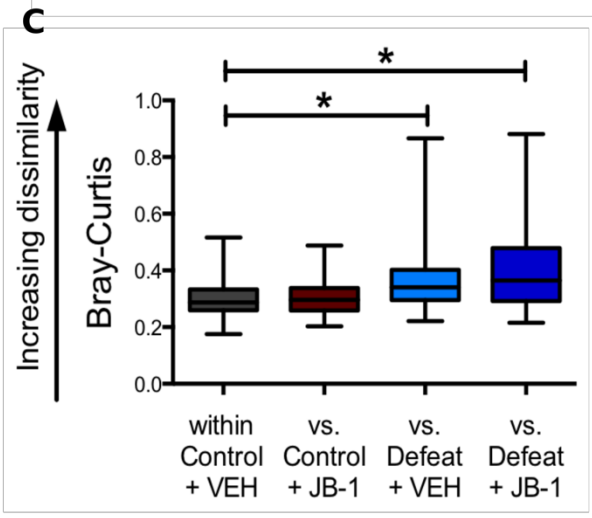
and gastrointestinal disorders, which are associated with persistent dysbiosis (Mayer, 2000). Thus, 3 weeks following the cessation of CSD, we examined the endurance of stress-induced microbial disruptions, and whether treatment facilitated recovery.

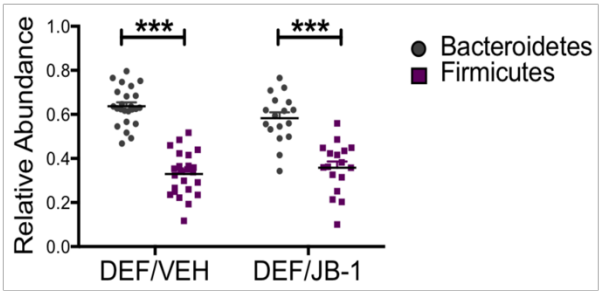
Group differences due to stress exposure were still evident at this time point (ANOSIM, R= 0.1307, p= 0.009). Within-group distances in CON/VEH were smaller than the distances versus DEF/VEH mice, indicating separation of CON/VEH and DEF/VEH groups due to stress exposure (Fig. 4E; Fig. S1F; Table S1 [Bonferroni-corrected non-parametric p= 0.022]). There was no statistically significant difference between vehicle- and JB-1-treated defeated mice.

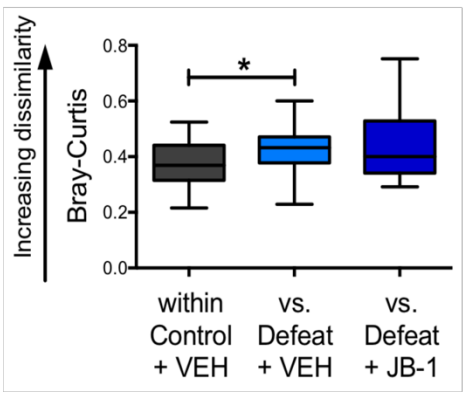
Similarly, comparison of community diversity and richness at the 3-week time point indicated differences only between CON/VEH and DEF/VEH groups (Fig. 4F, Phylogenetic diversity, $F_{2, 28}$= 7.893, p= 0.002; Chao1 richness, $F_{2, 28}$= 6.061, p= 0.007;). Thus, these data indicate that social defeat induced dysbiosis persisted for at least 3 weeks following stress exposure and the defeat-induced change in microbiome profile was not significantly altered by JB-1 treatment.


A
Phylogenetic Diversity
Control
Defeated
Richness Estimate
VEH
JB-1


B
Control
Defeated
PC2 (10%)
PC3 (8%)
PC1 (25%)
JB-1
Vehicle


C
Increasing dissimilarity
Bray-Curtis
within Control + VEH
vs. Control + JB-1
vs. Defeat + VEH
vs. Defeat + JB-1


D
Relative Abundance
DEF/VEH
DEF/JB-1
Bacteroidetes
Firmicutes


E
Increasing dissimilarity
Bray-Curtis
within Control + VEH
vs. Defeat + VEH
vs. Defeat + JB-1

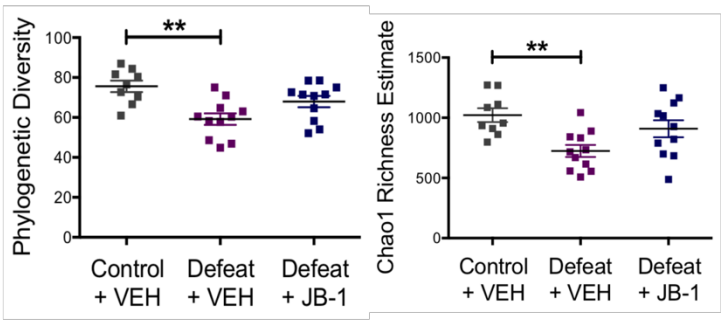

F
Phylogenetic Diversity
Chao1 Richness Estimate
Control + VEH
Defeat + VEH
Defeat + JB-1

Figure 4. JB-1 treatment does not affect stress-induced structural changes in the microbiota community. (A). Effect of chronic social defeat and JB-1 treatment on phylogenetic diversity and Chao1 richness estimates from the rarefied 16S rRNA data (n: CON/VEH= 18, CON/JB-1= 13, DEF/VEH= 24, DEF/JB-1= 17, 7923 reads/sample). **(B & C).** Principle coordinates analysis (PCoA) of Bray-Curtis distances from the average rarefied 16S rRNA data (n: CON/VEH= 18, CON/JB-1= 13, DEF/VEH= 24, DEF/JB-1= 17; n= 999 rarefactions, 6339 reads/sample) indicate a significant effect of social defeat on group clustering (p = 0.013), but no effect of JB-1 treatment (Median ± min/max). **(D).** Effect of chronic social defeat and JB-1 treatment on the taxonomic distribution of OTUs at the phylum level (n=17-24/group). **(E).** Bray-Curtis distances from the average rarefied 16S rRNA data (n= 999 rarefactions, 44,648 reads/sample) three weeks after stressor and treatment cessation indicate a persistent significant effect of social defeat on group clustering (p = 0.022), but no difference between the control group and defeated mice treated with JB-1 (Median ± min/max). **(F).** Phylogenetic diversity and Chao1 richness estimates from the rarefied 16S rRNA data (55,810 reads/sample) three weeks after stressor and treatment cessation indicate a persistent significant effect of social defeat, but no difference between the control group and defeated mice treated with JB-1 (n=9-11/group).
* (p < 0.05), ** (p <0.01), and *** (p < 0.001). Data are represented as mean ± SEM unless otherwise indicated.

4.3.5. The faecal metabolome is altered by exposure to chronic psychosocial stress and *L. rhamnosus* JB-1 treatment

Host and microbial metabolites play a contributory role in health and disease, including neural development and behaviour (Sharon et al., 2014). 621 metabolites were detected in the faeces of mice; 70 were significantly altered by stress exposure, (q < 0.1) many of which were associated with pathways previously predicted using *in silico* techniques: synthesis and metabolism of fatty acids, and tryptophan and tyrosine metabolism (Table S3) (Bharwani et al., 2016). Furthermore, 75 faecal metabolites were regulated by JB-1 treatment (Table S4).

Previous work has demonstrated that JB-1 signals the brain via the vagus (Bravo et al., 2011; Perez-Burgos et al., 2014, 2013). To investigate signals that play a role in

JB-1-driven vagal signalling, we explored for metabolites that were elevated exclusively in JB-1-treated stressed mice, however no such metabolites were detected ($q < 0.1$).

We investigated functional pathways that were altered by exposure to CSD, but not in JB-1-treated mice. This criterion ($q < 0.1$) yielded 15 metabolites (Fig. 5A; Fig. 5B; Table S5), including 1-methylnicotinamide—a vitamin B3-derivative with anti-inflammatory effects (Gębicki et al., 2003) and 4-hydroxybutryrate (GHB), a metabolite with neurotransmitter-like effects (Castelli et al., 2003; Gobaille et al., 2002; Maitre, Humbert, Kemmel, Aunis, & Andriamampandry, 2005). Other metabolites meeting this criterion include glutarate, N-acetylcitrulline, glycerate, lactobionate, 3-hydroxybutyrylcarnitine, 10-hydroxystearate, multiple metabolites derived from sphingolipid metabolism, alpha-muricholate, and lithocholate. However only for one metabolite that met this criteria, tyramine, a monoamine with sympathomimetic properties (Mundorf, Hochstetler, & Wightman, 1999; Schönfeld & Trendelenburg, 1989) did the difference between DEF/VEH and DEF/JB-1 reach statistical significance (Fig. 5C).

Stress increased the levels of kynurenine in both groups of defeated mice ($F_{1, 31}$= 5.839, $p = 0.022$) (Fig. 5D). Planned post-hoc analysis revealed significant differences between vehicle-treated defeated and control groups ($p < 0.05$), but none between JB-1-treated control and defeated mice ($p > 0.05$). Neither stress nor microbial treatment affected the kynurenine/tryptophan ratio—a sensitive estimate of cellular immunity (Fuchs et al., 1990; Werner-Felmayer et al., 1989).

These data demonstrate that CSD alters the levels of various faecal metabolites, some of which possess immunomodulatory and neuroactive properties, and that JB-1 treatment may modulate some of these changes.

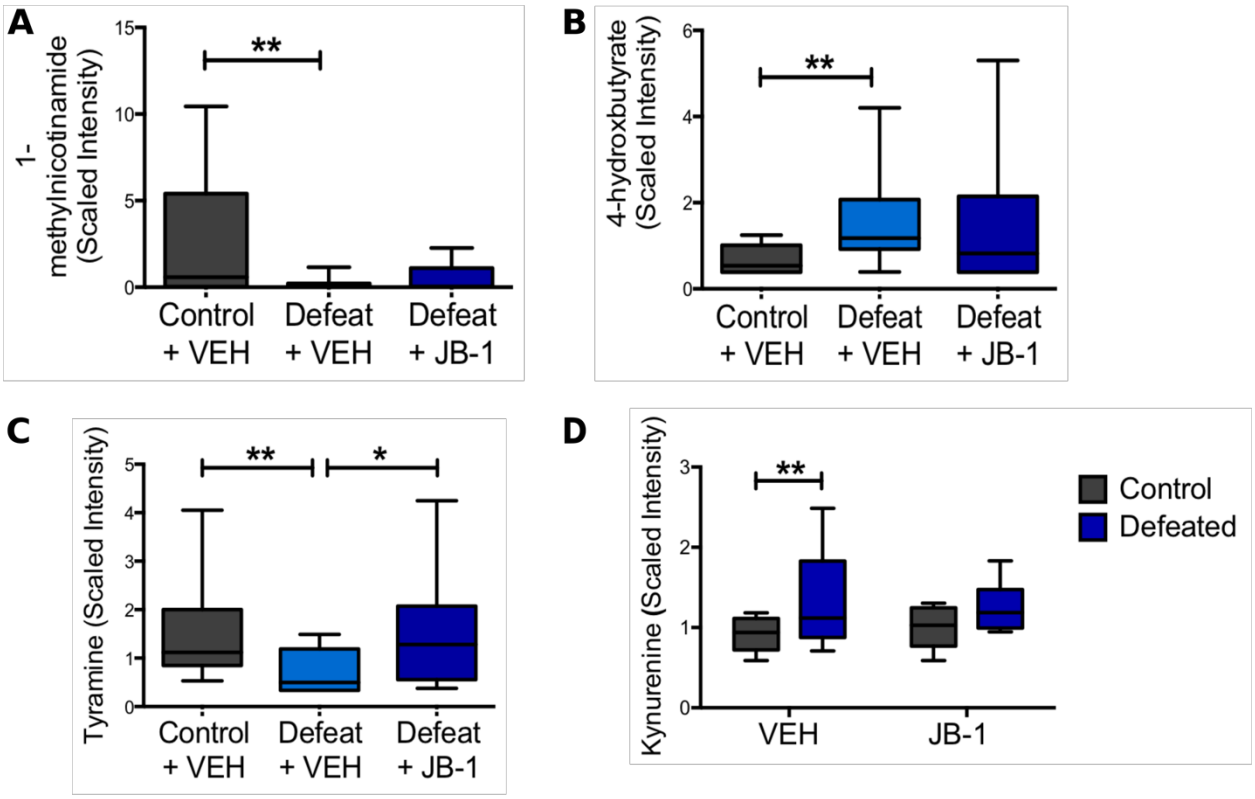


Figure 5. Effect of chronic stress and JB-1 treatment on the faecal metabolome. (A-D). Metabolites whose levels were altered by chronic stress but prevented in JB-1 treated mice (n: CON/VEH= 10, CON/JB-1= 5, DEF/VEH= 10, DEF/JB-1= 10). * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Data are represented as median ± min/max.

4.4. Discussion

Using a validated model of chronic stress and depression (Berton, McClung, et al., 2006; Krishnan et al., 2007), we demonstrate, for the first time, the influence of a single orally-administered bacteria strain, *Lactobacillus rhamnosus* JB-1, on behavioural

deficits and systemic immune alterations caused by chronic exposure to a psychosocial stressor. While we observed no effects on baseline behaviour, JB-1 attenuated stress-induced behavioural deficits, including changes in sociability and anxiety-like behaviour, and prevented immunoregulatory alterations associated with the stress phenotype. Notably, the tempering of stress-induced changes occurred in the absence of any effects of treatment on stress-related disruptions in the microbiota, suggesting JB-1 directly modulates gut-brain signalling pathways independently of the microbial community.

Following CSD, JB-1 treated stressed mice, as opposed to vehicle-treated, did not show avoidance of novel social stimuli, exhibited more frequent rearing behaviour in the OFT, and showed reduced aversion towards the light chamber (LD test). These data support the emerging literature suggesting that administration of specific bacterial strains decreases anxiety- and depressive-like behaviours (Bercik, Denou, et al., 2011; Bravo et al., 2011). Indeed, we have previously demonstrated that chronic administration of JB-1 in Balb/c mice altered baseline levels of anxiety-like behaviour (Bravo et al., 2011). That the effects of JB-1 here were limited to deficits produced by chronic stress and not baseline behaviour (Fig. 2), may be indicative of intrinsic differences between Balb/c and C57BL/6 mice, the latter of which exhibit reduced apprehension, neophobia, and anxiety-like behaviour on baseline behavioural assays (Ramos & Mormède, 1997). These mouse strain-specific effects may have implications for translational studies in humans suggesting that, in keeping with a recent report (Kelly et al., 2016), JB-1 would not be expected to have an anxiolytic effect in non-anxious

individuals. Similarly anti-depressants have very limited effects on healthy subjects (Serretti et al., 2010).

In models of psychiatric conditions, repeated aggression and defeat leads to persistent conditioned submissive behaviour and aversion towards social stimuli (Berton, McClung, et al., 2006; Huhman et al., 2003). These behavioural manifestations bear similarity to symptoms of social withdrawal in depression and phobic avoidance of trauma-related stimuli in PTSD (American Psychiatric Association, 2013). It is notable that the ameliorating effects of JB-1 on deficits in social behaviour were limited to interactions involving a non-threatening conspecific, while avoidance of the novel trauma-related stimulus was maintained. Previous research has suggested dissociation of social and non-social forms of anxiety-like behaviour (Liu & Smith, 2009). For instance, treatment with a human commensal organism, *Bacteroides fragilis*, in a model of autism spectrum disorder attenuated deficits in anxiety-like behaviour, but did not affect sociability (Hsiao et al., 2013). Our findings suggest that social anxiety may be further dissociated into discrete, differentially modulated behaviours expressed towards non-threatening versus threatening stimuli—the latter of which is experience-dependent (Berton, McClung, et al., 2006; Krishnan et al., 2007). Thus, the disparate effects of JB-1 on behaviours expressed by defeated mice may be due to independent underlying neural circuitry. Such dissociable circuitry has been indicated by work on the stimulation of nucleus accumbens afferents, which alters behaviour towards a novel aggressor, but not anxiety-like behaviour (Bagot et al., 2015). This concept is further supported and emphasized in the current study given the recovery of anxiety-like behaviour but not of aggressor avoidance behaviour three weeks post-defeat (Fig. 2H; Fig. 2I).

Consistent with the immunomodulatory role of gut bacteria (Forsythe & Bienenstock, 2010) and previous studies with JB-1 (Karimi et al., 2009), microbial treatment influenced systemic changes in the CSD-induced immune phenotype . Social defeat increased the population of activated splenic DCs—a shift completely prevented by JB-1. Furthermore, treatment with the bacteria-induced systemic expansion of Treg: a population that produces high levels of the anti-inflammatory cytokine, IL-10 (Groux et al., 1997). Coordination between multiple host systems—and dysregulation thereof—likely contributes to the phenotypic changes in stress and related psychiatric conditions, during which systemic disruptions and allostatic load accumulate over extended periods of time. For instance, a pro-inflammatory milieu and a decrease in Tregs are commonly observed in severe stress and PTSD (Lindqvist et al., 2014; Sommershof et al., 2009), and form the central premise of the inflammation theory of depression (Dantzer et al., 2008). Indeed, stress-induced trafficking of peripheral monocytes to the brain appears to play a crucial role in anxiety-like behaviour (Wohleb, Powell, Godbout, & Sheridan, 2013). Disruption of the host-microbiota relationship during chronic stress may contribute to exaggerated inflammation and immune dysregulation, and is associated with colitis and inflammatory bowel disease (Cámara, Gander, Begré, Von Känel, & Group, 2011; Reber et al., 2016). The observed acute increase in the Treg population (Fig. 3A) (Bharwani et al., 2016) following stress, may be a counteractive response to the pro-inflammatory shifts described in the literature upon stress induction (Bharwani et al., 2016; Wohleb, McKim, Sheridan, & Godbout, 2015; Wohleb et al., 2013)—such responses are a well-documented reaction to host inflammation in an attempt to restore homeostasis (O'Garra, Vieira, Vieira, & Goldfeld, 2004). Although this natural allostatic

mechanism does not prevent an inflammatory environment during maladaptive stress, JB-1-induced modulation of host-initiated immunoregulatory responses may be one mechanism contributing to the behavioural effects of the bacteria. Similar mechanisms were posited to explain the stress-mitigating effects of *Mycobacterium vaccae* immunization, which were demonstrated to depend on Tregs (Reber et al., 2016). These data suggest that recruitment of immune pathways in bottom-up (gut-to-brain) signalling is important, highlighting the need for a both a broader and more detailed examination of microbiota-immune-neural coordination and dysregulation of these systems in stress.

It has been proposed widely that modifying the resident intestinal bacteria in disease can reverse microbial dysbiosis and restore homeostatic function (Bruce-Keller et al., 2014; Buffington et al., 2016). Such an approach is especially relevant given evidence of microbiota disruption in severe stress and psychiatric conditions, and its association with adverse gastrointestinal outcomes (Mayer, 2000). Thus, we investigated whether the improved neurobehavioural phenotype due to microbial treatment was associated with alterations to the existing microbiota community. Prior to stress exposure, administration of JB-1 did not alter the profile of the microbiota—data that parallel observations in humans who were administered a different strain of *L. rhamnosus* (Eloe-fadrosh et al., 2015). Furthermore, in our study, microbial treatment did not prevent any of the shifts in the microbiota community due to stress exposure. JB-1 treatment also completely failed to restore the diversity and richness of the microbiota, or correct the relative abundances of specific OTUs altered by stress. Thus, the neuroactive properties of the beneficial microbe may be mediated independently of

restoring microbial community balance, and might be dependent on its functional activity and direct modulation of host signalling pathways. Not unexpectedly, the stress-induced dysbiosis was accompanied by a significant change in levels of various faecal metabolites while, perhaps more surprisingly, JB-1 treatment alone significantly modulated the levels of 75 metabolites, many of which have immunomodulatory and neuroactive properties. While the source of these metabolites, host or microbe, cannot be identified, these observations suggest that JB-1 could alter the function of the existing gut microbiota without influencing composition. Most notably, the reduction in tyramine levels induced by CSD was the only metabolite change significantly inhibited by JB-1 treatment. Tyramine is a monoaminergic neuromodulator, acting as an agonist for trace amine-associated receptor 1 (TAAR1) (Zucchi, Chiellini, Scanlan, & Grandy, 2006). Tyramine also causes the release of norepinephrine from sympathetic nerves, reversing re-uptake through the norepinephrine transporter and has been demonstrated to induce serotonin (5-HT) production by enterochromaffin cells (Kidd et al., 2008). Given that intestinal 5-HT (Yano et al., 2015) and catecholamines (Patterson et al., 2014) have been proposed as mediators of microbe-gut-brain signalling via modulation of the enteric nervous system, the impact of luminal tyramine levels on the gut-brain axis may warrant further investigation.

One limitation of the current study is that we only assessed the faecal microbiota and it is possible that JB-1 stabilized site-specific microbiota, for example in the small intestine or specifically associated with the epithelium elsewhere, that are involved in gut-brain signalling. However, a direct action of JB-1 on gut-brain signalling is further supported by previous studies using *in vivo* and *ex vivo* models that demonstrate it can

directly or indirectly activate the vagus nerve, and that an intact vagus is required to mediate the effects of this bacteria at least on baseline behaviour of Balb/c mice (Perez-Burgos et al., 2013). Collectively, these data suggest that JB-1, independently of changes in the microbiota, can recruit host signalling pathways such as vagal afferents, which mediate the effects of the bacteria on severe CSD-induced neurobehavioural changes.

Although numerous studies have demonstrated the effect of environmental adversity on disruption of gut microbiota (Bailey et al., 2011; Bharwani et al., 2016; Reber et al., 2016), there is very little evidence on the permanence of these changes in stress-related disorders, or whether microbial supplementation can facilitate the recovery of dysbiosis. A limited number of observations suggest a complex relationship between environmental factors and perturbations of the gut microbiota. Certain factors impart transient changes in the community while others, for instance antibiotic usage, leave behind a more persistent signature (David et al., 2014; Korpela et al., 2016). Furthermore, factors such as birth delivery mode have marked effects on the microbiota community during early-life that are no longer distinguishable in adulthood (Zhernakova et al., 2016). Our own observations suggest that stress-induced disruptions in the microbiota appear stable for a prolonged period following stress exposure. Examination of defeated mice three weeks following CSD revealed enduring structural changes in the faecal microbial community: defeated mice continued to show reduced diversity and richness in the variety of species represented, while exhibiting broad-scale changes in overall composition and profile. These long-term, stress-induced changes in the microbiome were not significantly altered with JB-1 treatment.

There have been increasing efforts to understand how large-scale disruptions and dynamic shifts in gut microbiota can drive phenotypic changes and disease states (Gilbert et al., 2016). This study represents a series of findings that further clarify the role of gut bacteria on neural function and behaviour. Although there continue to exist major gaps in our understanding of how disruptions in the microbiota contribute to neuropsychiatric conditions, the emerging theme underscores the intricate interactions between these systems in health and disease. Despite the complexity of the observed structural and functional changes in the microbial consortia, our data indicate that restoration of homeostasis can be facilitated using a single microbial strain. Given the diversity and inter-individual variability of the human gut microbiome (Costello et al., 2009; Eckburg et al., 2005; Lay et al., 2005), we propose that microbial-based interventions that bypass the microbiota to directly affect the host may possess greater therapeutic potential for the effective treatment of psychiatric conditions, or as an adjunct to current approaches.

Declarations

Ethics approval

All experiments followed Canadian Council on Animal Care guidelines and were approved by the McMaster Animal Research Ethics Board.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors report no biomedical financial interests or potential conflicts of interest. The sponsors had no role in study design; the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Funding

This research was funded by a grant from the Office of Naval Research (#N00014-14-1-0787). M.G.S. is supported as a Canada Research Chair.

Author's contributions

P.F., J.B., & A.B. conceptualized the study and designed the experiments. A.B. performed all animal experiments. A.B. and M.F.M prepared the samples and carried out FACS analysis. M.G.S. performed 16S rRNA DNA sequencing. A.B. acquired and analyzed the data, and wrote the initial draft of the manuscript. A.B., M.F.M., M.G.S., J.B., and P.F. contributed to data interpretation, P.F. revised the manuscript. All authors approved the final version of this article.

Acknowledgments

The authors would like to gratefully acknowledge Alimentary Health for their generous gift of *Lactobacillus rhamnosus* JB-1 bacteria, and Dr. Andrew Stanisz at the McMaster Brain-Body Institute for the preparation of bacterial treatments. The authors would also like to acknowledge Prof. Laure Bindels at the Université catholique de Louvain, Brussels, Belgium for the estimation of faecal content of *L. rhamnosus*.

4.5. Supplementary Figures & Tables

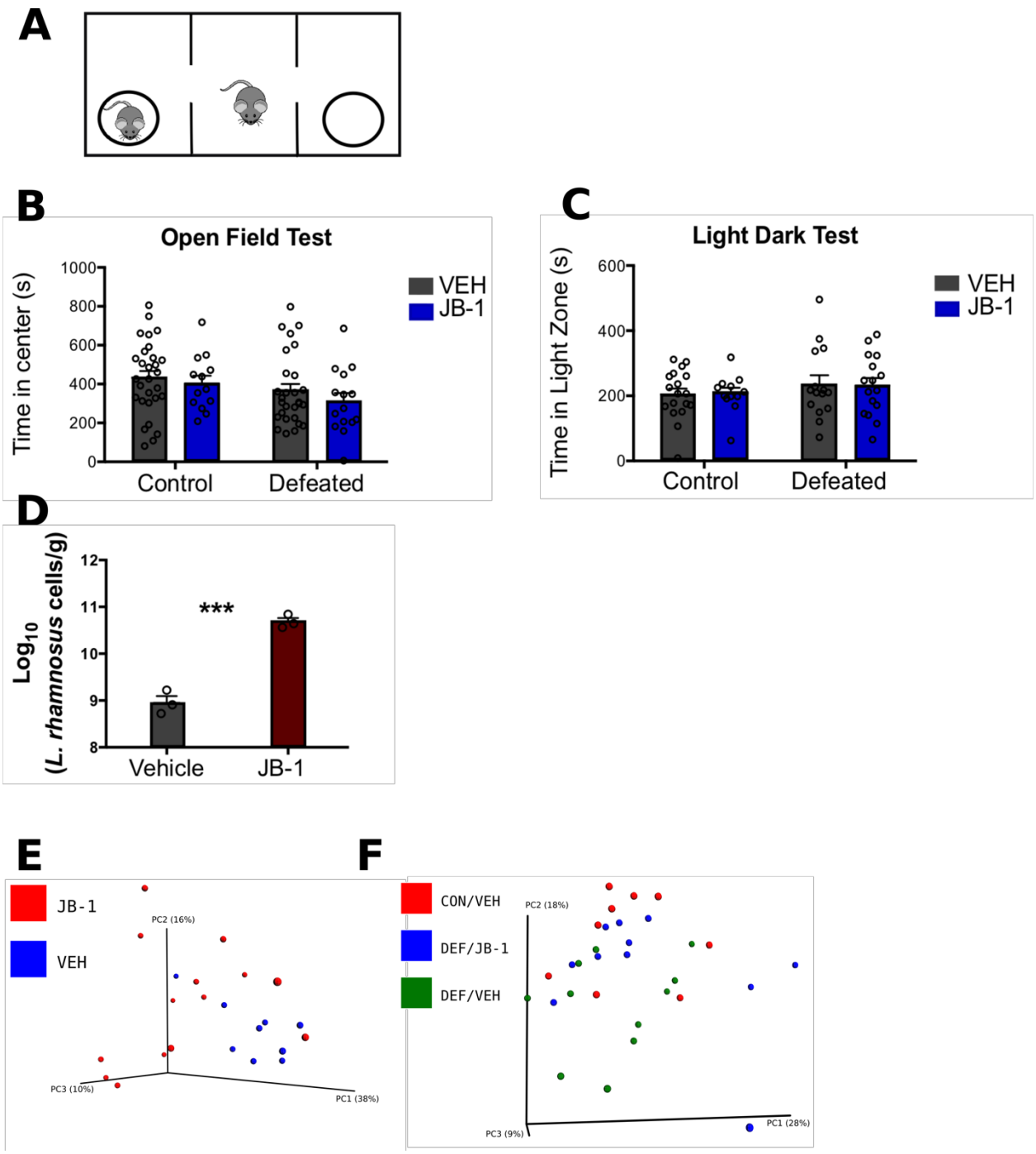


Figure S1. (A). Three-chamber sociability test paradigm. **(B & C).** Effect of chronic social defeat stress and JB-1 treatment on time spent in the center of the OFT and in the light chamber of the LD test. **(D).** Effect of JB-1 administration on detected levels of *Lactobacillus rhamnosus* cells in faecal samples. **(E).** Principle coordinates plots (PCoA)

of Bray-Curtis distances from the average rarefied 16S rRNA data (n= 999 rarefactions, 52,182 reads/sample) indicates no effect of JB-1 treatment after 18 days, prior to initiation of chronic social defeat stress. **(F).** Related to Fig. 4E: principle coordinates plots (PCoA) of Bray-Curtis distances from the average rarefied 16S rRNA data (n= 999 rarefactions, 44,648 reads/sample) three weeks after stressor and treatment cessation indicate a persistent significant effect of social defeat on group clustering (p = 0.022), but no difference between control and DEF/JB-1 groups.

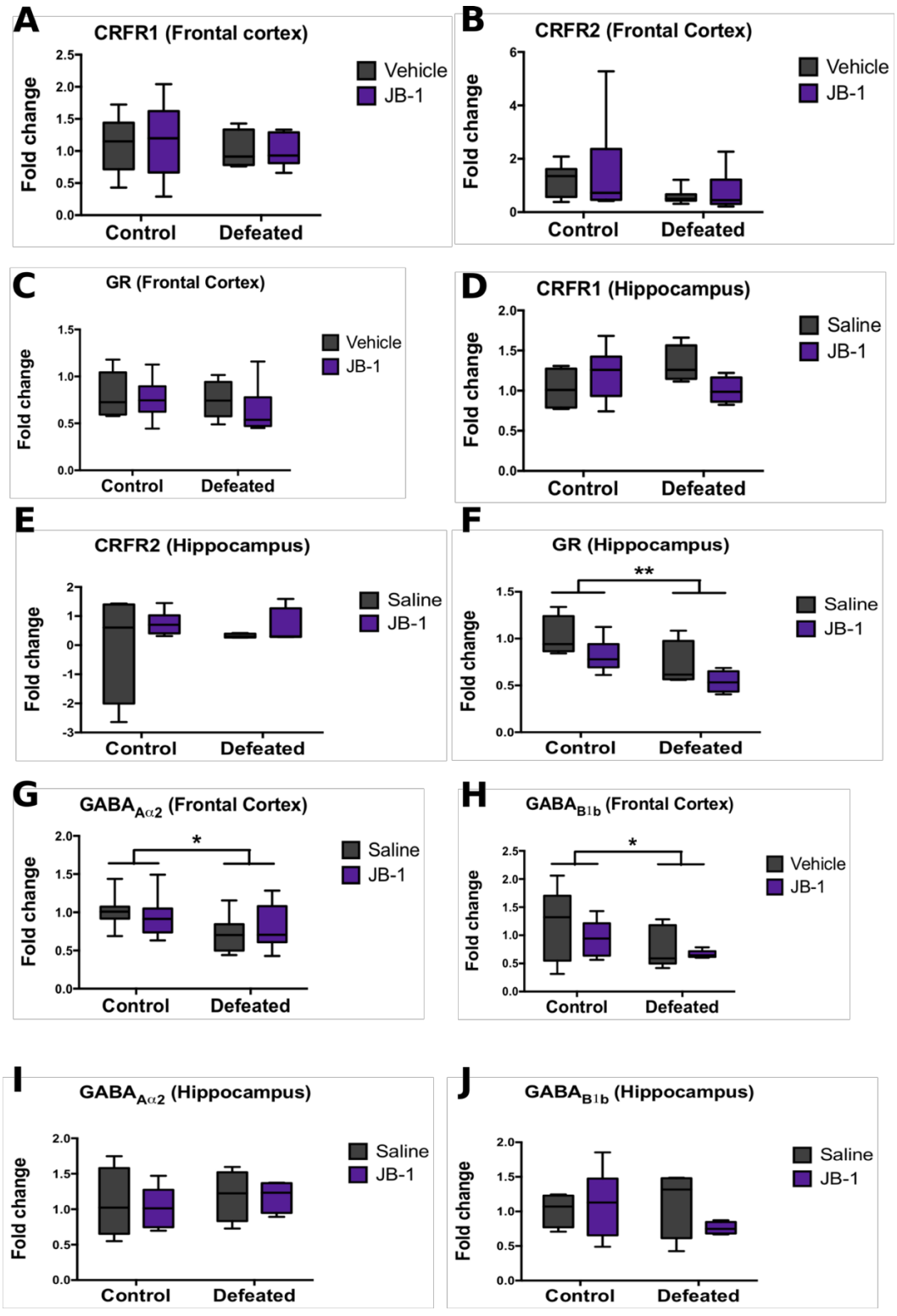


Figure S2. (A-J). Effect of chronic social defeat stress and JB-1 treatment on gene expression levels in the frontal cortex (n= 5-13/group) and the hippocampus (n= 4-8/group).

Table S1. Statistical comparisons of Bray-Curtis distances between *a priori* groupings. Related to Figure 4.

24 hours following CSD cessation:		
Intra-group Distance	**Inter-group Distance**	**Non-parametric *p*-value (Bonferroni-corrected)**
CON/VEH vs. CON/VEH	CON/VEH vs.DEF/VEH	0.013
CON/VEH vs. CON/VEH	CON/VEH vs. CON/JB-1	$p > 0.05$
CON/VEH vs. CON/VEH	CON/VEH vs. DEF/JB-1	0.013
DEF/VEH vs. DEF/VEH	DEF/VEH vs. DEF/JB-1	$p > 0.05$
CON/JB-1 vs. CON/JB-1	CON/JB-1 vs. DEF/JB-1	0.013
3 weeks following CSD cessation:		
Intra-group Distance	**Inter-group Distance**	**Non-parametric *p*-value (Bonferroni-corrected)**
CON/VEH vs. CON/VEH	CON/VEH vs.DEF/VEH	0.022
CON/VEH vs. CON/VEH	CON/VEH vs. DEF/JB-1	$p > 0.05$
DEF/VEH vs. DEF/VEH	DEF/VEH vs. DEF/JB-1	$p > 0.05$
DEF/JB-1 vs. DEF/JB-1	DEF/JB-1 vs. DEF/VEH	$p > 0.05$
CON/VEH vs. DEF/JB-1	CON/VEH vs. DEF/VEH	$p > 0.05$

Jackknife resampling was used to generate Bray-Curtis distances. (Dis)similarity between the microbiota profiles was assessed by comparing intra-group distances to inter-group distances using the Monte Carlo Permutation Procedure (MCPP) (999 permutations) and Bonferroni-corrected non-parametric t-tests. Null hypothesis: intra-group distances = inter-group distances. See Experimental Procedures for additional details.

Table S2. Discriminatory OTUs between undefeated saline- and JB-1-treated controls, and saline- and JB-1-treated defeated mice. Related to Figure 4.

OTU	Kruskal-Wallis Test Statistic	p-value	q-value (FDR)	Taxonomic Assignment
400	28.8534939 3	2.40E-06	0.00046 9867	Bacteroidetes|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
309	27.4775385 3	4.67E-06	0.00046 9867	Bacteroidetes|Bacteroidia|Bacteroidales
106	27.4252369 4	4.79E-06	0.00046 9867	Bacteroidetes|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
61	25.6310118 5	1.14E-05	0.00083 7442	Bacteroidetes|Bacteroidia|Bacteroidales
4	24.2872138 8	2.18E-05	0.00127 9363	Bacteroidetes|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
119	23.1167827 7	3.82E-05	0.00185 4143	Bacteroidetes|Bacteroidia|Bacteroidales|Prevotellaceae|Prevotella
43	22.8143257 7	4.41E-05	0.00185 4143	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Ruminococcus
17	18.9638237 9	0.00027 8148	0.01022 1949	Bacteroidetes|Bacteroidia|Bacteroidales
20	18.5818429 5	0.00033 359	0.01089 7268	Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus
29	17.3075541 1	0.00061 0914	0.01711 8763	Bacteroidetes|Bacteroidia|Bacteroidales
171	17.2077694 7	0.00064 0498	0.01711 8763	Firmicutes|Clostridia|Clostridiales
87	16.8316538 1	0.00076 5376	0.01875 1715	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Ruminococcus

337	16.4105473 3	0.000934073	0.020911138	Bacteroidetes|Bacteroidia|Bacteroidales
89	16.2752110 5	0.000995768	0.020911138	Proteobacteria|Alphaproteobacteria
18	16.0132721 3	0.001126901	0.021672506	Bacteroidetes|Bacteroidia|Bacteroidales
45	15.9167042 3	0.001179456	0.021672506	Bacteroidetes|Bacteroidia|Bacteroidales
63	15.1443327 3	0.00169737	0.029354512	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Oscillospira
222	14.3605027 5	0.002453338	0.040071193	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Clostridium

Discriminatory OTUs between the groups in the Kruskal-Wallis analysis (FDR <0.05). Green indicates a main effect of stress only. Blue indicates an additional main effect of treatment as well as a significant different between saline- and JB-1 treated unstressed animals in post-hoc analysis (*p*< 0.05). Post-hoc analysis on each of the discriminatory OTUs revealed a main effect of stress (*p* <0.05) but no significant differences between saline- and JB-1-treated defeated animals (*p*> 0.05).

Table S3. Faecal metabolites altered in response to stress (DEF/VEH versus CON/VEH). Related to Figure 5.

Super Pathway	**Sub-pathway**	**Metabolite**	**Fold Change**	***p*-value**	***q*-value**
Amino Acid	Glutamate Metabolism	Pyroglutamine	0.28	0.0081	0.0554
Amino Acid	Histidine Metabolism	1-methylhistidine	0.07	0.0002	0.0057
Amino Acid	Lysine Metabolism	Glutarate (pentanedioate)	1.94	0.004	0.0383
Amino Acid	Lysine Metabolism	N2,N6-diacetyllysine	0.47	0.0043	0.0392
Amino Acid	Phenylalanine and Tyrosine Metabolism	Phenylacetylglycine	0.07	0.0000	0.0029

Amino Acid	Phenylalanine and Tyrosine Metabolism	4-hydroxycinnamate	0.65	0.0022	0.0310
Amino Acid	Phenylalanine and Tyrosine Metabolism	Tyramine	0.49	0.0099	0.0631
Amino Acid	Phenylalanine and Tyrosine Metabolism	Phenol Sulfate	0.1	0.0048	0.0400
Amino Acid	Phenylalanine and Tyrosine Metabolism	p-cresol sulfate	0.29	0.0024	0.0315
Amino Acid	Phenylalanine and Tyrosine Metabolism	Phenylpropionylglycine	0.06	0.0000	0.0029
Amino Acid	Phenylalanine and Tyrosine Metabolism	3-(3-hydroxyphenyl)propionate sulfate	0.16	0.0105	0.0655
Amino Acid	Phenylalanine and Tyrosine Metabolism	2-hydroxyphenylacetate	0.34	0.0047	0.0399
Amino Acid	Phenylalanine and Tyrosine Metabolism	N-formylphenylalanine	1.99	0.0000	0.0034
Amino Acid	Tryptophan Metabolism	3-indoxyl sulfate	0.09	0.0001	0.0049
Amino Acid	Tryptophan Metabolism	Kynurenine	1.48	0.0034	0.0364
Amino Acid	Tryptophan Metabolism	Xanthurenate	0.23	0.0146	0.0806
Amino Acid	Tryptophan Metabolism	5-hydroxyindoleacetate	0.48	0.0121	0.0694
Amino Acid	Leucine, Isoleucine and Valine Metabolism	isovalerylglycine	0.07	0.0001	0.0041
Amino Acid	Leucine, Isoleucine and Valine Metabolism	3-methylcrotonylglycine	0.08	0.0001	0.0034
Amino Acid	Leucine, Isoleucine and Valine Metabolism	beta-hydroxyisovaleroylcarnitine	0.53	0.0109	0.0672
Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	N-acetylcysteine	0.27	0.0081	0.0554
Amino Acid	Urea cycle; Arginine and Proline Metabolism	Urea	0.11	0.0040	0.0383
Amino Acid	Urea cycle; Arginine and Proline Metabolism	N-acetylcitrulline	0.19	0.0122	0.0694
Amino Acid	Creatine Metabolism	Guanidinoacetate	0.13	0.0119	0.0694
Amino Acid	Guanidino and Acetamido Metabolism	Guanidinosuccinate	0.18	0.0032	0.0356

Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	glycerate	1.49	0.0112	0.0676
Carbohydrate	Disaccharides and Oligosaccharides	Lactobionate	4.13	0.0078	0.0554
Lipid	Fatty Acid Metabolism (BCAA Metabolism)	butyrylglycine	0.09	0.0027	0.0325
Lipid	Fatty Acid Metabolism (BCAA Metabolism)	propionylglycine	0.12	0.0052	0.0420
Lipid	Fatty Acid Metabolism(Acyl Glycine)	valerylglycine	0.14	0.0010	0.0177
Lipid	Fatty Acid Metabolism(Acyl Glycine)	hexanoylglycine	0.02	0.0000	0.0029
Lipid	Fatty Acid Metabolism(Acyl Carnitine)	3-hydroxybutyrylcarnitine (2)	0.45	0.0089	0.0588
Lipid	Fatty Acid Metabolism(Acyl Carnitine)	hexanoylcarnitine	0.59	0.0060	0.0476
Lipid	Carnitine Metabolism	carnitine	0.25	0.0037	0.0382
Lipid	Fatty Acid, Monohydroxy	4-hydroxybutyrate (GHB)	2.37	0.0075	0.0548
Lipid	Fatty Acid, Monohydroxy	10-hydroxystearate	0.54	0.0169	0.0919
Lipid	Phospholipid Metabolism	glycerophosphorylcholine (GPC)	2.41	0.0073	0.0548
Lipid	Phospholipid Metabolism	trimethylamine N-oxide	0.35	0.0079	0.0554
Lipid	Phospholipid Metabolism	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	2.70	0.0003	0.0082
Lipid	Phospholipid Metabolism	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	1.84	0.0120	0.0694
Lipid	Lysolipid	2-stearoyl-GPE (18:0)	1.96	0.0047	0.0399
Lipid	Monoacylglycerol	2-palmitoylglycerol (16:0)	1.47	0.0083	0.0558
Lipid	Monoacylglycerol	1-linolenoylglycerol (18:3)	1.74	0.0011	0.0177
Lipid	Monoacylglycerol	1-docosahexaenoylglycerol (22:6)	1.44	0.0043	0.0392

Lipid	Sphingolipid Metabolism	stearoyl sphingomyelin (d18:1/18:0)	2.08	0.0010	0.0174
Lipid	Sphingolipid Metabolism	sphingomyelin (d18:1/24:1, d18:2/24:0)*	2.75	0.0008	0.0168
Lipid	Sphingolipid Metabolism	behenoyl sphingomyelin (d18:1/22:0)	2.34	0.0040	0.0383
Lipid	Sphingolipid Metabolism	sphingomyelin (d18:1/20:0, d16:1/22:0)*	3.45	0.0002	0.0061
Lipid	Primary Bile Acid Metabolism	alpha-muricholate	0.50	0.0131	0.0735
Lipid	Primary Bile Acid Metabolism	tauro-beta-muricholate	2.85	0.0075	0.0548
Lipid	Primary Bile Acid Metabolism	cholate sulfate	0.42	0.0003	0.0082
Lipid	Secondary Bile Acid Metabolism	deoxycholate	0.39	0.0072	0.0548
Lipid	Secondary Bile Acid Metabolism	lithocholate	0.50	0.0011	0.0181
Lipid	Secondary Bile Acid Metabolism	hyodeoxycholate	0.49	0.0098	0.0631
Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	allantoin	0.11	0.0003	0.0082
Nucleotide	Pyrimidine Metabolism, Uracil containing	3-ureidopropionate	0.16	0.0021	0.0310
Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	quinolinate	0.66	0.0025	0.0315
Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	1-methylnicotinamide	0.08	0.0033	0.0356
Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	gulonic acid	0.14	0.0026	0.0324
Xenobiotics	Benzoate Metabolism	hippurate	0.08	0.0000	0.0034
Xenobiotics	Benzoate Metabolism	2-hydroxyhippurate (salicylurate)	0.13	0.0001	0.0037
Xenobiotics	Benzoate Metabolism	catechol sulfate	0.10	0.0009	0.0173
Xenobiotics	Benzoate Metabolism	4-methylcatechol sulfate	0.16	0.0047	0.0399

Xenobiotics	Benzoate Metabolism	4-ethylphenylsulfate	0.10	0.0009	0.0173
Xenobiotics	Food Component/Plant	gluconate	2.19	0.0028	0.0325
Xenobiotics	Food Component/Plant	cinnamoylglycine	0.06	0.0001	0.0037
Xenobiotics	Food Component/Plant	equol sulfate	0.25	0.0023	0.0310
Xenobiotics	Chemical	1-(3-aminopropyl)-2-pyrrolidone	0.43	0.0004	0.0086
Xenobiotics	Chemical	2-aminophenol sulfate	0.19	0.0022	0.0310
Xenobiotics	Chemical	S-(3-hydroxypropyl)mercapturic acid (HPMA)	0.16	0.0001	0.0037

Summary of faecal metabolites levels in SAL-treated defeated mice versus controls. Samples were analyzed using GC/LC-MS by Metabolon, Inc. Data were analyzed using two-way ANOVA with contrasts, followed by the Benjamini-Hochberg correction for multiple comparisons (FDR < 0.1). Additional details are provided in Experimental Procedures.

Table S4. Main effect of *L. rhamnosus (JB-1)* treatment on faecal metabolites. Related to Figure 5.

Super Pathway	Sub-pathway	Metabolite	Fold Change
Amino Acid	Glycine, Serine and Threonine Metabolism	dimethylglycine	0.528465971
Amino Acid	Glycine, Serine and Threonine Metabolism	O-acetylhomoserine	2.048110538
Amino Acid	Lysine Metabolism	6-oxopiperidine-2-carboxylic acid	0.785056295
Amino Acid	Phenylalanine and Tyrosine Metabolism	3-(4-hydroxyphenyl)propionate	0.775087018
Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	4-methylthio-2-oxobutanoate	0.739966294
Carbohydrate	Pentose Metabolism	ribose	0.932081839
Carbohydrate	Disaccharides and Oligosaccharides	lactobionate	0.700898487
Carbohydrate	Fructose, Mannose and Galactose Metabolism	mannitol/sorbitol	0.926302993
Carbohydrate	Aminosugar Metabolism	diacetylchitobiose	1.644910122

Carbohydrate	Aminosugar Metabolism	N-acetylmuramate	0.709766585
Lipid	Medium Chain Fatty Acid	caproate (6:0)	2.177107148
Lipid	Medium Chain Fatty Acid	heptanoate (7:0)	1.019036758
Lipid	Long Chain Fatty Acid	behenate (22:0)*	0.991121776
Lipid	Polyunsaturated Fatty Acid (n3 and n6)	docosapentaenoate (n3 DPA; 22:5n3)	1.345249422
Lipid	Polyunsaturated Fatty Acid (n3 and n6)	dihomo-linolenate (20:3n3 or n6)	1.489049628
Lipid	Polyunsaturated Fatty Acid (n3 and n6)	arachidonate (20:4n6)	1.287041565
Lipid	Fatty Acid, Branched	13-methylmyristate	1.072215026
Lipid	Fatty Acid, Keto	1-dihomo-linoleoylglycerol (20:2)	1.0209374
Lipid	Fatty Acid, Monohydroxy	3-hydroxyoctanoate	1.080411547
Lipid	Fatty Acid, Monohydroxy	3-hydroxypalmitate	1.161468959
Lipid	Endocannabinoid	oleoyl ethanolamide	0.88369415
Lipid	Endocannabinoid	palmitoyl ethanolamide	0.718847514
Lipid	Endocannabinoid	N-oleoyltaurine	1
Lipid	Endocannabinoid	N-stearoyltaurine	1.053209459
Lipid	Phospholipid Metabolism	1,2-dipalmitoyl-GPC (16:0/16:0)	1.371153846
Lipid	Phospholipid Metabolism	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	1.452778984
Lipid	Phospholipid Metabolism	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	1.417410714
Lipid	Phospholipid Metabolism	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	1.595
Lipid	Phospholipid Metabolism	1,2-dioleoyl-GPC (18:1/18:1)*	1.552163804
Lipid	Phospholipid Metabolism	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	1.467537408
Lipid	Phospholipid Metabolism	1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	1.531001389
Lipid	Phospholipid Metabolism	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	1.124132919

Lipid	Phospholipid Metabolism	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	1.352431712
Lipid	Phospholipid Metabolism	1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	1.509443861
Lipid	Phospholipid Metabolism	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	1.502653525
Lipid	Phospholipid Metabolism	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	1.483930711
Lipid	Phospholipid Metabolism	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	2.001454969
Lipid	Phospholipid Metabolism	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	1.893990755
Lipid	Phospholipid Metabolism	1-palmitoyl-2-alpha-linolenoyl-GPC (16:0/18:3n3)*	1.260792079
Lipid	Phospholipid Metabolism	1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	1.668453608
Lipid	Phospholipid Metabolism	1,2-dilinoleoyl-GPC (18:2/18:2)	1.483889045
Lipid	Phospholipid Metabolism	1,2-dilinoleoyl-GPE (18:2/18:2)*	1.481388135
Lipid	Lysolipid	1-palmitoyl-GPC (16:0)	1.230801119
Lipid	Lysolipid	1-stearoyl-GPC (18:0)	1.283551374
Lipid	Lysolipid	1-oleoyl-GPC (18:1)	1.389261745
Lipid	Lysolipid	1-linoleoyl-GPC (18:2)	1.363465602
Lipid	Lysolipid	1-lignoceroyl-GPC (24:0)	1.333777004
Lipid	Lysolipid	1-stearoyl-GPE (18:0)	1.430917529
Lipid	Lysolipid	1-oleoyl-GPE (18:1)	1.37662888
Lipid	Lysolipid	1-linoleoyl-GPE (18:2)*	1.298269312
Lipid	Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	1.085224641
Lipid	Plasmalogen	1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	1.671732523
Lipid	Lysoplasmalogen	1-(1-enyl-palmitoyl)-GPC (P-16:0)*	1.176681461

Lipid	Lysoplasmalogen	1-(1-enyl-palmitoyl)-GPE (P-16:0)*	1.201830065
Lipid	Lysoplasmalogen	1-(1-enyl-oleoyl)-GPE (P-18:1)*	1.196075353
Lipid	Monoacylglycerol	1-palmitoylglycerol (16:0)	1.070570742
Lipid	Monoacylglycerol	1-linoleoylglycerol (18:2)	1.155496303
Lipid	Monoacylglycerol	1-linolenoylglycerol (18:3)	1.523378792
Lipid	Monoacylglycerol	1-docosahexaenoylglycerol (22:6)	1.345331308
Lipid	Monoacylglycerol	1-dihomo-linolenylglycerol (20:3)	1.147470706
Lipid	Sphingolipid Metabolism	phytosphingosine	0.98797841
Lipid	Sphingolipid Metabolism	N-acetylsphingosine	0.552520723
Lipid	Primary Bile Acid Metabolism	chenodeoxycholate	0.575467595
Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	2'-deoxyinosine	0.741000163
Nucleotide	Purine Metabolism, Adenine containing	adenine	1.050200038
Nucleotide	Purine Metabolism, Adenine containing	2'-deoxyadenosine	0.603630862
Nucleotide	Pyrimidine Metabolism, Uracil containing	pseudouridine	0.791377043
Nucleotide	Pyrimidine Metabolism, Uracil containing	2'-deoxyuridine	0.58313253
Nucleotide	Pyrimidine Metabolism, Thymine containing	thymine	0.854961832
Cofactors and Vitamins	Riboflavin Metabolism	flavin adenine dinucleotide (FAD)	1.805010512
Cofactors and Vitamins	Tocopherol Metabolism	delta-tocopherol	1.200754401
Cofactors and Vitamins	Tocopherol Metabolism	gamma-tocopherol/beta-tocopherol	1.110886076
Cofactors and Vitamins	Vitamin A Metabolism	retinol (Vitamin A)	0.714665653
Xenobiotics	Benzoate Metabolism	3,4-dihydroxybenzoate	0.80562963
Xenobiotics	Chemical	sulfate*	0.769647878

Summary of the main effect of JB-1 treatment on faecal metabolites levels. Samples were analyzed using GC/LC-MS by Metabolon, Inc. Data were analyzed using two-way ANOVA with contrasts, followed by the Benjamini-Hochberg correction for multiple comparisons (FDR < 0.1). Additional details are provided in Experimental Procedures.

Table S5. Faecal metabolites altered by stress (DEF/VEH vs CON/VEH) but restored by JB-1 treatment (DEF/JB-1 vs CON/VEH). Related to Figure 5.

Super Pathway	**Sub-pathway**	**Metabolite**	**Mean Scaled Intensity in CON/VEH ± SD**	**Mean Scaled Intensity in DEF/JB-1 ± SD**	**Mean Scaled Intensity in DEF/VEH ± SD**
Amino Acid	Lysine Metabolism	glutarate (pentanedioate)	0.8588± 0.3989	1.1421± 0.5441	1.6694± 0.6053
Amino Acid	Phenylalanine and Tyrosine Metabolism	tyramine	1.52± 1.085	1.539± 1.17	0.7486± 0.4821
Amino Acid	Urea cycle: Arginine and Proline Metabolism	N-acetylcitrulline	2.7966± 2.8924	1.2584± 1.2868	0.5431± 0.4807
Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	glycerate	1.4014± 0.7765	1.6252± 0.5447	2.0827± 0.5813
Carbohydrate	Disaccharides and Oligosaccharides	lactobionate	1.2688± 0.4012	2.1238± 2.0977	5.2442± 10.0654
Lipid	Fatty Acid Metabolism (Acyl Carnitine)	3-hydroxybutyrylcarnitine (2)	0.8795± 0.3155	0.8191± 0.9963	0.3947± 0.2765
Lipid	Fatty Acid, Monohydroxy	4-hydroxybutyrate (GHB)	0.6713± 0.3389	1.591± 1.843	1.591± 1.112
Lipid	Fatty Acid, Monohydroxy	10-hydroxystearate	0.5619± 0.2018	0.5318± 0.4414	0.3033± 0.1655
Lipid	Sphingolipid Metabolism	stearoyl sphingomyelin (d18:1/18:0)	0.8860± 0.2344	1.4177± 1.0032	1.8429± 0.8086

Lipid	Sphingolipid Metabolism	sphingomyelin (d18:1/24:1, d18:2/24:0)*	0.8767± 0.4969	1.7348± 1.1928	2.4097± 1.1998
Lipid	Sphingolipid Metabolism	behenoyl sphingomyelin (d18:1/22:0)*	0.7665± 0.3955	1.4331± 1.0289	1.7909± 0.7421
Lipid	Sphingolipid Metabolism	sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.5735± 0.3629	1.2559± 1.1085	1.9790± 1.1147
Lipid	Primary Bile Acid Metabolism	alpha-muricholate	0.6263± 0.4049	0.4704± 0.2900	0.3142± 0.2307
Lipid	Secondary Bile Acid Metabolism	lithocholate	0.6566± 0.2543	0.4917± 0.3120	0.3278± 0.2064
Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	1-methylnicotinamide	2.695± 3.737	0.5261 ± 0.8623	0.215 ± 0.4037

Summary of faecal metabolites altered by stress but restored to control levels by JB-1 treatment. Samples were analyzed using GC/LC-MS by Metabolon, Inc. Data were analyzed using two-way ANOVA with contrasts, followed by the Benjamini-Hochberg correction for multiple comparisons (FDR < 0.1). Additional details are provided in Experimental Procedures.

4.6. References

Aaronson, S. T., Sears, P., Ruvuna, F., Bunker, M., Conway, C. R., Dougherty, D. D., … Zajecka, J. M. (2017). A 5-year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: comparison of response, remission, and suicidality. *American Journal of Psychiatry*, *174*(7), 640–648.

Aaronson, S. T., Sears, P., Ruvuna, F., Ph, D., Bunker, M., Pharm, D., & Conway, C. R. (2017). A 5-Year Observational Study of Patients With Treatment-Resistant Depression Treated With Vagus Nerve Stimulation or Treatment as Usual : Comparison of Response , Remission , and Suicidality, (July). https://doi.org/10.1176/appi.ajp.2017.16010034

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., … Tompkins, T. (2014). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology and Motility*, *26*(4), 510–520. https://doi.org/10.1111/nmo.12295

Al-Nedawi, K., Mian, M. F., Hossain, N., Karimi, K., Mao, Y.-K., Forsythe, P., … Bienenstock, J. (2014). Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 1–12. https://doi.org/10.1096/fj.14-259721

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association.

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., … Hen, R. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, 1. https://doi.org/10.1038/s41586-018-0262-4

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neuroscience and Biobehavioral Reviews*, *51*, 164–188. https://doi.org/10.1016/j.neubiorev.2015.01.018

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E., & Nestler, E. J. (2001). Induction of nuclear factor-κB in nucleus accumbens by chronic cocaine administration. *Journal of Neurochemistry*, *79*(1), 221–224.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., … Hase, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461), 232–236.

Avgustinovich, D. F., Kovalenko, I. L., & Kudryavtseva, N. N. (2005). A model of anxious depression: Persistence of behavioral pathology. *Neuroscience and Behavioral Physiology*, *35*(9), 917–924. https://doi.org/10.1007/s11055-005-0146-6

Baganz, N. L., & Blakely, R. D. (2012). A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chemical Neuroscience*, *4*(1), 48–63.

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., … Schöpf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486–496.

Bagot, R. C., Cates, H. M., Purushothaman, I., Vialou, V., Heller, E. A., Yieh, L., … Nestler, E. J. (2016). Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. *Biological Psychiatry*, *81*(4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012

Bagot, R. C., Parise, E. M., Pen, C. J., Zhang, H., Maze, I., Chaudhury, D., … Nestler, E. J. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms8062

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., & Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology*, *77*(11), 3846–3852.

Beas, B. S., Wright, B. J., Skirzewski, M., Leng, Y., Hyun, J. H., Koita, O., … Penzo, M. A. (2018). The locus coeruleus drives disinhibition in the midline thalamus via a dopaminergic mechanism. *Nature Neuroscience*, *21*(7), 963. https://doi.org/10.1038/s41593-018-0167-4

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., … Pamer, E. G. (2016). Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nature Medicine*, *22*(5), 516.

Bendtsen, K. M. B., Krych, L., Sørensen, D. B., Pang, W., Nielsen, D. S., Josefsen, K., … Hansen, A. K. (2012). Gut Microbiota Composition Is Correlated to Grid Floor Induced Stress and Behavior in the BALB/c Mouse. *PLoS ONE*, *7*(10), e46231. https://doi.org/10.1371/journal.pone.0046231

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., … Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609. https://doi.org/10.1053/j.gastro.2011.04.052

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., … Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and Motility*, *23*(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Bercik, P., Verdu, E. F., Foster, J. a, Macri, J., Potter, M., Huang, X., … Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102-2112.e1. https://doi.org/10.1053/j.gastro.2010.06.063

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience: Basic and Clinical*, *85*(1–3), 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0

Berton, O., Mcclung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress, (February), 864–869.

Berton, O., McClung, C. a, Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science (New York, N.Y.)*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural and functional consequences of chronic psychosocial stress on the microbiome and host. *Psychoneuroendocrinology*, *63*(2016), 217–227. https://doi.org/10.1016/j.psyneuen.2015.10.001

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 Medicine*, *15*(1), 7. https://doi.org/10.1186/s12916-016-0771-7

Blackshaw, L. A., Brookes, S. J. H., Grundy, D., & Schemann, M. (2007). Sensory transmission in the gastrointestinal tract. *Neurogastroenterology & Motility*, *19*, 1–19.

Boer, M. C., Joosten, S. A., & Ottenhoff, T. H. M. (2015). Regulatory T-cells at the interface between human host and pathogens in infectious diseases and vaccination. *Frontiers in Immunology*, *6*.

Bohórquez, DV, & Shahid, R. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *The Journal of …*, 1–5. https://doi.org/10.1172/JCI78361DS1

Bohórquez, Diego V., Samsa, L. A., Roholt, A., Medicetty, S., Chandra, R., & Liddle, R. A. (2014). An enteroendocrine cell - Enteric glia connection revealed by 3D electron microscopy. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0089881

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E., Taylor, C. M., Welsh, D. A., & Berthoud, H.-R. R. (2014). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry*, *77*(7), 607–615. https://doi.org/10.1016/j.biopsych.2014.07.012

Brynskikh, A., Warren, T., Zhu, J., & Kipnis, J. (2008). Adaptive immunity affects learning behavior in mice. *Brain, Behavior, and Immunity*, *22*(6), 861–869. https://doi.org/10.1016/j.bbi.2007.12.008

Buffington, S. A., Viana, G., Prisco, D., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., … Petrosino, J. F. (2016). Microbial Reconstitution Reverses Maternal Diet- Induced Social and Synaptic Deficits in Offspring Article Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell*, *165*(7), 1762–1775. https://doi.org/10.1016/j.cell.2016.06.001

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856.

Caenepeel, P. H., Janssens, J., Vantrappen, G., Eyssen, H., & Coremans, G. (1989). Interdigestive myoelectric complex in germ-free rats. *Digestive Diseases and Sciences*, *34*(8), 1180–1184.

Cámara, R. J. A., Gander, M.-L., Begré, S., Von Känel, R., & Group, S. I. B. D. C. S. (2011). Post-traumatic stress in Crohn's disease and its association with disease activity. *Frontline Gastroenterology*, *2*(1), 2–9.

Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012). The tail suspension test. *JoVE (Journal of Visualized Experiments)*, (59), e3769.

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., … Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal 1 – 3, 1236–1243. https://doi.org/10.3945/ajcn.2009.28095.The

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335

Carle, T. L., Ohnishi, Y. N., Ohnishi, Y. H., Alibhai, I. N., Wilkinson, M. B., Kumar, A., & Nestler, E. J. (2007). Absence of conserved C-terminal degron domain contributes to ΔFosB's unique stability. *Eur J Neurosci*, *25*, 3009–3019.

Castelli, M. P., Ferraro, L., Mocci, I., Carta, F., Carai, M. A. M., Antonelli, T., … Gessa, G. L. (2003). Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the

sedative/hypnotic effect of γ-hydroxybutyric acid. *Journal of Neurochemistry*, *87*(3), 722–732.

Castle, M., Comoli, E., & Loewy, A. D. (2005). Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience*, *134*(2), 657–669. https://doi.org/10.1016/j.neuroscience.2005.04.031

Chae, J., Nahas, Z., Lomarev, M., Denslow, S., Lorberbaum, J. P., Bohning, D. E., & George, M. S. (2003). A review of functional neuroimaging studies of vagus nerve stimulation (VNS), *37*, 443–455. https://doi.org/10.1016/S0022-3956(03)00074-8

Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., & Nestler, E. J. (1997). Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. *Journal of Neuroscience*, *17*(13), 4933–4941.

Chiu, R., Angel, P., & Karin, M. (1989). Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. *Cell*, *59*(6), 979–986. https://doi.org/10.1016/0092-8674(89)90754-X

Chung, L. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. *Development & Reproduction*, *19*(2), 61–67. https://doi.org/10.12717/DR.2015.19.2.061

Chunyu, J. P., Kyle, Z., Darrell, B., Dayanim, G., Bhatnagar, S., Luz, S., & Vigderman, A. S. (2019). The gut microbiome regulates the increases in depressive-type behaviors and in in fl ammatory processes in the ventral hippocampus of stress vulnerable rats. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0380-x

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., … Cryan, J. F. (2012). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences*, *103*(27), 10420–10425.

Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, *16*(2), 228–231.

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The Impact of the Gut Microbiota on Human Health: An Integrative View - 1-s2.0-S0092867412001043-main.pdf. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*(6), 2003–2014.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews. Microbiology*, *10*(11), 735–742. https://doi.org/10.1038/nrmicro2876

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science*, *326*(5960), 1694–1697.

Crawley, J. N. (1985). Exploratory behavior models of anxiety in mice. *Neuroscience & Biobehavioral Reviews*, *9*(1), 37–44.

Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V.,

… Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207–217. https://doi.org/10.1016/j.psyneuen.2014.01.014

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712.

Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009

Cunningham, J. T., Mifflin, S. W., Gould, G. G., & Frazer, A. (2008). Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. *Neuropsychopharmacology*, *33*(8), 1884–1895. https://doi.org/10.1038/sj.npp.1301570

Curran, T., & Franza Jr, B. R. (1988). Fos and Jun: the AP-1 connection. *Cell*, *55*(3), 395–397.

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. *Nature Reviews Neuroscience*, *9*(1), 46–56.

David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrotta, A., … Alm, E. J. (2014). Host lifestyle affects human microbiota on daily timescales. *Genome Biol*, *15*(7), R89.

de LeBlanc, A. de M., Dogi, C. A., Galdeano, C. M., Carmuega, E., Weill, R., & Perdigón, G. (2008). Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunology*, *9*(1), 27.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, a. J., Green, W., … Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*(August), 7735. https://doi.org/10.1038/ncomms8735

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Dinan, T G. (2005). Stress: the shared common component in major mental illnesses. *European Psychiatry*, *20*, S326–S328.

Dinan, Timothy G, Quigley, E. M. M., Ahmed, S. M. M., Scully, P., O'Brien, S., O'Mahony, L., … Keeling, P. W. N. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*, *130*(2), 304–311.

Dorward, D. W., Garon, C. F., & Judd, R. C. (1989). Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. *Journal of Bacteriology*,

171(5), 2499–2505.
Duffy, L. C., Zielezny, M. A., Marshall, J. R., Byers, T. E., Weiser, M. M., Phillips, J. F., … Graham, S. (1991). Relevance of major stress events as an indicator of disease activity prevalence in inflammatory bowel disease. *Behavioral Medicine*, *17*(3), 101–110.
Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.
Eloe-fadrosh, E. A., Brady, A., Crabtree, J., Drabek, E. F., Ma, B., Mahurkar, A., … Fraser, M. (2015). Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption. *MBio*, *6*(2), e00231-15. https://doi.org/10.1128/mBio.00231-15.Editor
Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, (October 2014). https://doi.org/10.1038/nn.4030
Erspamer, V. (1966). Occurrence of indolealkylamines in nature. In *5-Hydroxytryptamine and Related Indolealkylamines* (pp. 132–181). Springer.
Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., … Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, *110*(22), 9066–9071. https://doi.org/10.1073/pnas.1219451110
Foley, J. O., & DuBois, F. S. (1937). Quantitative studies of the vagus nerve in the cat. I. The ratio of sensory to motor fibers. *Journal of Comparative Neurology*, *67*(1), 49–67.
Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. *Immunological Investigations*, *39*(4–5), 429–448. https://doi.org/10.3109/08820131003667978
Forsythe, P., & Kunze, W. a. (2013). Voices from within: gut microbes and the CNS. *Cellular and Molecular Life Sciences : CMLS*, *70*(1), 55–69. https://doi.org/10.1007/s00018-012-1028-z
Forsythe, P., Kunze, W., & Bienenstock, J. (2016). Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? *BMC Medicine*, *14*(1), 58. https://doi.org/10.1186/s12916-016-0604-8
Forsythe, P., Sudo, N., Dinan, T., Taylor, V. H., & Bienenstock, J. (2010). Mood and gut feelings. *Brain, Behavior, and Immunity*, *24*(1), 9–16. https://doi.org/10.1016/j.bbi.2009.05.058
Forsythe, P., Wang, B., Khambati, I., & Kunze, W. a. (2012). Systemic effects of ingested lactobacillus rhamnosus: Inhibition of mast cell membrane potassium (IKCA) current and degranulation. *PLoS ONE*, *7*(7), 1–8. https://doi.org/10.1371/journal.pone.0041234
Frazer, A., & Benmansour, S. (2002). Delayed pharmacological effects of antidepressants. *Molecular Psychiatry*, *7*, S23–S28. https://doi.org/10.1038/sj.mp.4001015
Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., … Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central

homeostatic mechanism. *Nature Communications*, *5*, 3611. https://doi.org/10.1038/ncomms4611

Fuchs, D., Möller, A. A., Reibnegger, G., Stöckle, E., Werner, E. R., & Wachter, H. (1990). Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *3*(9), 873–876.

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling : What Happens in Vagus... *Neuron*, *101*, 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Furet, J.-P., Quénée, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology*, *97*(2), 197–207.

Furmaga, H., Sadhu, M., & Frazer, A. (2012). Comparison of FosB Immunoreactivity Induced by Vagal Nerve Stimulation with That Caused by Pharmacologically Diverse Antidepressants. *Journal of Pharmacology and Experimental Therapeutics*, *341*(2), 317–325. https://doi.org/10.1124/jpet.111.188953

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. In *Microbial endocrinology: The microbiota-gut-brain axis in health and disease* (pp. 39–71). Springer.

Furness, J. B., Kunze, W. A. A., & Clerc, N. (1999). II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *277*(5), G922–G928.

Galley, J. D., Yu, Z., Kumar, P., Dowd, S. E., Lyte, M., & Bailey, M. T. (2015). The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut Microbes*, *5*(6), 748–760. https://doi.org/10.4161/19490976.2014.972241

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., … Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515

Gärtner, K. (1990). A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Laboratory Animals*, *24*(1), 71–77.

Gębicki, J., Sysa-Jędrzejowska, A., Adamus, J., Woźniacka, A., Rybak, M., & Zielonka, J. (2003). 1-Methylnicotinamide: a potent anti-inflammatory agent of vitamin origin. *Pol. J. Pharmacol*, *55*, 109–112.

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., … Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*(7610), 94–103.

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: we have never been individuals. *The Quarterly Review of Biology*, *87*(4), 325–341.

Gobaille, S., Schleef, C., Hechler, V., Viry, S., Aunis, D., & Maitre, M. (2002). Gamma-hydroxybutyrate increases tryptophan availability and potentiates serotonin turnover in rat brain. *Life Sciences*, *70*(18), 2101–2112.

Goehler, L. E., Gaykema, R. P. A., Opitz, N., Reddaway, R., Badr, N., & Lyte, M. (2005). Activation in vagal afferents and central autonomic pathways: Early

responses to intestinal infection with Campylobacter jejuni. *Brain, Behavior, and Immunity*, *19*(4), 334–344. https://doi.org/10.1016/j.bbi.2004.09.002

Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols*, *6*(8), 1183–1191. https://doi.org/10.1038/nprot.2011.361

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 33–51.

Greenberg, M. E., Greene, L. A., & Ziff, E. B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *Journal of Biological Chemistry*, *260*(26), 14101–14110.

Greenberg, M. E., & Ziff, E. B. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature*, *311*(5985), 433.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., & Roncarolo, M. G. (1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*, *389*(6652), 737–742.

Groves, D. A., & Brown, V. J. (2005). Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neuroscience & Biobehavioral Reviews*, *29*(3), 493–500. https://doi.org/10.1016/j.neubiorev.2005.01.004

Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., … de Araujo, I. E. (2018). A Neural Circuit for Gut-Induced Reward. *Cell*, 1–14. https://doi.org/10.1016/j.cell.2018.08.049

He, B., Xu, W., Santini, P. A., Polydorides, A. D., Chiu, A., Estrella, J., … Plebani, A. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*, *26*(6), 812–826.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., … Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences*, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710.

Heitler, W. J. (2007). DataView: A Tutorial Tool for Data Analysis. Template-based Spike Sorting and Frequency Analysis. *Journal of Undergraduate Neuroscience Education*, *6*(1), A1.

Henningsen, P., Zimmermann, T., & Sattel, H. (2003). Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. *Psychosomatic Medicine*, *65*(4), 528–533.

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., … Warren, B. L. (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proceedings of the National Academy of Sciences*, *111*(52), 18799–18799. https://doi.org/10.1073/pnas.1423575112

Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Frontiers in Neuroendocrinology*, *14*(3), 173–213. https://doi.org/10.1006/frne.1993.1006

Holzer, P. (2011). Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacology & Therapeutics*, *131*(1), 142–170.

Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., … Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron*, *13*(5), 1235–1244.

Houlden, A., Goldrick, M., Brough, D., Vizi, E. S., Lénárt, N., Martinecz, B., … Denes, A. (2016). Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain, Behavior, and Immunity*, *57*, 10–20.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., … Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female Syrian hamsters. *Hormones and Behavior*, *44*(3), 293–299.

Husebye, E., Hellström, P. M., & Midtvedt, T. (1994). Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. *Digestive Diseases and Sciences*, *39*(5), 946–956.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuroimage*, *125*, 988–995.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Stanisz, G. J., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *NeuroImage*, *125*(2015), 988–995. https://doi.org/10.1016/j.neuroimage.2015.11.018

Jašarević, E., Howard, C. D., Misic, A. M., Beiting, D. P., & Bale, T. L. (2017). Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. *Scientific Reports*, *7*, 44182.

Jašarević, E., Howard, C. D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., … Bale, T. L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nature Neuroscience*. https://doi.org/10.1038/s41593-018-0182-5

Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., … Kheirbek, M. A. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron*, *0*(0), 1–14. https://doi.org/10.1016/j.neuron.2018.01.016

Jorissen, H. J. M. M., Ulery, P. G., Henry, L., Gourneni, S., Nestler, E. J., & Rudenko, G. (2007). Dimerization and DNA-binding properties of the transcription factor ΔFosB. *Biochemistry*, *46*(28), 8360–8372.

Jung, H. Y., Kim, W., Yoo, D. Y., Nam, S. M., Kim, J. W., Choi, J. H., … Hwang, I. K. (2014). Intragastric gavage with denatonium benzoate acutely induces neuronal activation in the solitary tract nucleus via the vagal afferent pathway. *Journal of*

Veterinary Science, *15*(4), 459–464. https://doi.org/10.4142/jvs.2014.15.4.459
Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B., Montoya, M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, *In Press*. https://doi.org/10.1126/science.aat5236
Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., … Bienenstock, J. (2006). Inhibitory effects of Lactobocillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, *55*(2), 191–196. https://doi.org/10.1136/gut.2005.070987
Karimi, K., Inman, M. D., Bienenstock, J., & Forsythe, P. (2009). Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *American Journal of Respiratory and Critical Care Medicine*, *179*(3), 186–193. https://doi.org/10.1164/rccm.200806-951OC
Karimi, K., Kandiah, N., Chau, J., Bienenstock, J., & Forsythe, P. (2012). A Lactobacillus rhamnosus Strain Induces a Heme Oxygenase Dependent Increase in Foxp3+ Regulatory T Cells. *PLoS ONE*, *7*(10), 1–12. https://doi.org/10.1371/journal.pone.0047556
Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., … Cryan, J. F. (2016). Lost in Translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.11.018
Kidd, M., Modlin, I. M., Gustafsson, B. I., Drozdov, I., Hauso, O., & Pfragner, R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *295*(2), G260–G272.
Kinsey, S. G., Bailey, M. T., Sheridan, J. F., Padgett, D. A., & Avitsur, R. (2007). Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. *Brain, Behavior, and Immunity*, *21*(4), 458–466.
Klooker, T. K., Braak, B., Painter, R. C., de Rooij, S. R., van Elburg, R. M., van den Wijngaard, R. M., … Boeckxstaens, G. E. (2009). Exposure to severe wartime conditions in early life is associated with an increased risk of irritable bowel syndrome: a population-based cohort study. *The American Journal of Gastroenterology*, *104*(9), 2250–2256.
König, H., Ponta, H., Rahmsdorf, U., Büscher, M., Schönthal, A., Rahmsdorf, H. J., & Herrlich, P. (1989). Autoregulation of fos: the dyad symmetry element as the major target of repression. *The EMBO Journal*, *8*(9), 2559–2566.
Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, *7*.
Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018
Kunze, W. A., Mao, Y., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., & Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and*

Molecular Medicine, *13*(8b), 2261–2270.
Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. a, … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676
Lay, C., Rigottier-Gois, L., Holmstrøm, K., Rajilic, M., Vaughan, E. E., de Vos, W. M., … Blaut, M. (2005). Colonic microbiota signatures across five northern European countries. *Applied and Environmental Microbiology*, *71*(7), 4153–4155.
Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-amram, H., … Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. https://doi.org/10.1038/ncomms15062
Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, *124*(4), 837–848.
Lindqvist, D., Wolkowitz, O. M., Mellon, S., Yehuda, R., Flory, J. D., Henn-Haase, C., … Neylan, T. C. (2014). Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. *Brain, Behavior, and Immunity*, *42*, 81–88.
Liu, Z.-H., & Smith, C. B. (2009). Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. *Neuroscience Letters*, *454*(1), 62–66.
Lucibello, F. C., Lowag, C., Neuberg, M., & Müller, R. (1989). Trans-repression of the mouse c-fos promoter: A novel mechanism of fos-mediated trans-regulation. *Cell*, *59*(6), 999–1007. https://doi.org/10.1016/0092-8674(89)90756-3
Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, *33*(8), 574–581.
Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathogens*, *9*(11).
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. *Physiology & Behavior*, *89*(3), 350–357. https://doi.org/10.1016/j.physbeh.2006.06.019
Lyte, M., Varcoe, J. J., & Bailey, M. T. (1998). Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiology and Behavior*, *65*(1), 63–68. https://doi.org/10.1016/S0031-9384(98)00145-0
Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system, *4*(June), 1626–1632.
Macpherson, A. J., & Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*, *303*(5664), 1662–1665.
Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *19*(1), 11–38.
Maitre, M., Humbert, J.-P., Kemmel, V., Aunis, D., & Andriamampandry, C. (2005). [A mechanism for gamma-hydroxybutyrate (GHB) as a drug and a substance of abuse]. *Medecine Sciences: M/S*, *21*(3), 284–289.

Malatynska, E., & Knapp, R. J. (2005). Dominant–submissive behavior as models of mania and depression. *Neuroscience & Biobehavioral Reviews*, *29*(4–5), 715–737.

Malkova, N. V, Collin, Z. Y., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, Behavior, and Immunity*, *26*(4), 607–616.

Mao, Y.-K., Kasper, D. L., Wang, B., Forsythe, P., Bienenstock, J., & Kunze, W. a. (2013). Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.*, *4*, 1465. https://doi.org/10.1038/ncomms2478

Mariat, D., Firmesse, O., Levenez, F., Guimarães, V., Sokol, H., Doré, J., … Furet, J.-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, *9*, 123. https://doi.org/10.1186/1471-2180-9-123

Mayer, E. A. (2000). The neurobiology of stress and gastrointestinal disease. *Gut*, *47*(6), 861–869.

Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195), 620.

Mccall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., Mcelligott, Z. A., & Stuber, G. D. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior, 1–23. https://doi.org/10.7554/eLife.18247

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain. *Molecular Brain Research*, *132*(January), 146–154. https://doi.org/10.1016/j.molbrainres.2004.05.014

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2005). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain, (May 2018). https://doi.org/10.1016/j.molbrainres.2004.05.014

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine*, *338*(3), 171–179.

McHenry, J. A., Robison, C. L., Bell, G. A., Vialou, V. V, Bolaños-Guzmán, C. A., Nestler, E. J., & Hull, E. M. (2016). The role of ΔfosB in the medial preoptic area: Differential effects of mating and cocaine history. *Behavioral Neuroscience*, *130*(5), 469.

McKinney, W. T., & Bunney, W. E. (1969). Animal model of depression: I. Review of evidence: implications for research. *Archives of General Psychiatry*, *21*(2), 240–248.

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility*, *25*(2), 183-e88.

Menard, C., Pfau, M. L., Hodes, G. E., Kana, V., Wang, V. X., Bouchard, S., … Russo, S. J. (2017). Social stress induces neurovascular pathology promoting depression. *Nature Neuroscience*, *20*(12), 1752–1760. https://doi.org/10.1038/s41593-017-0010-3

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … Cazaubiel, M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and

human subjects. *British Journal of Nutrition*, *105*(5), 755–764.
Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., … Wolf, S. A. (2016). Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports*, *15*(9), 1945–1956. https://doi.org/10.1016/j.celrep.2016.04.074
Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience*, *14*(1), 421–451.
Müller, R., Bravo, R., Burckhardt, J., & Curran, T. (1984). Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature*, *312*(5996), 716.
Mundorf, M. L., Hochstetler, S. E., & Wightman, R. M. (1999). Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *Journal of Neurochemistry*, *73*(6), 2397–2405.
Murphy, K., & Weaver, C. (2016). *Janeway's immunobiology*. Garland Science.
Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*(3), 695–700.
Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, *148*(1), 257–266.
Naritoku, D. K., Terry, W. J., & Helfert, R. H. (1995). Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Research*, *22*(1), 53–62. https://doi.org/10.1016/0920-1211(95)00035-9
Nemeroff, C. B., Mayberg, H. S., Krahl, S. E., McNamara, J., Frazer, A., Henry, T. R., … Brannan, S. K. (2006). VNS therapy in treatment-resistant depression: Clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology*, *31*(7), 1345–1355. https://doi.org/10.1038/sj.npp.1301082
Nestler, E. J. (2015). Δ FosB : A transcriptional regulator of stress and antidepressant responses. *European Journal of Pharmacology*, *753*, 66–72. https://doi.org/10.1016/j.ejphar.2014.10.034
Nestler, E. J., Barrot, M., & Self, D. W. (2001). ΔFosB: a sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences*, *98*(20), 11042–11046.
Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–265. https://doi.org/10.1111/j.1365-2982.2010.01620.x
Nishino, R., Mikami, K., Takahashi, H., Tomonaga, S., Furuse, M., Hiramoto, T., … Sudo, N. (2013). Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. *Neurogastroenterology & Motility*, *25*(6), 521-e371.
O'Garra, A., Vieira, P. L., Vieira, P., & Goldfeld, A. E. (2004). IL-10–producing and naturally occurring CD4+ Tregs: limiting collateral damage. *The Journal of Clinical Investigation*, *114*(10), 1372–1378.
O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M.

M. (2005). Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, L., Mccarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. M. (2005). Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., … Dinan, T. G. (2009a). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., … Dinan, T. G. (2009b). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., & Madsen, K. L. (2013). Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology*, *38*(9), 1738–1747.

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., & Hsiao, E. Y. (2018). The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell*, *173*(7), 1728-1741.e13. https://doi.org/10.1016/j.cell.2018.04.027

Padilla-coreano, N., Bolkan, S. S., Pierce, G. M., Spellman, T. J., Gordon, J. A., Padilla-coreano, N., … Hardin, W. D. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Article Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron*, *89*, 1–10. https://doi.org/10.1016/j.neuron.2016.01.011

Padmanabhan, P., Grosse, J., Asad, A. B. M. A., Radda, G. K., & Golay, X. (2013). Gastrointestinal transit measurements in mice with99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Research*, *3*(1), 1–8. https://doi.org/10.1186/2191-219X-3-60

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, *5*(7), e177.

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society*, *73*(04), 477–489.

Paxinos, G., & Franklin, K. B. J. (2004). *The mouse brain in stereotaxic coordinates*. Gulf professional publishing.

Perez-Burgos, A., Mao, Y.-K., Bienenstock, J., & Kunze, W. a. (2014). The gut-brain axis rewired: adding a functional vagal nicotinic "sensory synapse". *The FASEB Journal*, *28*(7), 3064–3074. https://doi.org/10.1096/fj.13-245282

Perez-Burgos, A., Wang, B., Mao, Y.-K., Mistry, B., McVey Neufeld, K.-A., Bienenstock, J., & Kunze, W. (2013). Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of*

Physiology. Gastrointestinal and Liver Physiology, *304*(2), G211-20. https://doi.org/10.1152/ajpgi.00128.2012

Perez-Burgos, A., Wang, L., McVey Neufeld, K., Mao, Y., Ahmadzai, M., Janssen, L. J., … Kunze, W. A. (2015). The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of Physiology*, *593*(17), 3943–3957.

Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., & Nestler, E. J. (2004). Induction of ΔFosB in reward-related brain structures after chronic stress. *Journal of Neuroscience*, *24*(47), 10594–10602.

Peyron, C., Luppi, P. H., Fort, P., Rampon, C., & Jouvet, M. (1996). Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *Journal of Comparative Neurology*, *364*(3), 402–413. https://doi.org/10.1002/(SICI)1096-9861(19960115)364:3<402::AID-CNE2>3.0.CO;2-8

Phillips, J. G. P. (1910). The Treatment of Melancholia by the Lactic Acid Bacillus. *The British Journal of Psychiatry*, *56*(234), 422-NP. https://doi.org/10.1192/bjp.56.234.422

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Yamada, T. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59.

Ramos, A., & Mormède, P. (1997). Stress and emotionality: a multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, *22*(1), 33–57.

Reber, S. O., Siebler, P. H., Donner, N. C., Morton, J. T., Smith, D. G., Kopelman, J. M., … Lowry, C. A. (2016). Immunization with a heat-killed preparation of the environmental bacterium *Mycobacterium vaccae* promotes stress resilience in mice. *Proceedings of the National Academy of Sciences*, 201600324. https://doi.org/10.1073/pnas.1600324113

Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., … Kashyap, P. C. (2014). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, *29*(4), 1395–1403.

Robison, A. J., Vialou, V., Mazei-Robison, M., Feng, J., Kourrich, S., Collins, M., … Neve, R. (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving ΔFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *Journal of Neuroscience*, *33*(10), 4295–4307.

Rolls, A., Shechter, R., London, A., Ziv, Y., Ronen, A., Levy, R., & Schwartz, M. (2007). Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biology*, *9*(9), 1081.

Rong, W., Hillsley, K., Davis, J. B., Hicks, G., Winchester, W. J., & Grundy, D. (2004). Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *The Journal of Physiology*, *560*(3), 867–881.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1), 529.

Sanderson, S., Boardman, W., Ciofi, C., & Gibson, R. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023.

https://doi.org/10.1038/nature4441021a
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676.
Schönfeld, C.-L., & Trendelenburg, U. (1989). The release of3H-noradrenaline by p-and m-tyramines and-octopamines, and the effect of deuterium substitution in α-position. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *339*(4), 433–440.
Schroeder, F. a, Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry*, *62*(1), 55–64. https://doi.org/10.1016/j.biopsych.2006.06.036
Schütte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J., & Minna, J. (1989). jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, *59*(6), 987–997. https://doi.org/10.1016/0092-8674(89)90755-1
Schwarz, J., Burguet, J., Rampin, O., Fromentin, G., Andrey, P., Tomé, D., … Darcel, N. (2010). Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. *PLoS ONE*, *5*(2), 13–15. https://doi.org/10.1371/journal.pone.0008974
Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, *164*(3), 337–340.
Serretti, A., Calati, R., Goracci, A., Di Simplicio, M., Castrogiovanni, P., & De Ronchi, D. (2010). Antidepressants in healthy subjects: what are the psychotropic/psychological effects? *European Neuropsychopharmacology*, *20*(7), 433–453.
Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2018). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron*, 1–14. https://doi.org/10.1016/j.neuron.2018.11.018
Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P. C., & Mazmanian, S. K. (2014). Perspective Specialized Metabolites from the Microbiome in Health and Disease. *Cell Metabolism*, *20*(5), 719–730. https://doi.org/10.1016/j.cmet.2014.10.016
Sharp, F. R., Sagar, S. M., Hicks, K., Lowenstein, D., & Hisanaga, K. (1991). c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. *Journal of Neuroscience*, *11*(8), 2321–2331.
Sibley, C. D., Grinwis, M. E., Field, T. R., Eshaghurshan, C. S., Faria, M. M., Dowd, S. E., … Surette, M. G. (2011). Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PloS One*, *6*(7), e22702.
Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.-M., … Kolassa, I.-T. (2009). Substantial reduction of naive and regulatory T cells following traumatic stress. *Brain, Behavior, and Immunity*, *23*(8), 1117–1124.
Stam, R., Akkermans, L. M., & Wiegant, V. M. (1997). Trauma and the gut: interactions between stressful experience and intestinal function. *Gut*, *40*(6), 704.
Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., … Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′Sialyllactose and 6′Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain, Behavior, and Immunity*, *50*, 166–177. https://doi.org/10.1016/j.bbi.2015.06.025

Thompson, J. A., Oliveira, R. A., Djukovic, A., Ubeda, C., & Xavier, K. B. (2015). Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota. *Cell Reports*, 1–11. https://doi.org/10.1016/j.celrep.2015.02.049

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … Mayer, E. a. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401, 1401.e1-4. https://doi.org/10.1053/j.gastro.2013.02.043

Torii, A., Torii, S., Fujiwara, S., Tanaka, H., Inagaki, N., & Nagai, H. (2007). Lactobacillus acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergology International*, *56*(3), 293–301.

Tsuji, M., Suzuki, K., Kinoshita, K., & Fagarasan, S. (2008). Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. In *Seminars in immunology* (Vol. 20, pp. 59–66). Elsevier.

Ulery, P. G., Rudenko, G., & Nestler, E. J. (2006). Regulation of ΔFosB stability by phosphorylation. *Journal of Neuroscience*, *26*(19), 5131–5142.

Umesaki, Y., Okada, Y., Matsumoto, S., Imaoka, A., & Setoyama, H. (1995). Segmented Filamentous Bacteria Are Indigenous Intestinal Bacteria That Activate Intraepithelial Lymphocytes and Induce MHC Class II Molecules and Fucosyl Asialo GM1 Glycolipids on the Small Intestinal Epithelial Cells in the Ex-Germ-Free Mouse. *Microbiology and Immunology*, *39*(8), 555–562.

van der Kleij, H., O'Mahony, C., Shanahan, F., O'Mahony, L., & Bienenstock, J. (2008). Protective effects of Lactobacillus reuteri and Bifidobacterium infantis in murine models for colitis do not involve the vagus nerve. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(4), R1131–R1137.

Vialou, V., Robison, A. J., Laplant, Q. C., Covington, H. E., Dietz, D. M., Ohnishi, Y. N., … Nestler, E. J. (2010). fosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neuroscience*, *13*(6), 745–752. https://doi.org/10.1038/nn.2551

Vialou, V., Thibault, M., Kaska, S., Cooper, S., Gajewski, P., Eagle, A., … Robison, A. J. (2015). Differential induction of FosB isoforms throughout the brain by fluoxetine and chronic stress. *Neuropharmacology*, *99*, 28–37. https://doi.org/10.1016/j.neuropharm.2015.07.005

Wang, B., Mao, Y.-K. K., Diorio, C., Pasyk, M., Wu, R. Y., Bienenstock, J., & Kunze, W. A. (2010). Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *The FASEB Journal*, *24*(10), 4078–4088. https://doi.org/10.1096/fj.09-153841

Wang, F. Bin, & Powley, T. L. (2000). Topographic inventories of vagal afferents in gastrointestinal muscle. *The Journal of Comparative Neurology*, *421*(3), 302–324. https://doi.org/10.1002/(SICI)1096-9861(20000605)421:3<302::AID-CNE2>3.0.CO;2-N [pii]

Wehner, S., Koscielny, A., Vilz, T. O., Stoffels, B., Engel, D. R., Kurts, C., & Kalff, J.

(2014). Measurement of gastrointestinal and colonic transit in mice, 1–9. https://doi.org/10.1038/protex.2011.219

Werner-Felmayer, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G., & Wachter, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells in vitro. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1012*(2), 140–147.

Whelan, F. J., Verschoor, C. P., Stearns, J. C., Rossi, L., Luinstra, K., Loeb, M., … Bowdish, D. M. E. (2014). The loss of topography in the microbial communities of the upper respiratory tract in the elderly. *Annals of the American Thoracic Society*, *11*(4), 513–521.

Wikoff, W. R., Anfora, A. T., Liu, J., Schultz, P. G., Lesley, S. A., Peters, E. C., & Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences*, *106*(10), 3698–3703.

Wohleb, E. S., McKim, D. B., Sheridan, J. F., & Godbout, J. P. (2015). Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Frontiers in Neuroscience*, *8*(January), 1–17. https://doi.org/10.3389/fnins.2014.00447

Wohleb, E. S., Powell, N. D., Godbout, J. P., & Sheridan, J. F. (2013). Stress-Induced Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(34), 13820–13833. https://doi.org/10.1523/JNEUROSCI.1671-13.2013

Wong, A. H. C., Gottesman, I. I., & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*, *14*(suppl_1), R11–R18.

Wu, J. C. Y. (2012). Psychological co-morbidity in functional gastrointestinal disorders: epidemiology, mechanisms and management. *Journal of Neurogastroenterology and Motility*, *18*(1), 13.

Wyss, M. T., Magistretti, P. J., Buck, A., & Weber, B. (2011). Labeled acetate as a marker of astrocytic metabolism. *Journal of Cerebral Blood Flow & Metabolism*, *31*(8), 1668–1674.

Yano, J. M. M., Yu, K., Donaldson, G. P. P., Shastri, G. G. G., Ann, P., Ma, L., … Hsiao, E. Y. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. https://doi.org/10.1016/j.cell.2015.02.047

Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., … Dileone, R. J. (2006). An essential role for ΔFosB in the nucleus accumbens in morphine action. *Nature Neuroscience*, *9*(2), 205.

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T., … Vieira-Silva, S. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, *352*(6285), 565–569.

Zijlmans, M. a. C., Korpela, K., Riksen-Walraven, J. M., de Vos, W. M., & de Weerth, C. (2015). Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, *53*, 233–245. https://doi.org/10.1016/j.psyneuen.2015.01.006

Zucchi, R., Chiellini, G., Scanlan, T. S., & Grandy, D. K. (2006). Trace amine-associated receptors and their ligands. *British Journal of Pharmacology*, *149*(8), 967–978.

CHAPTER 5.

Differential effects of acute and chronic bacterial treatment on c-Fos and ΔFosB expression through vagus nerve-dependent and independent pathways

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Abstract

Background: While the literature is replete with evidence of gut-brain signalling, the brain regions that are recruited in response to bacterial signals are unknown. Additionally, although several pathways have been proposed to mediate such interactions, it is unclear whether bacteria recruit multiple pathways that transmit information to distinct regions.

Methods: Male Balb/c mice were orally administered saline or *Lactobacillus rhamnosus* (JB-1) to measure acute c-Fos immunoreactivity and mesenteric vagal afferent fibre firing within 165 minutes, or ΔFosB following two weeks of treatment. Mice also underwent sub-diaphragmatic vagotomy or sham surgery to investigate whether severing the vagus abolished JB-1-induced c-Fos expression.

Results: Live, but not heat-killed bacteria significantly induced c-Fos expression in the basolateral and central amygdala, ventral hippocampus (vHpc), periaqueductal grey (PAG), dorsal raphe nucleus (DRN), and locus coeruleus (LC). Both live and heat-killed bacteria increased c-Fos expression in the paraventricular nucleus of the thalamus (PVT) and facilitated firing of vagal fibres absent behavioural changes. This is contrasted with chronic JB-1 treatment, which increased ΔFosB expression in the bed nucleus of the stria terminalis, PVT, dorsal hippocampus, vHpc, PAG, DRN, and LC. Severing the vagus prevented JB-1-induced c-Fos immunoreactivity in all regions except the vHpc and DRN.

Conclusions: These data identify regions that respond to bacteria-derived signals and indicate the recruitment of multiple signalling pathways. Future research will need to identify the targets of ΔFosB-mediated transcription in order to identify the molecular underpinnings of behavioural changes mediated by gut-brain signalling.

5.1. Introduction

Peripheral systems provide a rich source of sensory information for the central nervous system (CNS), integrating and encoding various stimuli to communicate with the CNS. The vast community of intestinal bacteria is one such source of peripheral signals, interacting with the brain through anatomical and other pathways that together comprise the 'gut-brain axis' (Collins et al., 2012; Forsythe et al., 2016). In rodent models, the presence of resident gut bacteria is critical for the appropriate development of the CNS, including memory (Gareau et al., 2011) and stress response systems (Sudo et al., 2004). Exposure to certain bacterial strains alters anxiety- and depression-like behaviours, along with facets of CNS physiology under normal conditions (Bercik et al., 2011; Bravo et al., 2011; Janik et al., 2016) and in states of chronic stress (Bharwani, Mian, Surette, Bienenstock, & Forsythe, 2017). In humans, consumption of a fermented milk product with specific bacterial strains produced alterations in the activity and connectivity of brain regions that process affective and sensory stimuli (Tillisch et al., 2013). Similarly, specific changes in brain activity have been measured by functional magnetic resonance after 4 weeks of probiotic treatment (Bagga et al., 2018). While such observations have added to a growing literature (Forsythe et al., 2016; Fülling et al., 2019), there remains much work to be done to address several glaring gaps in our

knowledge, including our understanding of the mechanisms underlying the detection, transmission, and processing of bacteria-derived signals in the brain. Although it is evident that the resident gut microbiota and certain exogenous strains can indeed influence behaviour and neural function, the precise regions in the brain that are recruited in response to such signals, as well as the changes in the pattern of neuronal response following acute versus chronic exposure to such signals remain unknown. Additionally, while several pathways have been proposed to mediate such interactions, including neural, immune, and humoural signals, it is unclear whether bacteria recruit multiple pathways, and whether these transmit information to distinct regions of the brain.

c-Fos and ΔFosB are part of the Fos-Jun family of transcription factors that are expressed in response to various stimuli and bind to sites on gene promoters (reviewed in Mcclung et al., 2004; Nestler, 2015; Nestler, Barrot, & Self, 2001). *c-Fos* is an immediate early gene marker of neuronal activity (Morgan & Curran, 1991). c-Fos expression is closely linked temporally with *c-Fos* transcription (Sharp et al., 1991), is maximal at 1-2 hours following a stimulus, and degrades within 6 hours (Jung et al., 2014; Sharp et al., 1991), thus providing a useful indicator of an acute neuronal response. ΔFosB is a truncated splice variant of FosB, a group of proteins that peak at 6 hours following a stimulus (Chen et al., 1997; Hope et al., 1994). However, unlike the full-length protein and other splice variants, the 35-37kD ΔFosB isoform possesses unique properties that render it stable and allow it to accumulate at high levels following repeated exposure to the stimulus. ΔFosB has been studied in several brain regions, notably the nucleus accumbens and dorsal striatum, for its role in mediating behavioural

changes through persistent neural adaptations, especially in models of chronic stress, chronic drug use, and repeated natural rewards (Nestler, 2015).

In the present study, we examined for evidence of neuronal activity in the brain shortly after oral administration of a specific bacterial strain, *Lactobacillus rhamnosus* (JB-1), previously demonstrated to modulate anxiety and depression-like behaviours in mice (Bharwani et al., 2017; Bravo et al., 2011). We observed increased c-Fos expression in distributed brain regions within 165 minutes and in the absence of changes in behaviour in the tail suspension test. While both the live and heat-killed bacteria increased firing of mesenteric vagal fibres, the effects on c-Fos expression in the CNS were mostly limited to the live strain. Sub-diaphragmatic sectioning of vagus nerve abolished c-Fos expression in most but not all regions, further underscoring the role of the vagus and indicating the recruitment of additional, vagal-independent signalling pathways. Finally, only chronic bacterial treatment induced ΔFosB expression in distinct brain regions, along with changes in behaviour.

5.2. Experimental procedures

5.2.1. Animals. Adult male BALB/c (BALB/cAnNCrl) mice aged 6-8 weeks were obtained from Charles River (Montreal, QC, Canada) and allowed to habituate to the animal facility for at least 1 week. Mice were maintained on a 12 h light/dark cycle (lights on at 5am) with *ad libitum* access to food and water. Prior to all experiments, mice were habituated and handled by the researcher. All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by McMaster University's Animal Research Ethics Board.

5.2.2. Preparation and treatment with bacteria. *Lactobacillus rhamnosus* (JB-1) bacteria were prepared as described previously (Bravo et al., 2011). Briefly, bacteria from stock were suspended in tubes filled with Man-Rogosa-Sharpe (MRS) medium for 48-h under anaerobic conditions. Following this, bacteria were harvested, washed with sterile phosphate buffer saline (PBS) to the desired concentration, re-suspended in MRS broth, and stored at -80°C in 1ml aliquots at 10^{10} colony-forming units (CFUs)/ml. Heat-killed bacteria were prepared as previously described (Kamiya et al., 2006; Mao et al., 2013), by heating 10^{10} CFU aliquots of viable bacteria for 20 minutes at 80°C. No bacterial growth was detected after 72 hours under anaerobic conditions at 37°C.

Animals were orally gavaged with 200µl of treatment, comprising either PBS or $2x10^9$ colony forming units (CFU) of live or heat-killed of *Lactobacillus rhamnosus* (JB-1) washed and re-suspended in PBS. For experiments involving acute treatment, animals were gavaged with a single dose ~165 minutes prior to the administration of anesthesia for transcardial perfusions. 165 minutes was the chosen time-point given previous work demonstrating that the gastrointestinal transit time to the stomach and small intestine in mice is ~1 hour (Padmanabhan, Grosse, Asad, Radda, & Golay, 2013; Wehner et al., 2014), and that c-Fos immunoreactivity in the brain peaks between 1-2 hours (Jung et al., 2014; Nestler et al., 2001). For experiments involving chronic treatment, animals were gavaged daily over a period of 14 days, with the final treatment occurring 20-24 hours prior to perfusions. This time-point was chosen in an effort to ensure complete degradation of stimulus-induced FosB, which occurs within 18 hours, and ensure labeling of only the stable ΔFosB isoform (McHenry et al., 2016; Perrotti et al., 2004). Control groups in this experiment were treated with oral saline for two weeks (saline

group) or oral saline for 13 days followed by a single dose of the bacteria on the final day (acute JB-1).

5.2.3. Vagotomy. Animals underwent surgery for sub-diaphragmatic vagotomy as previously described (Bravo et al., 2011; van der Kleij, O'Mahony, Shanahan, O'Mahony, & Bienenstock, 2008). Animals were allowed to recover for 14 days prior to treatment and perfusion. Sham vagotomy was also performed on surgical control animals.

5.2.4. Mesenteric nerve recording. Tissue was prepared from mice as previously described (McVey Neufeld et al., 2013). Briefly, 2 cm fresh segments of jejunum were placed in a 2 mL recording dish lined with Sylgard and filled with Krebs. The oral and anal ends were cannulated and flushed with plastic tubing, and the mesentery was pinned to isolate nerve bundles by microdissection. The serosal compartment was separately perfused with prewarmed Krebs/nicardipine (3 μM). The nerve bundle was gently sucked into a glass pipette with an attached electrode and extracellular multiunit nerve recordings were made using a Multi-Clamp 700B amplifier and Digidata 1440A signal converter (Molecular Devices). For intraluminal administration experiments, baseline recordings were collected for 20 minutes with luminal perfusion of the Krebs prior to adding treatments at equivalent doses to the respective oral treatments identified in methods. For *in vivo* administration experiments, recordings were made from mice that were administered a single dose of saline, live, or heat-killed JB-1, as described above. Electrical signals were bandpass-filtered at 2 kHz and sampled at 20 kHz. Single units representing discharge from individual single vagal fibres were discriminated and identified by their unique spike waveform shape and amplitude (Perez-Burgos et al., 2013; Rong et al., 2004) using Dataview computer software (Heitler, 2007), which uses

principal component analysis to sort the recorded multiunit spikes into single unit categories according to shape and amplitude.

5.2.5. Tail Suspension Test. 165 minutes following administration of a single dose of treatment or 1 day following a 14-day course of oral treatments, animals were tested for depression-like behaviour with the tail suspension test (TST). Mice were transferred from the housing room to the behavioural testing room and allowed to habituate for 30 minutes. Following habituation, mice were suspended by the tail using 17 cm laboratory tape (Can et al., 2012) from a suspension bar. 2 cm of the tape was affixed to the mouse tail and the remainder of the tape used for suspension. Animals were suspended for a total of 6 minutes. Behaviour was video recorded and scored by a blinded observer. Freezing behaviour was measured and calculated as a percentage of the total time suspended.

5.2.6. Immunohistochemistry. Mice were anaesthetized with a mixture of ketamine/xylazine solution and transcardially perfused with 0.01 M ice-cold heparinized phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Brains were extracted and placed overnight in 4% PFA at 4°C before being rinsed with PBS and stored in solutions of 10% sucrose followed by 30% sucrose in PBS until the brains sank. Fixed brains were snap frozen with isopentane and dry ice, and stored at -80°C until sectioning. We obtained 50 µm coronal sections using a Microm HM550 Thermo Scientific Cryostat at -20°C and stored the slices in 24-well plates containing PBS at 4°C. To visualize c-Fos and ΔFosB, floating sections were washed 3x for 5 minutes in PBS and blocked for 2h in 0.2% Triton-X 100/0.01 M PBS (PBS-T) containing 5% (v/v) normal goat serum. We incubated sections overnight at 4°C with a primary c-Fos antibody in blocking buffer (1:5000, rabbit anti-c-Fos, Synaptic Systems, 226 003), a

primary FosB/ΔFosB antibody (1:2000, rabbit anti-FosB, Abcam, ab184938), or a primary TPH2 antibody (1:500, rabbit anti-TPH2, Abcam, ab184505). Unlike the stable ΔFosB isoform, stimulus-induced FosB is degraded by 18 hr. Thus, all cells labeled with the pan-FosB antibody were considered to reflect ΔFosB (McHenry et al., 2016; Perrotti et al., 2004). The following morning, sections were washed 3x for 10 minutes in PBS-T, then incubated with AlexaFluor 488-conjugated goat anti-rabbit secondary antibody (1:300, Thermo Fisher Scientific, A-11034) for 1h at room temperature. Sections were washed again 3x for 10 minutes in PBS-T, floated onto clear slides, and mounted and coverslipped using Vectashield with DAPI (Vector Labs).

5.2.7 Fos analysis. To quantify c-Fos$^+$ immunoreactive nuclei, sections containing the regions of interest (ROI) were imaged on an Olympus VS120 Virtual Slide microscope or a Zeiss AxioImager Z1 microscope (AxioCam MRm3) at the Research Institute of St. Joe's, Hamilton. All groups were imaged using identical scanning parameters (gain, exposure time) and laser power. Using ImageJ Fiji (Rueden et al., 2017; Schindelin et al., 2012), images were converted to 16-bit, background was subtracted, and ROIs were traced using recognizable landmarks from The Mouse Brain Atlas, fourth edition (Paxinos & Franklin, 2004): nucleus accumbens (NAc; approximate bregma AP +0.97 mm), bed nucleus of the stria terminalis (BNST; approximate bregma AP +0.01 mm), paraventricular nucleus of the hypothalamus (PVH; approximate bregma AP -0.82 mm), paraventricular nucleus of the thalamus (PVT; approximate bregma AP -1.43 mm), dorsal hippocampus (dHpc; approximate bregma AP -1.67 mm), basolateral and central amygdala (BLA, CeA; approximate bregma AP -1.67 mm), ventral hippocampus (vHpc; approximate bregma AP -3.15 mm), periaqueductal grey (PAG; approximate bregma

AP -4.23 mm), rostral dorsal raphe nucleus (DRN; approximate bregma AP -4.23 mm), locus coeruleus (LC; approximate bregma AP -5.41 mm), nucleus tractus solitarius (NTS; approximate bregma AP -6.59 mm). Fos immunoreactivity was quantified using automated thresholding and particle analysis using consistent parameters. Fos^+ cell nuclei density was calculated by the number of Fos^+ cells divided by the ROI area (cells/mm^2).

5.2.8 Statistical analysis. Sample sizes were initially determined based on prior work employing similar methodology; no formal techniques were used to predetermine sample sizes. Certain brain areas could not be quantified for specific subjects due to damage incurred during extraction and processing (please consult figure legend for the *n* of each analysed region). In all experiments, animals were randomly assigned to treatment and surgery groups. Data were analyzed in GraphPad Prism 6 using a two-tailed student's *t*-test (or a Mann-Whitney U test when assumptions of a normal distribution were violated), and univariate ANOVA (or Kruskal-Wallis test when assumptions for equality of group variances were violated) followed by Tukey's or Dunn's multiple comparisons test when significant main effects were observed. Data are shown as mean ± standard error except where otherwise indicated. For all analyses, statistical significance was set a $p < 0.05$.

5.3. Results

5.3.1. A single orally administered dose of live bacteria induces c-Fos immunoreactivity throughout the brain.

Given multiple converging lines of evidence demonstrating the influence of intestinal bacteria and gut-brain signalling on neural function and behaviour (Collins et

al., 2012; Cryan & Dinan, 2012; Forsythe et al., 2016), we interrogated the brain regions recruited by acute oral exposure to *Lactobacillus rhamnosus* (JB-1)—a strain that we have previously demonstrated to be physiologically active (Bharwani et al., 2017; Bravo et al., 2011; Perez-Burgos et al., 2014, 2013). Male BALB/c mice were orally administered a single dose of either the live or heat-killed bacteria and were perfused 165 minutes later in order to test for expression of c-Fos—an immediate early gene that is a secondary marker of *in vivo* neuronal activity (Morgan & Curran, 1991). Seven of the 16 regions examined exhibited robust c-Fos expression only in response to the live but not heat-killed bacteria or saline. Presented from rostral to caudal, this included the BLA and CeA at AP level -1.67 mm, which showed an overall main effect of treatment (Fig. 1A: BLA, $F_{(2,18)}$= 6.616, p= 0.007; Fig.1B: CeA, $F_{(2,18)}$= 16.5, p< 0.0001). Tukey post-hoc comparisons revealed that both the BLA (p < 0.05) and CeA (p < 0.001) show a significantly greater response to treatment with the live versus the heat-killed bacteria. In the vHpc (AP -3.15 mm), there was a significant main effect of treatment in both, the ventral CA1 (Fig. 1C: vCA1, $F_{(2,18)}$= 5.769, p= 0.0116) and the ventral CA3 (Fig. 1D: vCA3, $F_{(2,18)}$= 4.756, p= 0.022). The vCA1 showed significantly elevated c-Fos expression in response to the live bacteria only, relative to administration of the heat-killed strain (Tukey's multiple comparisons test, p < 0.05). In the vCA3, relative to saline treatment, there was a significant increase in c-Fos$^+$ nuclei density in mice that were administered the live bacteria (Tukey's multiple comparisons test, p < 0.05), but no statistically significant difference between live and heat-killed treatment groups. Examination of the PAG and DRN, both at bregma AP -4.23 mm, revealed a significant effect of treatment (Fig. 1E: PAG, $F_{(2,18)}$= 12.33, p= 0.0004; Fig. 1F-H: DRN, $F_{(2,18)}$=

9.602, p= 0.0015). Both regions showed significantly elevated c-Fos expression in response to administration of the live bacteria relative to the heat-killed strain (p< 0.01). Similarly, there was a main effect of treatment in the LC (Fig. 1I: LC, $F_{(2,18)}$= 9.542, p= 0.0015), with mice that were administered the live bacteria showing significantly greater c-Fos expression relative to saline (p< 0.05) and heat-killed treatment groups (p< 0.01). Of the 18 regions examined, only the PVT exhibited a significant c-Fos response to administration of both, live and heat-killed JB-1 (Fig. 1J: PVT, $F_{(2,18)}$= 5.164, p= 0.0169) relative to saline treatment (Tukey's multiple comparison test; live JB-1 vs saline, p< 0.05; heat-killed JB-1 vs saline, p< 0.05; live vs heat-killed JB-1, p> 0.05).

In contrast, eight of the 16 regions exhibited no significant c-Fos response to either the live or heat-killed bacteria at 165 minutes following treatment. This included the NAc at bregma AP +0.97 mm (Fig. 2A: NAc, $H_{(2,18)}$= 4.275, p= 0.1183); the BNST at bregma AP +0.01 mm (Fig. 2B: BNST, $F_{(2,18)}$= 1.832, p= 0.1888); the PVH at bregma AP -0.82 mm (Fig. 2C: PVH, $F_{(2,18)}$= 0.0830, p= 0.9207); the dorsal CA1, CA3, and dentate gyrus at bregma AP -1.67 mm (Fig. 2D: dCA1, no c-Fos detected; Fig. 2E: dCA3, $F_{(2,18)}$= 0.0021, p= 0.9979; Fig. 2F: dDG, $F_{(2,18)}$= 1.073, p= 0.3627); the ventral dentate gyrus at bregma AP -3.15 mm (Fig. 2G: vDG, $F_{(2,18)}$= 0.8120, p= 0.4596); and the nucleus tractus solitarius at bregma AP -6.59 mm (Fig. 2H: NTS, $F_{(2,17)}$= 1.405, p= 0.2724). Taken together, these data demonstrate that oral administration of a specific bacterial strain is sufficient to activate multiple regions throughout the brain within 165 minutes, and indicate the specific nature of this signalling given the limited response to administration of heat-killed bacteria of the same strain.

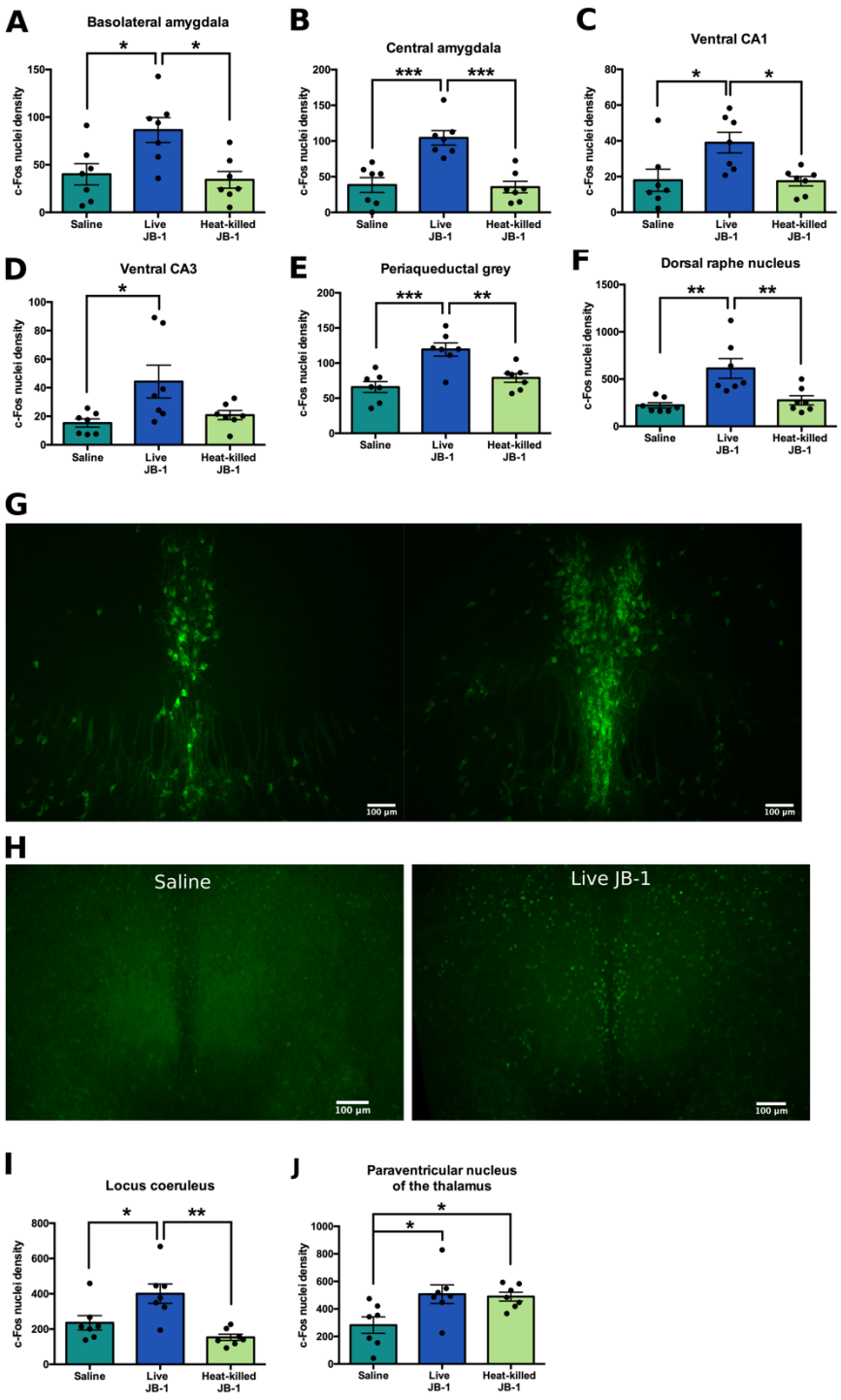

Figure 1. A single oral dose of live bacteria causes an increase in c-Fos expression in specific regions. (A-F, I-J) c-Fos$^+$ cell density (cells/mm^2), quantified 165 minutes following administration of saline, live JB-1, or heat-killed JB-1 (n=7 for all groups). **(G)** Images of tryptophan hydroxylase 2 staining in the DRN at locations representative of c-Fos quantification. **(H)** Representative images of c-Fos staining in the DRN * p<0.05, ** p<0.01, ***p<0.001.

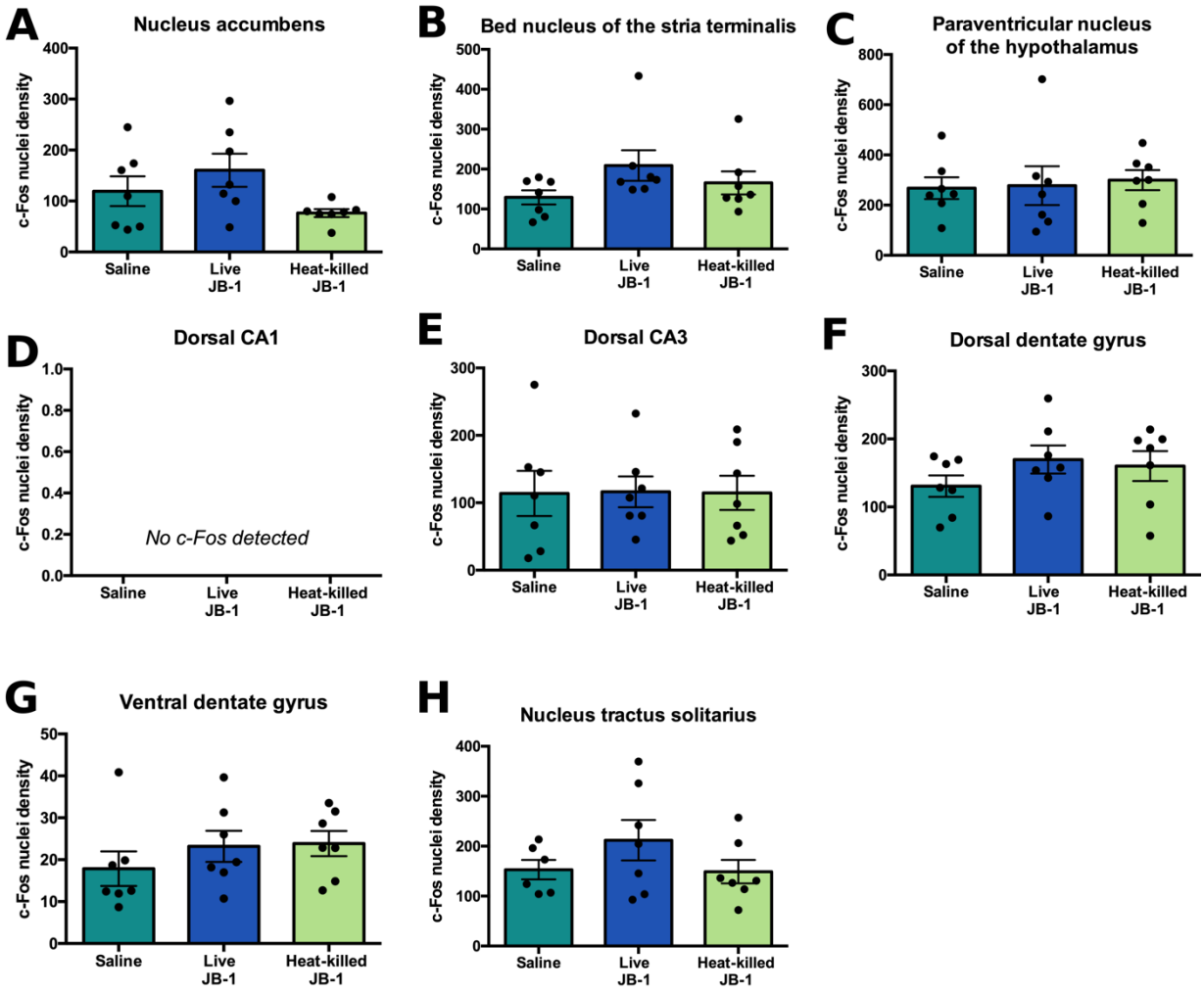

Figure 2. A single oral dose of live bacteria does not affect c-Fos expression in certain regions. c-Fos^{+} cell density (cells/mm^{2}), quantified 165 minutes following administration of saline, live JB-1, or heat-killed JB-1. There were no significant differences in c-Fos levels between treatment groups **(A-H)**. n=7 for all groups, with the exception of the NTS of the saline control group (n=6) **(H)**, due to damage incurred during dissection and tissue processing.

5.3.2. Chronic but not acute bacterial treatment alters behaviour in the tail suspension test.

Given that chronic treatment with JB-1 alters anxiety- and depression-like behaviours in mice (Bharwani et al., 2017; Bravo et al., 2011), and that a single administration of the bacteria increased neuronal activity in various brain regions (Fig. 1), we examined whether acute administration was also associated with behavioural changes. To test this, a separate group of mice were administered either saline or the live bacteria at 165-minutes preceding the tail suspension test—a model for assessing depression-like behaviour (Cryan, Mombereau, & Vassout, 2005). There were no significant differences in time spent immobile between treatment groups (Fig. 3A; $t_{(18)}$= 0.5727, p= 0.5738). Following chronic treatment however, mice treated with JB-1 exhibited reduce time spent immobile (Fig. 3B; $t_{(20)}$= 2.161, p= 0.0430), in accordance with changes in depression-like behaviour during the forced swim test described in previous work (Bravo et al., 2011). These data suggest acute bacterial treatment is insufficient to alter behaviour within 165 minutes of administration, despite widespread neuronal activity.

5.3.3. Live and heat-killed bacteria facilitate firing of vagal afferent fibres.

We have previously demonstrated that live JB-1 increases the firing of vagal afferents via a nicotinic synapse (Perez-Burgos et al., 2014, 2013), and that an intact vagus nerve is necessary for the effects of chronic JB-1 treatment on neural and behavioural changes (Bravo et al., 2011). Given the observation that heat-killed JB-1 increased neuronal activity in the PVT (Fig. 1G), we wondered whether the effects of the heat-killed bacteria could also be mediated, at least partly, by vagal signalling. To

investigate this, we recorded afferent vagal fibre activity from the mesenteric nerve bundle after adding either the live or heat-killed bacteria directly to the gut lumen in separate preparations, at equivalent doses to the oral treatment. Exposure to either treatment significantly decreased the interval between spikes (interspike interval), indicating increased vagal firing (Fig. 3C: live JB-1, $t_{(31)}$=4.606, p< 0.0001; Fig. 3D: heat-killed JB-1, $t_{(29)}$= 4.173, p= 0.0002). Comparison of treatments showed no significant difference in the change in interspike interval between treatment groups (Fig. 3E; U= 420, p= 0.6671); however, there was a significant difference in the variance between treatment groups ($F_{(28,30)}$= 4.507, p= 0.0001). Next, we measured vagal fibre activity in mice that were orally administered saline, live, or heat-killed JB-1. Only vagal fibres in the live bacterial treatment group exhibited significantly lower interspike interval 165 minutes following treatment, indicating increased vagal activity relative to saline treatment (Fig. 3F; ; $F_{(2,86)}$= 6.113, p= 0.0033; Tukey's multiple comparison test, saline versus live JB-1 groups, p< 0.01, saline versus heat-killed JB-1 groups, p> 0.05). These data suggest that while both live and heat-killed JB-1 facilitate vagal firing, there may be differences in the pattern and responsivity of individual vagal fibres, which may account for differences in the c-Fos response.

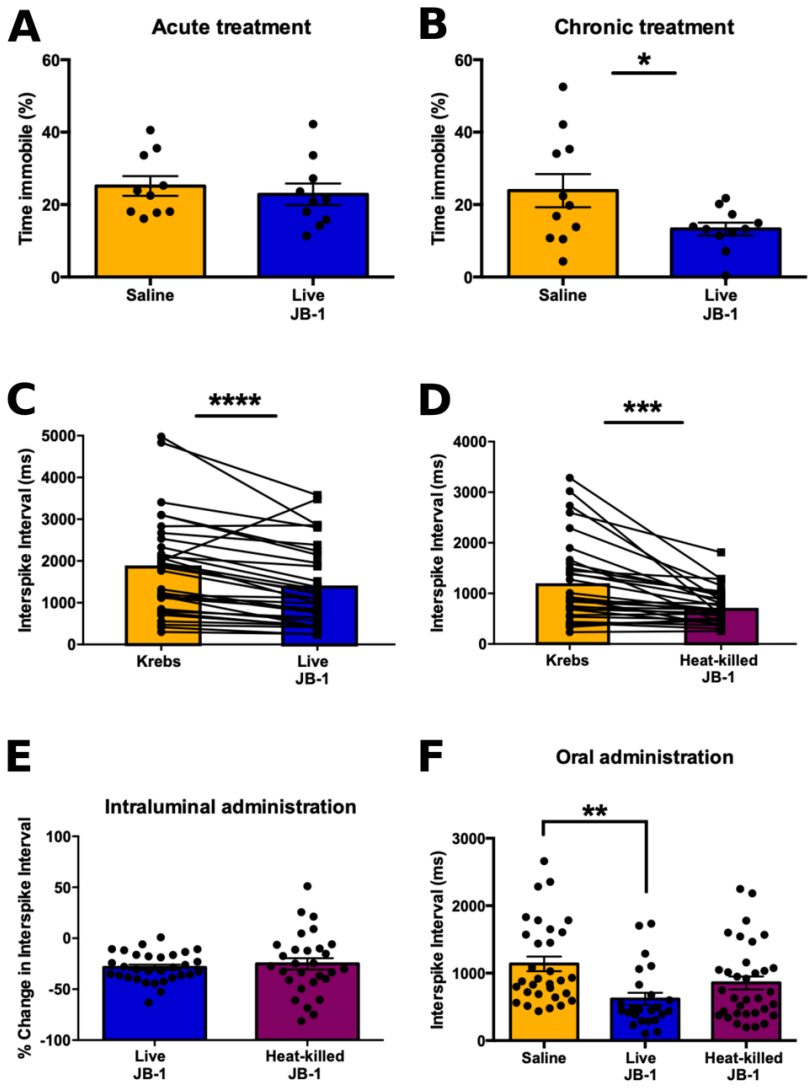

Figure 3. Effect of bacteria exposure on behaviour and mesenteric vagal afferent fibre activity. (A) Percentage of time spent immobile during a six-minute tail suspension test, measured 165 minutes following treatment administration. **(B)** Percentage of time spent immobile during a six-minute tail suspension test, measured following chronic bacterial treatment. **(C & D)** Change in time interval between vagal spike firing following direct, acute exposure of the jejunal lumen to **(C)** the live (n=5 animals, 32 fibres) or **(D)** heat-killed bacteria (n=5 animals, 30 fibers) at equivalent dose to the oral treatment. **(E)** Percentage change in time interval between vagal spike firing in jejunal segments directly exposed to live versus heat-killed bacteria treatment. **(F)** Change in time interval between vagal spike firing in jejunal segments, measured 165 minutes following oral administration of saline (n=5 animals, 31 fibers), live (n=5 animals, 24 fibers), or heat-killed bacteria (n= 5 animals, 34 fibers). * $p<0.05$, ** $p<0.01$, ***$p<0.001$

5.3.4. Sub-diaphragmatic vagotomy prevents c-Fos immunoreactivity in certain brain regions.

We have previously shown that the vagus nerve is necessary for the effects of JB-1 on anxiety- and depression-like behaviours, and on the expression of GABA receptors in the brain (Bravo et al., 2011). However, several pathways have been proposed to mediate gut-brain signalling (Collins et al., 2012), and it is unclear whether any of these are recruited in addition to the vagus nerve to transmit JB-1-derived signals to the brain. Thus, we examined whether severing the vagus completely abolished JB-1-induced c-Fos immunoreactivity in the aforementioned regions (Fig. 1). Mice that underwent a sub-diaphragmatic vagotomy (Vx) or sham surgery were orally administered the live bacteria or saline to measure c-Fos expression in the brain after 165 minutes. In the amygdala, Vx prevented the effects of JB-1 on c-Fos expression in the BLA (Fig. 4A; $F_{(2,18)}$= 5.785, p= 0.0115; Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, p< 0.05, Vx JB-1 mice versus sham saline mice, p> 0.05) and in the CeA (Fig. 4B; $F_{(2,18)}$= 9.643, p= 0.0014; Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, p< 0.05, Vx JB-1 mice versus sham saline mice, p> 0.05). In the PVT, Vx animals treated with the live bacteria failed to show an increase in c-Fos levels (Fig. 4C; $F_{(2,18)}$= 8.062, p= 0.0032; Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, p< 0.05, Vx JB-1 mice versus sham saline mice, p> 0.05). Similar effects were observed in the PAG (Fig. 4D; $F_{(2,18)}$= 4.333, p= 0.0291; Tukey's multiple comparison test, Vx JB-1 mice versus mice sham saline mice, p> 0.05) and the LC (Fig. 4E; $F_{(2,18)}$= 19.37, p< 0.00001;

Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, $p< 0.001$, Vx JB-1 mice versus sham saline mice, $p> 0.05$).

In contrast, analysis of c-Fos expression in the ventral hippocampus revealed that severing the vagus did not prevent an increase in c-Fos$^+$ nuclei density in the ventral CA3 (Fig. 4F; $F_{(2,18)}= 10.80$, $p= 0.0008$; Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, $p> 0.05$, Vx JB-1 mice versus sham saline mice, $p< 0.05$) and in the ventral CA1 (Fig. 4G; $F_{(2,18)}= 4.493$, $p= 0.0261$), although post-hoc comparisons in the vCA1 failed to show a significant difference between Vx mice administered the live bacteria and sham surgery mice administered saline (Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, $p> 0.05$, Vx JB-1 mice versus sham saline mice, $p> 0.05$). In the DRN, both sham and Vx groups administered the live bacteria showed significantly increased c-Fos immunoreactivity (Fig. 4H; $F_{(2,18)}= 5.604$, $p= 0.0128$; Tukey's multiple comparison test, JB-1 treatment in sham versus Vx groups, $p> 0.05$, Vx JB-1 mice versus sham saline mice, $p< 0.05$). These observations suggest that while signals to certain regions of the brain are entirely vagal-dependent, other regions may receive signals via additional vagal-independent pathways.

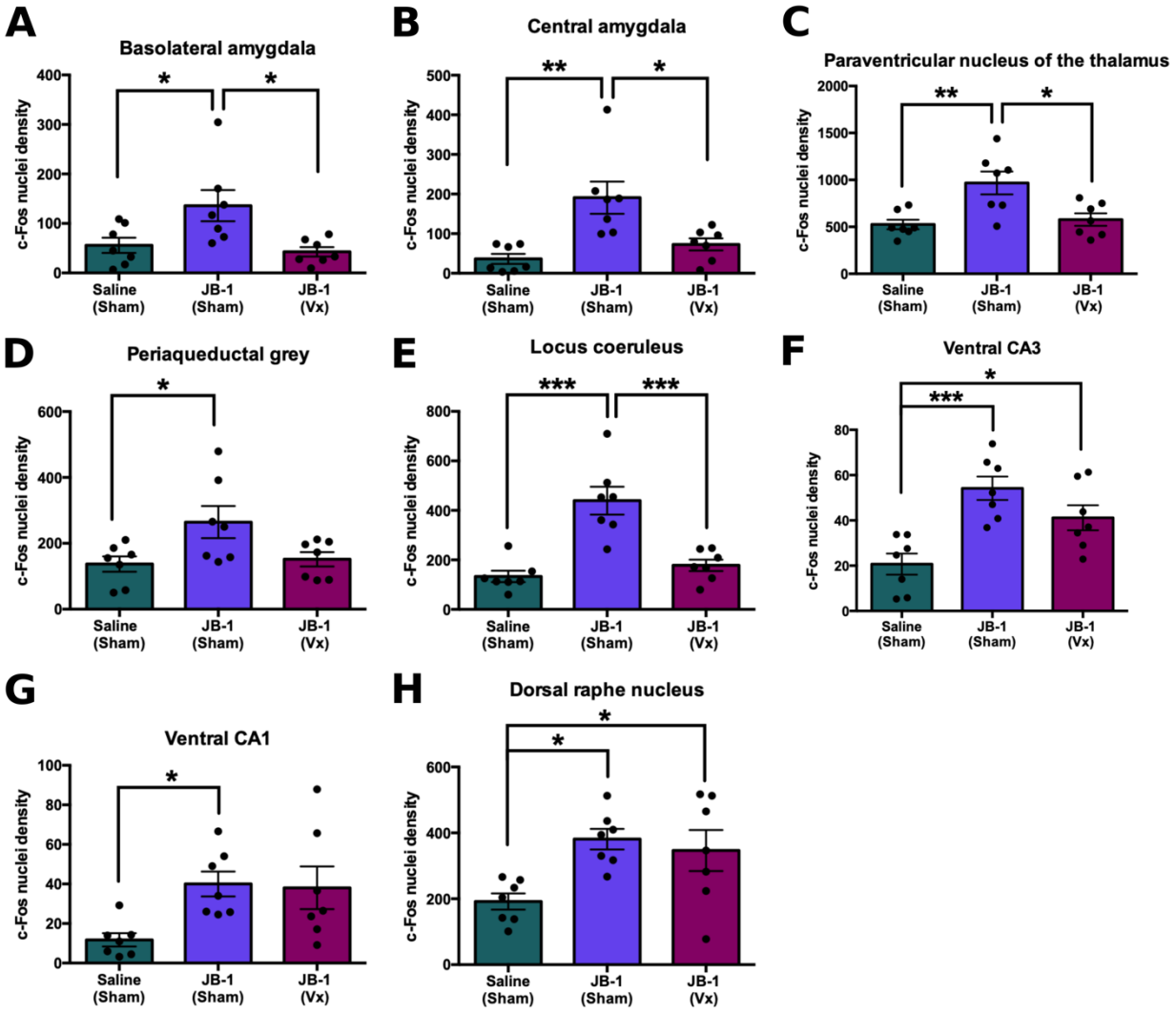

Figure 4. Effect of vagotomy on JB-1-induced c-Fos expression. Mean ± SEM c-Fos$^+$ cell density (cells/mm^2), quantified 165 minutes following administration of saline or live JB-1 to mice that underwent sham surgery or vagotomy (n=7 for all groups and regions). In all regions **(A-H)**, there was an overall main effect of treatment. Tukey post-hoc comparisons revealed that in most regions **(A-E)**, severing the vagus abolished JB-1-induced c-Fos expression. In certain regions however, severing the vagus did not affect c-Fos levels: in the vCA3 **(F)** and DRN **(H)**. In both regions, c-Fos levels in vagotomised animals administered the live bacteria were significantly greater than sham surgery mice administered saline, and no significantly different from sham surgery mice administered the live bacteria. * p<0.05, ** p<0.01, ***p<0.001.

5.3.5. Chronic but not acute bacteria administration induces ΔFosB expression in distinct regions.

ΔFosB (deltaFosB) is a member of the Fos family of transcription factors that is unique in its properties that allow it to accumulate in the brain in response to chronic drug administration, chronic stress exposure, and repeated natural rewards, reflecting long-term neural adaptations (Mcclung et al., 2004; Nestler et al., 2001). Given that only chronic and not acute JB-1 treatment engenders behavioural changes (Fig. 3A; Bravo et al., 2011), we investigated the pattern of ΔFosB expression following acute versus chronic bacteria treatment.

Chronic bacteria administration induced ΔFosB expression in distinct brain regions, unlike acute bacteria and saline treatment. In the BNST, there was an overall main effect of treatment (Fig. 5A; BNST, H= 7.311, p= 0.0259). Post-hoc comparisons revealed significantly greater ΔFosB$^+$ nuclei density in chronic treated mice relative to the acute treatment group (Dunn's multiple comparison test, p<0.05). Similar effects were observed in the dorsal CA1 and dorsal CA3 (Fig. 5B: dCA1, $F_{(2,18)}$= 5.463, p= 0.0140; Fig. 5C: dCA3, $F_{(2,18)}$= 7.786, p= 0.0037). Chronically treated mice exhibited greater ΔFosB levels relative to the saline treatment group in the dCA1 (p< 0.05), and relative to both acute (p< 0.05) and the saline treatment groups (p< 0.01) in the dCA3. In the PVT, significantly greater ΔFosB levels were observed following chronic treatment (Fig. 5D: PVT, $F_{(2,18)}$= 19.35, p< 0.0001; Tukey's multiple comparison test, p< 0.001 relative to acute and saline treatment groups). In the ventral hippocampus, increased ΔFosB expression was observed in the ventral DG (Fig. 5E: vDG, $F_{(2,18)}$= 4.633, p= 0.0238; Tukey's multiple comparison test, p< 0.05 relative to acute and saline

treatment), vCA1 (Fig. 5F & 5G: vCA1, $F_{(2,18)}$= 9.452, p= 0.0016; Tukey's multiple comparison test, p< 0.01 relative to acute treatment and p< 0.05 relative to saline treatment), and vCA3 (Fig. 5H: vCA3, $F_{(2,18)}$= 11.21, p= 0.0007; Tukey's multiple comparison test, p< 0.001 relative to acute treatment and p< 0.05 relative to saline treatment). Finally, similar effects of chronic treatment were observed on ΔFosB levels were observed in the PAG (Fig. 5I: PAG, $F_{(2,18)}$= 8.881, p= 0.0021; Tukey's multiple comparison test, p< 0.01 relative to acute and saline treatment), the DRN (Fig. 5J: DRN, H= 13.45, p= 0.0012; Dunn's multiple comparison test, p< 0.01 relative to acute and saline treatment), and the LC (Fig. 5K: LC, $F_{(2,18)}$= 13.78, p= 0.0002; Tukey's multiple comparison test, p< 0.001 relative to acute treatment and p< 0.01 saline treatment).

In contrast, there was no effect of bacteria treatment on $\Delta FosB^{+}$ nuclei density in the NAc (Fig. 6A; NAc, $F_{(2,18)}$= 2.811, p= 0.0866); the PVH (Fig. 6B; PVH, $F_{(2,16)}$= 2.537, p= 0.1104); the dorsal DG (Fig. 6C; dDG, $F_{(2,18)}$= 2.548, p= 0.1061); the BLA (Fig. 6D; BLA, $F_{(2,17)}$= 2.864, p= 0.0848); and the CeA (Fig. 6E; CeA, $F_{(2,17)}$= 2.945, p= 0.7487).

Together, these data demonstrate that long-term oral treatment with a specific bacterial strain is necessary to induce ΔFosB expression in distinct regions throughout the brain.

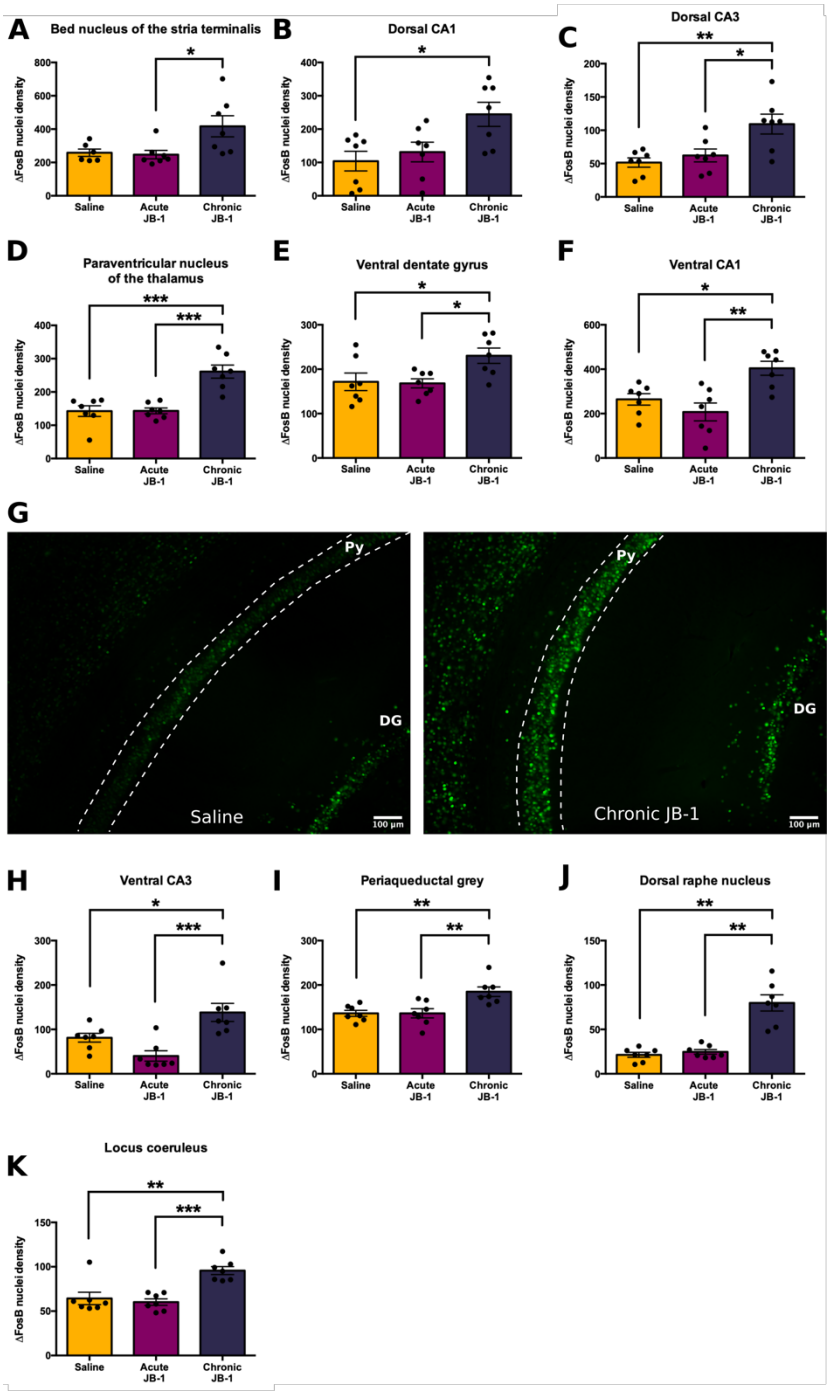

Figure 5. Chronic administration of JB-1 over two weeks induces ΔFosB expression in specific regions. (A-F, H-K) $\Delta FosB^+$ cell density (cells/mm^2), quantified 20-24h following two weeks of treatment (n=7 for all groups, with the exception of the BNST of the saline control group (n=6) **(A)**, due to damage incurred during dissection and tissue processing). **(H)** Representative images of ΔFosB staining in the vHpc * $p<0.05$, ** $p<0.01$, ***$p<0.001$. DG: dentate gyrus; Py: pyramidal cell layer, CA1.

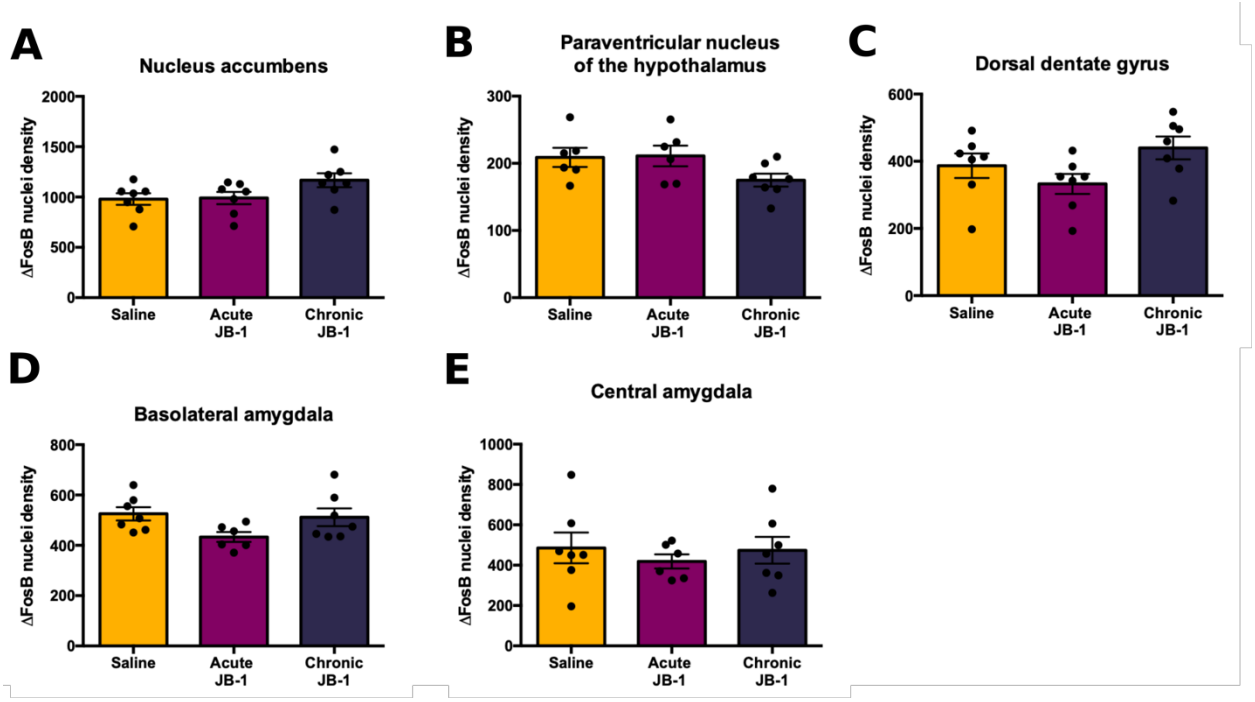

Figure 6. Chronic administration of JB-1 over two weeks does not affect ΔFosB expression in specific regions. **(A-E)** ΔFosB $^{+}$ cell density (cells/mm^2), quantified 20-24h following two weeks of treatment (n=7 for all groups, with the exception of the PVH of the saline and acute treatment group, and the BLA and CeA of the acute treatment group, due to damage incurred during dissection and tissue processing). There were no significant differences in ΔFosB levels between groups.

5.4. Discussion

There exist multiple lines of evidence demonstrating the influence of intestinal bacteria and associated signals on the CNS and behaviour, via a proposed 'gut-brain axis' (Collins et al., 2012; Cryan & Dinan, 2012; Forsythe et al., 2016). However, very little is known about the brain regions that are recruited by this form of signalling, the changes in the response pattern following acute versus chronic exposure to such signals, and the pathways that mediate these interactions. Here we show that oral administration of a specific bacterial strain induces expression of Fos gene products throughout the brain, within 165 minutes, and that the specific pattern of expression and

behavioural effects differ between acute and chronic treatment. Although both live and heat-killed bacteria promote vagal firing, the effects on neuronal activity, measured here by c-Fos density, are mostly limited to the live bacteria. Additionally, severing the vagus nerve prevented c-Fos expression in many but not all previously responsive brain regions, suggesting a critical role for the vagus but indicating the presence of additional signalling pathways.

Within 165 minutes, oral administration of the live bacteria increased expression of c-Fos in the BLA, CeA, PVT, vCA1, vCA3, PAG, DRN, and LC (Fig. 1). Administration of heat-killed bacteria only increased c-Fos expression in the PVT. We chose the 165-minute time-point given that transit time to the mouse stomach and small intestine is ~1 hour (Padmanabhan et al., 2013; Wehner et al., 2014), both of which are heavily innervated by the vagus nerve (F. Bin Wang & Powley, 2000), and that c-Fos immunoreactivity in the brain peaks between 1-2 hours following acute drug exposure (Jung et al., 2014; Nestler et al., 2001). While the data herein does not necessarily suggest that all of these distributed brain areas are integral to the effects of the bacteria, it does align with previous works outlining regions that receive direct or indirect projections from the vagus nerve (Groves & Brown, 2005; Han et al., 2018), and those that are affected by vagal nerve stimulation (VNS)—an approved and successful therapy in the treatment of refractory depression (Aaronson, Sears, Ruvuna, Bunker, et al., 2017; Chae et al., 2003; Cunningham, Mifflin, Gould, & Frazer, 2008; Furmaga, Sadhu, & Frazer, 2012; Naritoku, Terry, & Helfert, 1995; Nemeroff et al., 2006). This is particularly relevant given the critical role of the vagus in mediating the behavioural effects of this particular bacterial strain (Bravo et al., 2011; Perez-Burgos et al., 2014).

These observations are further underlined by studies showing that the regions exhibiting a c-Fos response to bacterial treatment and VNS also play a role in stress and mood-related processes, including the ventral hippocampus (Anacker et al., 2018; Bagot et al., 2015; Jimenez et al., 2018; Padilla-coreano et al., 2016), the paraventricular nucleus of the thalamus (Beas et al., 2018), and the locus coeruleus (Mccall et al., 2017). Surprisingly, in the acute treatment experiments we did not observe increased c-Fos expression in the NTS, which is innervated by fibres from the nodose ganglia—a collection of the cell bodies of afferent vagal fibres (Foley & DuBois, 1937; Nemeroff et al., 2006). This may have been a result of the chosen timepoint in these experiments. It is also possible this was due to saline-induced distention of the gut, which also increases single- and multi-unit firing of mesenteric vagal afferents (Perez-Burgos et al., 2013) and consequently c-Fos expression in the NTS (Jung et al., 2014; Schwarz et al., 2010), thus obscuring any potential differences between treatment groups; or perhaps, cell groups in the NTS that maximally respond to gut-derived vagal are located more caudally in the brainstem (Han et al., 2018; Schwarz et al., 2010).

Severing vagal fibres below the diaphragm abolished acute JB-1-induced c-Fos expression in the BLA, CeA, PVT, PAG, and the LC, while sparing c-Fos induction in the vHpc and DRN (Fig. 4). While we have shown that sub-diaphragmatic vagotomy abolishes the effects of JB-1 on anxiety- and depression-like behaviours (Bravo et al., 2011), it was unclear if severing the vagus would also abolish the acute c-Fos response to bacterial treatment, or whether there exist additional pathways that transmit bacteria-derived signals, but which alone remain insufficient to drive behavioural changes. The data here demonstrate that vagotomy does indeed abolish the c-Fos response in certain

brain regions, while others continue to respond to bacterial administration in the absence of an intact vagus nerve. Bacteria-induced c-Fos expression was no longer observed in regions that receive vagal afferents and are also activated by VNS: BLA, CeA, PVT, and LC (Chae et al., 2003; Cunningham et al., 2008; Han et al., 2018; Naritoku et al., 1995). In contrast, the DRN and vHpc continued to exhibit elevated c-Fos levels following treatment in vagotomised mice. While both regions receive visceral afferents from the NTS, either through direct projections or via a multi-synaptic pathway (Berthoud & Neuhuber, 2000; Castle et al., 2005; Peyron, Luppi, Fort, Rampon, & Jouvet, 1996), neither exhibits a c-Fos response to vagal nerve stimulation as well (Cunningham et al., 2008; Naritoku et al., 1995). This further implicates a role for vagal-independent pathways in mediating JB-1-related signals to these regions, at least in acute settings. Putative signalling pathways include spinal afferent fibres, the activity of which can be modulated through distinct mechanisms by different bacterial strains (Kamiya et al., 2006; Perez-Burgos et al., 2015). Additional and alternative pathways include various soluble signalling mediators such as cytokines, short-chain fatty acids, and bacteria- and host-derived metabolites and humoural factors (Forsythe et al., 2016). Future work should examine which of these pathways are readily recruited within the timeframe described in this study. Bacteria also form and shed microvesicles, which contain a variety of molecules, including DNA and RNA (Dorward et al., 1989). In the case of JB-1, its microvesicles can replicate the immune and enteric effects of the parent bacteria, resulting in increased levels of regulatory T cells and IL-10^{+} dendritic cells, as well as increased firing of intrinsic primary afferent neurons (Al-Nedawi et al., 2014). The latter phenomenon is likely mediated via the intestinal epithelium given the

absence of effects when microvesicles were placed in direct contact with myenteric plexus neurons, but present when placed on the apical surface of the epithelium.

Chronic but not acute bacterial treatment altered behaviour during the TST (Fig. 3A-B) and increased ΔFosB expression in distinct regions throughout the brain (Fig. 5). Although previous studies have demonstrated induction of ΔFosB in response to acute stimulation (Mcclung et al., 2004; Nestler et al., 2001; Perrotti et al., 2004), we did not observe any such response following administration of a single bacterial dose. Of the ten regions characterized by greater ΔFosB levels following chronic treatment, some also exhibited robust c-Fos levels following acute treatment (Fig. 1), while others demonstrated no such response (Fig. 2: BNST, dorsal CA1 and CA3). Conversely, chronic treatment had no effect on ΔFosB levels in the BLA and CeA, which previously demonstrated treatment-induced c-Fos expression, suggesting that despite an acute response, not all regions may undergo long-term adaptations in response to chronic treatment. The targets of FosB proteins have been studied extensively, notably in the NAc and the dorsal striatum, for their role in models of chronic drug use and mood disorders, including regulation of AMPA and NMDA glutamate receptor subunits (Mcclung et al., 2004; Vialou et al., 2010), NF-κB (Ang et al., 2001), dynorphin (Zachariou et al., 2006), and Ca^{2+}/calmodulin-dependent protein kinase II (Robison et al., 2013). While all of the distributed regions exhibiting elevated Fos levels may not be integral to the behavioural effects of the bacteria, the stability of ΔFosB and its response to chronic treatment uniquely position it as a potential mediator of neural changes, and its accumulating levels may thus reflect the presence of long-term adaptations (Mcclung et al., 2004; Nestler, 2015). Therefore, determining the distinct targets of ΔFosB in

different brain regions may be critical to understanding its role in the behavioural changes associated with gut-brain signalling.

Within minutes of application to a jejunal segment, live and heat-killed bacteria increased the firing frequency of afferent vagal fibres (Fig. 3C-E). This change is driven by an increase in the firing of individual fibres rather than an increase in the number of active fibres (Mao et al., 2013; Perez-Burgos et al., 2014, 2013). Following acute oral treatment, increased vagal firing was only seen after administration of live bacteria (Fig. 3F). Additionally, while it did not induce widespread c-Fos immunoreactivity in the CNS, oral administration of the heat-killed bacteria did increase c-Fos expression in the PVT (Fig. 1G), further suggesting that the heat-killed strain is not physiologically inert. This is in line with work demonstrating that although heat-killed JB-1 fails to evoke sensory responses in intrinsic primary afferent neurons or increase their excitability (Mao et al., 2013), it does inhibit the single fibre unit activity of the dorsal root ganglion and reduces visceral pain in a model of colorectal distention (Kamiya et al., 2006). Given that both live and heat-killed bacteria facilitate vagal firing but only the former induces widespread neuronal activity suggests intrinsic differences in signal encoding. This aligns with our data at 165 minutes following oral treatment, where we observed elevated firing frequency of vagal fibres only in mice treated with the live bacteria (Fig. 3F). Thus, it is possible these differences in signal encoding exist either in the form and pattern of the elicited neuronal spike trains (Furness, Kunze, & Clerc, 1999), or via additional signals provided by complementary pathways, as discussed above.

This study demonstrates that the CNS responds rapidly to a single oral administration of a specific bacterial strain. The widespread CNS responses are limited

to the live bacteria, and there is a differential pattern of Fos expression following acute versus chronic administration. Severing the vagus nerve prevents the acute response to bacterial treatment in certain regions that receive vagal projections. Given that vagus nerve-mediated signalling is critical for the neural and behavioural effects of this bacteria (Bravo et al., 2011) and other bacteria (Bercik et al., 2011), some of these regions that are no longer responsive following vagotomy likely play a critical role in mediating the observed behavioural changes. Based on these data, we also propose that signals from gut bacteria are relayed by additional, vagal-independent pathways along the gut-brain axis, thus enabling rich interactions between the periphery and the CNS. Future research will need to describe the precise nature of the bacteria-derived signals that trigger responses from these pathways, as well as the gene targets of ΔFosB in such models in order to understand the molecular underpinnings of gut-brain signalling.

5.5. References

Aaronson, S. T., Sears, P., Ruvuna, F., Bunker, M., Conway, C. R., Dougherty, D. D., … Zajecka, J. M. (2017). A 5-year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: comparison of response, remission, and suicidality. *American Journal of Psychiatry*, *174*(7), 640–648.

Aaronson, S. T., Sears, P., Ruvuna, F., Ph, D., Bunker, M., Pharm, D., & Conway, C. R. (2017). A 5-Year Observational Study of Patients With Treatment-Resistant Depression Treated With Vagus Nerve Stimulation or Treatment as Usual : Comparison of Response , Remission , and Suicidality, (July). https://doi.org/10.1176/appi.ajp.2017.16010034

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., … Tompkins, T. (2014). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology and Motility*, *26*(4), 510–520. https://doi.org/10.1111/nmo.12295

Al-Nedawi, K., Mian, M. F., Hossain, N., Karimi, K., Mao, Y.-K., Forsythe, P., …

Bienenstock, J. (2014). Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 1–12. https://doi.org/10.1096/fj.14-259721

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association.

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., … Hen, R. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, 1. https://doi.org/10.1038/s41586-018-0262-4

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neuroscience and Biobehavioral Reviews*, *51*, 164–188. https://doi.org/10.1016/j.neubiorev.2015.01.018

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E., & Nestler, E. J. (2001). Induction of nuclear factor-κB in nucleus accumbens by chronic cocaine administration. *Journal of Neurochemistry*, *79*(1), 221–224.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., … Hase, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461), 232–236.

Avgustinovich, D. F., Kovalenko, I. L., & Kudryavtseva, N. N. (2005). A model of anxious depression: Persistence of behavioral pathology. *Neuroscience and Behavioral Physiology*, *35*(9), 917–924. https://doi.org/10.1007/s11055-005-0146-6

Baganz, N. L., & Blakely, R. D. (2012). A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chemical Neuroscience*, *4*(1), 48–63.

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., … Schöpf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486–496.

Bagot, R. C., Cates, H. M., Purushothaman, I., Vialou, V., Heller, E. A., Yieh, L., … Nestler, E. J. (2016). Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. *Biological Psychiatry*, *81*(4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012

Bagot, R. C., Parise, E. M., Pen, C. J., Zhang, H., Maze, I., Chaudhury, D., … Nestler, E. J. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms8062

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., & Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology*, *77*(11), 3846–3852.

Beas, B. S., Wright, B. J., Skirzewski, M., Leng, Y., Hyun, J. H., Koita, O., … Penzo, M. A. (2018). The locus coeruleus drives disinhibition in the midline thalamus via a dopaminergic mechanism. *Nature Neuroscience*, *21*(7), 963. https://doi.org/10.1038/s41593-018-0167-4

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., … Pamer, E. G. (2016). Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nature Medicine*, *22*(5), 516.

Bendtsen, K. M. B., Krych, L., Sørensen, D. B., Pang, W., Nielsen, D. S., Josefsen, K., … Hansen, A. K. (2012). Gut Microbiota Composition Is Correlated to Grid Floor Induced Stress and Behavior in the BALB/c Mouse. *PLoS ONE*, *7*(10), e46231. https://doi.org/10.1371/journal.pone.0046231

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., … Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609. https://doi.org/10.1053/j.gastro.2011.04.052

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., … Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and Motility*, *23*(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Bercik, P., Verdu, E. F., Foster, J. a, Macri, J., Potter, M., Huang, X., … Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102-2112.e1. https://doi.org/10.1053/j.gastro.2010.06.063

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience: Basic and Clinical*, *85*(1–3), 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0

Berton, O., Mcclung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress, (February), 864–869.

Berton, O., McClung, C. a, Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science (New York, N.Y.)*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural and functional consequences of chronic psychosocial stress on the microbiome and host. *Psychoneuroendocrinology*, *63*(2016), 217–227. https://doi.org/10.1016/j.psyneuen.2015.10.001

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 Medicine*, *15*(1), 7. https://doi.org/10.1186/s12916-016-0771-7

Blackshaw, L. A., Brookes, S. J. H., Grundy, D., & Schemann, M. (2007). Sensory transmission in the gastrointestinal tract. *Neurogastroenterology & Motility*, *19*, 1–19.

Boer, M. C., Joosten, S. A., & Ottenhoff, T. H. M. (2015). Regulatory T-cells at the interface between human host and pathogens in infectious diseases and

vaccination. *Frontiers in Immunology*, *6*.
Bohórquez, DV, & Shahid, R. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *The Journal of …*, 1–5. https://doi.org/10.1172/JCI78361DS1
Bohórquez, Diego V., Samsa, L. A., Roholt, A., Medicetty, S., Chandra, R., & Liddle, R. A. (2014). An enteroendocrine cell - Enteric glia connection revealed by 3D electron microscopy. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0089881
Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108
Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E., Taylor, C. M., Welsh, D. A., & Berthoud, H.-R. R. (2014). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry*, *77*(7), 607–615. https://doi.org/10.1016/j.biopsych.2014.07.012
Brynskikh, A., Warren, T., Zhu, J., & Kipnis, J. (2008). Adaptive immunity affects learning behavior in mice. *Brain, Behavior, and Immunity*, *22*(6), 861–869. https://doi.org/10.1016/j.bbi.2007.12.008
Buffington, S. A., Viana, G., Prisco, D., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., … Petrosino, J. F. (2016). Microbial Reconstitution Reverses Maternal Diet- Induced Social and Synaptic Deficits in Offspring Article Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell*, *165*(7), 1762–1775. https://doi.org/10.1016/j.cell.2016.06.001
Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856.
Caenepeel, P. H., Janssens, J., Vantrappen, G., Eyssen, H., & Coremans, G. (1989). Interdigestive myoelectric complex in germ-free rats. *Digestive Diseases and Sciences*, *34*(8), 1180–1184.
Cámara, R. J. A., Gander, M.-L., Begré, S., Von Känel, R., & Group, S. I. B. D. C. S. (2011). Post-traumatic stress in Crohn's disease and its association with disease activity. *Frontline Gastroenterology*, *2*(1), 2–9.
Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012). The tail suspension test. *JoVE (Journal of Visualized Experiments)*, (59), e3769.
Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., … Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal 1 – 3, 1236–1243. https://doi.org/10.3945/ajcn.2009.28095.The
Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335
Carle, T. L., Ohnishi, Y. N., Ohnishi, Y. H., Alibhai, I. N., Wilkinson, M. B., Kumar, A., & Nestler, E. J. (2007). Absence of conserved C-terminal degron domain contributes

to ΔFosB's unique stability. *Eur J Neurosci*, *25*, 3009–3019.
Castelli, M. P., Ferraro, L., Mocci, I., Carta, F., Carai, M. A. M., Antonelli, T., … Gessa, G. L. (2003). Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of γ-hydroxybutyric acid. *Journal of Neurochemistry*, *87*(3), 722–732.
Castle, M., Comoli, E., & Loewy, A. D. (2005). Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience*, *134*(2), 657–669. https://doi.org/10.1016/j.neuroscience.2005.04.031
Chae, J., Nahas, Z., Lomarev, M., Denslow, S., Lorberbaum, J. P., Bohning, D. E., & George, M. S. (2003). A review of functional neuroimaging studies of vagus nerve stimulation (VNS), *37*, 443–455. https://doi.org/10.1016/S0022-3956(03)00074-8
Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., & Nestler, E. J. (1997). Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. *Journal of Neuroscience*, *17*(13), 4933–4941.
Chiu, R., Angel, P., & Karin, M. (1989). Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. *Cell*, *59*(6), 979–986. https://doi.org/10.1016/0092-8674(89)90754-X
Chung, L. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. *Development & Reproduction*, *19*(2), 61–67. https://doi.org/10.12717/DR.2015.19.2.061
Chunyu, J. P., Kyle, Z., Darrell, B., Dayanim, G., Bhatnagar, S., Luz, S., & Vigderman, A. S. (2019). The gut microbiome regulates the increases in depressive-type behaviors and in in fl ammatory processes in the ventral hippocampus of stress vulnerable rats. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0380-x
Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., … Cryan, J. F. (2012). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77
Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences*, *103*(27), 10420–10425.
Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, *16*(2), 228–231.
Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The Impact of the Gut Microbiota on Human Health: An Integrative View - 1-s2.0-S0092867412001043-main.pdf. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035
Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*(6), 2003–2014.
Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews. Microbiology*, *10*(11), 735–742. https://doi.org/10.1038/nrmicro2876
Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time.

Science, *326*(5960), 1694–1697.
Crawley, J. N. (1985). Exploratory behavior models of anxiety in mice. *Neuroscience & Biobehavioral Reviews*, *9*(1), 37–44.
Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., … Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207–217. https://doi.org/10.1016/j.psyneuen.2014.01.014
Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712.
Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009
Cunningham, J. T., Mifflin, S. W., Gould, G. G., & Frazer, A. (2008). Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. *Neuropsychopharmacology*, *33*(8), 1884–1895. https://doi.org/10.1038/sj.npp.1301570
Curran, T., & Franza Jr, B. R. (1988). Fos and Jun: the AP-1 connection. *Cell*, *55*(3), 395–397.
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. *Nature Reviews Neuroscience*, *9*(1), 46–56.
David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrotta, A., … Alm, E. J. (2014). Host lifestyle affects human microbiota on daily timescales. *Genome Biol*, *15*(7), R89.
de LeBlanc, A. de M., Dogi, C. A., Galdeano, C. M., Carmuega, E., Weill, R., & Perdigón, G. (2008). Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunology*, *9*(1), 27.
De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, a. J., Green, W., … Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*(August), 7735. https://doi.org/10.1038/ncomms8735
Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65
Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005
Dinan, T G. (2005). Stress: the shared common component in major mental illnesses. *European Psychiatry*, *20*, S326–S328.
Dinan, Timothy G, Quigley, E. M. M., Ahmed, S. M. M., Scully, P., O'Brien, S., O'Mahony, L., … Keeling, P. W. N. (2006). Hypothalamic-pituitary-gut axis

dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*, *130*(2), 304–311.

Dorward, D. W., Garon, C. F., & Judd, R. C. (1989). Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. *Journal of Bacteriology*, *171*(5), 2499–2505.

Duffy, L. C., Zielezny, M. A., Marshall, J. R., Byers, T. E., Weiser, M. M., Phillips, J. F., … Graham, S. (1991). Relevance of major stress events as an indicator of disease activity prevalence in inflammatory bowel disease. *Behavioral Medicine*, *17*(3), 101–110.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.

Eloe-fadrosh, E. A., Brady, A., Crabtree, J., Drabek, E. F., Ma, B., Mahurkar, A., … Fraser, M. (2015). Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption. *MBio*, *6*(2), e00231-15. https://doi.org/10.1128/mBio.00231-15.Editor

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, (October 2014). https://doi.org/10.1038/nn.4030

Erspamer, V. (1966). Occurrence of indolealkylamines in nature. In *5-Hydroxytryptamine and Related Indolealkylamines* (pp. 132–181). Springer.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., … Cani, P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, *110*(22), 9066–9071. https://doi.org/10.1073/pnas.1219451110

Foley, J. O., & DuBois, F. S. (1937). Quantitative studies of the vagus nerve in the cat. I. The ratio of sensory to motor fibers. *Journal of Comparative Neurology*, *67*(1), 49–67.

Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. *Immunological Investigations*, *39*(4–5), 429–448. https://doi.org/10.3109/08820131003667978

Forsythe, P., & Kunze, W. a. (2013). Voices from within: gut microbes and the CNS. *Cellular and Molecular Life Sciences : CMLS*, *70*(1), 55–69. https://doi.org/10.1007/s00018-012-1028-z

Forsythe, P., Kunze, W., & Bienenstock, J. (2016). Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? *BMC Medicine*, *14*(1), 58. https://doi.org/10.1186/s12916-016-0604-8

Forsythe, P., Sudo, N., Dinan, T., Taylor, V. H., & Bienenstock, J. (2010). Mood and gut feelings. *Brain, Behavior, and Immunity*, *24*(1), 9–16. https://doi.org/10.1016/j.bbi.2009.05.058

Forsythe, P., Wang, B., Khambati, I., & Kunze, W. a. (2012). Systemic effects of ingested lactobacillus rhamnosus: Inhibition of mast cell membrane potassium (IKCA) current and degranulation. *PLoS ONE*, *7*(7), 1–8. https://doi.org/10.1371/journal.pone.0041234

Frazer, A., & Benmansour, S. (2002). Delayed pharmacological effects of

antidepressants. *Molecular Psychiatry*, *7*, S23–S28. https://doi.org/10.1038/sj.mp.4001015

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., … Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, *5*, 3611. https://doi.org/10.1038/ncomms4611

Fuchs, D., Möller, A. A., Reibnegger, G., Stöckle, E., Werner, E. R., & Wachter, H. (1990). Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *3*(9), 873–876.

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling : What Happens in Vagus... *Neuron*, *101*, 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Furet, J.-P., Quénée, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology*, *97*(2), 197–207.

Furmaga, H., Sadhu, M., & Frazer, A. (2012). Comparison of FosB Immunoreactivity Induced by Vagal Nerve Stimulation with That Caused by Pharmacologically Diverse Antidepressants. *Journal of Pharmacology and Experimental Therapeutics*, *341*(2), 317–325. https://doi.org/10.1124/jpet.111.188953

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. In *Microbial endocrinology: The microbiota-gut-brain axis in health and disease* (pp. 39–71). Springer.

Furness, J. B., Kunze, W. A. A., & Clerc, N. (1999). II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *277*(5), G922–G928.

Galley, J. D., Yu, Z., Kumar, P., Dowd, S. E., Lyte, M., & Bailey, M. T. (2015). The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut Microbes*, *5*(6), 748–760. https://doi.org/10.4161/19490976.2014.972241

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., … Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515

Gärtner, K. (1990). A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Laboratory Animals*, *24*(1), 71–77.

Gębicki, J., Sysa-Jędrzejowska, A., Adamus, J., Woźniacka, A., Rybak, M., & Zielonka, J. (2003). 1-Methylnicotinamide: a potent anti-inflammatory agent of vitamin origin. *Pol. J. Pharmacol*, *55*, 109–112.

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., … Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*(7610), 94–103.

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: we have never been individuals. *The Quarterly Review of Biology*, *87*(4), 325–341.

Gobaille, S., Schleef, C., Hechler, V., Viry, S., Aunis, D., & Maitre, M. (2002). Gamma-

hydroxybutyrate increases tryptophan availability and potentiates serotonin turnover in rat brain. *Life Sciences*, *70*(18), 2101–2112.

Goehler, L. E., Gaykema, R. P. A., Opitz, N., Reddaway, R., Badr, N., & Lyte, M. (2005). Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with Campylobacter jejuni. *Brain, Behavior, and Immunity*, *19*(4), 334–344. https://doi.org/10.1016/j.bbi.2004.09.002

Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols*, *6*(8), 1183–1191. https://doi.org/10.1038/nprot.2011.361

Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 33–51.

Greenberg, M. E., Greene, L. A., & Ziff, E. B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *Journal of Biological Chemistry*, *260*(26), 14101–14110.

Greenberg, M. E., & Ziff, E. B. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature*, *311*(5985), 433.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., & Roncarolo, M. G. (1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*, *389*(6652), 737–742.

Groves, D. A., & Brown, V. J. (2005). Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neuroscience & Biobehavioral Reviews*, *29*(3), 493–500. https://doi.org/10.1016/j.neubiorev.2005.01.004

Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., … de Araujo, I. E. (2018). A Neural Circuit for Gut-Induced Reward. *Cell*, 1–14. https://doi.org/10.1016/j.cell.2018.08.049

He, B., Xu, W., Santini, P. A., Polydorides, A. D., Chiu, A., Estrella, J., … Plebani, A. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*, *26*(6), 812–826.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., … Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences*, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710.

Heitler, W. J. (2007). DataView: A Tutorial Tool for Data Analysis. Template-based Spike Sorting and Frequency Analysis. *Journal of Undergraduate Neuroscience Education*, *6*(1), A1.

Henningsen, P., Zimmermann, T., & Sattel, H. (2003). Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. *Psychosomatic Medicine*, *65*(4), 528–533.

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., … Warren, B. L. (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proceedings of the National Academy of Sciences*, *111*(52), 18799–18799.

https://doi.org/10.1073/pnas.1423575112
Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Frontiers in Neuroendocrinology*, *14*(3), 173–213. https://doi.org/10.1006/frne.1993.1006
Holzer, P. (2011). Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacology & Therapeutics*, *131*(1), 142–170.
Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., … Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron*, *13*(5), 1235–1244.
Houlden, A., Goldrick, M., Brough, D., Vizi, E. S., Lénárt, N., Martinecz, B., … Denes, A. (2016). Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain, Behavior, and Immunity*, *57*, 10–20.
Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., … Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024
Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female Syrian hamsters. *Hormones and Behavior*, *44*(3), 293–299.
Husebye, E., Hellström, P. M., & Midtvedt, T. (1994). Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. *Digestive Diseases and Sciences*, *39*(5), 946–956.
Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuroimage*, *125*, 988–995.
Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Stanisz, G. J., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *NeuroImage*, *125*(2015), 988–995. https://doi.org/10.1016/j.neuroimage.2015.11.018
Jašarević, E., Howard, C. D., Misic, A. M., Beiting, D. P., & Bale, T. L. (2017). Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. *Scientific Reports*, *7*, 44182.
Jašarević, E., Howard, C. D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., … Bale, T. L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nature Neuroscience*. https://doi.org/10.1038/s41593-018-0182-5
Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., … Kheirbek, M. A. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron*, *0*(0), 1–14. https://doi.org/10.1016/j.neuron.2018.01.016
Jorissen, H. J. M. M., Ulery, P. G., Henry, L., Gourneni, S., Nestler, E. J., & Rudenko, G. (2007). Dimerization and DNA-binding properties of the transcription factor

ΔFosB. *Biochemistry*, *46*(28), 8360–8372.
Jung, H. Y., Kim, W., Yoo, D. Y., Nam, S. M., Kim, J. W., Choi, J. H., … Hwang, I. K. (2014). Intragastric gavage with denatonium benzoate acutely induces neuronal activation in the solitary tract nucleus via the vagal afferent pathway. *Journal of Veterinary Science*, *15*(4), 459–464. https://doi.org/10.4142/jvs.2014.15.4.459
Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B., Montoya, M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, *In Press*. https://doi.org/10.1126/science.aat5236
Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., … Bienenstock, J. (2006). Inhibitory effects of Lactobocillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, *55*(2), 191–196. https://doi.org/10.1136/gut.2005.070987
Karimi, K., Inman, M. D., Bienenstock, J., & Forsythe, P. (2009). Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *American Journal of Respiratory and Critical Care Medicine*, *179*(3), 186–193. https://doi.org/10.1164/rccm.200806-951OC
Karimi, K., Kandiah, N., Chau, J., Bienenstock, J., & Forsythe, P. (2012). A Lactobacillus rhamnosus Strain Induces a Heme Oxygenase Dependent Increase in Foxp3+ Regulatory T Cells. *PLoS ONE*, *7*(10), 1–12. https://doi.org/10.1371/journal.pone.0047556
Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., … Cryan, J. F. (2016). Lost in Translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2016.11.018
Kidd, M., Modlin, I. M., Gustafsson, B. I., Drozdov, I., Hauso, O., & Pfragner, R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *295*(2), G260–G272.
Kinsey, S. G., Bailey, M. T., Sheridan, J. F., Padgett, D. A., & Avitsur, R. (2007). Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. *Brain, Behavior, and Immunity*, *21*(4), 458–466.
Klooker, T. K., Braak, B., Painter, R. C., de Rooij, S. R., van Elburg, R. M., van den Wijngaard, R. M., … Boeckxstaens, G. E. (2009). Exposure to severe wartime conditions in early life is associated with an increased risk of irritable bowel syndrome: a population-based cohort study. *The American Journal of Gastroenterology*, *104*(9), 2250–2256.
König, H., Ponta, H., Rahmsdorf, U., Büscher, M., Schönthal, A., Rahmsdorf, H. J., & Herrlich, P. (1989). Autoregulation of fos: the dyad symmetry element as the major target of repression. *The EMBO Journal*, *8*(9), 2559–2566.
Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, *7*.
Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404.

https://doi.org/10.1016/j.cell.2007.09.018
Kunze, W. A., Mao, Y., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., & Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and Molecular Medicine*, *13*(8b), 2261–2270.
Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. a, … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676
Lay, C., Rigottier-Gois, L., Holmstrøm, K., Rajilic, M., Vaughan, E. E., de Vos, W. M., … Blaut, M. (2005). Colonic microbiota signatures across five northern European countries. *Applied and Environmental Microbiology*, *71*(7), 4153–4155.
Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-amram, H., … Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. https://doi.org/10.1038/ncomms15062
Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, *124*(4), 837–848.
Lindqvist, D., Wolkowitz, O. M., Mellon, S., Yehuda, R., Flory, J. D., Henn-Haase, C., … Neylan, T. C. (2014). Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. *Brain, Behavior, and Immunity*, *42*, 81–88.
Liu, Z.-H., & Smith, C. B. (2009). Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. *Neuroscience Letters*, *454*(1), 62–66.
Lucibello, F. C., Lowag, C., Neuberg, M., & Müller, R. (1989). Trans-repression of the mouse c-fos promoter: A novel mechanism of fos-mediated trans-regulation. *Cell*, *59*(6), 999–1007. https://doi.org/10.1016/0092-8674(89)90756-3
Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, *33*(8), 574–581.
Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathogens*, *9*(11).
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. *Physiology & Behavior*, *89*(3), 350–357. https://doi.org/10.1016/j.physbeh.2006.06.019
Lyte, M., Varcoe, J. J., & Bailey, M. T. (1998). Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiology and Behavior*, *65*(1), 63–68. https://doi.org/10.1016/S0031-9384(98)00145-0
Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system, *4*(June), 1626–1632.
Macpherson, A. J., & Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*, *303*(5664), 1662–1665.
Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*,

19(1), 11–38.
Maitre, M., Humbert, J.-P., Kemmel, V., Aunis, D., & Andriamampandry, C. (2005). [A mechanism for gamma-hydroxybutyrate (GHB) as a drug and a substance of abuse]. *Medecine Sciences: M/S*, *21*(3), 284–289.
Malatynska, E., & Knapp, R. J. (2005). Dominant–submissive behavior as models of mania and depression. *Neuroscience & Biobehavioral Reviews*, *29*(4–5), 715–737.
Malkova, N. V, Collin, Z. Y., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, Behavior, and Immunity*, *26*(4), 607–616.
Mao, Y.-K., Kasper, D. L., Wang, B., Forsythe, P., Bienenstock, J., & Kunze, W. a. (2013). Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.*, *4*, 1465. https://doi.org/10.1038/ncomms2478
Mariat, D., Firmesse, O., Levenez, F., Guimarăes, V., Sokol, H., Doré, J., … Furet, J.-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, *9*, 123. https://doi.org/10.1186/1471-2180-9-123
Mayer, E. A. (2000). The neurobiology of stress and gastrointestinal disease. *Gut*, *47*(6), 861–869.
Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195), 620.
Mccall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., Mcelligott, Z. A., & Stuber, G. D. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior, 1–23. https://doi.org/10.7554/eLife.18247
Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain. *Molecular Brain Research*, *132*(January), 146–154. https://doi.org/10.1016/j.molbrainres.2004.05.014
Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2005). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain, (May 2018). https://doi.org/10.1016/j.molbrainres.2004.05.014
McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine*, *338*(3), 171–179.
McHenry, J. A., Robison, C. L., Bell, G. A., Vialou, V. V, Bolaños-Guzmán, C. A., Nestler, E. J., & Hull, E. M. (2016). The role of ΔfosB in the medial preoptic area: Differential effects of mating and cocaine history. *Behavioral Neuroscience*, *130*(5), 469.
McKinney, W. T., & Bunney, W. E. (1969). Animal model of depression: I. Review of evidence: implications for research. *Archives of General Psychiatry*, *21*(2), 240–248.
McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility*, *25*(2), 183-e88.
Menard, C., Pfau, M. L., Hodes, G. E., Kana, V., Wang, V. X., Bouchard, S., … Russo, S. J. (2017). Social stress induces neurovascular pathology promoting depression. *Nature Neuroscience*, *20*(12), 1752–1760. https://doi.org/10.1038/s41593-017-

0010-3
Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … Cazaubiel, M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition*, *105*(5), 755–764.
Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., … Wolf, S. A. (2016). Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports*, *15*(9), 1945–1956. https://doi.org/10.1016/j.celrep.2016.04.074
Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience*, *14*(1), 421–451.
Müller, R., Bravo, R., Burckhardt, J., & Curran, T. (1984). Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature*, *312*(5996), 716.
Mundorf, M. L., Hochstetler, S. E., & Wightman, R. M. (1999). Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *Journal of Neurochemistry*, *73*(6), 2397–2405.
Murphy, K., & Weaver, C. (2016). *Janeway's immunobiology*. Garland Science.
Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*(3), 695–700.
Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, *148*(1), 257–266.
Naritoku, D. K., Terry, W. J., & Helfert, R. H. (1995). Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Research*, *22*(1), 53–62. https://doi.org/10.1016/0920-1211(95)00035-9
Nemeroff, C. B., Mayberg, H. S., Krahl, S. E., McNamara, J., Frazer, A., Henry, T. R., … Brannan, S. K. (2006). VNS therapy in treatment-resistant depression: Clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology*, *31*(7), 1345–1355. https://doi.org/10.1038/sj.npp.1301082
Nestler, E. J. (2015). Δ FosB : A transcriptional regulator of stress and antidepressant responses. *European Journal of Pharmacology*, *753*, 66–72. https://doi.org/10.1016/j.ejphar.2014.10.034
Nestler, E. J., Barrot, M., & Self, D. W. (2001). ΔFosB: a sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences*, *98*(20), 11042–11046.
Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–265. https://doi.org/10.1111/j.1365-2982.2010.01620.x
Nishino, R., Mikami, K., Takahashi, H., Tomonaga, S., Furuse, M., Hiramoto, T., … Sudo, N. (2013). Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. *Neurogastroenterology & Motility*, *25*(6), 521-e371.

O'Garra, A., Vieira, P. L., Vieira, P., & Goldfeld, A. E. (2004). IL-10–producing and naturally occurring CD4+ Tregs: limiting collateral damage. *The Journal of Clinical Investigation*, *114*(10), 1372–1378.

O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. (2005). Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, L., Mccarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. M. (2005). Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., … Dinan, T. G. (2009a). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., … Dinan, T. G. (2009b). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., & Madsen, K. L. (2013). Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology*, *38*(9), 1738–1747.

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., & Hsiao, E. Y. (2018). The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell*, *173*(7), 1728-1741.e13. https://doi.org/10.1016/j.cell.2018.04.027

Padilla-coreano, N., Bolkan, S. S., Pierce, G. M., Spellman, T. J., Gordon, J. A., Padilla-coreano, N., … Hardin, W. D. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Article Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron*, *89*, 1–10. https://doi.org/10.1016/j.neuron.2016.01.011

Padmanabhan, P., Grosse, J., Asad, A. B. M. A., Radda, G. K., & Golay, X. (2013). Gastrointestinal transit measurements in mice with99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Research*, *3*(1), 1–8. https://doi.org/10.1186/2191-219X-3-60

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, *5*(7), e177.

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society*, *73*(04), 477–489.

Paxinos, G., & Franklin, K. B. J. (2004). *The mouse brain in stereotaxic coordinates*. Gulf professional publishing.

Perez-Burgos, A., Mao, Y.-K., Bienenstock, J., & Kunze, W. a. (2014). The gut-brain axis rewired: adding a functional vagal nicotinic "sensory synapse". *The FASEB*

Journal, *28*(7), 3064–3074. https://doi.org/10.1096/fj.13-245282

Perez-Burgos, A., Wang, B., Mao, Y.-K., Mistry, B., McVey Neufeld, K.-A., Bienenstock, J., & Kunze, W. (2013). Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *304*(2), G211-20. https://doi.org/10.1152/ajpgi.00128.2012

Perez-Burgos, A., Wang, L., McVey Neufeld, K., Mao, Y., Ahmadzai, M., Janssen, L. J., … Kunze, W. A. (2015). The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of Physiology*, *593*(17), 3943–3957.

Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., & Nestler, E. J. (2004). Induction of ΔFosB in reward-related brain structures after chronic stress. *Journal of Neuroscience*, *24*(47), 10594–10602.

Peyron, C., Luppi, P. H., Fort, P., Rampon, C., & Jouvet, M. (1996). Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *Journal of Comparative Neurology*, *364*(3), 402–413. https://doi.org/10.1002/(SICI)1096-9861(19960115)364:3<402::AID-CNE2>3.0.CO;2-8

Phillips, J. G. P. (1910). The Treatment of Melancholia by the Lactic Acid Bacillus. *The British Journal of Psychiatry*, *56*(234), 422-NP. https://doi.org/10.1192/bjp.56.234.422

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Yamada, T. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59.

Ramos, A., & Mormède, P. (1997). Stress and emotionality: a multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, *22*(1), 33–57.

Reber, S. O., Siebler, P. H., Donner, N. C., Morton, J. T., Smith, D. G., Kopelman, J. M., … Lowry, C. A. (2016). Immunization with a heat-killed preparation of the environmental bacterium *Mycobacterium vaccae* promotes stress resilience in mice. *Proceedings of the National Academy of Sciences*, 201600324. https://doi.org/10.1073/pnas.1600324113

Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., … Kashyap, P. C. (2014). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, *29*(4), 1395–1403.

Robison, A. J., Vialou, V., Mazei-Robison, M., Feng, J., Kourrich, S., Collins, M., … Neve, R. (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving ΔFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *Journal of Neuroscience*, *33*(10), 4295–4307.

Rolls, A., Shechter, R., London, A., Ziv, Y., Ronen, A., Levy, R., & Schwartz, M. (2007). Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biology*, *9*(9), 1081.

Rong, W., Hillsley, K., Davis, J. B., Hicks, G., Winchester, W. J., & Grundy, D. (2004). Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *The Journal of Physiology*, *560*(3), 867–881.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., &

Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1), 529.

Sanderson, S., Boardman, W., Ciofi, C., & Gibson, R. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/nature4441021a

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676.

Schönfeld, C.-L., & Trendelenburg, U. (1989). The release of3H-noradrenaline by p-and m-tyramines and-octopamines, and the effect of deuterium substitution in α-position. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *339*(4), 433–440.

Schroeder, F. a, Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry*, *62*(1), 55–64. https://doi.org/10.1016/j.biopsych.2006.06.036

Schütte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J., & Minna, J. (1989). jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, *59*(6), 987–997. https://doi.org/10.1016/0092-8674(89)90755-1

Schwarz, J., Burguet, J., Rampin, O., Fromentin, G., Andrey, P., Tomé, D., … Darcel, N. (2010). Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. *PLoS ONE*, *5*(2), 13–15. https://doi.org/10.1371/journal.pone.0008974

Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, *164*(3), 337–340.

Serretti, A., Calati, R., Goracci, A., Di Simplicio, M., Castrogiovanni, P., & De Ronchi, D. (2010). Antidepressants in healthy subjects: what are the psychotropic/psychological effects? *European Neuropsychopharmacology*, *20*(7), 433–453.

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2018). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron*, 1–14. https://doi.org/10.1016/j.neuron.2018.11.018

Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P. C., & Mazmanian, S. K. (2014). Perspective Specialized Metabolites from the Microbiome in Health and Disease. *Cell Metabolism*, *20*(5), 719–730. https://doi.org/10.1016/j.cmet.2014.10.016

Sharp, F. R., Sagar, S. M., Hicks, K., Lowenstein, D., & Hisanaga, K. (1991). c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. *Journal of Neuroscience*, *11*(8), 2321–2331.

Sibley, C. D., Grinwis, M. E., Field, T. R., Eshaghurshan, C. S., Faria, M. M., Dowd, S. E., … Surette, M. G. (2011). Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PloS One*, *6*(7), e22702.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.-M., … Kolassa, I.-T. (2009). Substantial reduction of naive and regulatory T cells following traumatic stress. *Brain, Behavior, and Immunity*, *23*(8), 1117–1124.

Stam, R., Akkermans, L. M., & Wiegant, V. M. (1997). Trauma and the gut: interactions between stressful experience and intestinal function. *Gut*, *40*(6), 704.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., … Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′Sialyllactose and 6′Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain, Behavior, and Immunity*, *50*, 166–177. https://doi.org/10.1016/j.bbi.2015.06.025

Thompson, J. A., Oliveira, R. A., Djukovic, A., Ubeda, C., & Xavier, K. B. (2015). Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota. *Cell Reports*, 1–11. https://doi.org/10.1016/j.celrep.2015.02.049

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … Mayer, E. a. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401, 1401.e1-4. https://doi.org/10.1053/j.gastro.2013.02.043

Torii, A., Torii, S., Fujiwara, S., Tanaka, H., Inagaki, N., & Nagai, H. (2007). Lactobacillus acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergology International*, *56*(3), 293–301.

Tsuji, M., Suzuki, K., Kinoshita, K., & Fagarasan, S. (2008). Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. In *Seminars in immunology* (Vol. 20, pp. 59–66). Elsevier.

Ulery, P. G., Rudenko, G., & Nestler, E. J. (2006). Regulation of ΔFosB stability by phosphorylation. *Journal of Neuroscience*, *26*(19), 5131–5142.

Umesaki, Y., Okada, Y., Matsumoto, S., Imaoka, A., & Setoyama, H. (1995). Segmented Filamentous Bacteria Are Indigenous Intestinal Bacteria That Activate Intraepithelial Lymphocytes and Induce MHC Class II Molecules and Fucosyl Asialo GM1 Glycolipids on the Small Intestinal Epithelial Cells in the Ex-Germ-Free Mouse. *Microbiology and Immunology*, *39*(8), 555–562.

van der Kleij, H., O'Mahony, C., Shanahan, F., O'Mahony, L., & Bienenstock, J. (2008). Protective effects of Lactobacillus reuteri and Bifidobacterium infantis in murine models for colitis do not involve the vagus nerve. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(4), R1131–R1137.

Vialou, V., Robison, A. J., Laplant, Q. C., Covington, H. E., Dietz, D. M., Ohnishi, Y. N., … Nestler, E. J. (2010). fosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neuroscience*, *13*(6), 745–752. https://doi.org/10.1038/nn.2551

Vialou, V., Thibault, M., Kaska, S., Cooper, S., Gajewski, P., Eagle, A., … Robison, A. J. (2015). Differential induction of FosB isoforms throughout the brain by fluoxetine and chronic stress. *Neuropharmacology*, *99*, 28–37. https://doi.org/10.1016/j.neuropharm.2015.07.005

Wang, B., Mao, Y.-K. K., Diorio, C., Pasyk, M., Wu, R. Y., Bienenstock, J., & Kunze, W. A. (2010). Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *The FASEB Journal*, *24*(10), 4078–4088. https://doi.org/10.1096/fj.09-153841

Wang, F. Bin, & Powley, T. L. (2000). Topographic inventories of vagal afferents in

gastrointestinal muscle. *The Journal of Comparative Neurology*, *421*(3), 302–324. https://doi.org/10.1002/(SICI)1096-9861(20000605)421:3<302::AID-CNE2>3.0.CO;2-N [pii]

Wehner, S., Koscielny, A., Vilz, T. O., Stoffels, B., Engel, D. R., Kurts, C., & Kalff, J. (2014). Measurement of gastrointestinal and colonic transit in mice, 1–9. https://doi.org/10.1038/protex.2011.219

Werner-Felmayer, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G., & Wachter, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells in vitro. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1012*(2), 140–147.

Whelan, F. J., Verschoor, C. P., Stearns, J. C., Rossi, L., Luinstra, K., Loeb, M., … Bowdish, D. M. E. (2014). The loss of topography in the microbial communities of the upper respiratory tract in the elderly. *Annals of the American Thoracic Society*, *11*(4), 513–521.

Wikoff, W. R., Anfora, A. T., Liu, J., Schultz, P. G., Lesley, S. A., Peters, E. C., & Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences*, *106*(10), 3698–3703.

Wohleb, E. S., McKim, D. B., Sheridan, J. F., & Godbout, J. P. (2015). Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Frontiers in Neuroscience*, *8*(January), 1–17. https://doi.org/10.3389/fnins.2014.00447

Wohleb, E. S., Powell, N. D., Godbout, J. P., & Sheridan, J. F. (2013). Stress-Induced Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(34), 13820–13833. https://doi.org/10.1523/JNEUROSCI.1671-13.2013

Wong, A. H. C., Gottesman, I. I., & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*, *14*(suppl_1), R11–R18.

Wu, J. C. Y. (2012). Psychological co-morbidity in functional gastrointestinal disorders: epidemiology, mechanisms and management. *Journal of Neurogastroenterology and Motility*, *18*(1), 13.

Wyss, M. T., Magistretti, P. J., Buck, A., & Weber, B. (2011). Labeled acetate as a marker of astrocytic metabolism. *Journal of Cerebral Blood Flow & Metabolism*, *31*(8), 1668–1674.

Yano, J. M. M., Yu, K., Donaldson, G. P. P., Shastri, G. G. G., Ann, P., Ma, L., … Hsiao, E. Y. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. https://doi.org/10.1016/j.cell.2015.02.047

Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., … Dileone, R. J. (2006). An essential role for ΔFosB in the nucleus accumbens in morphine action. *Nature Neuroscience*, *9*(2), 205.

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T., … Vieira-Silva, S. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, *352*(6285), 565–569.

Zijlmans, M. a. C., Korpela, K., Riksen-Walraven, J. M., de Vos, W. M., & de Weerth, C. (2015). Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, *53*, 233–245. https://doi.org/10.1016/j.psyneuen.2015.01.006

Zucchi, R., Chiellini, G., Scanlan, T. S., & Grandy, D. K. (2006). Trace amine-associated receptors and their ligands. *British Journal of Pharmacology*, *149*(8), 967–978.

CHAPTER 6. DISCUSSION

6.1. Summary of Findings

This dissertation examines the bidirectional interactions between peripheral systems—specifically the gut microbiota—and the central nervous system, in chronic stress, and probes for existing biological pathways that enable periphery-CNS signalling. In the publication that comprises chapter 3, we employed an ethologically valid model of chronic social stress (Malatynska & Knapp, 2005) in concert with 16S rRNA sequencing techniques to study the top-down effects of chronic stress on the gut microbiota. We demonstrated that chronic social stress causes changes in social and exploratory behaviours of mice, along with changes in the peripheral immunoregulatory phenotype. These changes are associated with alterations in the structure of the bacterial community, specifically reduced diversity and richness, and changes in the community composition. We also observed distinct shifts in the levels of specific operational taxonomic units across phyla, along with predicted functional changes in the microbiota, including altered metabolism of SCFAs, tyrosine, and tryptophan. In the published article that comprises chapter 4, we followed up upon this work by demonstrating that chronic treatment with a specific bacterial strain, *L. rhamnosus* (JB-1), attenuates the effects of chronic stress on anxiety-like and social behaviour, while preventing systemic inflammation. Notably, we observed that treatment did not affect

the gut microbiota community structure: treated mice continued to exhibit reduced diversity and richness along with structural alterations in the gut microbial community following stress exposure. These data suggest that it is likely that JB-1 exerts its effect on gut-brain pathways independent of inducing structural changes in the gut microbial community. Finally, in chapter 5 of this dissertation, we investigated the response of brain regions to acute versus chronic bacterial signals, and examined the role of the vagus nerve in mediating such interactions. We observed that a single oral dose of live but not heat-killed JB-1 induced expression of c-Fos—a marker of neuronal activity (Morgan & Curran, 1991)—in distributed brain regions within 165 minutes, while both live and heat-killed treatments elicited vagal firing. Severing the vagus nerve did not abolish c-Fos response in all of these regions, suggesting a critical role for the vagus while indicating the presence of additional signalling pathways. Furthermore, only chronic but not acute treatment induced the expression of ΔFosB—a marker of long-term neural adaptations (Mcclung et al., 2005; Nestler et al., 2001)—in various brain regions, some of which were previously unresponsive to acute bacterial administration.

6.2. Bidirectional gut-brain interactions in chronic stress

The last decade has seen a growing list of studies observing evidence of disruptions in the microbiota in various diseases and its association with adverse health outcomes. In chapter 3, we demonstrate that chronic social stress induces complex community-wide and species-level shifts in the microbiota (Bharwani et al., 2016). Stressed mice exhibit a pronounced decrease in the diversity and richness of the community, along with a shift in the overall profile. Additionally, we observed both elevations and reductions in the proportions of specific bacterial species that comprise

the major phyla. Along with changes in behaviour, this was associated with phenotypic immunoregulatory changes and elevated markers of inflammation. This parallels previous observations demonstrating top-down effects of stress on the microbiota (Bailey et al., 2011; O'Mahony et al., 2009a); however few studies had previously examined the specific nature of these structural alterations at the OTU level and described the functional consequences of these changes. Our *in silico* metagenomic analysis predicted differential expression of 145 functional pathways between control and chronically stressed mice, including those involved in the synthesis of tryptophan, tyrosine—precursors to serotonin and dopamine respectively—and short chain fatty acids. This is in agreement with observations that more than 90% of the total serotonin content is synthesized in the gut (Erspamer, 1966); that germ-free mice exhibit altered levels of plasma and hippocampal serotonin (Clarke et al., 2012; Wikoff et al., 2009); and that short chain fatty acids play a role in gut-brain signalling (Erny et al., 2015; Schroeder et al., 2007; Yano et al., 2015). Although sex differences were not investigated in these experiments, it is highly likely that the microbiota of male and female mice would exhibit unique differences in response to chronic stress given previous evidence of sex differences in gut-brain signalling in the literature (Clarke et al., 2012; Jašarević, Howard, Misic, Beiting, & Bale, 2017). Thus, these data implicate specific peripheral mechanisms through which changes in the gut microbiota community might influence facets of host physiology.

It has been widely proposed that preventing or reversing a state of microbiota "dysbiosis" may restore homeostasis (Bruce-Keller et al., 2014; Buffington et al., 2016; Olson et al., 2018). In chapter 4, we demonstrate that chronic treatment with JB-1

attenuates the effects of chronic social stress. Relative to saline-treated stressed mice, JB-1-treated stressed mice did not avoid novel conspecifics, exhibited more frequent transitions into the light chamber during the LD test—an assay for anxiety-like behaviour (Crawley, 1985)—and exhibit more frequent exploratory behaviour during the OFT. Bacterial treatment also prevented stress-induced activation of splenic dendritic cells and caused a systemic expansion of IL-10+ Treg cells. Notably, these bottom-up effects appeared to be mediated independently of the microbiota structure: chronic bacterial treatment did not alter the diversity or composition of gut bacteria in unstressed or stressed mice. Similar observations have been made in humans consuming a different *L. rhamnosus* strain (Eloe-fadrosh et al., 2015). It is thus likely that these effects are a result of direct modulation of gut-brain signalling pathways. Given that an intact vagus nerve is critical for the behavioural effects of this particular bacterial strain (Bravo et al., 2011), and given the absence of evidence for its translocation across the intestinal epithelium (Perez-Burgos et al., 2013), these data implicate the role of intervening pathways between bacteria and vagal synapses. The enteric nervous system (ENS) is one such candidate: more than 50% of vagal afferent fibres receive sensory input via an anatomical synapse with intrinsic primary afferent neurons (IPANs) of the ENS (Perez-Burgos et al., 2014)—the activity of which can be modulated by JB-1 (Mao et al., 2013). While the identity of the signalling mediator that carries JB-1-related signals from the epithelium to host IPANs is unknown, enteroendocrine cells can release a variety of chemical mediators and even form synapses with sensory neurons (Blackshaw, Brookes, Grundy, & Schemann, 2007; Diego V. Bohórquez et al., 2014; Kaelberer et al., 2018). JB-1 also sheds microvesicles that can replicate its effects on the immune and

enteric nervous systems (Al-Nedawi et al., 2014). Additional promising mediators include various soluble signalling factors such as short-chain fatty acids, and bacteria- and host-derived metabolites and humoural factors (Forsythe et al., 2016). Collectively, these data demonstrate the presence of bidirectional gut-brain signalling in chronic stress, and suggests that bacteria-mediated gut-to-brain signalling can occur independently of structural changes in the microbiota.

6.3. Differential response of brain regions to acute versus chronic bacteria treatment

Although there is abundant evidence for the effects of bacterial treatment on neural function and behaviour (Bercik, Park, et al., 2011; Bharwani et al., 2017; Bravo et al., 2011), these studies have focused primarily on long-term treatment, with time-points that span three to four weeks in duration. There is little information about the specific brain regions that are rapidly recruited in response to an initial acute exposure. Furthermore, it is unclear how this response pattern evolves over the duration of chronic treatment, and its association with the onset of behavioural changes. In chapter 5 we demonstrate that within 165 minutes of oral administration, the live bacteria rapidly induced c-Fos expression in regions throughout the brain, including BLA, CeA, PVT, vCA1, vCA3, PAG, DRN, and LC. This expression was specific and limited to the live bacteria, as administration of heat-killed JB-1 only induced c-Fos expression in the PVT. c-Fos is an immediate early gene and its expression is closely linked temporally with gene transcription (Sharp et al., 1991). c-Fos expression peaks at 1-2 hours following a stimulus and degrades within 6 hours (Jung et al., 2014; Sharp et al., 1991), thus providing a useful indicator of acute neuronal activity (Morgan & Curran, 1991). Similar

c-Fos responses have been observed in a model of caecal infection with *Campylobacter jejuni* (Goehler et al., 2005), albeit on slightly longer timescales (6 hours post-infection). That c-Fos expression in this infection model occurred prior to the induction of circulating cytokines further implicates the role of neuronal gut-brain signalling. Given that we did not observe behavioural changes on the TST following administration of a single bacterial dose, it is unlikely that all of these regions are integral to the effects of the bacteria. However, the expression pattern does reveal a network of brain regions that are likely involved in rapid sensory responses to bacteria.

Conversely, we also show in chapter 5 that only chronic, not acute bacterial treatment induced ΔFosB expression in distributed brain regions. ΔFosB is part of a group of transcription factor proteins that, unlike c-Fos, peak at 6 hours following stimulation (Chen et al., 1997; Hope et al., 1994). Unlike other members of this protein family however, ΔFosB is a truncated splice variant that is stable and accumulates at high levels following repeated stimulation due to phosphorylation of its serine residue sites and the lack of a C-terminus (Mcclung et al., 2004). These properties make it a good candidate for a mediator of long-term neural adaptations in response to chronic treatment. In regions that previously exhibited an acute c-Fos response, we observed increased ΔFosB expression following chronic treatment in the PVT, vHpc, PAG, DRN, and LC. Additionally, we also observed increased ΔFosB levels in two regions previously unresponsive to a single dose: the BNST and dHpc. Chronic treatment had no effect on ΔFosB expression in the BLA and CeA, which previously demonstrated an acute c-Fos response. Thus, not all responsive regions undergo long-term adaptations in response to chronic treatment. Given that altered ΔFosB levels were temporally

associated with changes in time spent immobile during the TST, determining the distinct targets of ΔFosB may be critical to understanding its role in the behavioural changes associated with gut-brain signalling, especially in regions that have been observed to play a role in stress and mood-related processes, including the vHpc (Anacker et al., 2018; Bagot et al., 2015; Jimenez et al., 2018; Padilla-coreano et al., 2016), the PVT (Beas et al., 2018), and the LC (Mccall et al., 2017). The targets of FosB proteins have also been studied extensively in models of chronic drug use and mood disorders. Putative targets include the regulation of AMPA and NMDA glutamate receptor subunits (Mcclung et al., 2004; Vialou et al., 2010), NF-κB (Ang et al., 2001), dynorphin (Zachariou et al., 2006), and Ca^{2+}/calmodulin-dependent protein kinase II (Robison et al., 2013).

6.4. The vagus nerve plays a critical role in gut-brain signalling

The vagus nerve, or cranial nerve X, is a mixed nerve that carries both afferent and efferent signals to and from the brain, enabling incessant communication between the brain and the viscera to maintain homeostasis as part of the parasympathetic arm of the autonomic nervous system. 80% of vagal fibres carry afferent signals to cell bodies in the nodose ganglia, which in turn innervate the NTS (Foley & DuBois, 1937; Nemeroff et al., 2006). Numerous brain regions receive direct and indirect projections from the NTS, including the LC, DRN, PAG, vHpc, and PVT (Castle et al., 2005; Groves & Brown, 2005; Han et al., 2018). Traditionally studied for its role in hunger and satiety, there is increasing evidence for the role of the vagus in mood and reward-related processes (Fülling et al., 2019; Han et al., 2018). For instance, stimulation of the vagus nerve is employed as an approved and successful therapy in the treatment of refractory

MDD (Aaronson, Sears, Ruvuna, Ph, et al., 2017). Furthermore, studies have demonstrated the role of vagus in bacteria-mediated gut-brain signalling. The anxiolytic and antidepressant-like effects of specific bacterial strains are dependent on the integrity of the vagus nerve (Bercik, Park, et al., 2011; Bravo et al., 2011), while certain bacterial strains facilitate vagal firing through an increase in the firing of individual fibres (Perez-Burgos et al., 2013). In chapter 5, we show that an intact vagus nerve is critical for the widespread c-Fos response to acute JB-1 treatment. Severing vagal fibres below the diaphragm prevented c-Fos expression in the BLA, CeA, PVT, PAG, and the LC, all of which receive vagal innervation and are activation by VNS (Chae et al., 2003; Cunningham et al., 2008; Han et al., 2018; Naritoku et al., 1995). In contrast, the DRN and vHpc continued to exhibit elevated c-Fos levels following treatment in vagotomised mice. These regions are also unresponsive to VNS (Cunningham et al., 2008; Naritoku et al., 1995), suggesting the presence of vagal-independent pathways that transmit bacteria-related signals.

Given that both live and heat-killed bacteria induce c-Fos expression in the PVT, but only the live bacteria induced widespread c-Fos expression suggests intrinsic differences in signal transmission. We thus wondered whether live and heat-killed treatments differentially affected vagal activity. Within minutes of application to a jejunal segment, live and heat-killed bacteria increased the firing frequency of afferent vagal fibres. This change is driven by an increase in the firing of individual fibres rather than an increase in the number of active fibres (Mao et al., 2013; Perez-Burgos et al., 2014, 2013). However, there was a significant difference in the variance between live and heat-killed groups. Moreover, at 165-minutes following oral treatment, we only observed

elevated firing frequency of vagal fibres in mice treated with the live bacteria. Other notable differences include that unlike the live bacteria, heat-killed JB-1 fails to evoke sensory responses in IPANs or increase their excitability (Mao et al., 2013). Thus, it is possible that the differential effects on c-Fos expression are due to differences in signal encoding in the precise form and pattern of the elicited vagal spike trains (Furness et al., 1999).

6.5. Conclusions

Although our understanding of the influence of gut bacteria has grown considerably since the days of George Porter Phillips, who observed that melancholic individuals treated with a lactic acid bacillus “wear a happier expression” (Phillips, 1910), there remain glaring gaps in our knowledge. While the literature is now replete with evidence demonstrating that chronic stress is associated with disruptions to the microbiota community, it is not clear whether these changes are merely consequence of systemic alterations in host physiology, or whether they mediate the behavioural and neural changes associated with chronic stress. Using GF rodents and models that alter the microbiota with antibiotics or exogenous bacterial strains, it is certainly evident that changes to the “normal” microbiota can drive gut-brain signalling. However, these data do not fully establish whether top-down, stress-induced changes in the microbiota feed back to influence the behavioural response to stress under homeostatic or clinically-relevant situations. Moreover, not all individuals react identically to stressors. Whether the microbiota plays a role in stress-induced susceptibility to MDD and whether the community profile can be used to identify at-risk individuals is unknown.

Gut bacteria can recruit multiple signalling pathways such as the vagus nerve and the immune system, perhaps even simultaneously by the same strain as suggested in chapter 5. But given that these bacteria do not cross the epithelial barrier under normal conditions (Perez-Burgos et al., 2013), there must exist intermediate signals that bridge microbes to pathways along the gut-brain axis. Furthermore, since not all bacteria have physiological effects when administered, and not all physiologically active bacteria utilize the same signalling pathways, there must also exist mechanisms that allow the host to distinguish between bacteria and elicit an appropriate response. These mechanisms, which may ostensibly include surface components and chemical mediators, must be sufficiently unique to also allow differential responses to live and heat-killed preparations of the same strain, which often have different effects, as demonstrated in chapter 5 and elsewhere (Mao et al., 2013). It is possible such differences are a result of the precise pattern of the elicited neural discharge (Furness et al., 1999), or due to interactions between signals provided by different pathways.

Finally, while research over the last decade has enumerated the various neural changes that occur alongside behavioural alterations, how precisely the former translates to expression of the latter is not clear. Thus, future work will need to build upon the observations detailed in chapter 5 by clarifying the precise targets of ΔFosB in distinct brain regions, the long-term adaptations that these engender in neurons, and how such adaptations drive the expression of altered behavioural phenotypes, in order to better understand the molecular underpinnings of interactions between bacteria and the CNS.

CHAPTER 7. REFERENCES

Aaronson, S. T., Sears, P., Ruvuna, F., Bunker, M., Conway, C. R., Dougherty, D. D., … Zajecka, J. M. (2017). A 5-year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: comparison of response, remission, and suicidality. *American Journal of Psychiatry*, *174*(7), 640–648.

Aaronson, S. T., Sears, P., Ruvuna, F., Ph, D., Bunker, M., Pharm, D., & Conway, C. R. (2017). A 5-Year Observational Study of Patients With Treatment-Resistant Depression Treated With Vagus Nerve Stimulation or Treatment as Usual : Comparison of Response , Remission , and Suicidality, (July). https://doi.org/10.1176/appi.ajp.2017.16010034

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., … Tompkins, T. (2014). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology and Motility*, *26*(4), 510–520. https://doi.org/10.1111/nmo.12295

Al-Nedawi, K., Mian, M. F., Hossain, N., Karimi, K., Mao, Y.-K., Forsythe, P., … Bienenstock, J. (2014). Gut commensal microvesicles reproduce parent bacterial signals to host immune and enteric nervous systems. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 1–12. https://doi.org/10.1096/fj.14-259721

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)*. Washington, DC: American Psychiatric Association.

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., … Hen, R. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, 1. https://doi.org/10.1038/s41586-018-0262-4

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neuroscience and Biobehavioral Reviews*, *51*, 164–188. https://doi.org/10.1016/j.neubiorev.2015.01.018

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E., & Nestler, E. J. (2001). Induction of nuclear factor-κB in nucleus accumbens by chronic cocaine administration. *Journal of Neurochemistry*, *79*(1), 221–224.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., … Hase, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461), 232–236.

Avgustinovich, D. F., Kovalenko, I. L., & Kudryavtseva, N. N. (2005). A model of anxious depression: Persistence of behavioral pathology. *Neuroscience and Behavioral Physiology*, *35*(9), 917–924. https://doi.org/10.1007/s11055-005-0146-6

Baganz, N. L., & Blakely, R. D. (2012). A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chemical Neuroscience*, *4*(1), 48–63.

Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., … Schöpf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486–496.

Bagot, R. C., Cates, H. M., Purushothaman, I., Vialou, V., Heller, E. A., Yieh, L., … Nestler, E. J. (2016). Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. *Biological Psychiatry*, *81*(4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012

Bagot, R. C., Parise, E. M., Pen, C. J., Zhang, H., Maze, I., Chaudhury, D., … Nestler, E. J. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nature Communications*, *6*. https://doi.org/10.1038/ncomms8062

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., & Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology*, *77*(11), 3846–3852.

Beas, B. S., Wright, B. J., Skirzewski, M., Leng, Y., Hyun, J. H., Koita, O., … Penzo, M. A. (2018). The locus coeruleus drives disinhibition in the midline thalamus via a dopaminergic mechanism. *Nature Neuroscience*, *21*(7), 963. https://doi.org/10.1038/s41593-018-0167-4

Benakis, C., Brea, D., Caballero, S., Faraco, G., Moore, J., Murphy, M., … Pamer, E. G. (2016). Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. *Nature Medicine*, *22*(5), 516.

Bendtsen, K. M. B., Krych, L., Sørensen, D. B., Pang, W., Nielsen, D. S., Josefsen, K., … Hansen, A. K. (2012). Gut Microbiota Composition Is Correlated to Grid Floor Induced Stress and Behavior in the BALB/c Mouse. *PLoS ONE*, *7*(10), e46231. https://doi.org/10.1371/journal.pone.0046231

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., … Collins, S. M. (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology*, *141*(2), 599–609. https://doi.org/10.1053/j.gastro.2011.04.052

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., … Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and Motility*, *23*(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Bercik, P., Verdu, E. F., Foster, J. a, Macri, J., Potter, M., Huang, X., … Collins, S. M. (2010). Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology*, *139*(6), 2102-2112.e1. https://doi.org/10.1053/j.gastro.2010.06.063

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience: Basic and Clinical*, *85*(1–3), 1–17. https://doi.org/10.1016/S1566-0702(00)00215-0

Berton, O., Mcclung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress, (February), 864–869.

Berton, O., McClung, C. a, Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science (New York, N.Y.)*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural and functional consequences of chronic psychosocial stress on the microbiome and host. *Psychoneuroendocrinology*, *63*(2016), 217–227. https://doi.org/10.1016/j.psyneuen.2015.10.001

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 Medicine*, *15*(1), 7. https://doi.org/10.1186/s12916-016-0771-7

Blackshaw, L. A., Brookes, S. J. H., Grundy, D., & Schemann, M. (2007). Sensory transmission in the gastrointestinal tract. *Neurogastroenterology & Motility*, *19*, 1–19.

Boer, M. C., Joosten, S. A., & Ottenhoff, T. H. M. (2015). Regulatory T-cells at the interface between human host and pathogens in infectious diseases and vaccination. *Frontiers in Immunology*, *6*.

Bohórquez, DV, & Shahid, R. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *The Journal of …*, 1–5. https://doi.org/10.1172/JCI78361DS1

Bohórquez, Diego V., Samsa, L. A., Roholt, A., Medicetty, S., Chandra, R., & Liddle, R. A. (2014). An enteroendocrine cell - Enteric glia connection revealed by 3D electron microscopy. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0089881

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Bruce-Keller, A. J., Salbaum, J. M., Luo, M., Blanchard, E., Taylor, C. M., Welsh, D. A., & Berthoud, H.-R. R. (2014). Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. *Biological Psychiatry*, *77*(7), 607–615. https://doi.org/10.1016/j.biopsych.2014.07.012

Brynskikh, A., Warren, T., Zhu, J., & Kipnis, J. (2008). Adaptive immunity affects learning behavior in mice. *Brain, Behavior, and Immunity*, *22*(6), 861–869. https://doi.org/10.1016/j.bbi.2007.12.008

Buffington, S. A., Viana, G., Prisco, D., Auchtung, T. A., Ajami, N. J., Petrosino, J. F., … Petrosino, J. F. (2016). Microbial Reconstitution Reverses Maternal Diet- Induced Social and Synaptic Deficits in Offspring Article Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell*, *165*(7), 1762–

1775. https://doi.org/10.1016/j.cell.2016.06.001
Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856.
Caenepeel, P. H., Janssens, J., Vantrappen, G., Eyssen, H., & Coremans, G. (1989). Interdigestive myoelectric complex in germ-free rats. *Digestive Diseases and Sciences*, *34*(8), 1180–1184.
Cámara, R. J. A., Gander, M.-L., Begré, S., Von Känel, R., & Group, S. I. B. D. C. S. (2011). Post-traumatic stress in Crohn's disease and its association with disease activity. *Frontline Gastroenterology*, *2*(1), 2–9.
Can, A., Dao, D. T., Terrillion, C. E., Piantadosi, S. C., Bhat, S., & Gould, T. D. (2012). The tail suspension test. *JoVE (Journal of Visualized Experiments)*, (59), e3769.
Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., … Delzenne, N. M. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal 1 – 3, 1236–1243. https://doi.org/10.3945/ajcn.2009.28095.The
Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335
Carle, T. L., Ohnishi, Y. N., Ohnishi, Y. H., Alibhai, I. N., Wilkinson, M. B., Kumar, A., & Nestler, E. J. (2007). Absence of conserved C-terminal degron domain contributes to ΔFosB's unique stability. *Eur J Neurosci*, *25*, 3009–3019.
Castelli, M. P., Ferraro, L., Mocci, I., Carta, F., Carai, M. A. M., Antonelli, T., … Gessa, G. L. (2003). Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of γ-hydroxybutyric acid. *Journal of Neurochemistry*, *87*(3), 722–732.
Castle, M., Comoli, E., & Loewy, A. D. (2005). Autonomic brainstem nuclei are linked to the hippocampus. *Neuroscience*, *134*(2), 657–669. https://doi.org/10.1016/j.neuroscience.2005.04.031
Chae, J., Nahas, Z., Lomarev, M., Denslow, S., Lorberbaum, J. P., Bohning, D. E., & George, M. S. (2003). A review of functional neuroimaging studies of vagus nerve stimulation (VNS), *37*, 443–455. https://doi.org/10.1016/S0022-3956(03)00074-8
Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y., & Nestler, E. J. (1997). Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. *Journal of Neuroscience*, *17*(13), 4933–4941.
Chiu, R., Angel, P., & Karin, M. (1989). Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. *Cell*, *59*(6), 979–986. https://doi.org/10.1016/0092-8674(89)90754-X
Chung, L. (2015). A Brief Introduction to the Transduction of Neural Activity into Fos Signal. *Development & Reproduction*, *19*(2), 61–67. https://doi.org/10.12717/DR.2015.19.2.061
Chunyu, J. P., Kyle, Z., Darrell, B., Dayanim, G., Bhatnagar, S., Luz, S., & Vigderman, A. S. (2019). The gut microbiome regulates the increases in depressive-type

behaviors and in in fl ammatory processes in the ventral hippocampus of stress vulnerable rats. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0380-x

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., … Cryan, J. F. (2012). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences*, *103*(27), 10420–10425.

Clarke, T. B., Davis, K. M., Lysenko, E. S., Zhou, A. Y., Yu, Y., & Weiser, J. N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature Medicine*, *16*(2), 228–231.

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The Impact of the Gut Microbiota on Human Health: An Integrative View - 1-s2.0-S0092867412001043-main.pdf. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Collins, S. M., & Bercik, P. (2009). The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology*, *136*(6), 2003–2014.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews. Microbiology*, *10*(11), 735–742. https://doi.org/10.1038/nrmicro2876

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science*, *326*(5960), 1694–1697.

Crawley, J. N. (1985). Exploratory behavior models of anxiety in mice. *Neuroscience & Biobehavioral Reviews*, *9*(1), 37–44.

Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., … Rabot, S. (2014). Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, *42*, 207–217. https://doi.org/10.1016/j.psyneuen.2014.01.014

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712.

Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009

Cunningham, J. T., Mifflin, S. W., Gould, G. G., & Frazer, A. (2008). Induction of c-Fos and DeltaFosB immunoreactivity in rat brain by Vagal nerve stimulation. *Neuropsychopharmacology*, *33*(8), 1884–1895. https://doi.org/10.1038/sj.npp.1301570

Curran, T., & Franza Jr, B. R. (1988). Fos and Jun: the AP-1 connection. *Cell*, *55*(3), 395–397.

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. *Nature Reviews Neuroscience*, *9*(1), 46–56.

David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrotta, A., … Alm, E. J. (2014). Host lifestyle affects human microbiota on daily timescales. *Genome Biol*, *15*(7), R89.

de LeBlanc, A. de M., Dogi, C. A., Galdeano, C. M., Carmuega, E., Weill, R., & Perdigón, G. (2008). Effect of the administration of a fermented milk containing Lactobacillus casei DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunology*, *9*(1), 27.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, a. J., Green, W., … Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*(August), 7735. https://doi.org/10.1038/ncomms8735

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry*, *19*(2), 146–148. https://doi.org/10.1038/mp.2013.65

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179–1188. https://doi.org/10.1016/j.neuroscience.2010.08.005

Dinan, T G. (2005). Stress: the shared common component in major mental illnesses. *European Psychiatry*, *20*, S326–S328.

Dinan, Timothy G, Quigley, E. M. M., Ahmed, S. M. M., Scully, P., O'Brien, S., O'Mahony, L., … Keeling, P. W. N. (2006). Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? *Gastroenterology*, *130*(2), 304–311.

Dorward, D. W., Garon, C. F., & Judd, R. C. (1989). Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. *Journal of Bacteriology*, *171*(5), 2499–2505.

Duffy, L. C., Zielezny, M. A., Marshall, J. R., Byers, T. E., Weiser, M. M., Phillips, J. F., … Graham, S. (1991). Relevance of major stress events as an indicator of disease activity prevalence in inflammatory bowel disease. *Behavioral Medicine*, *17*(3), 101–110.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.

Eloe-fadrosh, E. A., Brady, A., Crabtree, J., Drabek, E. F., Ma, B., Mahurkar, A., … Fraser, M. (2015). Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption. *MBio*, *6*(2), e00231-15. https://doi.org/10.1128/mBio.00231-15.Editor

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., … Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*, (October 2014). https://doi.org/10.1038/nn.4030

Erspamer, V. (1966). Occurrence of indolealkylamines in nature. In *5-Hydroxytryptamine and Related Indolealkylamines* (pp. 132–181). Springer.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., … Cani,

P. D. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences*, *110*(22), 9066–9071. https://doi.org/10.1073/pnas.1219451110

Foley, J. O., & DuBois, F. S. (1937). Quantitative studies of the vagus nerve in the cat. I. The ratio of sensory to motor fibers. *Journal of Comparative Neurology*, *67*(1), 49–67.

Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. *Immunological Investigations*, *39*(4–5), 429–448. https://doi.org/10.3109/08820131003667978

Forsythe, P., & Kunze, W. a. (2013). Voices from within: gut microbes and the CNS. *Cellular and Molecular Life Sciences : CMLS*, *70*(1), 55–69. https://doi.org/10.1007/s00018-012-1028-z

Forsythe, P., Kunze, W., & Bienenstock, J. (2016). Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? *BMC Medicine*, *14*(1), 58. https://doi.org/10.1186/s12916-016-0604-8

Forsythe, P., Sudo, N., Dinan, T., Taylor, V. H., & Bienenstock, J. (2010). Mood and gut feelings. *Brain, Behavior, and Immunity*, *24*(1), 9–16. https://doi.org/10.1016/j.bbi.2009.05.058

Forsythe, P., Wang, B., Khambati, I., & Kunze, W. a. (2012). Systemic effects of ingested lactobacillus rhamnosus: Inhibition of mast cell membrane potassium (IKCA) current and degranulation. *PLoS ONE*, *7*(7), 1–8. https://doi.org/10.1371/journal.pone.0041234

Frazer, A., & Benmansour, S. (2002). Delayed pharmacological effects of antidepressants. *Molecular Psychiatry*, *7*, S23–S28. https://doi.org/10.1038/sj.mp.4001015

Frost, G., Sleeth, M. L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., … Bell, J. D. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Communications*, *5*, 3611. https://doi.org/10.1038/ncomms4611

Fuchs, D., Möller, A. A., Reibnegger, G., Stöckle, E., Werner, E. R., & Wachter, H. (1990). Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *3*(9), 873–876.

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling : What Happens in Vagus... *Neuron*, *101*, 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Furet, J.-P., Quénée, P., & Tailliez, P. (2004). Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology*, *97*(2), 197–207.

Furmaga, H., Sadhu, M., & Frazer, A. (2012). Comparison of FosB Immunoreactivity Induced by Vagal Nerve Stimulation with That Caused by Pharmacologically Diverse Antidepressants. *Journal of Pharmacology and Experimental Therapeutics*, *341*(2), 317–325. https://doi.org/10.1124/jpet.111.188953

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. In *Microbial endocrinology: The microbiota-gut-brain axis in health and disease* (pp.

39–71). Springer.
Furness, J. B., Kunze, W. A. A., & Clerc, N. (1999). II. The intestine as a sensory organ: neural, endocrine, and immune responses. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *277*(5), G922–G928.
Galley, J. D., Yu, Z., Kumar, P., Dowd, S. E., Lyte, M., & Bailey, M. T. (2015). The structures of the colonic mucosa-associated and luminal microbial communities are distinct and differentially affected by a prolonged murine stressor. *Gut Microbes*, *5*(6), 748–760. https://doi.org/10.4161/19490976.2014.972241
Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., … Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307–317. https://doi.org/10.1136/gut.2009.202515
Gärtner, K. (1990). A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Laboratory Animals*, *24*(1), 71–77.
Gębicki, J., Sysa-Jędrzejowska, A., Adamus, J., Woźniacka, A., Rybak, M., & Zielonka, J. (2003). 1-Methylnicotinamide: a potent anti-inflammatory agent of vitamin origin. *Pol. J. Pharmacol*, *55*, 109–112.
Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., … Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*(7610), 94–103.
Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: we have never been individuals. *The Quarterly Review of Biology*, *87*(4), 325–341.
Gobaille, S., Schleef, C., Hechler, V., Viry, S., Aunis, D., & Maitre, M. (2002). Gamma-hydroxybutyrate increases tryptophan availability and potentiates serotonin turnover in rat brain. *Life Sciences*, *70*(18), 2101–2112.
Goehler, L. E., Gaykema, R. P. A., Opitz, N., Reddaway, R., Badr, N., & Lyte, M. (2005). Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with Campylobacter jejuni. *Brain, Behavior, and Immunity*, *19*(4), 334–344. https://doi.org/10.1016/j.bbi.2004.09.002
Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols*, *6*(8), 1183–1191. https://doi.org/10.1038/nprot.2011.361
Gower, J. C. (1975). Generalized procrustes analysis. *Psychometrika*, *40*(1), 33–51.
Greenberg, M. E., Greene, L. A., & Ziff, E. B. (1985). Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *Journal of Biological Chemistry*, *260*(26), 14101–14110.
Greenberg, M. E., & Ziff, E. B. (1984). Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature*, *311*(5985), 433.
Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., & Roncarolo, M. G. (1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*, *389*(6652), 737–742.
Groves, D. A., & Brown, V. J. (2005). Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neuroscience & Biobehavioral Reviews*, *29*(3), 493–500. https://doi.org/10.1016/j.neubiorev.2005.01.004
Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., … de Araujo, I.

E. (2018). A Neural Circuit for Gut-Induced Reward. *Cell*, 1–14. https://doi.org/10.1016/j.cell.2018.08.049

He, B., Xu, W., Santini, P. A., Polydorides, A. D., Chiu, A., Estrella, J., … Plebani, A. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*, *26*(6), 812–826.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., … Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences*, *108*(7), 3047–3052. https://doi.org/10.1073/pnas.1010529108

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693–710.

Heitler, W. J. (2007). DataView: A Tutorial Tool for Data Analysis. Template-based Spike Sorting and Frequency Analysis. *Journal of Undergraduate Neuroscience Education*, *6*(1), A1.

Henningsen, P., Zimmermann, T., & Sattel, H. (2003). Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. *Psychosomatic Medicine*, *65*(4), 528–533.

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., … Warren, B. L. (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proceedings of the National Academy of Sciences*, *111*(52), 18799–18799. https://doi.org/10.1073/pnas.1423575112

Hoffman, G. E., Smith, M. S., & Verbalis, J. G. (1993). c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Frontiers in Neuroendocrinology*, *14*(3), 173–213. https://doi.org/10.1006/frne.1993.1006

Holzer, P. (2011). Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacology & Therapeutics*, *131*(1), 142–170.

Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., … Nestler, E. J. (1994). Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron*, *13*(5), 1235–1244.

Houlden, A., Goldrick, M., Brough, D., Vizi, E. S., Lénárt, N., Martinecz, B., … Denes, A. (2016). Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production. *Brain, Behavior, and Immunity*, *57*, 10–20.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., … Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024

Huhman, K. L., Solomon, M. B., Janicki, M., Harmon, A. C., Lin, S. M., Israel, J. E., & Jasnow, A. M. (2003). Conditioned defeat in male and female Syrian hamsters. *Hormones and Behavior*, *44*(3), 293–299.

Husebye, E., Hellström, P. M., & Midtvedt, T. (1994). Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral

propagation of migrating myoelectric complex. *Digestive Diseases and Sciences*, *39*(5), 946–956.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *Neuroimage*, *125*, 988–995.

Janik, R., Thomason, L. A. M., Stanisz, A. M., Forsythe, P., Stanisz, G. J., Bienenstock, J., & Stanisz, G. J. (2016). Magnetic resonance spectroscopy reveals oral Lactobacillus promotion of increases in brain GABA, N-acetyl aspartate and glutamate. *NeuroImage*, *125*(2015), 988–995. https://doi.org/10.1016/j.neuroimage.2015.11.018

Jašarević, E., Howard, C. D., Misic, A. M., Beiting, D. P., & Bale, T. L. (2017). Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. *Scientific Reports*, *7*, 44182.

Jašarević, E., Howard, C. D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., … Bale, T. L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nature Neuroscience*. https://doi.org/10.1038/s41593-018-0182-5

Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., … Kheirbek, M. A. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron*, *0*(0), 1–14. https://doi.org/10.1016/j.neuron.2018.01.016

Jorissen, H. J. M. M., Ulery, P. G., Henry, L., Gourneni, S., Nestler, E. J., & Rudenko, G. (2007). Dimerization and DNA-binding properties of the transcription factor ΔFosB. *Biochemistry*, *46*(28), 8360–8372.

Jung, H. Y., Kim, W., Yoo, D. Y., Nam, S. M., Kim, J. W., Choi, J. H., … Hwang, I. K. (2014). Intragastric gavage with denatonium benzoate acutely induces neuronal activation in the solitary tract nucleus via the vagal afferent pathway. *Journal of Veterinary Science*, *15*(4), 459–464. https://doi.org/10.4142/jvs.2014.15.4.459

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B., Montoya, M., Shen, X., & Bohórquez, D. V. (2018). A gut-brain neural circuit for nutrient sensory transduction. *Science*, *In Press*. https://doi.org/10.1126/science.aat5236

Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., … Bienenstock, J. (2006). Inhibitory effects of Lactobocillus reuteri on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut*, *55*(2), 191–196. https://doi.org/10.1136/gut.2005.070987

Karimi, K., Inman, M. D., Bienenstock, J., & Forsythe, P. (2009). Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *American Journal of Respiratory and Critical Care Medicine*, *179*(3), 186–193. https://doi.org/10.1164/rccm.200806-951OC

Karimi, K., Kandiah, N., Chau, J., Bienenstock, J., & Forsythe, P. (2012). A Lactobacillus rhamnosus Strain Induces a Heme Oxygenase Dependent Increase in Foxp3+ Regulatory T Cells. *PLoS ONE*, *7*(10), 1–12. https://doi.org/10.1371/journal.pone.0047556

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., … Cryan, J. F. (2016). Lost in Translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects.

Brain, Behavior, and Immunity. https://doi.org/10.1016/j.bbi.2016.11.018

Kidd, M., Modlin, I. M., Gustafsson, B. I., Drozdov, I., Hauso, O., & Pfragner, R. (2008). Luminal regulation of normal and neoplastic human EC cell serotonin release is mediated by bile salts, amines, tastants, and olfactants. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *295*(2), G260–G272.

Kinsey, S. G., Bailey, M. T., Sheridan, J. F., Padgett, D. A., & Avitsur, R. (2007). Repeated social defeat causes increased anxiety-like behavior and alters splenocyte function in C57BL/6 and CD-1 mice. *Brain, Behavior, and Immunity*, *21*(4), 458–466.

Klooker, T. K., Braak, B., Painter, R. C., de Rooij, S. R., van Elburg, R. M., van den Wijngaard, R. M., … Boeckxstaens, G. E. (2009). Exposure to severe wartime conditions in early life is associated with an increased risk of irritable bowel syndrome: a population-based cohort study. *The American Journal of Gastroenterology*, *104*(9), 2250–2256.

König, H., Ponta, H., Rahmsdorf, U., Büscher, M., Schönthal, A., Rahmsdorf, H. J., & Herrlich, P. (1989). Autoregulation of fos: the dyad symmetry element as the major target of repression. *The EMBO Journal*, *8*(9), 2559–2566.

Korpela, K., Salonen, A., Virta, L. J., Kekkonen, R. A., Forslund, K., Bork, P., & de Vos, W. M. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nature Communications*, *7*.

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Kunze, W. A., Mao, Y., Wang, B., Huizinga, J. D., Ma, X., Forsythe, P., & Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *Journal of Cellular and Molecular Medicine*, *13*(8b), 2261–2270.

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. a, … Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676

Lay, C., Rigottier-Gois, L., Holmstrøm, K., Rajilic, M., Vaughan, E. E., de Vos, W. M., … Blaut, M. (2005). Colonic microbiota signatures across five northern European countries. *Applied and Environmental Microbiology*, *71*(7), 4153–4155.

Leclercq, S., Mian, F. M., Stanisz, A. M., Bindels, L. B., Cambier, E., Ben-amram, H., … Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. https://doi.org/10.1038/ncomms15062

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, *124*(4), 837–848.

Lindqvist, D., Wolkowitz, O. M., Mellon, S., Yehuda, R., Flory, J. D., Henn-Haase, C., … Neylan, T. C. (2014). Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. *Brain, Behavior, and Immunity*, *42*, 81–88.

Liu, Z.-H., & Smith, C. B. (2009). Dissociation of social and nonsocial anxiety in a

mouse model of fragile X syndrome. *Neuroscience Letters*, *454*(1), 62–66.
Lucibello, F. C., Lowag, C., Neuberg, M., & Müller, R. (1989). Trans-repression of the mouse c-fos promoter: A novel mechanism of fos-mediated trans-regulation. *Cell*, *59*(6), 999–1007. https://doi.org/10.1016/0092-8674(89)90756-3
Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *Bioessays*, *33*(8), 574–581.
Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathogens*, *9*(11).
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. *Physiology & Behavior*, *89*(3), 350–357. https://doi.org/10.1016/j.physbeh.2006.06.019
Lyte, M., Varcoe, J. J., & Bailey, M. T. (1998). Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiology and Behavior*, *65*(1), 63–68. https://doi.org/10.1016/S0031-9384(98)00145-0
Macpherson, A. J., & Harris, N. L. (2004). Interactions between commensal intestinal bacteria and the immune system, *4*(June), 1626–1632.
Macpherson, A. J., & Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*, *303*(5664), 1662–1665.
Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *19*(1), 11–38.
Maitre, M., Humbert, J.-P., Kemmel, V., Aunis, D., & Andriamampandry, C. (2005). [A mechanism for gamma-hydroxybutyrate (GHB) as a drug and a substance of abuse]. *Medecine Sciences: M/S*, *21*(3), 284–289.
Malatynska, E., & Knapp, R. J. (2005). Dominant–submissive behavior as models of mania and depression. *Neuroscience & Biobehavioral Reviews*, *29*(4–5), 715–737.
Malkova, N. V, Collin, Z. Y., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, Behavior, and Immunity*, *26*(4), 607–616.
Mao, Y.-K., Kasper, D. L., Wang, B., Forsythe, P., Bienenstock, J., & Kunze, W. a. (2013). Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.*, *4*, 1465. https://doi.org/10.1038/ncomms2478
Mariat, D., Firmesse, O., Levenez, F., Guimarães, V., Sokol, H., Doré, J., … Furet, J.-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, *9*, 123. https://doi.org/10.1186/1471-2180-9-123
Mayer, E. A. (2000). The neurobiology of stress and gastrointestinal disease. *Gut*, *47*(6), 861–869.
Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195), 620.
Mccall, J. G., Siuda, E. R., Bhatti, D. L., Lawson, L. A., Mcelligott, Z. A., & Stuber, G. D. (2017). Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior, 1–23. https://doi.org/10.7554/eLife.18247

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2004). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain. *Molecular Brain Research*, *132*(January), 146–154. https://doi.org/10.1016/j.molbrainres.2004.05.014

Mcclung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O., & Nestler, E. J. (2005). ΔFosB : A molecular switch for long-term adaptation in the brain D FosB : a molecular switch for long-term adaptation in the brain, (May 2018). https://doi.org/10.1016/j.molbrainres.2004.05.014

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine*, *338*(3), 171–179.

McHenry, J. A., Robison, C. L., Bell, G. A., Vialou, V. V, Bolaños-Guzmán, C. A., Nestler, E. J., & Hull, E. M. (2016). The role of ΔfosB in the medial preoptic area: Differential effects of mating and cocaine history. *Behavioral Neuroscience*, *130*(5), 469.

McKinney, W. T., & Bunney, W. E. (1969). Animal model of depression: I. Review of evidence: implications for research. *Archives of General Psychiatry*, *21*(2), 240–248.

McVey Neufeld, K. A., Mao, Y. K., Bienenstock, J., Foster, J. A., & Kunze, W. A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology & Motility*, *25*(2), 183-e88.

Menard, C., Pfau, M. L., Hodes, G. E., Kana, V., Wang, V. X., Bouchard, S., … Russo, S. J. (2017). Social stress induces neurovascular pathology promoting depression. *Nature Neuroscience*, *20*(12), 1752–1760. https://doi.org/10.1038/s41593-017-0010-3

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … Cazaubiel, M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *British Journal of Nutrition*, *105*(5), 755–764.

Möhle, L., Mattei, D., Heimesaat, M. M., Bereswill, S., Fischer, A., Alutis, M., … Wolf, S. A. (2016). Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports*, *15*(9), 1945–1956. https://doi.org/10.1016/j.celrep.2016.04.074

Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience*, *14*(1), 421–451.

Müller, R., Bravo, R., Burckhardt, J., & Curran, T. (1984). Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature*, *312*(5996), 716.

Mundorf, M. L., Hochstetler, S. E., & Wightman, R. M. (1999). Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *Journal of Neurochemistry*, *73*(6), 2397–2405.

Murphy, K., & Weaver, C. (2016). *Janeway's immunobiology*. Garland Science.

Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, *59*(3), 695–700.

Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of

bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*, *148*(1), 257–266.

Naritoku, D. K., Terry, W. J., & Helfert, R. H. (1995). Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Research*, *22*(1), 53–62. https://doi.org/10.1016/0920-1211(95)00035-9

Nemeroff, C. B., Mayberg, H. S., Krahl, S. E., McNamara, J., Frazer, A., Henry, T. R., … Brannan, S. K. (2006). VNS therapy in treatment-resistant depression: Clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology*, *31*(7), 1345–1355. https://doi.org/10.1038/sj.npp.1301082

Nestler, E. J. (2015). Δ FosB : A transcriptional regulator of stress and antidepressant responses. *European Journal of Pharmacology*, *753*, 66–72. https://doi.org/10.1016/j.ejphar.2014.10.034

Nestler, E. J., Barrot, M., & Self, D. W. (2001). ΔFosB: a sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences*, *98*(20), 11042–11046.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology and Motility*, *23*(3), 255–265. https://doi.org/10.1111/j.1365-2982.2010.01620.x

Nishino, R., Mikami, K., Takahashi, H., Tomonaga, S., Furuse, M., Hiramoto, T., … Sudo, N. (2013). Commensal microbiota modulate murine behaviors in a strictly contamination-free environment confirmed by culture-based methods. *Neurogastroenterology & Motility*, *25*(6), 521-e371.

O'Garra, A., Vieira, P. L., Vieira, P., & Goldfeld, A. E. (2004). IL-10–producing and naturally occurring CD4+ Tregs: limiting collateral damage. *The Journal of Clinical Investigation*, *114*(10), 1372–1378.

O'Mahony, L., McCarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. (2005). Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, L., Mccarthy, J., Kelly, P., Hurley, G., Luo, F., Chen, K., … Quigley, E. M. M. M. (2005). Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*, *128*(3), 541–551. https://doi.org/10.1053/j.gastro.2004.11.050

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., … Dinan, T. G. (2009a). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., … Dinan, T. G. (2009b). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., & Madsen, K. L. (2013). Effects of Lactobacillus helveticus on murine behavior are dependent on

diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology*, *38*(9), 1738–1747.

Olson, C. A., Vuong, H. E., Yano, J. M., Liang, Q. Y., Nusbaum, D. J., & Hsiao, E. Y. (2018). The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell*, *173*(7), 1728-1741.e13. https://doi.org/10.1016/j.cell.2018.04.027

Padilla-coreano, N., Bolkan, S. S., Pierce, G. M., Spellman, T. J., Gordon, J. A., Padilla-coreano, N., … Hardin, W. D. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Article Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron*, *89*, 1–10. https://doi.org/10.1016/j.neuron.2016.01.011

Padmanabhan, P., Grosse, J., Asad, A. B. M. A., Radda, G. K., & Golay, X. (2013). Gastrointestinal transit measurements in mice with99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Research*, *3*(1), 1–8. https://doi.org/10.1186/2191-219X-3-60

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., & Brown, P. O. (2007). Development of the human infant intestinal microbiota. *PLoS Biology*, *5*(7), e177.

Patterson, E., Cryan, J. F., Fitzgerald, G. F., Ross, R. P., Dinan, T. G., & Stanton, C. (2014). Gut microbiota, the pharmabiotics they produce and host health. *Proceedings of the Nutrition Society*, *73*(04), 477–489.

Paxinos, G., & Franklin, K. B. J. (2004). *The mouse brain in stereotaxic coordinates*. Gulf professional publishing.

Perez-Burgos, A., Mao, Y.-K., Bienenstock, J., & Kunze, W. a. (2014). The gut-brain axis rewired: adding a functional vagal nicotinic “sensory synapse”. *The FASEB Journal*, *28*(7), 3064–3074. https://doi.org/10.1096/fj.13-245282

Perez-Burgos, A., Wang, B., Mao, Y.-K., Mistry, B., McVey Neufeld, K.-A., Bienenstock, J., & Kunze, W. (2013). Psychoactive bacteria Lactobacillus rhamnosus (JB-1) elicits rapid frequency facilitation in vagal afferents. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *304*(2), G211-20. https://doi.org/10.1152/ajpgi.00128.2012

Perez-Burgos, A., Wang, L., McVey Neufeld, K., Mao, Y., Ahmadzai, M., Janssen, L. J., … Kunze, W. A. (2015). The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic Lactobacillus reuteri DSM 17938. *The Journal of Physiology*, *593*(17), 3943–3957.

Perrotti, L. I., Hadeishi, Y., Ulery, P. G., Barrot, M., Monteggia, L., Duman, R. S., & Nestler, E. J. (2004). Induction of ΔFosB in reward-related brain structures after chronic stress. *Journal of Neuroscience*, *24*(47), 10594–10602.

Peyron, C., Luppi, P. H., Fort, P., Rampon, C., & Jouvet, M. (1996). Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. *Journal of Comparative Neurology*, *364*(3), 402–413. https://doi.org/10.1002/(SICI)1096-9861(19960115)364:3<402::AID-CNE2>3.0.CO;2-8

Phillips, J. G. P. (1910). The Treatment of Melancholia by the Lactic Acid Bacillus. *The British Journal of Psychiatry*, *56*(234), 422-NP. https://doi.org/10.1192/bjp.56.234.422

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Yamada, T. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59.

Ramos, A., & Mormède, P. (1997). Stress and emotionality: a multidimensional and genetic approach. *Neuroscience & Biobehavioral Reviews*, *22*(1), 33–57.

Reber, S. O., Siebler, P. H., Donner, N. C., Morton, J. T., Smith, D. G., Kopelman, J. M., … Lowry, C. A. (2016). Immunization with a heat-killed preparation of the environmental bacterium *Mycobacterium vaccae* promotes stress resilience in mice. *Proceedings of the National Academy of Sciences*, 201600324. https://doi.org/10.1073/pnas.1600324113

Reigstad, C. S., Salmonson, C. E., Rainey III, J. F., Szurszewski, J. H., Linden, D. R., Sonnenburg, J. L., … Kashyap, P. C. (2014). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *The FASEB Journal*, *29*(4), 1395–1403.

Robison, A. J., Vialou, V., Mazei-Robison, M., Feng, J., Kourrich, S., Collins, M., … Neve, R. (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving ΔFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *Journal of Neuroscience*, *33*(10), 4295–4307.

Rolls, A., Shechter, R., London, A., Ziv, Y., Ronen, A., Levy, R., & Schwartz, M. (2007). Toll-like receptors modulate adult hippocampal neurogenesis. *Nature Cell Biology*, *9*(9), 1081.

Rong, W., Hillsley, K., Davis, J. B., Hicks, G., Winchester, W. J., & Grundy, D. (2004). Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice. *The Journal of Physiology*, *560*(3), 867–881.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*(1), 529.

Sanderson, S., Boardman, W., Ciofi, C., & Gibson, R. (2006). Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023. https://doi.org/10.1038/nature4441021a

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676.

Schönfeld, C.-L., & Trendelenburg, U. (1989). The release of3H-noradrenaline by p-and m-tyramines and-octopamines, and the effect of deuterium substitution in α-position. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *339*(4), 433–440.

Schroeder, F. a, Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry*, *62*(1), 55–64. https://doi.org/10.1016/j.biopsych.2006.06.036

Schütte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J., & Minna, J. (1989). jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. *Cell*, *59*(6), 987–997. https://doi.org/10.1016/0092-8674(89)90755-1

Schwarz, J., Burguet, J., Rampin, O., Fromentin, G., Andrey, P., Tomé, D., … Darcel, N. (2010). Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. *PLoS ONE*, *5*(2), 13–15. https://doi.org/10.1371/journal.pone.0008974

Sender, R., Fuchs, S., & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell*, *164*(3), 337–340.

Serretti, A., Calati, R., Goracci, A., Di Simplicio, M., Castrogiovanni, P., & De Ronchi, D. (2010). Antidepressants in healthy subjects: what are the psychotropic/psychological effects? *European Neuropsychopharmacology*, *20*(7), 433–453.

Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. (2018). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron*, 1–14. https://doi.org/10.1016/j.neuron.2018.11.018

Sharon, G., Garg, N., Debelius, J., Knight, R., Dorrestein, P. C., & Mazmanian, S. K. (2014). Perspective Specialized Metabolites from the Microbiome in Health and Disease. *Cell Metabolism*, *20*(5), 719–730. https://doi.org/10.1016/j.cmet.2014.10.016

Sharp, F. R., Sagar, S. M., Hicks, K., Lowenstein, D., & Hisanaga, K. (1991). c-fos mRNA, Fos, and Fos-related antigen induction by hypertonic saline and stress. *Journal of Neuroscience*, *11*(8), 2321–2331.

Sibley, C. D., Grinwis, M. E., Field, T. R., Eshaghurshan, C. S., Faria, M. M., Dowd, S. E., … Surette, M. G. (2011). Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PloS One*, *6*(7), e22702.

Sommershof, A., Aichinger, H., Engler, H., Adenauer, H., Catani, C., Boneberg, E.-M., … Kolassa, I.-T. (2009). Substantial reduction of naive and regulatory T cells following traumatic stress. *Brain, Behavior, and Immunity*, *23*(8), 1117–1124.

Stam, R., Akkermans, L. M., & Wiegant, V. M. (1997). Trauma and the gut: interactions between stressful experience and intestinal function. *Gut*, *40*(6), 704.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., … Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(Pt 1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′Sialyllactose and 6′Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain, Behavior, and Immunity*, *50*, 166–177. https://doi.org/10.1016/j.bbi.2015.06.025

Thompson, J. A., Oliveira, R. A., Djukovic, A., Ubeda, C., & Xavier, K. B. (2015). Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota. *Cell Reports*, 1–11. https://doi.org/10.1016/j.celrep.2015.02.049

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … Mayer, E. a. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394–1401, 1401.e1-4. https://doi.org/10.1053/j.gastro.2013.02.043

Torii, A., Torii, S., Fujiwara, S., Tanaka, H., Inagaki, N., & Nagai, H. (2007). Lactobacillus acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergology International*, *56*(3), 293–301.

Tsuji, M., Suzuki, K., Kinoshita, K., & Fagarasan, S. (2008). Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis. In *Seminars in immunology* (Vol. 20, pp. 59–66). Elsevier.

Ulery, P. G., Rudenko, G., & Nestler, E. J. (2006). Regulation of ΔFosB stability by

phosphorylation. *Journal of Neuroscience*, *26*(19), 5131–5142.
Umesaki, Y., Okada, Y., Matsumoto, S., Imaoka, A., & Setoyama, H. (1995). Segmented Filamentous Bacteria Are Indigenous Intestinal Bacteria That Activate Intraepithelial Lymphocytes and Induce MHC Class II Molecules and Fucosyl Asialo GM1 Glycolipids on the Small Intestinal Epithelial Cells in the Ex-Germ-Free Mouse. *Microbiology and Immunology*, *39*(8), 555–562.
van der Kleij, H., O'Mahony, C., Shanahan, F., O'Mahony, L., & Bienenstock, J. (2008). Protective effects of Lactobacillus reuteri and Bifidobacterium infantis in murine models for colitis do not involve the vagus nerve. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(4), R1131–R1137.
Vialou, V., Robison, A. J., Laplant, Q. C., Covington, H. E., Dietz, D. M., Ohnishi, Y. N., … Nestler, E. J. (2010). fosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neuroscience*, *13*(6), 745–752. https://doi.org/10.1038/nn.2551
Vialou, V., Thibault, M., Kaska, S., Cooper, S., Gajewski, P., Eagle, A., … Robison, A. J. (2015). Differential induction of FosB isoforms throughout the brain by fluoxetine and chronic stress. *Neuropharmacology*, *99*, 28–37. https://doi.org/10.1016/j.neuropharm.2015.07.005
Wang, B., Mao, Y.-K. K., Diorio, C., Pasyk, M., Wu, R. Y., Bienenstock, J., & Kunze, W. A. (2010). Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. *The FASEB Journal*, *24*(10), 4078–4088. https://doi.org/10.1096/fj.09-153841
Wang, F. Bin, & Powley, T. L. (2000). Topographic inventories of vagal afferents in gastrointestinal muscle. *The Journal of Comparative Neurology*, *421*(3), 302–324. https://doi.org/10.1002/(SICI)1096-9861(20000605)421:3<302::AID-CNE2>3.0.CO;2-N [pii]
Wehner, S., Koscielny, A., Vilz, T. O., Stoffels, B., Engel, D. R., Kurts, C., & Kalff, J. (2014). Measurement of gastrointestinal and colonic transit in mice, 1–9. https://doi.org/10.1038/protex.2011.219
Werner-Felmayer, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G., & Wachter, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells in vitro. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1012*(2), 140–147.
Whelan, F. J., Verschoor, C. P., Stearns, J. C., Rossi, L., Luinstra, K., Loeb, M., … Bowdish, D. M. E. (2014). The loss of topography in the microbial communities of the upper respiratory tract in the elderly. *Annals of the American Thoracic Society*, *11*(4), 513–521.
Wikoff, W. R., Anfora, A. T., Liu, J., Schultz, P. G., Lesley, S. A., Peters, E. C., & Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences*, *106*(10), 3698–3703.
Wohleb, E. S., McKim, D. B., Sheridan, J. F., & Godbout, J. P. (2015). Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. *Frontiers in Neuroscience*, *8*(January), 1–17. https://doi.org/10.3389/fnins.2014.00447
Wohleb, E. S., Powell, N. D., Godbout, J. P., & Sheridan, J. F. (2013). Stress-Induced

Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(34), 13820–13833. https://doi.org/10.1523/JNEUROSCI.1671-13.2013

Wong, A. H. C., Gottesman, I. I., & Petronis, A. (2005). Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Human Molecular Genetics*, *14*(suppl_1), R11–R18.

Wu, J. C. Y. (2012). Psychological co-morbidity in functional gastrointestinal disorders: epidemiology, mechanisms and management. *Journal of Neurogastroenterology and Motility*, *18*(1), 13.

Wyss, M. T., Magistretti, P. J., Buck, A., & Weber, B. (2011). Labeled acetate as a marker of astrocytic metabolism. *Journal of Cerebral Blood Flow & Metabolism*, *31*(8), 1668–1674.

Yano, J. M. M., Yu, K., Donaldson, G. P. P., Shastri, G. G. G., Ann, P., Ma, L., … Hsiao, E. Y. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*, *161*(2), 264–276. https://doi.org/10.1016/j.cell.2015.02.047

Zachariou, V., Bolanos, C. A., Selley, D. E., Theobald, D., Cassidy, M. P., Kelz, M. B., … Dileone, R. J. (2006). An essential role for ΔFosB in the nucleus accumbens in morphine action. *Nature Neuroscience*, *9*(2), 205.

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T., … Vieira-Silva, S. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science*, *352*(6285), 565–569.

Zijlmans, M. a. C., Korpela, K., Riksen-Walraven, J. M., de Vos, W. M., & de Weerth, C. (2015). Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology*, *53*, 233–245. https://doi.org/10.1016/j.psyneuen.2015.01.006

Zucchi, R., Chiellini, G., Scanlan, T. S., & Grandy, D. K. (2006). Trace amine-associated receptors and their ligands. *British Journal of Pharmacology*, *149*(8), 967–978.