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Master Essay

Interkingdom Signaling Between Bacterial and Mammalian Cells

Influence of signaling compounds on cognition, behavior and pathogenicity

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Abstract

From the moment we are born, we are bombarded by the countless microorganism in our environment. Eukaryotes and prokaryotes have evolved side by side for billions of years, thus it is not surprising that they are able to detect and respond to each other's chemical signals. By intercepting and reacting to each other's signaling molecules, organisms engage in a finely tuned interplay which has far reaching impacts on their biology. But what kinds of chemical signals is interkingdom communication based on, and how do prokaryotes and eukaryotes recognize and respond to each other's signals? In this report, we will explore the influence of bacterial signaling compounds on a eukaryotic host's cognition and behavior, and examine the effects of eukaryotic hormones on microbiota. Additionally, we will talk about potential applications for microbiome engineering and discuss the implications behind interkingdom signaling and the Microbiota-Gut-Brain axis.

Contents

1 Introduction

1.1 Signaling systems of prokaryotes and eukaryotes

Eukaryotes make use of chemicals known as hormones to regulate various aspects of their physiology (Pacheco & Sperandio, 2009). Three general categories of hormones are found in humans, namely steroids, peptides or proteins, and amines or amino acid derivatives, examples of which are shown in Figure 1 (Hughes & Sperandio, 2008). Peptide and protein hormones are typically composed of 3–200 amino acids, and are often subjected to post-translational modification. This is the largest class of hormones, and includes insulin and epidermal growth factor. Steroid hormones, such as progesterone, are synthesized from cholesterol, and due to their hydrophobic nature are able to cross plasma membranes and interact with receptors inside the cell. In contrast, amine hormones (including dopamine, epinephrine and norepinephrine) and peptide hormones are unable to cross the cell membrane. As a result, such hormones bind primarily to cell surface receptors such as G-protein-coupled receptors (GPCRs) and receptor kinases. Thus, a hormone's structure determines the location of its receptor (Hughes & Sperandio, 2008).

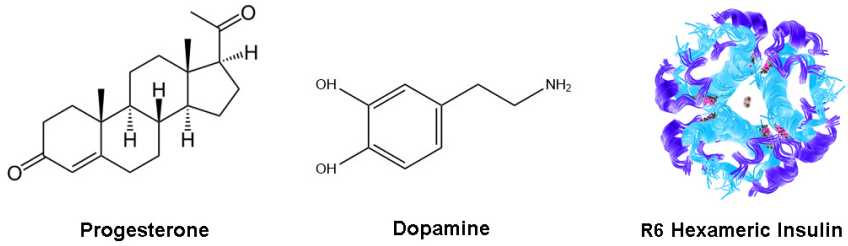

Figure 1 – Progesterone, Dopamine and R6 Hexameric Insulin (Chang et al., 1997)

Prokaryotic intercellular communication systems are in some ways similar to those of eukaryotes due to their common evolutionary origin (Hughes & Sperandio, 2008). The most obvious parallel is the use of signaling compounds to regulate and synchronize various activities within a group of cells. Whereas eukaryotic cells communicate with hormones, prokaryotic cells make use of small, hormone-like molecules known as autoinducers to monitor and respond to changes in the population (Bassler, 1999; Fuqua, Winans, & Greenberg, 1994). These compounds are constantly secreted and detected by bacteria, and trigger gene expression when a certain threshold population density (a quorum) has been reached (Fuqua et al., 1994), (Bassler, 1999). This communication system, known as quorum sensing (QS), allows bacteria to coordinate population-wide gene expression, endowing them with behaviors normally associated with multicellular organisms (Bassler, 1999), (Pacheco & Sperandio, 2009). This phenomenon is illustrated in Figure 2. A wide variety of responses are regulated by QS, including motility, biofilm formation and the production of virulence factors (Pacheco & Sperandio, 2009). Behaviors which are beneficial when expressed in a group, as opposed to a single bacterium are often under QS control (Waters & Bassler, 2005).

Due to its central role in bacterial group biology, QS also plays an important role in symbiosis and pathogenesis (de Kievit & Iglewski, 2000). The first described example

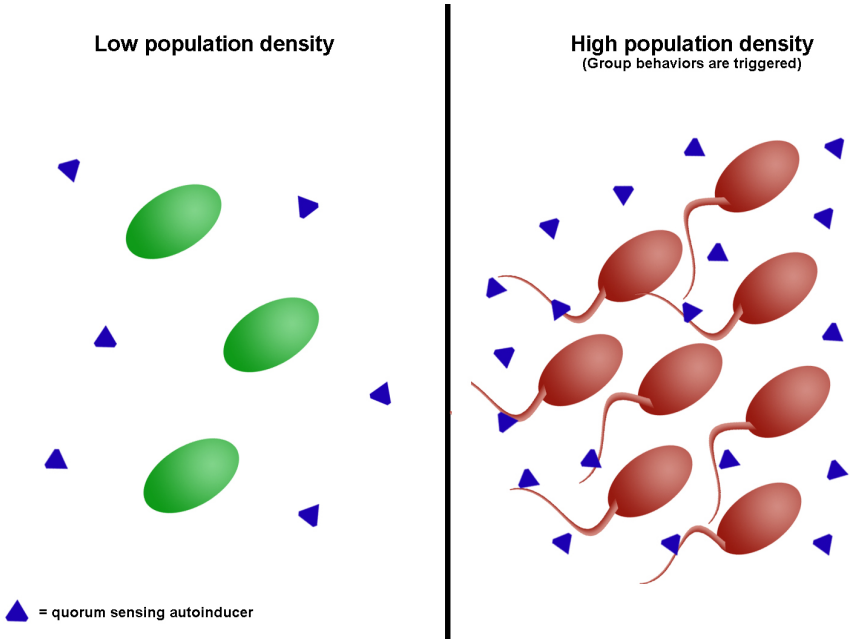

Figure 2 – Quorum Sensing allows for synchronized gene expression

of QS in nature comes from a study carried out by Nealson, Platt, and Hastings (1970), where they investigated the regulation of bioluminescence in the marine bacterium *Vibrio fischeri* . While planktonic *Vibrio fischeri* does not emit luminescence, when present in high concentrations (such as those found in the light organ of the Hawaiian bobtail squid, *Euprymna scolopes*) the QS autoinducer acyl-homoserine-lactone (AHL) activates the LuxRI QS system, initiating the transcription of *Lux* genes required for bioluminescence (Verma & Miyashiro, 2013). Colonization of the light organ is necessary for the correct development of its epithelial cells (Visick, Foster, Doino, McFall-Ngai, & Ruby, 2000). The organ itself serves to camouflage the squid by eliminating its shadow from the water column, while providing an ideal growth environment for the bacteria (Verma & Miyashiro, 2013). This fascinating case of interkingdom symbiosis is a perfect example of a QS system directing bacteria toward either a planktonic or a colonial lifestyle, depending on population density (Pacheco & Sperandio, 2009).

1.2 Interkingdom signaling

During their long coexistence with eukaryotic hosts, bacteria have evolved receptors for detecting eukaryotic signals (P. Freestone, 2013). These signals tell the bacterium that a host is nearby, and allow for optimally timed expression of genes required for colonization (P. Freestone & Lyte, 2010). For a long time it was thought that bacterial growth and virulence are dependent only on local environmental conditions, such as pH, temperature and the availability of nutrients (P. Freestone, 2013). However, recent investigations by P. Freestone and Lyte (2010), and P. Freestone (2013) revealed that host hormone signals also have a significant impact on bacterial behavior. Accumulating evidence shows that eukaryotic cells possess receptors which allow them to intercept and react to bacterial autoinducers, much like they would with hormones

(Pacheco & Sperandio, 2009). Likewise, hormones can be picked up and interpreted by bacteria. Having evolved in such close proximity, it is not surprising that the activities of one affect the activities of the other. The emerging field of interkingdom signaling is based on these discoveries. This type of communication mediates pathogenic, commensal and symbiotic relationships between microorganisms and their eukaryotic hosts (Pacheco & Sperandio, 2009). The chemical communication systems that make such cross-talk possible are thought to be as ancient as the relationship between host and bacteria (Pacheco & Sperandio, 2009). In this article we will focus on the interactions between bacteria and mammalian cells. Although extensive plant-microbe interactions also exist, these are beyond the scope of this text (Mine, Sato, & Tsuda, 2014).

1.3 What is the gut microbiome and what is its significance?

The intestine of a human adult is the most densely colonized organ in the body, containing an estimated 10^{14} bacterial cells, exceeding the number of eukaryotic cells making up the entire body by an order of magnitude (Balish, 1999). Furthermore, the entire human microbiome (occupying the oral, nasal and otic cavities, the surface of the skin and the urogenital and gastrointestinal (GI) tracts) is estimated to encode approximately 150 times more genes than the human host (Bhattacharjee & Lukiw, 2013), (Hill, Bhattacharjee, Pogue, & Lukiw, 2014). Although the colonization of the intestine begins in the prenatal period, densities of bacterial populations in neonates are still low, and are subject to further development after birth (Jiménez et al., 2005). Out of the 55 currently known bacterial phyla, the two prominent members of gut microbiota are *Bacteroidetes* and *Firmicutes*, making up approximately 48% and 51% of the phylotypes found in the GI-tract (Hill et al., 2014). A variety of bacteria make up the remaining 1%, including *Cyanobacteria*, *Proteobacteria*, *Spirochetes* and *Actinobacteria*, as well as a number of fungi, protozoa and viruses (Hill et al., 2014).

Humans live in a symbiotic association with their intestinal microbiota, which carries out critical protective, structural, and metabolic functions (Belizario & Napolitano, 2015). The roles of the gut microbiota range from processing of indigestible polysaccharides to the synthesis of vitamins, and as a whole, help maintain the health of the host (Belizario & Napolitano, 2015). The gut microbiome also protects the host from infection by preventing pathogens from attaching to gut binding sites (Sudo et al., 2004). Even the development of an infant's brain is influenced by the microbiome (M, E, & J, 2013).

The gut microbiome is known to produce hormone-like compounds that find their way into the bloodstream and bind to receptors in different parts of the body, including the brain (G. Clarke et al., 2014). Thus, the metabolic products of gut microbiota have far reaching influence on the host, well beyond the GI-tract. Furthermore, specific members of the intestinal microbiome can detect and respond to the host's hormones (Lyte, 2004). The cross-talk between bacteria and host becomes even more significant when we consider the indirect role that gut microbiota plays in the regulation of endocrine networks (G. Clarke et al., 2014).

Despite the obvious physiological differences between bacterial and host cells, the gut microbiome's specialized functions and its ability to operate collectively make it comparable to an organ. Additionally, the microbiome is host-specific, partially heritable, and can be altered through surgery, antibiotics and dietary changes, all hallmarks of

an organ (Marchesi et al., 2015). This notion is given further support if we consider the critical interactions of the microbiome with cells and organs of the host which affect nearly all aspects of the host's physiology (Marchesi et al., 2015). Given that many of these interaction take place at distal sites, the gut microbiota most closely resembles an endocrine organ (Forsythe, Sudo, Dinan, Taylor, & Bienenstock, 2010). Taken together, these characteristics have prompted researchers to term the gut microbiome a "virtual organ" (O'Hara & Shanahan, 2006).

The notion that our microbial partners maintain and promote human health is not new, however, the extent of their influence is only beginning to be unraveled. Recent technological advancements in the fields of genetic sequencing, proteomics, and bioinformatics, have driven a growing appreciation of the importance of gut microbiota to psychological and physiological well-being (Belizario & Napolitano, 2015). Studies have implicated microbiota in a number of disorders, including obesity (Turnbaugh et al., 2006), depression (Bercik et al., 2010) and irritable bowel syndrome (Ringel & Ringel-Kulka, 2015), however the mode of action is not well understood. Accumulating evidence suggests a two-way interaction between the gut, its microbiota and the brain, via a so-called Microbiome-Gut-Brain (MGB) axis (Forsythe et al., 2010), (Bravo et al., 2011) (G. Clarke et al., 2014). This communication system evolved to serve several vital functions, such as discriminating between useful, useless and dangerous substances in food, promoting the consumption of appropriate nutrients, and maintaining homeostasis in the gut (Holzer & Farzi, 2014). Dysregulation of the MGB axis has been implicated in a number of disorders, and has been demonstrated to affect the Hypothalamic-Pituitary-Adrenal (HPA) axis which mediates responses to stress (Sekirov, Russell, Antunes, & Finlay, 2010).

Interkingdom signaling is a relatively new field of study, and as such, many aspects of this phenomenon are still under investigation. Although most of the mechanisms behind such interactions have not yet been elucidated, a number of studies have demonstrated intriguing connections between host and microbiota. The potential influence that gut microbiota may exert on the host's behavior and cognition is a particularly exciting topic that has attracted considerable interest in the recent years (Lyte, 2013). How do bacteria communicate with their human host, and what impact does this bidirectional communication have on the host's psychological and physiological well-being? How are the host's cells able to communicate with bacteria, and in what ways does this affect the bacterial community? These are some of the question we will address in this article.

2 Influence of microbiota on host behavior and cognition

2.1 Interkingdom axes of communication

Several axes of interkingdom communication have been discovered, including the MGB and HPA axes (Forsythe et al., 2010), (G. Clarke et al., 2014), (Foster, Lyte, Meyer, & Cryan, 2015), (Petra et al., 2015). As shown in in Figure 3, a wide variety of factors are involved in conveying signals from gut to brain, including cytokines, antibiotics, intestinal neurotransmitters, pathogens, and sensory vagal fibers (Petra et al., 2015). The HPA axis, together with the neuropeptides released from sensory nerve fibers, influences the composition of the gut microbiota either directly, or via nutrient availability. Thus, taken together, the MGB and HPA axes serve as a communication link to convey information from the gut to the brain, and vice versa. This interaction has been implicated in several disorders where inflammation is thought to play a role, such as multiple sclerosis, obesity, autism-spectrum disorders (Petra et al., 2015). Early evidence supporting the concept of the MGB axis was provided by studies comparing behaviors of germ-free and colonized mice. These studies revealed that gut microbiota may play a role in the pathogenesis of non-GI disorders. Germ-free mice exhibit an increased caloric intake in order to maintain body weight, impaired cardiac output, altered levels of norepinephrine and an exaggerated stress response (Sekirov et al., 2010). These symptoms are believed to stem from dysregulation of the HPA axis (Sekirov et al., 2010). The results of these studies indicate that the HPA axis is a key component of the bidirectional communication system relaying signals between gut and brain, and can be influenced by changes in the enteric microbiota (Bravo et al., 2011). However, the mechanism by which microbiota influence the HPA axis has not yet been elucidated.

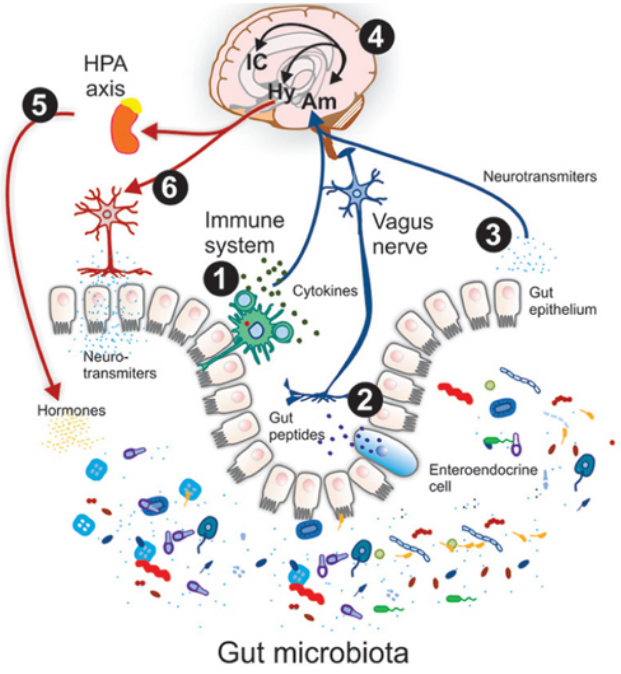

Figure 3 – An overview of the Microbiota-Gut-Brain axis (Montiel-Castro et al., 2013)

2.2 Mechanism behind the Microbiota-Gut-Brain axis - the vagus nerve

The mechanism behind the communication of gut microbiota with the brain via the MGB axis was the subject of an investigation carried out by Goehler et al. (2005), where they attempted to determine whether local infection in the gut activates vagal sensory neurons. The work carried out by this group demonstrated that the introduction of just one particular bacterial strain into the mouse gut leads to the development of anxiety-like behavior. This investigation also revealed that signals sent via the vagus nerve from gut to brainstem in response to the composition of gut microbiota, activated specific regions of the brain (Goehler et al., 2005). These conclusions were drawn based on experiments where the expression of c-Fos (a neuronal activation marker) in the vagal sensory ganglia and in the nucleus of the solitary tract (the main sensory relay nucleus of the vagus nerve, also known as nTS) was assessed. The mice used for this study were orally inoculated with live *Campylobacter jejuni* (*C. jejuni*) or with saline, in the case of the control group. Mice inoculated with *C. jejuni* exhibited a significant increase in the expression of c-Fos in the neurons bilateral to the vagal ganglia. Additionally, activation of the neurons in the nTS has been show to take place (Goehler et al., 2005)

Furthermore, treatment with *C. jejuni* resulted in the activation of brain regions tied to primary viscerosensory pathways, as well as the central autonomic network and immune-responsive regions. These results contribute evidence to the notion that vagal sensory neurons relay signals to the brain concerning potential pathogens, and most likely contribute to the host's defense responses (Goehler et al., 2005). The activation of vagal sensory neurons in response to an inoculation of live bacteria supports the premise of peripheral neurons monitoring the changes in local conditions brought about by gut microbiota, and suggests that signaling through particular neural pathways might provide the brain with information relevant to host defense, such as the site of infection and the type of pathogen involved (Goehler et al., 2005).

2.3 Serotonin and gut microbiota

Gut microbiota has been shown to influence exploratory, anxiety-like and social behaviors in mice via the psychobiotic effect (Dinan, Stanton, & Cryan, 2013). The ability of microbiota to influence the host's behavior is based on their ability to produce and detect neurochemicals (Lyte, 2013). Both prokaryotes and eukaryotic microorganisms are known to possess a wide range of neurohormones, as well as receptors for neurohormones (Lenard, 1992). A study performed by Asano et al. (2012) revealed that gut microbiota is capable of producing neurochemicals (such as dopamine and norepinephrine) in situ, in quantities that are sufficiently high to result in neurophysiological changes in the host. Thus, it is not surprising that the production of these chemicals by bacteria in the gut lumen can affect host-specific neural receptors, thereby influencing host behavior (Bravo et al., 2011), (Asano et al., 2012). Studies investigating the psychobiotic effects of bacterial species making up the gut microbiome often focus on *Lactobacillus* or *Bifidobacterium*, as many members of these species are known to be prolific producers of neurochemicals (Foster et al., 2015).

Changing the composition of the gut microbiome alters plasma concentrations of tryptophan (G. Clarke et al., 2014). This essential amino acid serves as a precursor to serotonin

(see Figure 4), a vital neurotransmitter responsible for modulating a range of behaviors and physiological processes including anxiety, obsessive behaviors (Ho et al., 2015), blood pressure (Bogdanski, Weissbach, & Udenfriend, 1958) and breathing (Manzke et al., 2010). Disruptions in the serotonergic system have been implicated in various disorders, such as coronary artery disease (Vikenes, Farstad, & Nordrehaug, 1999), depression (Svenningsson et al., 2006) (Drevets et al., 2007), and obsessive-compulsive disorder (Ho et al., 2015), (G. Clarke et al., 2014). Serotonin may also affect the immune system, creating a regulatory feedback loop where changes in serotonin concentrations induced by the microbiota influence the way the host deals with pathogenic or commensal microorganisms, thus affecting the metabolism and ecology of the gut microbiota, and possibly leading to further changes in serotonin concentrations (Ridaura & Belkaid, 2015).

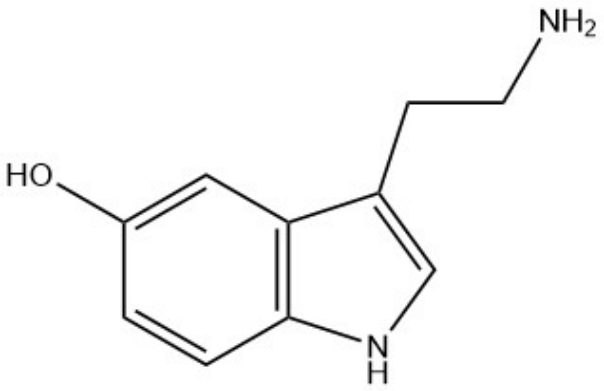

Figure 4 – Structure of serotonin

Most antidepressant drugs work by increasing serotonin concentration in the brain, however, research shows that serotonin is also synthesized by gut microbiota *in vivo* (Patterson et al., 2014). A study by Wikoff et al. (2009) provides evidence to support this. Their investigation revealed that plasma serotonin levels in germ-free mice were nearly three times lower compared to control mice. Neonatal stress has been shown to induce changes in the gut microbiota, and is a known risk factor for depression (O'Mahony et al., 2009). The results of Wikoff's investigation are complemented by the findings of Bailey and Coe (1999) who demonstrated that neonatal stress in rhesus monkeys reduces the amount of *Bifidobacteria* and *Lactobacilli* in their gut. Infant rhesus monkeys had significantly less fecal bacteria (*Lactobacilli* in particular) after just three days of maternal separation (Bailey & Coe, 1999). This decrease in gut microbiota correlated with behaviors indicative of stress. Furthermore, infants who exhibited these behaviors were also found to be more susceptible to opportunistic bacterial infections (Bailey & Coe, 1999). The results of this study show that early neonatal stress caused by maternal separation might not only result in psychological problems later in life, but also increase vulnerability to infectious disease.

2.4 γ-aminobutyric acid and gut microbiota

γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, and is responsible for regulating a variety of psychological and physiological processes (Cryan & Kaupmann, 2005). The chemical structure of GABA is given in Figure 5. Metabotropic

GABA receptors play an important role in the maintenance of normal behavior, and past studies have implicated $GABA_B$ receptors (heterodimeric GPCRs) in the pathophysiology of several neuropsychiatric conditions, such as anxiety, depression and mood disorders (Cryan & Kaupmann, 2005), (Bravo et al., 2011). Furthermore, GABAergic neurotransmission in the hippocampus has been linked to various memory processes, and is necessary for contextual conditioning (Crestani et al., 1999). This premise is supported by evidence showing that spatial working memory is improved upon administration of $GABA_B$ antagonists (Helm et al., 2005).

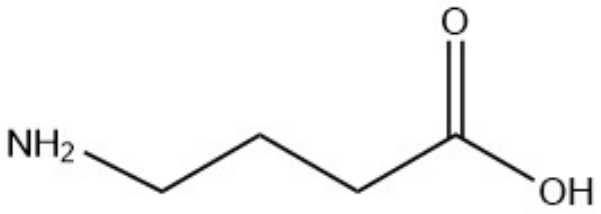

Figure 5 – Structure of γ-aminobutyric acid (GABA)

In a study carried out by Bravo et al. (2011), anxiety and depressive-like behaviors in mice were alleviated after oral administration of *L. rhamnosus*. Subsequently, changes in $GABA_A\alpha_1$, $GABA_A\alpha_2$, and $GABA_{B1b}$ mRNA levels were detected in regions of the brain associated with these behaviors. The differences observed were consistent with behavioral responses. Expression of $GABA_{B1b}$ mRNA was found to increase in the prefrontal cortex after treatment with *L. rhamnosus* (Bravo et al., 2011). This observation is in line with previous studies which linked reductions in $GABA_B$ receptor expression in frontal cortices to depression (Cryan & Kaupmann, 2005). Analysis of $GABA_{B1b}$ mRNA in the amygdala and hippocampus revealed a reduced expression, consistent with the antidepressant-like effect of $GABA_B$ receptor antagonists (Slattery, Desrayaud, & Cryan, 2005), (Bravo et al., 2011). Treated mice exhibited enhanced memory towards an aversive stimulus, compared to control mice, consistent with studies reporting the role of $GABA_{B1b}$ in learning processes, particularly those related to fear (L. H. Jacobson, Kelly, Bettler, Kaupmann, & Cryan, 2007), (Jacobson, 2007). Further support for these findings comes from studies showing that administration of particular bacterial strains improves memory function in mice (Gareau et al., 2011). Enhancement of cognitive abilities resulting from treatment with certain bacteria has also been demonstrated in humans (Messaoudi et al., 2011), suggesting that probiotic treatment can improve memory function and learning, as well as ameliorate behavioral and cognition problems stemming from an exaggerated stress response.

2.5 Brain-Derived Neurotrophic Factor and gut microbiota

Neurotrophins are a family of proteins that regulate various aspects of neural function, including neural development, maintenance, plasticity, axon growth, and dendrite pruning (Huang & Reichardt, 2001). They are also involved in regulating cell fate decisions and in the expression of neurotransmitters, ion channels and other proteins necessary for normal neural function (Huang & Reichardt, 2001).

Brain-Derived Neurotrophic Factor (BDNF), shown in Figure 6, is one of four neurotrophins found in mammals, and has been shown to play a crucial role in the normal

neural development of mice (Ernfors, Kucera, Lee, Loring, & Jaenisch, 1995). BDNF is known to be particularly important for long-term potentiation, a strengthening of synapses based on patterns of neural activity which serves as a basis for the process of learning and long-term memory formation (Yamada & Nabeshima, 2003). An increase in BDNF mRNA expression has been associated with enhanced memory acquisition and consolidation, as has the activation of its receptor TrkB (Yamada & Nabeshima, 2003). Furthermore, deprivation of either BDNF or TrkB has been shown to impair memory formation and recall. It is thought that BDNF maintains elevated levels of neuronal excitation by interfering with GABAergic signaling. An investigation led by Henneberger, Juttner, Rothe, and Grantyn (2002) revealed that BDNF-induced GABAergic inhibition is a result of acute downregulation of $GABA_A$ receptors.

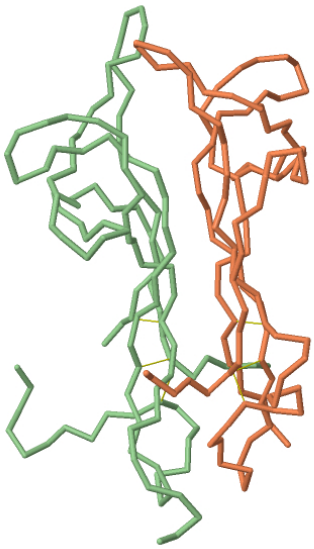

Figure 6 – Structure of Brain-Derived Neurotrophic Factor (BDNF) (Robinson et al., 1999)

Considering the numerous important roles played by BDNF, it is not surprising that disruptions in BDNF signaling pathways can result in a variety of disorders. BDNF has been implicated in schizophrenia (Xiong et al., 2014) and drug addiction (Vargas-Perez et al., 2009), and linked to depression (Warner-Schmidt & Duman, 2006), epilepsy (Gall, Lauterborn, Bundman, Murray, & Isackson, 1991), and Alzheimer's disease (Mattson, 2008), among others.

The relationship between gut bacteria and expression of BDNF has been explored by Savignac et al. (2013). Expression of BDNF is reduced in the absence of gut bacteria, but can be rescued with the oral administration of probiotics. The increase in BDNF levels in the brain manifests in the form of anxiolytic effects (Savignac et al., 2013). In their study, Savignac et al. (2013) investigated whether prebiotic compounds known to stimulate the gut microbiota also influence the levels of BDNF in the brain. Rats that were fed large amounts of fructo-oligosaccharides (FOS) or galacto-oligosaccharides (GOS) for five weeks had increased levels of BDNF in the hippocampus, compared to the control group. These results support the notion that the composition of the gut microbiome can be mediated by prebiotics, and that gut microbiota influence BDNF expression in

the brain. For this reason, the use of prebiotics in the treatment of neuropsychiatric disorders and in the maintenance of psychological health is a topic that warrants further investigation.

The results of studies discussed above support the idea that bacteria are capable of mediating mood and behavior of the host via the production of neurochemicals which act on the MGB and HPA axes, relaying signals pertaining to the state of intestinal microbiota from gut to brain. It has been demonstrated that pathological changes in the modulation of the HPA axis can be reversed by treatment with *Lactobacillus* or *Bifidobacterium* (Gareau, Jury, MacQueen, Sherman, & Perdue, 2007), (Bravo et al., 2011). Studies investigating the production and detection of neurochemicals by microorganisms have helped shed light on the mechanisms by which microorganisms can affect host behavior, and represent an important foundation in the emerging field of microbial endocrinology (Lenard, 1992), (Lyte, 2010), (Asano et al., 2012).

2.6 A single strain can influence cognition

A growing body of evidence shows that the introduction of even a single bacterial strain can have a profound impact on the host's cognitive processes. An investigation by Sudo et al. (2004) revealed an exaggerated hypothalamic-pituitary response to mild restraint stress in germ-free mice, which was diminished after colonization by selected probiotic bacteria. Bercik et al. (2010) were the first to demonstrate a change in behavior upon introduction of a particular gut bacterium. This study showed that the introduction of the parasite *Trichuris muris* in the mouse gut resulted in gut inflammation and anxiety-like behavior (as demonstrated by a light/dark preference test), and that behavior is alleviated by the introduction of *Bifidobacterium longum* (Bercik et al., 2010). Another study carried out by Desbonnet et al. (2010) revealed that the administration of *Bifidobacterium infantis* (*B. infantis*), alleviated anxiety-like behaviors in rats that were deprived of maternal contact at an early age. Rats subjected to maternal deprivation exhibited a reduced swim behavior in the forced swim test, and decreased norepinephrine levels in the brain (Desbonnet et al., 2010). Probiotic treatment with *B. infantis* restored norepinephrine concentration in the brain and reversed the behavioral deficits (Desbonnet et al., 2010). Another study investigating the antidepressant effects imparted by *B. infantis* revealed that ingestion of this bacterium led to increased levels of tryptophan in the blood (G. Clarke et al., 2014). If *B. infantis* is able to influence tryptophan metabolism, it is possible that it could also have an impact on serotonin levels.

These studies not only demonstrate the link between the gut microbiome and host behavior, but also reveal that a single bacterial strain may lead to beneficial or detrimental changes in the host's behavior. The psychobiotic effects of individual bacterial strains are a hot topic in current investigations. Understanding which microorganisms are able to influence which behavior will bring us one step closer to precise therapeutic targeting of microbiota for the treatment of stress-related neuropsychiatric disorders (G. Clarke et al., 2014).

2.7 Fecal Microbiota Transplantation

Further evidence to confirm the significance of the gut microbiome's influence on host behavior was provided by studies demonstrating the adoptive transfer of behavioral phenotypes between mice of varying behavioral profiles (Collins, Kassam, & Bercik, 2013). This discovery raised the possibility of bacterial transplantation as a form of therapeutic treatment for disorders of the central nervous system, and prompted a renewed interest in Fecal Microbiota Transplantation (FMT) (Bercik et al., 2010). This treatment strategy restores the diversity of the gut microbiome by introducing fecal matter from a healthy donor. Although first reported in 1958, this method has only recently come into clinical use for the treatment of recurrent *Clostridium difficile* infections (Eiseman, Silen, Bascom, & Kauvar, 1958), (Bakken et al., 2011). Animal studies carried out in the past few years indicate that FMT could be useful in treating a number of other diseases, including neurological disorders. The beneficial effect of FMT on non-GI disorders was first reported by Borody, Campbell, and Torres (2011) who observed a remission of idiopathic thrombocytopenic purpura in a patient undergoing FMT for the treatment of ulcerative colitis. This serendipitous discovery brought FMT into the spotlight. Since then, accumulating evidence has shown FMT to be promising in the treatment of various other non-GI disorders such as myoclonus dystonia (Borody, Rosen, Torres, & Nowak, 2011) and Parkinson's disease (Ananthaswamy, 2011).

In addition to altering host behavior, the phenomenon of adoptive phenotype transfer has also been demonstrated in the context of liver disease (Le Roy et al., 2013) and obesity (Turnbaugh et al., 2006) in mice, and insulin sensitivity in humans (Vrieze et al., 2012), suggesting that FMT can potentially be used in the treatment of these disorders. However, careful FMT donor screening is necessary to exclude donors with medical conditions that could be passed on to the recipient (Collins et al., 2013). This includes GI disorders such as inflammatory bowel disease, and CNS disorders such as depression.

Taken together, the available evidence supports the notion that the composition of the gut microbiome has a significant impact on neural function and host behavior. This impact is mediated directly when bacterial metabolites act on the brain, and indirectly, by influencing host metabolism of neuro-active chemicals and their precursors (Cryan & Dinan, 2012). Dysregulation of the MGB and HPA axes of communication has been implicated in a number of GI and psychological disorders, highlighting the need for further research into interkingdom signaling.

3 Influence of host hormones on microbiota

3.1 Epinephrine and norepinephrine

When an organism encounters a stressful situation, several hormones are released in response. The catecholamine hormone epinephrine, and its demethylated counterpart norepinephrine (also known as adrenaline and noradrenaline) play a central role in the response to stress (Pignatelli, Magalhaes, & Magalhaes, 1998). The structures of these hormones are given in Figure 7. Norepinephrine is produced by the adrenergic neurons of the enteric nervous system (Furness, 2000). For this reason it is thought to be the predominant signal in the GI-tract under normal conditions (Furness, 2000), (Hughes & Sperandio, 2008). In contrast, epinephrine is secreted directly in to the bloodstream by the CNS and the medulla of the adrenal gland. This allows it to reach all parts of the body quickly in times of stress, so that it can trigger responses necessary to deal with the situation. Both of these hormones play a crucial part in modulating the HPA axis and sympatho-adrenomedullary system (Pignatelli et al., 1998). Some immune system cells (including macrophages, mast cells and endothelial cells) have been implicated in tuning the stress response by regulating the adrenal cortex, suggesting a link between the immune system and stress (Pignatelli et al., 1998). The norepinephrine system has been implicated in the pathophysiology of depression, and may play a role in ADHD (Dell'Osso, Palazzo, Oldani, & Altamura, 2011), (del Campo, Chamberlain, Sahakian, & Robbins, 2011).

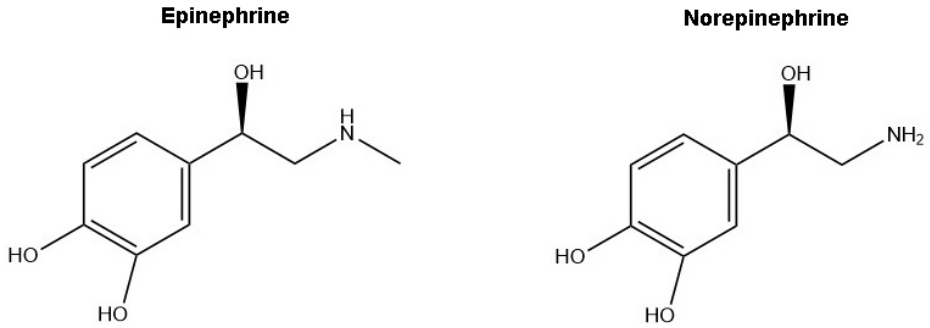

Figure 7 – Structures of epinephrine and norepinephrine

Evidence shows that ligand-binding sites for epinephrine and norepinephrine in a human adrenergic receptor are similar, suggesting that both are recognized by the same type of receptor. (Freddolino et al., 2004). The physiological effects of these two hormones are transduced by β_2-adrenergic receptors (β_2-ARs) (Elenkov, Wilder, Chrousos, & Vizi, 2000). Mammalian adrenergic receptors are the largest class of GPCRs (Hughes & Sperandio, 2008). The β_2-AR is expressed by a wide variety of cells, and acts as the central mediator of the stress response (Elenkov et al., 2000), (Kolmus, Tavernier, & Gerlo, 2015). Activation of these receptors on skeletal muscle, smooth muscle and liver cells relaxes the airways, redirects blood flow towards the muscles, inhibits the secretion of insulin and stimulates glucagon production (Black, 2002). Taken together, these effects prepare the organism for action by increasing blood glucose levels and oxygen concentration (Black, 2002). Furthermore, both of these hormones play important

biological roles in the GI-tract, where they modulate chloride and potassium secretion, submucosal blood flow, and intestinal smooth-muscle contraction (Horger, Schultheiss, & Diener, 1998). Although bacteria do not express adrenergic receptors, accumulating evidence shows that they do respond to epinephrine and norepinephrine (Sperandio, Torres, Jarvis, Nataro, & Kaper, 2003), (M. B. Clarke, Hughes, Zhu, Boedeker, & Sperandio, 2006), (Pacheco & Sperandio, 2009).

Soon after coming into clinical use, reports of adrenaline-associated sepsis began to surface (Renaud M, 1930), (Lyte, 2004). Since that time, numerous studies have linked these hormones with infectious disease (Lyte, 2004). Most of these studies assumed that the association between stress hormones and infection arose due to immune suppressing properties of catecholamines (Reiche, Nunes, & Morimoto, 2004), (P. Freestone, 2013). It was not until 1992 that convincing evidence was obtained to prove that bacteria recognize catecholamine hormones (Lyte & Ernst, 1992). The research group of Lyte demonstrated a dramatic increase in growth rate of *Escherichia coli*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* in the presence of epinephrine or norepinephrine (Lyte & Ernst, 1992). The term "microbial endocrinology", ascribed to the phenomenon of microorganisms recognizing eukaryotic hormones, was coined by Lyte (1993) shortly after this landmark study.

Numerous reports describing the impact of catecholamine stress hormones on the growth of different bacterial strains were published in the years that followed (P. Freestone, 2013). One study by P. P. Freestone, Haigh, Williams, and Lyte (1999) demonstrated that recognition of catecholamines was a widespread phenomenon among both Gram-negative and Gram-positive bacteria. Another investigation by Halang et al. (2015) revealed that epinephrine and norepinephrine enhanced swimming motility and virulence factor production by *Vibrio cholera*, contributing to its pathogenicity. Thus, even though catecholamine stress hormones enhance the host's ability to respond to its environment, they are also detected by bacteria, allowing them to gauge the psychophysiological state of the host and exploit a weakened immune system (Hughes & Sperandio, 2008).

3.2 Epi/NE/AI-3 signaling system enhances enterohaemorrhagic *E. coli* pathogenicity

Bacteria regulate the expression of a multitude of phenotypes by detecting and responding to epinephrine and norepinephrine (Hughes & Sperandio, 2008). The latter, for example, was shown to induce the expression of fimbriae and toxins in pathogenic *E. coli* (Lyte & Ernst, 1992). The role that epinephrine and norepinephrine play in bacterial infection was conclusively proven by Sperandio et al. (2003) in a revolutionary study which demonstrated that both hormones induce the expression of flagella and type III secretion system (T3SS) in enterohaemorrhagic *E. coli* (*EHEC*) O157:H7. *EHEC* is a food-borne pathogen that is responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (Kaper, 1998). Once it enters the GI-tract, host epinephrine and norepinephrine activate the expression of virulence genes, promoting colonization (Sperandio et al., 2003). As the bacterium colonizes the large intestine, it triggers the formation of attaching and effacing lesions on epithelial cells. The result is the destruction of microvilli, and the formation of pedestal-like structures that hold individual bacterial cells (Moon, Whipp, Argenzio, Levine, & Giannella, 1983). The genes governing this process

are encoded by the locus of enterocyte effacement (LEE) chromosomal pathogenicity island (McDaniel, Jarvis, Donnenberg, & Kaper, 1995). The genes encoding T3SS are found in the LEE region (Jarvis et al., 1995). The flagellar regulon is activated simultaneously with the LEE region, enhancing motility and promoting colonization (Sperandio, Mellies, Nguyen, Shin, & Kaper, 1999). *EHEC* also carries *stx* genes encoding the Shiga toxin, which damages vascular, intestinal and renal cells (Loukiadis, Kerouredan, Beutin, Oswald, & Brugere, 2006). Hemolytic uremic syndrome is caused by the destruction of epithelial cells in the urinary tract, while a similar process in the GI-tract results in diarrhea (Karmali, Petric, Lim, Fleming, & Steele, 1983).

The virulence genes of *EHEC* are activated by one of three chemical signals; bacterial QS autoinducer 3 (AI-3) produced by the gut microbiota, as well as epinephrine and norepinephrine produced by the host (Sperandio et al., 2003). This is known as the Epi/NE/AI-3 signaling system (M. B. Clarke et al., 2006). All three are agonistic signals, the responses to which can be blocked by adrenergic antagonists such as phentolamine (M. B. Clarke et al., 2006), (Pacheco & Sperandio, 2009). α-adrenergic agonists are commonly used to treat chronic diarrhea, suggesting that AI-3 might play a role in maintaining homeostasis in the GI-tract (Hughes & Sperandio, 2008). Although the complete chemical structure of AI-3 has not yet been elucidated, it is known to be an aminated aromatic compound (Pacheco & Sperandio, 2009). AI-3 has been reported to be somewhat hydrophobic, suggesting that it cannot cross the cell membrane, and thus most likely binds to membrane-bound receptors on the surface of the cell (Hughes & Sperandio, 2008). Recognition of these signaling molecules by histidine sensor kinases embedded in the membrane of *EHEC* is essential for the expression of virulence in vivo, and triggers a complex regulatory cascade which culminates in the expression of LEE and *stx* genes (Sperandio et al., 2003), (M. B. Clarke et al., 2006).

3.3 Epi/NE/AI-3 is mediated by QseBC

One notable mediator of this signaling pathway is the sensor kinase QseC, which binds directly to epinephrine and norepinephrine, as well as AI-3 (M. B. Clarke et al., 2006). Studies using rabbit infection models have demonstrated that the virulence of mutants with non-functional QseC is attenuated, suggesting that this sensor kinase plays a key role in the in vivo pathogenesis of *EHEC* (M. B. Clarke et al., 2006), (Waldor & Sperandio, 2007). Evidence shows that QseC is part of a two-component signal transduction system where QseC is the sensor kinase and QseB is its cognate response regulator (Pacheco & Sperandio, 2009). When *EHEC* enters the GI-tract, epinephrine or norepinephrine bind to QseC, activating it. QseC then undergoes autophosphorylation and transduces the signal to QseB. As a result, the transcription of the primary regulator of the flagella regulon (*flhDC*) is initiated (Rasko et al., 2008), (Pacheco & Sperandio, 2009). This signaling system, known as AI-3/Epi/NE, is thought to operate in a number of other strains besides *EHEC*. *In silico* analysis have revealed QseC homologues in a variety of other bacteria, including *Shigella flexneri*, *Haemophilus influenza* and *Ralstonia eutropha* (Rasko et al., 2008).

The discovery of the QseC sensor is an important step in the exploration of interkingdom signaling. Recognition of epinephrine and norepinephrine by a receptor for a bacterial QS compound suggest that these host and bacterial signals are interchangeable

(Sperandio et al., 2003). It is the first example of a receptor that responds to chemical signals from both host and bacteria, and serves to integrate bacteria-host signaling at the biochemical level (Hughes & Sperandio, 2008).

3.4 Dynorphin: endogenous opioids are hijacked by pathogenic bacteria

Since Lyte and Ernst's groundbreaking study on bacterial responses to eukaryotic stress neurohormones, accumulating evidence has revealed that bacterial sensitivity to eukaryotic signaling molecules is far broader than originally suspected (Lesouhaitier et al., 2009). It now appears that bacteria are also capable of sensing other hormones such as somatostatin (Yamashita et al., 1998), and insulin, (Woods, Jones, & Hill, 1993) as well as immunomodulatory and natriuretic peptides (Porat, Clark, Wolff, & Dinarello, 1991), (Veron et al., 2007). In addition to the release of epinephrine and norepinephrine, an organism undergoing stress also produces endogenous opioids. These neurotransmitters carry out a broad range of functions, such as immune modulation, secretion, and maintenance of epithelial barrier function (Zaborina et al., 2007). They are secreted by some mammalian tissues (including those within the gut, and at sites of inflammation) and immune cells in response to stress, and are classified into three main families, based on their affinity to δ-, μ-, and κ-opioid receptors (Sternini, Patierno, Selmer, & Kirchgessner, 2004). One such endogenous opioid, belonging to the κ-opioid family is dynorphin.

When released during stress, endogenous opioids have been demonstrated to act as paracrine and autocrine signals (Peterson, Molitor, & Chao, 1998). Given the broad distribution of endogenous opioids in the intestinal mucosa, and that host-derived signaling molecules can be hijacked by bacteria to increase their virulence, it is possible that pathogens monitor dynorphin levels to gauge the host's susceptibility to infection (Neudeck, Loeb, & Buck, 2003), (Sternini et al., 2004). Evidence showing that neutrophils synthesize and release opioids at sites of inflammation suggests that bacteria are exposed to opioids during the course of infection, further substantiating this hypothesis (Sternini et al., 2004).

A study carried out by Zaborina et al. (2007) explored the influence dynorphin has on the pathogenicity of *Pseudomonas aeruginosa* (*P. aeruginosa*). *P. aeruginosa* is an opportunistic pathogen commonly found in the human microbiome (de Bentzmann & Plésiat, 2011). It is known for causing potentially fatal infections in critically ill, and immunocompromised patients, with mortality rates approaching 60% (Aliaga, Mediavilla, & Cobo, 2002). In fact, up to 50% of all hospitalized patients are estimated to carry this bacterium (Marshall, Christou, & Meakins, 1993). The main colonization site in such patients is the GI-tract (Marshall et al., 1993). Evidence suggests that the degree of host stress is a critical determinant in the outcome of the infection, where stress positively correlates with mortality (Blot, Vandewoude, Hoste, & Colardyn, 2003).

In their investigation, Zaborina et al. (2007) exposed *P. aeruginosa* to dynorphin and a synthetic κ-agonist (U-50,488), using pyocyanin production as a proxy for virulence. Pyocyanin is a blue-green phenazine which is toxic to mammalian cells, and a variety of microorganisms (Young, 1947). The results of their *in vivo* experiments on mice demonstrate that dynorphin is released from the gut mucosa into the lumen after intestinal

ischemia, and binds to desquamated epithelia and intestinal *P. aeruginosa* (Zaborina et al., 2007). Once bound by *P. aeruginosa*, dynorphin activates quinolone signaling, which leads to copious pyocyanin production (Zaborina et al., 2007).

Pseudomonas quinolone signaling (PQS) is a regulatory system linking the LasRI and RhlRI QS systems (McKnight, Iglewski, & Pesci, 2000). PQS has been implicated in the pathogenesis of *P. aeruginosa* in nematodes (Mahajan-Miklos, Tan, Rahme, & Ausubel, 1999), mice (Lau, Ran, Kong, Hassett, & Mavrodi, 2004) and plants (Rahme et al., 1997). Zaborina's investigation found that dynorphin penetrates the bacterial membrane and directly induces the expression of the *pqsABCDE* operon controlled by the multiple virulence factor regulator (*MvfR*) (Zaborina et al., 2007). The binding of dynorphin to *P. aeruginosa* was confirmed via direct antibody staining, while its penetration into the bacterial cytosol was demonstrated by immunoelectron microscopy (Zaborina et al., 2007). Additionally, their results suggest that dynorphin synergizes with PQS to increase *pqsABCDE* expression even further (Zaborina et al., 2007). Expression of the *pqsABCDE* operon leads to enhanced production of three QS-assosiated signals: 2-heptyl-4-hydroxyquinoline N-oxide (HQNO), 4-hydroxy-2-heptylquinoline (HHQ), and PQS (Deziel et al., 2004).

The results of this investigation showed that both U-50,488 and dynorphin enhanced *P. aeruginosa* virulence, which then suppressed the growth of beneficial *Lactobacilli*, revealing yet another potential mechanism linking disturbances in the gut microbiota to stress and changes in cognition (Zaborina et al., 2007). Data from this study also suggests that dynorphin might enhance *P. aeruginosa* virulence not just in the GI-tract, but also at other sites of injury and inflammation, as such sites are heavily innervated and highly populated by macrophages and neutrophils (Stein et al., 1990). For this reason, the authors of this study suspect that dynorphin could be responsible for *P. aeruginosa* virulence in all inflammation-associated infections, including burns, implanted medical devices, and lung infections in cystic fibrosis (CF) patients (Zaborina et al., 2007).

Numerous reports show that exposure to extreme stress results in increased mortality upon subsequent bacterial challenge in animals (Alverdy et al., 2000). The findings of Zaborina et al. (2007) shed light on the processes behind this phenomenon. The data they acquired contributes further evidence supporting the idea that compounds secreted by the host during stress and immune activation can directly interact with pathways regulating bacterial virulence (Sperandio et al., 2003), (Zaborina et al., 2007). Their research demonstrates that *P. aeruginosa* can intercept endogenous opioids released by a host under stress, and integrate them in its quorum sensing circuitry, thereby enhancing its virulence (Zaborina et al., 2007). This comes about as a result of enhanced PQS production which activates QS circuits. Additionally, HQNO and pyocyanin produced in response to dynorphin, reduce the populations of beneficial probiotic bacteria, giving *P. aeruginosa* a competitive advantage (Zaborina et al., 2007).

These discoveries suggest that opportunistic pathogens such as *P. aeruginosa* have the means of monitoring their host by "listening in" on the host's communications, which allows them to take advantage of a weakened host under stress (Zaborina et al., 2007). This behavior further highlights the truly opportunistic nature of this bacterium (Zaborina et al., 2007). The evidence gathered in this study has significant clinical implications, given that intestinal ischemia tends to accompany severe physiological stress (such as surgery) and has been linked to fatal *P. aeruginosa* infections in the GI-tract

(Yale & Balish, 1972). Furthermore, endogenous opioids have been shown to accumulate at sites of inflammation, which may help explain the resilience and severe pathogenicity of *P. aeruginosa* infecting the lungs of CF patients (Pacheco & Sperandio, 2009). Taken together, the evidence reveals a sophisticated connection linking eukaryotic host stress signaling to bacterial QS and pathogenesis. The existence of such a system implies that stress responses are among the most basic physiological functions in both prokaryotes and eukaryotes, and play a central role in interkingdom communication (Hughes & Sperandio, 2008).

4 Discussion and Conclusion

4.1 Overview

Our understanding of the human microbiome and its functions has come a long way over the last decade. Much of the progress can be attributed to the development of new technologies which allow researchers to analyze the genomes of entire microbial communities, and explore interactions between microorganism and host on a molecular level. Metagenomic analyses have shown that the microbiome is comparable in function to a "virtual organ", responsible for mediating a broad variety of physiological and psychological processes (O'Hara & Shanahan, 2006). As such, the microbiome may represent a novel target for therapeutic intervention.

In our discussion of interkingdom signaling between the gut microbiota and host, we explored the influence that intestinal bacteria exert on the host's physiology and cognition via the MGB and HPA axes. We also examined the impact of severe psychological and physiological stress on gut microbiota. Evidence shows that the composition of the gut microbiota exerts a strong influence on the host's response to stress. The neurotransmitters serotonin and GABA have been implicated in anxiety and depression, and their production is mediated in part by the gut microbiota (Svenningsson et al., 2006), (Bravo et al., 2011), (Asano et al., 2012). The production of neurotrophin BDNF (involved in memory acquisition and consolidation) is also influenced by intestinal bacteria (Savignac et al., 2013). These findings suggest that the gut microbiota plays a key role in maintaining psychological and physiological health, and that altering the composition of the microbiome (through prebiotics, probiotics or antibiotics) can lead to beneficial or detrimental changes in the host. This premise is supported by evidence from studies examining the influence of particular bacterial strains on health and cognition. These studies revealed that administration of probiotic bacteria, such as *Bifidobacteria* and *Lactobacilli*, alleviated behaviors associated with anxiety and depression in rodents via a psychobiotic effect (Bercik et al., 2010), (Desbonnet et al., 2010), (Dinan et al., 2013). Furthermore, the transfer of gut microbiota from one host to another via FMT has been shown to induce expression of the donor phenotype in the recipient (Collins et al., 2013). This discovery suggests that FMT can be used to treat both GI and non-GI disorders. Ultimately, an individual's microbiome may be used to devise personalized healthcare strategies, leading to more efficient and targeted therapeutic treatments.

The neurohormones epinephrine and norepinephrine have been shown to be produced by gut bacteria in vivo, in significant quantities (Asano et al., 2012). Abnormal production of these signaling molecules has been linked to the dysregulation of the HPA axis and may play a role in anxiety, depression and ADHD (Sekirov et al., 2010) (Dell'Osso et al., 2011), (del Campo et al., 2011). A number of bacteria are known to detect and respond to epinephrine and norepinephrine, including *E. coli* and *V. cholera* (Sperandio et al., 2003). Endogenous opioids, such as dynorphin are also recognized by some bacteria (Zaborina et al., 2007). Evidence shows that each of these hormones enhances bacterial virulence through the activation of QS pathways, which lead to the expression of flagella, toxin production and increased growth rates (Zaborina et al., 2007). Thus, although these hormones serve to prepare the host for an adverse situation, they also reveal the host's weakened state to pathogens, thereby promoting colonization. Taken together, these

findings reveal a cross-talk between bacteria and host which mediates a wide variety of processes ranging from digestion to cognition.

4.2 Potential applications

The widespread prevalence of interkingdom signaling, and the extensive range of processes it mediates carries some fascinating implications. Given the importance of the microbiome composition to the health of the host, it stands to reason that many disorders are a result of "dysbiosis", a pathological alteration of the microbiome (Ringel & Ringel-Kulka, 2015). As such, these conditions could be treated by targeting specific microorganisms, and either promoting or repressing their growth. This could be achieved with probiotics or prebiotics, and represents a potential shift in therapeutic strategies from localized treatment towards a more global approach. In other words, physical and psychological well-being could be maintained by a personalized regimen of prebiotic and probiotic supplements. Additionally, these types of supplements could also be used to treat existing conditions. Such therapeutic targeting is thought to be particularly useful in treating metabolic and stress-related disorders (G. Clarke et al., 2014). A study by Ko, Lin, and Tsai (2013) found that soy milk enriched with GABA produced by *L. brevis* was as effective an antidepressant as fluoxetine (a selective serotonin re-uptake inhibitor commonly prescribed to treat depression). Although it is tempting to speculate over the potential therapeutic approaches involving the microbiome, more research is needed to unravel the complex interactions between bacteria and host.

Manipulation of the microbiome could be harnessed to modulate microbial colonization in infants, thereby promoting the development of a healthy gut microbiota in early childhood. The benefits of a healthy gut microbiome in early childhood are likely to extend well into adulthood, and could reduce the occurrence of various disorders (Belizario & Napolitano, 2015).

4.3 Future research

Although much progress has been made in the fields of microbial endocrinology and interkingdom signaling, a lot more research is needed to take the newly discovered host-microbiome interactions from laboratory investigations to practical applications. Individual bacterial components that mediate interkingdom signaling need to be investigated in order to conclusively prove that the signaling is direct (bacteria produces a neurochemical that binds to host receptor) rather than indirect (fragment of bacterial cell wall from a dead cell leads to a cascade of signals). Furthermore, the roles of eukaryotic, fungal and viral microbiota need to be determined, as most studies examining the microbiome have focused on bacteria. Another line of inquiry could explore the influence of products we regularly come in contact with (such as pharmaceuticals, cosmetics, cleaning products and pesticides) on the microbiome to determine whether they are safe for our microbial partners.

Most studies involving the gut microbiota have been carried out in rodents, and further human studies are needed to definitively prove the presence of this effect in humans. Whereas the focus of most early studies was limited to the influence of microbiota on stress responses, future studies can broaden their focus to explore the role of microbiota

in other conditions, including eating disorders, substance addiction, and schizophrenia (Foster et al., 2015).

Numerous microbiome engineering methods have been proposed, including CRISPR/Cas systems, quorum sensing systems, prebiotics, probiotics, synbiotics, and phage therapy (Belizario & Napolitano, 2015). Engineering of pathogen-specific bacteriophages is a particularly promising technique that could eventually allow for therapeutic fine-tuning of an individual's microbiome, and represents an important step towards personalized medical treatments involving microbiota (Belizario & Napolitano, 2015). For example, given the link between infertility and seminal microbiota, such research could one day lead to novel fertility treatments (Weng et al., 2014). Deciphering the cross-talk between microbiota and host will provide valuable insights into our physiology and cognition, while microbiome engineering could lead to novel therapeutic strategies for treating or preventing GI disorders, bacterial infections, and neuropsychiatric conditions (Kelly et al., 2015), (P. Freestone, 2013).

4.4 Unanswered questions

Despite all the data collected in the last decade pertaining to host-microbiome interaction, many questions remain unanswered. As we have seen, bacteria can bind and respond to epinephrine and norepinephrine using the QseC sensor kinase and its cognate response regulator QseB. Since the discovery of the QseBC system, a homologous QseEF adrenergic receptor system has been found (Reading, Rasko, Torres, & Sperandio, 2009). This prompts the question: are there more bacterial adrenergic receptors, and what processes do they mediate? We have also discussed the cross-talk between bacteria and host via the Epi/NE/AI-3 signaling system. Although there is convincing evidence showing that epinephrine and norepinephrine are recognized by certain bacteria, it is unclear if AI-3 binds to any host receptors. Given its structural similarity to host hormones, it does not seem unlikely that AI-3 acts as a chemical signal. If this is indeed the case, is this signaling dependent on adrenergic receptors? Furthermore, do bacterial cells recognize steroid and lipidic mammalian hormones? Do bacterial peptide hormones influence host signaling, and do host peptide hormones influence bacterial quorum sensing? Are there mammalian receptors for AHLs, and what processes are they involved in? These are the kind of questions we can hope to answer in the coming years.

Many recreational drugs have structures resembling mammalian signaling molecules. As shown in Figure 8, amphetamine, 2C-B and MDMA have structural similarities (benzene ring with a two carbon chain, terminating with an amine) to dopamine and (nor)epinephrine. As we have seen, these signaling molecules have a significant impact on bacterial pathogenicity and the composition of the microbiome. Could chronic drug use eventually reshape the microbiome? Support for this notion comes from research showing that morphine can directly activate the expression of virulence in *Pseudomonas aeruginosa*, with chronic exposure leading to lethal gut-derived sepsis (Babrowski et al., 2012). The microbiome has been implicated in substance addiction (Leclercq et al., 2014). If drug use leads to an altered microbiome, could this contribute to some of the symptoms associated with addiction and could the microbiome be targeted for the treatment of addiction? A better understanding of the relationship between substance addiction and the microbiome could lead to more effective therapies.

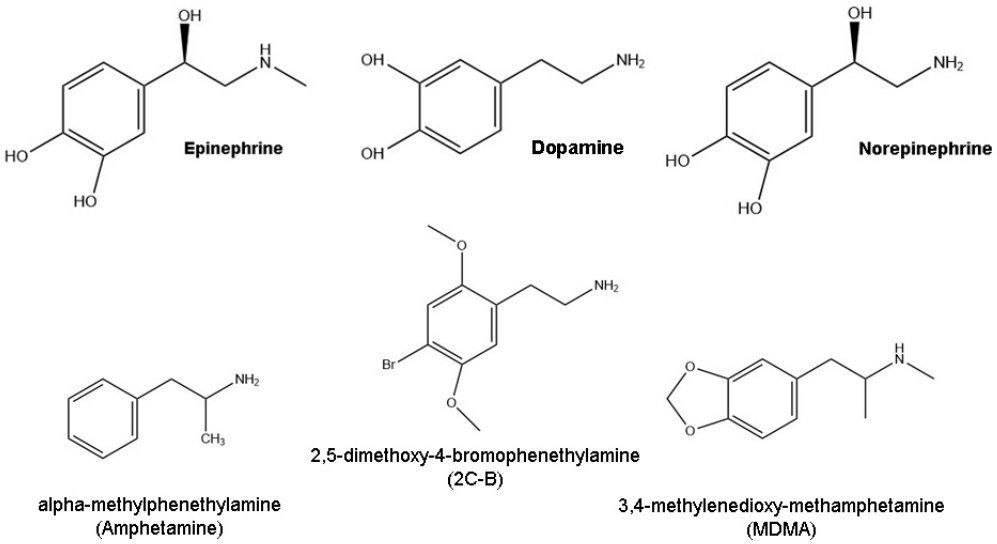

Figure 8 – Structures of psychoactive drugs resemble the structures of signaling molecules.

Recreational and prescription drug overdoses are a sadly common phenomenon. According to the United Nations World Drug Report 2014, an estimated 183,000 lives were claimed by fatal illicit drug overdoses in 2012 (United Nations Office on Drugs and Crime, 2014). One potential line of future investigation could address the contribution of gut microbiota to differing drug metabolism between individuals. Such research could answer whether specific gut microbiomes make people more predisposed to a drug overdose, and whether an individual's microbiota needs to be taken into account when prescribing medication (Houkai, Jiaojiao, & Wei, 2015).

A better grasp of host-microbiome interaction and the underlying factors may also shed light on the influence of lifestyle on physical and mental well-being. Lifestyle factors such as diet and exercise are likely to play a role in the composition of the microbiome (Machado & de Sá Filho, 2015), and may thereby contribute to overall health of the host. Given the role that gut microbiota play in the response to stress, it might also play a role in shaping the character of its host. For example, a host carrying microbiota that ameliorates the stress response may be more optimistic and agreeable. If this anxiolytic effect blunts the unpleasant effects of norepinephrine, then such a host may also be more likely to enjoy extreme sports. Studies investigating the anxiolytic effects of intestinal bacteria provide support for this notion (Ko et al., 2013), (Savignac et al., 2013). Differences in genetics, environment, diet and lifestyle between distantly separated populations result in differing microbiomes, and as such, the microbiome may be partially responsible for some cultural differences. Meditation has been shown to influence norepinephrine levels and alleviate stress (Amihai & Kozhevnikov, 2015). Since epinephrine and norepinephrine affect the growth of particular bacteria, could regular meditation sessions influence the composition of the microbiome? If so, could this partially explain the health benefits associated with meditation?

Could traumatic events trigger the release of large quantities of stress hormones, and

could this have long-lasting effects on the microbiome? Given that studies have linked early life stress with adverse changes in the gut microbiota (Bailey & Coe, 1999), (Desbonnet et al., 2010), could this be a factor in stress-related conditions such as PTSD?

The discoveries of interkingdom signaling and the human microbiome also give rise to intriguing existential questions. If the microbiome (made up of many separate entities, carrying far more genes than our own human cells) has such significant and far-reaching effects on the cognition and physiology of its host, then what does it mean to be an individual? If we cannot function without them, is there even such a thing as an individual person, or are we more like a collective, a "superorganism"?

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