General and Comparative Endocrinology 292 (2020) 113437

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Regulation of endocrine systems by the microbiome: Perspectives from comparative animal models

Candace L. Williams[a,*], Natàlia Garcia-Reyero[b], Christopher J. Martyniuk[c], Christopher W. Tubbs[a], Joseph H. Bisesi Jr.[d]

[a] Reproductive Sciences, San Diego Zoo Global Institute for Conservation Research, Escondido, CA 92027, USA
[b] Environmental Laboratory, US Army Engineer Research & Development Center, Vicksburg, MS 39180, USA
[c] Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida Genetics Institute, Interdisciplinary Program in Biomedical Sciences Neuroscience, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA
[d] Department of Environmental and Global Health and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL 32611, USA

ARTICLE INFO

Keywords:
Microbiome
Reproduction
Stress
Animal behavior
Endocrine disruption
Conservation endocrinology

ABSTRACT

The microbiome regulates endocrine systems and influences many aspects of hormone signaling. Using examples from different animal taxa, we highlight the state of the science in microbiome research as it relates to endocrinology and endocrine disruption research. Using a comparative approach discussing fish, birds, and mammals, we demonstrate the bidirectional interaction between microbiota and hormone systems, presenting concepts that include (1) gastrointestinal microbiome regulation of the neuroendocrine feeding axis; (2) stress hormones and microbial communities; (3) the role of site-specific microbiota in animal reproduction; (4) microbiome effects on the neuroendocrine systems and behavior; and (5) novel mechanisms of endocrine disruption through the microbiome. This mini-review demonstrates that hormones can directly affect the richness and diversity of microbiota and conversely, microbiota can influence hormone production and mediate their functions in animals. In addition, microbiota can influence the action of a diverse range of neurotransmitters and neuropeptides in the central nervous system, which can lead to behavioral disruptions. As many animals have species-specific reproductive behaviors, it is important to understand how shifts in the microbiota relate to these complex interactions between sexes. This is especially important for captive animals on specialized diets, and there are significant implications for microbiome research in conservation and reproductive biology. For example, microbial metabolites may modify motility of gametes or modulate hormone-receptor interactions in reproductive tissues. Thus, efforts to incorporate metabolomics into the science of microbiome-endocrine relationships, both those produced by the host and those generated from microbial metabolism, are increasingly needed. These concepts have fostered an exciting emerging era in comparative endocrinology.

1. Introduction

For most animals, microorganisms play a critical role in their survival (McFall-Ngai et al., 2013). These symbiotic relationships are numerous and diverse, begin during development, and are modified throughout an organism's lifetime (Milani et al., 2017). Although different anatomical niches of an organism (e.g., skin, reproductive tract, etc.) can host unique microbiomes, the majority of microbiota within an organism reside in the gastrointestinal (GI) tract and play fundamental roles in a number of physiological processes. For example, gut microbiota are critical to the development and maintenance of the host immune system (Hooper et al., 2012; Lozupone, 2018) while also excluding pathogens via colonization resistance (Sorbara and Pamer, 2019). Additionally, members of the gut microbiota are important for nutrient acquisition. This is especially true for herbivores, in which microbes are entirely responsible for the breakdown of fibrous plant material to host-accessible nutrients (Bergman, 1990; Flint et al., 2012).

There is growing evidence that microbiota interact with their host's endocrine function and thus have the potential to influence, or be influenced by, the myriad of physiological processes the endocrine system regulates (reviewed in Garcia-Reyero (2017)). For example, associations between feeding, growth and metabolism, the stress response and reproduction, and gut microbiota have all been established. Insights into potential mechanisms are equally diverse, as differences in microbial communities have been correlated with differences in hormone metabolism (Kwa et al., 2016; Ridlon et al., 2013), circulating levels of

* Corresponding author at: Institute for Conservation Research, San Diego Zoo Global, 15600 San Pasqual Valley Road, Escondido, CA 92027, USA.
E-mail address: cwilliams@sandiegozoo.org (C.L. Williams).

https://doi.org/10.1016/j.ygcen.2020.113437
Received 14 October 2019; Received in revised form 29 January 2020; Accepted 10 February 2020
Available online 12 February 2020
0016-6480/

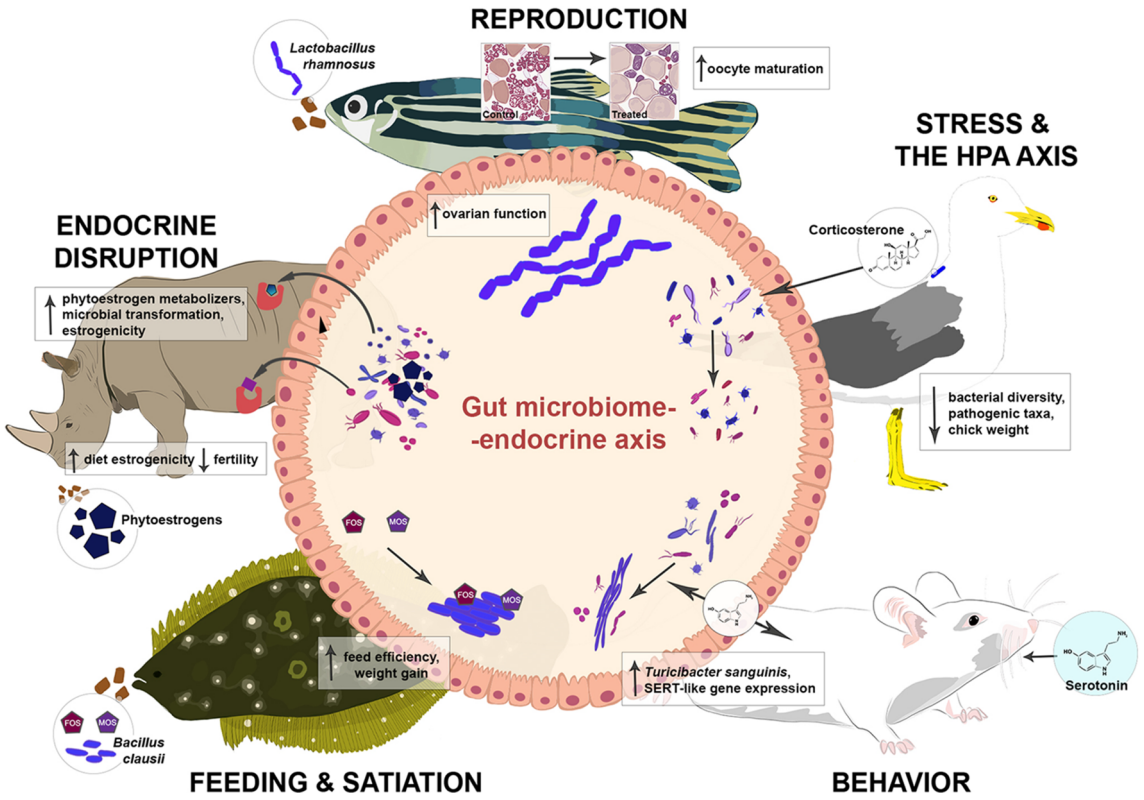

Fig. 1. Highlighted examples of known interactions between gut microbiota and the endocrine system, particularly those associated with reproduction (Carnevali et al., 2013), stress and the HPA axis (Noguera et al., 2018), behavior (Fung et al., 2019), feeding and satiation [fructooligosaccharide (FOS) & mannan-oligosaccharide (MOS)] (Ye et al., 2011), and endocrine disruption (Tubbs et al., 2016; Williams et al., 2019a).

hormones (Antwis et al., 2019; Miller et al., 2017), behavior (Dinan et al., 2015), and even altered gene expression in endocrine tissues (Martin et al., 2019). Similarly, there is strong evidence for bi-directional relationships between microbiota and exogenous endocrine disrupting compounds, with microbiota serving as targets for EDCs (Rosenfeld, 2017), but also transforming EDCs to affect host function (Williams et al., 2019a).

Although our knowledge about the interactions between microbiota and vertebrate endocrine systems is growing rapidly, the field is still in its infancy. As such, most of what is known comes from studies conducted in humans or rodents, and in many cases, causal relationships between microbiota and endocrine function are not clearly established. Nevertheless, these studies clearly demonstrate important linkages between microorganisms and hormone systems, and it is likely that similar relationships exist across vertebrates. The goal of this mini-review is to highlight what is currently known regarding the interface between microbiota and endocrine systems. Where possible, work in comparative models will be reviewed, however the majority of findings presented are from laboratory species and humans. It is our hope that this review will compel comparative endocrinologists to consider the potential contributions of microbiota to the hormonal regulation of the species they study. To facilitate this, potential future directions and considerations for future research are presented.

2. Interactions of microbiota and the endocrine axis in animals

2.1. The gastrointestinal microbiome and the regulation of feeding and satiation

Upon ingestion of a meal, nutrients in the GI tract stimulate a complex set of hormones, peptides, and neurotransmitters that are responsible for bidirectional signaling along the gut-brain axis (GBA). Much of this bidirectional communication takes place via enteroendocrine cells (EEC)–specialized cells in the GI epithelium that are responsible for excretion of important signaling molecules and peptides (Sandhu et al., 2017). Among these hormones are cholecystokinin (CCK) and peptide YY (PYY), which are responsible for signaling satiation either through direct EEC-nerve communication or indirect paracrine mechanisms (Batterham and Bloom, 2003; Butt and Volkoff, 2019; Sandhu et al., 2017). EECs can also release glucagon-like peptide-1 (GLP1), subsequently stimulating insulin secretion. Release of these important neuropeptides is controlled in part by the presence of specific luminal content. The apical membranes of EECs express numerous G protein-coupled receptors (GPCR) including GPR41 and GPR43, both of which bind short chain fatty acids (SCFAs) (Lin et al., 2012; Tolhurst et al., 2012). Among the species in the gut microbiota, members of the phylum Bacteroidetes are known to produce the SCFAs acetate and propionate, whereas the Firmicutes are primarily responsible for the production of butyrate (Kau et al., 2011). While SCFAs have been shown to influence functions that include inflammatory responses and metabolism, they also impact neuroendocrine hormone release through interactions with EEC surface receptors (Cani et al., 2013). The gut microbiome can also impact bile acid synthesis and the formation of secondary bile acids, both of which influence the release of EEC neuropeptides through interaction with apical bile acid GPCR, TGR5, as well as the farnesoid X receptor (FXR), a nuclear receptor responsible for maintaining glucose tolerance and insulin sensitivity (Cani et al., 2013; Sandhu et al., 2017). Multiple studies have linked a number of metabolic disorders including obesity and diabetes mellitus to alterations in SCFA and bile acid production following changes in the gut microbiome (den Besten et al., 2013; Samuel et al., 2008).

While the majority of research on the mechanisms underlying microbial control of GBA signaling is focused on human, mouse, and rat models, evidence suggests that the influence of the microbiome on neuroendocrine signaling is conserved across numerous animal taxa. In fish, few studies have examined the direct mechanism of microbiota-

gut-brain axis signaling, but studies in zebrafish (*Danio rerio*) have indicated that microbial colonization is necessary for normal epithelial absorption of fatty acids, as well as lipid accumulation and metabolism (Sheng et al., 2018). Colonization of Japanese flounder (*Paralichthys olivaceus*) with the beneficial bacteria *Bacillus clausii* and the administration of the prebiotics fructooligosaccharide (FOS) and mannan-oligosaccharide (MOS) also resulted in increased weight gain, feed efficiency, and growth (Fig. 1). This was attributed to increased food intake and nutrient digestion, both of which are under the control of enteric endocrine signaling (Ye et al., 2011). A recent review of the contributions of the microbiome to livestock show similar results, as alterations in microbial communities affected feeding and satiation among numerous species (O'Callaghan et al., 2016).

While peptide hormones involved in feeding and satiation including CCK, peptide YY, and GLP1 have been shown to be influenced by the gut microbiome, there are also neurotransmitters including serotonin and gamma-aminobutyric acid (GABA) that are involved in these functions. Multiple studies in humans have found that gut microbiota are capable of producing the neurotransmitters serotonin, GABA, melatonin, acetylcholine, and histamine and that these microbially derived neurotransmitters can access the central nervous system via enterochromaffin cells and/or the enteric nervous system (Sandhu et al., 2017; Tillisch, 2014). In dogs, decreased circulating levels of serotonin were found to be associated with decreased gut microbial diversity in obese animals. While decreased circulating serotonin is associated with increased appetite, which may help to explain the association with obesity, it is unclear whether the loss of microbial taxa directly impacts circulating serotonin due to decreases in microbially derived serotonin (Park et al., 2015). More research is needed to understand the role of microbially derived neurotransmitters on feeding and satiation in humans and animals.

2.2. Microbiota, stress, and the hypothalamic–pituitaryadrenal (HPA) axis

For several years, questions surrounding the role of the microbiome in the regulation of the hypothalamic–pituitaryadrenal (HPA) axis have been proposed. It has been learned that the composition of the maternal microbiome, as well as the timing and progression of initial colonization, are intimately tied to childhood development and the HPA axis (de Weerth, 2017). These early interactions influence an individual's ability to physiologically respond to and cope with stress. The microbiome-HPA axis interaction was explored by Sodu et al. (Sudo et al., 2004), finding that the HPA response of germ-free (GF) mice (raised in the absence of microorganisms) was more sensitive to restraint stress than that of mice raised to have a normal functional microbiota, but were lacking specific pathogens (specific pathogen free mice, SPF). GF mice also showed reduced expression levels of cortical glucocorticoid receptor mRNA and elevated corticotropin releasing factor (CRF) mRNA and protein levels in the hypothalamus compared to SPF mice (Sudo et al., 2004). Moreover, plasma adrenocorticotropic hormone (ACTH) and corticosterone elevation in response to restraint stress was substantially higher in GF mice than in SPF mice. Taken together, the HPA axis of GF mice appeared to be hypersensitive to certain types of stress. These aberrant responses in GF mice were ameliorated to some degree with oral inoculation of the microbiota from SPF mice. Following studies such as this, evidence has mounted that there is bi-directional communication between the gut microbiome and the HPA axis (Dinan and Cryan, 2012; Foster et al., 2017; Morris and Ridlon, 2017) indicating that commensal microbes regulate the development and function of an individual's HPA stress axis.

Despite this understanding, there is little research in non-rodent/human animal models that characterize the communication between the HPA axis and the microbiome, creating an important knowledge gap. Communication between the HPA axis and the microbiome is expected to differ in animals, as the primary stress hormones vary across taxa (e.g. cortisol plays a dominant role in the human, fish, and most mammals stress response while corticosterone prevails in rodents, birds, amphibians and reptiles) (Baker et al., 2013). The few data available in comparative animal models indeed point to a bidirectional relationship between the stress axis and the microbiome. In a novel approach, Noguera et al. (2018) manipulated glucocorticoid levels in yellow-legged gulls (*Larus michahellis*) to discern how elevated stress-related hormones would impact the composition and diversity of the gut microbiota. Birds were treated with corticosteroid implants, and 16S sequencing was conducted on their fecal samples (Fig. 1). Intriguingly, the study revealed that several potential avian pathogenic taxa (e.g., *Microvigas*, *Helicobacter*, and *Pseudomonas*) were underrepresented in the gut following corticosterone implants. Commensal microbiota (e.g., Firmicutes) were also underrepresented in the birds with the corticosteroid implant. The study contradicted popular thought that increased levels of stress hormones contributes to an increased risk of pathogenic bacteria proliferation. However, chick weight was lower in implanted birds, suggesting there may be a negative trade-off. These conflicting results highlight the need for further understanding of the connection between the HPA axis and the microbiome. In another study in birds, broiler chickens were heat stressed and growth and cortisol measured in addition to the microbiome response (Shi et al., 2019). With heat stress, the levels of cortisol increased from 10 to 30%, which was measured on multiple days throughout the study (1, 7, 14, 28 days). The rise in cortisol also correlated to a shift in the gut microbiota composition in the caecum (Shi et al., 2019). Heat stress for 7 days increased the Firmicutes and the Tenericutes and decreased the prevalence of the Bacteroidetes by approximately 30%. Although it was not possible to discern whether the cortisol was modifying the microbe composition directly, the study proposes an interesting link between heat stress and the gut microbiota.

Other experiments using non-rodent models have revealed a potential role for cortisol in mediating the relationship between the GBA. In a study in male Yorkshire piglets, correlative relationships between serum serotonin, serum cortisol, colon volatile fatty acids, and the microbiome were investigated (Mudd et al., 2017). The study revealed that the *Ruminococcus* were negatively correlated with cortisol. This was significant because the authors proposed that cortisol may be the candidate signal mediating the interactions between *Ruminococcus* and brain N-acetylaspartate, an amino acid derivative that plays a crucial role in protecting neuronal development and function from injury. Petrosus et al. (2018) assessed cortisol's potential role in modulating gut microbiota more directly. By orally administering cortisol at 73.2 mg of hydrocortisone acetate to piglets twice on the first day of the experiment, blood cortisol levels increased as anticipated and were accompanied by a shift in the intestinal environment to favor aerobes and pathogens. The authors observed a high proportion of *Escherichia coli* and a low proportion of *Lactobacillus* with higher levels of cortisol, proposing a mechanism for the onset of opportunistic infections resulting from cortisol-induced immunosuppression.

Taken together, these studies in different animals have revealed that several types of stressors (restraint stress, environmental stressors) and hormonal manipulation of the HPA axis can significantly alter the gut microbiota. As there exist strong links between stress and behavior, researchers have begun to address questions about whether these interactions are modulated by microbes specifically, and experiments have been conducted in both zebrafish (Davis et al., 2016) and mammals (Crumeyrolle-Arias et al., 2014). It is noteworthy that the bacterial community can play a significant role in metabolizing steroid hormones, and some communities may be able to convert steroid precursors such as dietary cholesterol into active glucocorticoids. For example, Ridlon et al. (2013) revealed a high capacity of *Clostridium scindens* to convert glucocorticoids into androgens in the gut. Undoubtedly, the interaction among different hormone axes in relation to the gut microbiome will be complex, and it will remain challenging to discern microbiota responses unique to specific hormones. Knowledge gaps to pursue include discerning the roles of corticosteroids in

modulating gut microbiota of amphibians and reptiles, and cortisol in fishes. To the best of our knowledge, these investigations have not been conducted in these animal taxa.

2.3. Microbiota and the reproductive axis: fish, baboons, and everything in between

Defining clear, mechanistic relationships between microbiota, the endocrine system, and the hormonal control of reproduction is challenging, and a limited number of studies have been conducted in this area. To date, most investigations into the role microbiota may play in reproduction have been descriptive studies examining changes in microbial communities within specific body niches (i.e., male and female reproductive tracts) throughout the reproductive cycle (Moreno and Simon, 2018). For example, in wild baboons (*Papio cynocephalus*) and captive macaques (*Macaca fascicularis*), using 16S rRNA gene sequencing, reproductive/hormonal state was determined to be a strong indicator of vaginal microbial community composition (Miller et al., 2017; Nugeyre et al., 2019) These findings largely reflect those in humans, with one distinct difference. In humans, the vaginal microbiome is dominated by lactobacilli (Moreno and Simon, 2018), whereas in these two non-human primates, other lactic acid producing taxa were dominant community members (Miller et al., 2017). Together these studies highlight a likely influence of hormones on vaginal microbial communities, and/or vice versa. In addition, they demonstrate that, while microbiomes may differ in terms of community composition, functional niches are often conserved allowing distinct microbial communities to serve similar roles across species.

Relatively fewer studies have investigated the male reproductive tract microbiome and its potential role in reproduction. It is well known that dysbiosis of the male reproductive tract can have significant effects on fertility, though not necessarily via endocrine-mediated mechanisms (Gimenes et al., 2014). A recent study in humans demonstrated that semen from men exhibiting good sperm quality (i.e., high motility and normal morphology) had enriched abundance of *Staphylococcus* spp. and *Lactobacillus* spp., respectively, and in general, male and female reproductive tract microbiomes are similar (Baud et al., 2019). In comparative models, and in particular fish, the role of hormones in sperm maturation within the male reproductive tract (Miura et al., 1992) and the acquisition of sperm motility (Tan et al., 2019) are well established. However, it is intriguing to hypothesize how microbiota might contribute to these processes, potentially through the synthesis of biomolecules with signaling potential, or via other mechanisms. Investigating such relationships present a potentially rich area of future research.

Gut microbiota can potentially influence the endocrine control of reproduction by directly transforming hormones, thereby altering their bioavailability and efficacy (Kunc et al., 2016). This is possible because members of the gut microbiota commonly express a number of enzymes capable of transforming hormones, and in particular conjugated steroids, such as β-glucosidases, β-glucoronidases and hydroxysteroid dehydrogenases (Kunc et al., 2016; Kwa et al., 2016). Enzymatic transformation by gut microbiota has been demonstrated for all steroid classes (Kunc et al., 2016). However, given our growing understanding of the role hormones and gut microbiota play in breast cancer development in humans, the most comprehensive understanding of steroid-microbiota interactions involve estrogens (Kwa et al., 2016; Plottel and Blaser, 2011). Following hepatic conjugation, estrogens (estrone, estradiol and estriol) are excreted in the bile can become deconjugated by gut microbiota, making them available to re-enter the circulation (Kwa et al., 2016). It has been proposed that this deconjugation and increased availability contributes to the excess levels of circulating of estrogens associated with the development and progression of breast cancer (Kwa et al., 2016; Plottel and Blaser, 2011). Presumably, reproductive processes could be also affected by the deconjugating activity of microbiota on estrogens, in addition to other steroids involved in the hormonal control of reproduction, though in-depth studies are lacking.

In terms of overall fertility, there are a few studies that have investigated associations between reproductive success, alterations in endocrine function and differences in gut microbiota. In humans, differences in gut microbial communities, or therapeutic treatments that result in community shifts, have implicated gut microbiota in the increased incidence of reproductive pathologies that can affect fertility (Baker et al., 2017). These include obesity, polycystic ovary syndrome, endometriosis, and endometrial hyperplasia. It is hypothesized that microbe-mediated increases in circulating estrogen levels contribute to these phenomena (Baker et al., 2017). Early studies comparing GF and microbially-colonized rodents have shown that colonized individuals excreted significantly higher levels of reproductive steroids (Eriksson et al., 1969) and demonstrated higher reproductive capacity (Shimizu et al., 1998). Probiotic treatment of zebrafish (*Danio rerio*) with *Lactobacillus rhamnosus* resulted in increased ovarian function, which was associated with increased ovarian expression of genes positively associated with oocyte maturation and ovulation and a down regulation of negatively associated genes (Carnevali et al., 2013) (Fig. 1). In black rhinoceros (*Diceros bicornis michaeli*), breeding success and elevated fecal progestagen production (i.e., evidence of ovarian activity) were associated with the increased abundance of four relatively rare microbial taxa (Antwis et al., 2019). Similarly, low abundance microbiota have been suggested to contribute to fertility differences in southern white rhinoceros (*Ceratotherium simum simum*) (Williams et al., 2019a). Taken together, these studies demonstrate that both overall community structure, as well as the increased presence of rare gut microbiota, can influence reproductive capacity, likely through the modulation of various levels of reproductive control.

2.4. The interplay between the microbiome, behavior, and (neuro)endocrine systems

The multitude of cells that form the microbiota develop and establish extremely complex communication and biofeedback networks not only with other microbes, but also within the host. Through different communication paths, evidence points to a microbial role in the development of the central nervous system, neurotransmission, and behavior (Dinan and Cryan, 2017; Dinan et al., 2015). Several mechanisms have been proposed to explain how the gut microbiome might influence the brain (Fig. 2). Those include the production of metabolites such as lipopolysaccharides (LPS) and short chain fatty acids (SCFAs) and the induction of inflammatory mediators (e.g., cytokines) that can interact with the enteric nervous system (neurons in the intestine) locally, or signal through the vagus nerve to impact the neuroendocrine system (reviewed by Cussotto et al. (2018)). Microbiota are also capable of transmitting signals both short and long distances through electrochemical means, including ion channel and signaling among neurons in a human brain (Prindle et al., 2015) to affect hosts, which in return, can send feedback to the microbial community.

As mentioned above, studies suggest that the gut microbiome can affect behavior through interactions with the host neuroendocrine system. Some of those behaviors include stress-related behavior, social behavior, sexual behavior, cognition and addiction, all of which are modulated by neuroendocrine pathways (reviewed in Cussotto et al. (2018)). One mechanism used by microbiota to modulate the neuroendocrine function involves the production of SCFAs in the gut but can travel far from their production location (Macfarlane and Macfarlane, 2012; Vijay and Morris, 2014). Butyrate and propionate can affect dopamine and noradrenaline synthesis, and propionic acid also is suspected to modulate serotonergic neurotransmission, as well as GABA, dopamine, and serotonin levels (El-Ansary et al., 2012; Nankova et al., 2014; Stilling et al., 2016), explaining their potential effects on behavior. Specifically, lower levels of fecal butyrate and decreased abundance of butyrate-producing taxa were found in the microbiome of autistic individuals, suggesting that there may be a potential

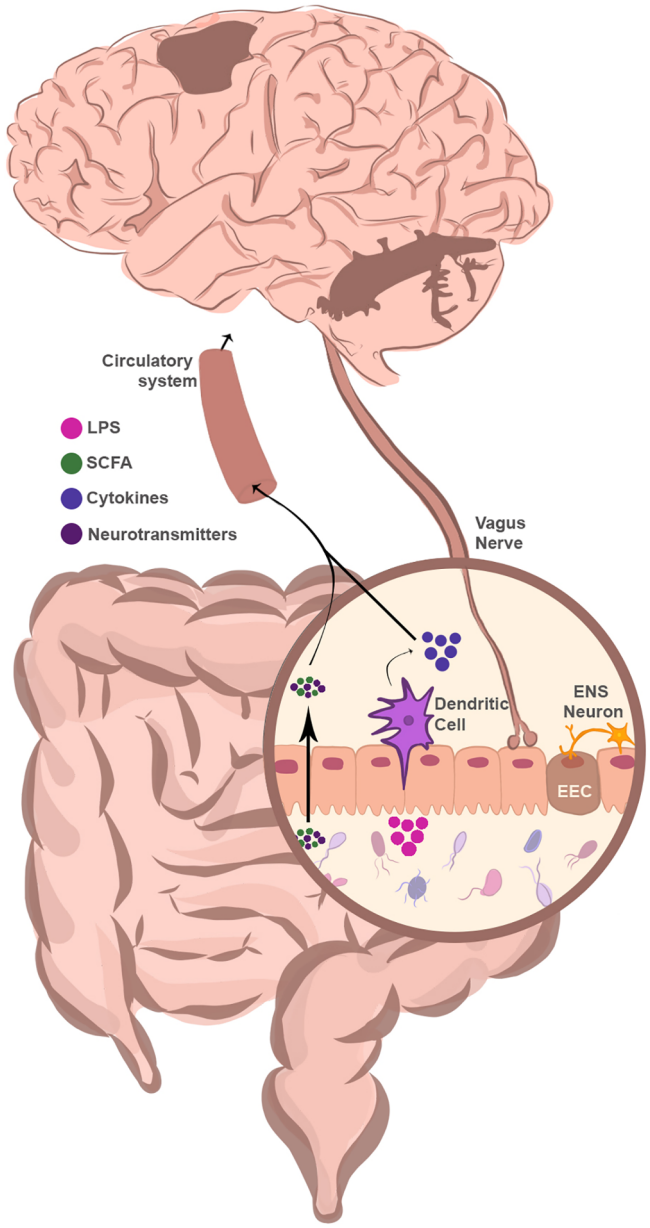

Fig. 2. Gut microbiota may influence neuroendocrine function through several actions, including the production of metabolites like lipopolysaccharides (LPS) short chain fatty acids (SCFAs) and neurotransmitters, the induction of inflammatory meditators such as cytokines, and the interaction with the enteric nervous system (ENS) and enteroendocrine cells (EECs), locally, or systemically through the vagus nerve.

relationship between these SCFAs and the neurodevelopmental and behavioral effects of autism spectrum disorder (Liu et al., 2019).

Bacteria have been shown to produce and/or use a wide range of mammalian neurotransmitters, including dopamine, acetylcholine, norepinephrine, GABA, or serotonin (Strandwitz, 2018), suggesting they can influence processes driven by those neurotransmitters. For example, administration of *Lactobacillus rhamnosus* altered the expression of GABA receptors in the brain, leading to a decrease of anxiety and depression-like behavior in mice (Bravo et al., 2011). Serotonin synthesized in the gastrointestinal tract by enterochromaffin cells, accounts for >90% of the body's content (Hata et al., 2017). While it is believed that about 50% of that gut-derived serotonin is regulated by the gut microbiota, the actual mechanisms and function of such regulation are largely unknown, but are believed to be largely performed by spore-forming bacteria dominated by the Clostridiaceae and Turicibacteraceae (Yano et al., 2015). Fung et al. (2019) recently demonstrated that elevated levels of intestinal serotonin increased the relative abundance of spore-forming bacteria. They identified *Turicibacter sanguinis* as expressing a neurotransmitter sodium symporter-related (SERT) protein with homology to the mammalian protein (Fig. 1).

Multiple studies have indicated the gastrointestinal microbiome is also capable of regulating sex hormones including testosterone. Utilizing non-obese Type 1 diabetes mouse models, studies have indicated that colonization with commensal bacteria in male mice increases circulating testosterone and transfer of male gut microbiota to females also conferred this increase in testosterone (Markle, et al., 2013). It is also clear that this effect is bi-directional, with host testosterone levels modulating gastrointestinal microbiota in favor of species that are involved in anti-inflammatory responses (Yurkovetskiy et al., 2013). Results of these studies have led investigators to attribute the sex-biased nature of auto-immune diseases to the role of the gastrointestinal microbiota in circulating testosterone (An and Kasper, 2013). This bi-directional control of circulating testosterone between host and microbiota may also provide a novel mechanism for endocrine disrupting chemicals to impact host health. Androgenic and anti-androgenic chemicals are regularly found in surface water samples around the world and these chemicals are likely to influence the delicate balance of host, microbiome, and circulating testosterone (Baker, 2014).

The microbiome has also been linked to social behavior, presumably including interactions with the neuroendocrine system. While the exact signaling mechanisms have not been clearly established, there are a few examples highlighting the interplay of the microbiota and behavior. For instance, Amato et al. (2017) explored the effect of host kinship and time spent in social contact on the gut microbiota of wild, black howler monkeys (*Alouatta pigra*), showing that closely related individuals had less similar gut microbial communities than non-related individuals. Similar relationships were found in the gelada monkey (*Theropithecus gelada*), in which social organization and diet played a role in structuring the gut microbiota (Trosvik et al., 2018). In lemurs (*Propithecus verreauxi*), Perofsky et al. (2017) found that 58% of the individual variation in the gut microbiome was attributed to social group membership, even when controlling for confounding factors such as diet, genetic relatedness, or spatial proximity. The interactions between behavior, the (neuro)endocrine system and the microbiota are still largely unknown. Future studies are needed across different species using multidisciplinary approaches to further elucidate these intricate and often unexpected connections to link the microbiota and neuroendocrine control of behavior.

2.5. The microbiome: novel mechanisms of endocrine disruption

Exposure to endocrine disrupting chemicals (EDCs), may also impact host microbiota and endocrine function. The term EDC refers to the compound's ability to disrupt normal endocrine function within hosts, typically due to the structural similarity of the EDCs and endogenously produced compounds (Colborn et al., 1993; McLachlan, 2016). These chemicals include natural compounds, like phytoestrogens, and those of anthropogenic origins, like plastics, and exposure routes range from oral ingestion to contact through skin or inhalation and transfer through placenta or milk to offspring (see Diamanti-Kandarakis et al. (2009) and Gore et al. (2015) for review). Since microbiota interface with chemicals at these sites of exposure, EDCs themselves can target microbiota leading to systemic effects, but microbiota can also influence EDC severity through chemical transformations. Due to the integral role of the endocrine system and the microbiome in homeostasis, any dysfunction in either can lead to various negative host outcomes (Guillette, 2006), such as metabolic disorders (Velmurugan et al., 2017), infertility (Adams, 1995) and cancer (Diamanti-Kandarakis et al., 2009). Below we highlight examples of these bidirectional interactions between microbiota and EDCs and their

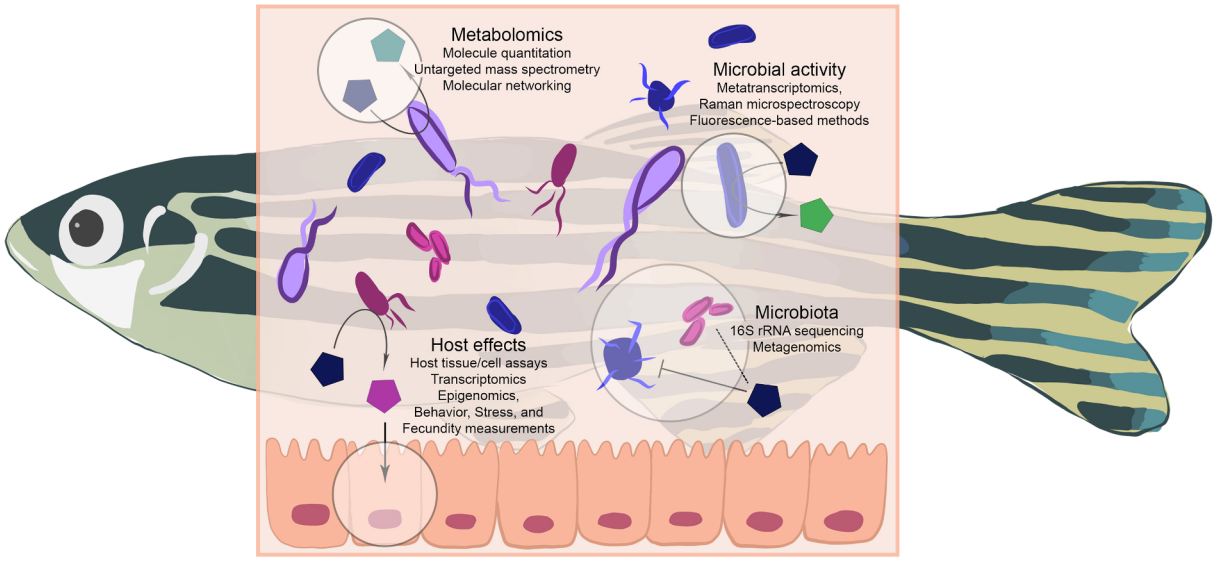

Fig. 3. The study of the microbiome-endocrine interactions requires multi-dimensional approaches both *in vitro* and *in vivo*, including the characterization of microbiota and the metabolome, microbial activity assessments, and investigations into the effect on hosts. Pentagon shapes represent endogenous or exogenous endocrine molecules that may act on or be acted on by members of the gut microbiota.

potential to affect hosts.

Microplastics and chemicals used to protect plastics have garnered significant attention recently. Some of these plastics are endocrine disruptors, acting as aryl-hydrocarbon receptor activators or reproductive toxicants in many cases (i.e. anti-androgens, estrogens) (Krüger et al., 2008; Ohtani et al., 2000). Recent evidence indicates that the phthalate plasticizer diethylhexyl phthalate (DEHP) can disrupt expression of peroxisome proliferator activated receptor alpha (PPARα) in the gastrointestinal system of zebrafish–a receptor that is sensitive to microbially derived SCFAs (Buerger et al., 2019b). Additionally, DEHP has been shown to decrease microbial diversity and disrupt important microbial functions related to nutrient processing including lipid and carbohydrate metabolism (Buerger et al., 2019a). Combined, these effects can extend beyond the microbiome, leading to several negative host outcomes, including inflammation.

However, some microbiota can degrade phthalates to chemical moieties, reducing these negative outcomes. For example, Asian carp (*Hypophthalmichthys* spp.) gut microbiota reduced the estrogenicity of phthalates (Kolb et al., 2019). Specifically, several gut microbiota displayed bioremediation potential individually, including *Rhodococcus ruber*, an isolate whose growth was promoted by DMP (dimethyl phthalate) and DBP (dibutyl phthalate), but inhibited by DEP (diethyl phthalate), and *Achromobacter aegrifaciens* SKTGEO1 whose aggregates increased during log phase growth (Kolb et al., 2019). However, the entire microbiota displayed more rapid growth on phthalate mixture compared to individual isolates (Kolb et al., 2019), indicating that interactions among microbiota may be important in degrading phthalates.

Although microbiota can transform and degrade EDCs to reduce endocrine disruption potential, this is not always the case. One example is that of phytoestrogens where the microbial transformation of the isoflavone daidzein to equol (Atkinson et al., 2005) has been associated with infertility in various species, but is best characterized in ewes. Exposure to daidzein-rich clover is associated with the development of reproductive pathologies and infertility (Adams, 1995), but it is the microbial metabolite equol that is thought to be the driver of this effect (Adams, 1995). Similar to ewes, captive southern white rhinoceros (SWR) display similar pathologies, erratic or absent luteal activity, and reduced fertility (Hermes et al., 2006; Patton et al., 1999; Tubbs et al., 2016). SWR's highly estrogenic captive diet has been implicated in this phenomenon, as diet estrogenicity was significantly correlated to infertility in captive-born females (Tubbs et al., 2016), and previous work has shown how phytoestrogens (daidzein, equol, genistein, coumestrol) may disrupt SWR endocrine function using *in vitro* estrogen receptor assays (Tubbs et al., 2012).

Linking microbiota's role in endocrine dysfunction is challenging. By integrating 16S rRNA sequencing, targeted mass spectrometry, *in vitro* estrogen receptor assays, and fertility measurements, Williams et al. found that SWR females that excrete the highest levels of equol display the highest level of fertility (Williams et al., 2019a) (Fig. 1). In addition, members of another phytoestrogen class, the coumestans, were quantified, finding that despite high levels of both methoxycoumestrol and coumestrol in the diet, very little of these compounds were excreted (Williams et al., 2019a), indicating possible microbial transformation. Interestingly, no previously identified phytoestrogen metabolizer was correlated to the concentration of any phytoestrogen metabolite, but members of the Coriobacteraceae and *Eubacterium* spp. (Braune and Blaut, 2016) were found in low relative abundance in SWR. Their involvement in this phenomenon is possible, as rare taxa have previously been associated with fertility in captive eastern black rhinoceros (Antwis et al., 2019). Combined, these results suggest that microbiota play an important role in phytoestrogen-associated infertility in SWR, potentially mediated by novel microbiota and metabolites. However, determining which members are involved in this process and the mechanism by which they influence fertility remain elusive at this time.

3. Future directions: linking microbiota function to host endocrine responses

Both the endocrine system and microbiome drive physiological processes across systems through various means. Since these modifications do not occur in a single direction, it is important that we evaluate these interactions thorough multi-dimensional approaches (Fig. 3). Investigating the entire microbiome means not only characterizing microbial members, but identifying their functions within the system, typically through the production of small molecules (Melnik et al., 2017). Therefore, future work should study the entire microbiome, evaluating the microbiota and the suite of molecules they create, through microbiological and mass spectrometry analyses, respectively.

Several new methods may aid in understanding these interactions,

however the strategic use of long-standing methodology is also promising. Most studies examining microbial interactions and the endocrine system rely solely on the use of microbial sequencing; specifically, 16S rRNA amplicon sequencing methods. Although this method is useful, it is compositional, only reporting changes in relative abundance, leading to several biases, primarily from the selection of primers and data analysis tools due to its amplification-dependent approach (Steen et al., 2019). Despite abundance being an important factor when identifying overall microbiome function, this is not always the case. In many ecosystems, rare taxa may exert a disproportionate functional role (Hausmann et al., 2019), which can be missed in compositional analyses. Metagenomics may be useful in these situations, but alone has its own limitations since it lacks the capability of measuring *in situ* microbial activity. Meta-transcriptomics provides this information, almost in real time, but results rely heavily on sequencing depth and the success of rRNA depletion (Singer et al., 2017).

Microbiota have complex relationships (Oliphant and Allen-Vercoe, 2019), high rates of horizontal gene transfer (Bonham et al., 2017), and in many systems are poorly classified (Steen et al., 2019). Therefore, identifying which microbiota are involved in these systems can be difficult. Microbial activity-based measures may help (Berry et al., 2015; Hatzenpichler et al., 2014). Of greater help would be the ability to isolate active microbiota for further evaluation (Lee et al., 2019) and specifically, investigating metabolically active microbiota using culture-based methods (Lagier et al., 2018) to discover microbially-mediated small molecule production. Like the microbiota in non-human systems, small molecules are also poorly classified, and using new methods, like untargeted mass spectrometry and molecular networking (Quinn et al., 2017; Wang et al., 2016), would allow for the identification and classification of novel natural products. Identifying which microbiota and small molecules play an active role in these processes would provide a better understanding of how the microbiome may modulate endocrine function.

Determining how microbially-derived compounds impact host processes is also difficult. *In vitro* methods, such as co-culture of microbiota and host tissues/cells, may provide some insight. Richards et al. (2019) measured host transcriptional response to the exposure of a healthy microbiota, revealing that several genes that were differentially expressed were linked to obesity and colorectal cancer. Using a similar approach, these types of analyses could be useful to understanding host-microbiome-endocrine interactions in various healthy and disease states, but also especially informative in endocrine disruption research. After thorough vetting, *in vivo* approaches, such as administration of certain microbiota and/or their microbially-derived natural products and measurement of host outcomes may also provide further information regarding the link between the microbiome and its role in endocrine function within hosts. These types of approaches are important for determining the underlying mechanism of how the microbiome modulates and, in some cases, controls endocrine function *in vivo*. Studies like these are integral for identifying how we can direct microbial functions for alternative endpoints leading to remediation or therapeutic options to control or prevent negative host-outcomes (Vázquez-Baeza et al., 2018; Williams et al., 2019b).

4. Conclusions

A considerable amount of evidence has been gathered that demonstrates complex, often bi-directional, interactions between gut microbiota and host endocrine systems. As research in this field continues, similar relationships are certain to be established between gut microbiota and hormone systems not discussed in depth here (e.g., thyroid hormones, aryl hydrocarbon receptor pathways, etc.), in addition to the interactions between the endocrine system and other site-specific microbiomes. By carefully integrating multi-disciplinary approaches, we stand to identify clearer mechanistic relationships between microbiota and endocrine function. Doing so will undoubtedly clarify already established microbiota-endocrine relationships, elucidate novel mechanisms, molecules and microbes critical to endocrine function and disruption, and broaden our understanding of the field of comparative endocrinology as a whole.

CRediT authorship contribution statement

Candace L. Williams: Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Natàlia Garcia-Reyero:** Conceptualization, Writing - original draft, Writing - review & editing. **Christopher J. Martyniuk:** Conceptualization, Writing - original draft, Writing - review & editing. **Christopher W. Tubbs:** Conceptualization, Writing - original draft, Writing - review & editing. **Joseph H. Bisesi:** Conceptualization, Writing - original draft, Writing - review & editing.

Acknowledgements

The authors have no conflicts of interest to declare. The concepts for this review article were developed at NASCE and we thank all members of the NASCE community for fostering new ideas among its participants. We would also like to thank Barbara Durrant for her careful review of the manuscript. Statements, opinions or conclusions contained in this article do not necessarily represent those of the United States Government. J.H.B. received support from The National Science Foundation (CBET Award Number 1605119).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2020.113437.

References

Adams, N.R., 1995. Organizational and activational effects of phytoestrogens on the reproductive tract of the ewe. P. Soc. Exp. Biol. Med. Society for Experimental Biology and Medicine (New York, N.Y.). 208, 87–91.
Amato, K.R., Van Belle, S., Di Fiore, A., Estrada, A., Stumpf, R., White, B., et al., 2017. Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate. Microb. Ecol. 74, 250–258.
An, D., Kasper, D.L., 2013. Testosterone: more than having the guts to win the Tour de France. Immunity 39 (2), 208–210.
Antwis, R.E., Edwards, K.L., Unwin, B., Walker, S.L., Shultz, S., 2019. Rare gut microbiota associated with breeding success, hormone metabolites and ovarian cycle phase in the critically endangered eastern black rhino. Microbiome 7, 27.
Atkinson, C., Frankenfeld, C.L., Lampe, J.W., 2005. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp. Biol. Med. 230, 155–170.
Baker, M.E., et al., 2013. Evolution of hormone selectivity in glucocorticoid and mineralocorticoid receptors. J. Steroid Biochem. Mol. Biol. 137, 57–70.
Baker, M.E., 2014. The microbiome as a target for endocrine disruptors: novel chemicals may disrupt androgen and microbiome-mediated autoimmunity. Endocrine Disruptors 2 (1), e964539.
Baker, J.M., Al-Nakkash, L., Herbst-Kralovetz, M.M., 2017. Estrogen–gut microbiome axis: physiological and clinical implications. Maturitas 103, 45–53.
Batterham, R.L., Bloom, S.R., 2003. The gut hormone peptide YY regulates appetite. Ann. N Y. Acad. Sci. 994, 162–168.
Baud, D., Pattaroni, C., Vulliemoz, N., Castella, V., Marsland, B.J., Stojanov, M., 2019. Sperm microbiota and its impact on semen parameters. Front. Microbiol. 10, 234.
Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70, 567–590.
Berry, D., Mader, E., Lee, T.K., Woebken, D., Wang, Y., Zhu, D., et al., 2015. Tracking heavy water incorporation for identifying and sorting active microbial cells. Proc. Natl. Acad. Sci. U.S.A. 112, E194–E203.
Bonham, K.S., Wolfe, B.E., Dutton, R.J., 2017. Extensive horizontal gene transfer in cheese-associated bacteria. eLife 6, e22144.
Braune, A., Blaut, M., 2016. Bacterial species involved in the conversion of dietary flavonoids in the human gut. Gut Microbes 7, 216–234.
Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., et al., 2011. Ingestion of lactobacillus strain regulates emotional behavior and central gaba receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. U.S.A. 108, 16050–16055.
Buerger, A.N., Dillon, D.T., Schmidt, J., Yang, T., Zubcevic, J., Martyniuk, C.J., et al., 2019. Gastrointestinal dysbiosis following diethylhexyl phthalate exposure in Danio rerio (Zebrafish): Altered microbial diversity, functionality, and network connectivity Environ. Poll. (in review).

Buerger, A.N., Schmidt, J., Chase, A., Paxaio, C., Patel, T.N., Brumback, B.A., et al., 2019b. Examining the responses of the zebrafish (*Danio rerio*) gastrointestinal system to the suspected obesogen diethylhexyl phthalate. Environ. Pollut. 245, 1086–1094.
Butt, R.L., Volkoff, H., 2019. Gut microbiota and energy homeostasis in fish. Front. Endocrinol. (Lausanne) 10, 9.
Cani, P.D., Everard, A., Duparc, T., 2013. Gut microbiota, enteroendocrine functions and metabolism. Curr. Opin. Pharmacol. 13, 935–940.
Carnevali, O., Avella, M.A., Gioacchini, G., 2013. Effects of probiotic administration on zebrafish development and reproduction. Gen. Comp. Endocrinol. 188, 297–302.
Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101 (5), 378–384.
Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Daugé, V., et al., 2014. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. Psychoneuroendocrinology 42, 207–217.
Cussotto, S., Sandhu, K.V., Dinan, T.G., Cryan, J.F., 2018. The neuroendocrinology of the microbiota-gut-brain axis: a behavioural perspective. Front. Neuroendocrinol. 51, 80–101.
Davis, D.J., Bryda, E.C., Gillespie, C.H., Ericsson, A.C., 2016. Microbial modulation of behavior and stress responses in zebrafish larvae. Behav. Brain Res. 311, 219–227.
de Weerth, C., 2017. Do bacteria shape our development? Crosstalk between intestinal microbiota and hpa axis. Neurosci. Biobehav. Rev. 83, 458–471.
den Besten, G., van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.-J., Bakker, B.M., 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J. Lipid Res. 54, 2325–2340.
Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. 30 (4), 293–342.
Dinan, T.G., Cryan, J.F., 2012. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology 37, 1369–1378.
Dinan, T.G., Cryan, J.F., 2017. Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. J. Physiol. 595, 489–503.
Dinan, T.G., Stilling, R.M., Stanton, C., Cryan, J.F., 2015. Collective unconscious: how gut microbes shape human behavior. J. Psychiatri. Res. 63, 1–9.
El-Ansary, A.K., Bacha, A.B., Kotb, M., 2012. Etiology of autistic features: the persisting neurotoxic effects of propionic acid. J. Neuroinflamm. 9, 74.
Eriksson, H., Gustafsson, J.Å., Sjövall, J., 1969. Steroids in germfree and conventional rats. Euro. J. Biochem. 9, 550–554.
Flint, H.J., Scott, K.P., Louis, P., Duncan, S.H., 2012. The role of the gut microbiota in nutrition and health. Nat. Rev. Gastroenterol. Hepatol. 9, 577.
Foster, J.A., Rinaman, L., Cryan, J.F., 2017. Stress & the gut-brain axis: regulation by the microbiome. Neurobiol. Stress 7, 124–136.
Fung, T.C., Vuong, H.E., Luna, C.D., Pronovost, G.N., Aleksandrova, A.A., Riley, N.G., et al., 2019. Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. Nat. Microbiol. 1–10.
Garcia-Reyero, N., 2017. The clandestine organs of the endocrine system. Gen. Comp. Endocinol. 257, 264–271.
Gimenes, F., et al., 2014. Male infertility: a public health issue caused by sexually transmitted pathogens. Nat. Rev. Urol. 11, 672.
Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36 (6), E1–E150.
Guillette, L.J., 2006. Endocrine disrupting contaminants—beyond the dogma. Environ. Health Perspect. 114 (S1), 9–12.
Hata, T., Asano, Y., Yoshihara, K., Kimura-Todani, T., Miyata, N., Zhang, X.-T., et al., 2017. Regulation of gut luminal serotonin by commensal microbiota in mice. PLoS One 12, e0180745.
Hatzenpichler, R., Scheller, S., Tavormina, P.L., Babin, B.M., Tirrell, D.A., Orphan, V.J., 2014. In situ visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. Environ. Microbiol. 16, 2568–2590.
Hausmann, B., Pelikan, C., Rattei, T., Loy, A., Pester, M., 2019. Long-term transcriptional activity at zero growth of a cosmopolitan rare biosphere member. mBio 10, e02189–02118.
Hermes, R., Hildebrandt, T.B., Walzer, C., Goritz, F., Patton, M.L., Silinski, S., et al., 2006. The effect of long non-reproductive periods on the genital health in captive female white rhinoceroses (*Ceratotherium simum simum, C.s. Cottoni*). Theriogenology 65, 1492–1515.
Hooper, L.V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. Science 336, 1268–1273.
Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., Gordon, J.I., 2011. Human nutrition, the gut microbiome and the immune system. Nature 474, 327.
Kolb, S.A., O'Loughlin, E.J., Gsell, T.C., 2019. Data on the characterization of phthalate-degrading bacteria from asian carp microbiomes and riverine sediments. Data in Brief 25, 104375.
Krüger, T., Long, M., Bonefeld-Jørgensen, E.C., 2008. Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology 246, 112–123.
Kunc, M., Gabrych, A., Witkowski, J.M., 2016. Microbiome impact on metabolism and function of sex, thyroid, growth and parathyroid hormones. Acta Biochim. Pol. 63, 189–201.
Kwa, M., Plottel, C.S., Blaser, M.J., Adams, S., 2016. The intestinal microbiome and estrogen receptor-positive female breast cancer. J. Natl. Cancer Inst. 108, djw029.
Lagier, J.-C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., et al., 2018. Culturing the human microbiota and culturomics. Nat. Rev. Microbiol. 16, 540–550.
Lee, K.S., Palatinszky, M., Pereira, F.C., Nguyen, J., Fernandez, V.I., Mueller, A.J., et al., 2019. An automated raman-based platform for the sorting of live cells by functional properties. Nat. Microbiol. 4, 1035–1048.
Lin, H.V., Frassetto, A., Kowalik Jr, E.J., Nawrocki, A.R., Lu, M.M., Kosinski, J.R., et al., 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One 7, e35240.
Liu, S., Li, E., Sun, Z., Fu, D., Duan, G., Jiang, M., et al., 2019. Altered gut microbiota and short chain fatty acids in chinese children with autism spectrum disorder. Sci. Rep. 9, 287.
Lozupone, C.A., 2018. Unraveling interactions between the microbiome and the host immune system to decipher mechanisms of disease. mSystems 3, e00183–00117.
Macfarlane, G.T., Macfarlane, S., 2012. Bacteria, colonic fermentation, and gastrointestinal health. J. AOAC Int. 95, 50–60.
Markle, J.G., Frank, D.N., Mortin-Toth, S., Robertson, C.E., Feazel, L.M., Rolle-Kampczyk, U., Von Bergen, M., McCoy, K.D., Macpherson, A.J., Danska, J.S., 2013. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339 (6123), 1084–1088.
Martin, A.M., Sun, E.W., Rogers, G.B., Keating, D.J., 2019. The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. Front. Physiol. 10, 428.
McFall-Ngai, M., Hadfield, M.G., Bosch, T.C.G., Carey, H.V., Domazet-Lošo, T., Douglas, A.E., et al., 2013. Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. U.S.A. 110, 3229–3236.
McLachlan, J.A., 2016. Environmental signaling: from environmental estrogens to endocrine-disrupting chemicals and beyond. Andrology 4 (4), 684–694.
Melnik, A.V., da Silva, R.R., Hyde, E.R., Aksenov, A.A., Vargas, F., Bouslimani, A., et al., 2017. Coupling targeted and untargeted mass spectrometry for metabolome-microbiome-wide association studies of human fecal samples. Anal. Chem. 89, 7549–7559.
Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., et al., 2017. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol. Mol. Biol. Rev. 81, e00036–00017.
Miller, E.A., Livermore, J.A., Alberts, S.C., Tung, J., Archie, E.A., 2017. Ovarian cycling and reproductive state shape the vaginal microbiota in wild baboons. Microbiome 5, 8.
Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y., 1992. The role of hormones in the acquisition of sperm motility in salmonid fish. J. Exp. Zool. 261 (3), 359–363.
Moreno, I., Simon, C., 2018. Deciphering the effect of reproductive tract microbiota on human reproduction. Reprod. Med. Biol. 18, 40–50.
Morris, D.J., Ridlon, J.M., 2017. Glucocorticoids and gut bacteria: "The galf hypothesis" in the metagenomic era. Steroids 125, 1–13.
Mudd, A.T., Berding, K., Wang, M., Donovan, S.M., Dilger, R.N., 2017. Serum cortisol mediates the relationship between fecal ruminococcus and brain n-acetylaspartate in the young pig. Gut Microbes 8, 589–600.
Nankova, B.B., Agarwal, R., MacFabe, D.F., La Gamma, E.F., 2014. Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including creb-dependent catecholaminergic neurotransmission, in pc12 cells-possible relevance to autism spectrum disorders. PLoS One 9, e103740.
Noguera, J.C., Aira, M., Pérez-Losada, M., Domínguez, J., Velando, A., 2018. Glucocorticoids modulate gastrointestinal microbiome in a wild bird. R. Soc. Open Sci. 5, 171743.
Nugeyre, M.-T., Tchitchek, N., Adapen, C., Cannou, C., Contreras, V., Benjelloun, F., et al., 2019. Dynamics of vaginal and rectal microbiota over several menstrual cycles in female cynomolgus macaques. Front. Cell Infect. Microbiol. 9, 188.
O'Callaghan, T., Ross, R., Stanton, C., Clarke, G., 2016. The gut microbiome as a virtual endocrine organ with implications for farm and domestic animal endocrinology. Domes. Anim. Endocrinol. 56, S44–S55.
Ohtani, H., Miura, I., Ichikawa, Y., 2000. Effects of dibutyl phthalate as an environmental endocrine disruptor on gonadal sex differentiation of genetic males of the frog rana rugosa. Environ. Health Perspec. 108, 1189–1193.
Oliphant, K., Allen-Vercoe, E., 2019. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. Microbiome 7, 91.
Park, H.J., Lee, S.E., Kim, H.B., Isaacson, R., Seo, K.W., Song, K.H., 2015. Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. J. Vet. Intern. Med. 29, 43–50.
Patton, M.L., Swaisgood, R.R., Czekala, N.M., White, A.M., Fetter, G.A., Montagne, J.P., et al., 1999. Reproductive cycle length and pregnancy in the southern white rhinoceros (*Ceratotherium simum simum*) as determined by fecal pregnane analysis and observations of mating behavior. Zoo Biol. 18, 111–127.
Perofsky, A.C., Lewis, R.J., Abondano, L.A., Di Fiore, A., Meyers, L.A., 2017. Hierarchical social networks shape gut microbial composition in wild verreaux's sifaka. Proc. R. Soc. Lond. B. Biol. Sci. 284, 20172274.
Petrosus, E., Silva, E.B., Lay Jr, D., Eicher, S.D., 2018. Effects of orally administered cortisol and norepinephrine on weanling piglet gut microbial populations and salmonella passage. J. Anim. Sci. 96, 4543–4551.
Plottel, C.S., Blaser, M.J., 2011. Microbiome and malignancy. Cell. Host Microbe. 10, 324–335.
Prindle, A., Liu, J., Asally, M., Ly, S., Garcia-Ojalvo, J., Süel, G.M., 2015. Ion channels enable electrical communication in bacterial communities. Nature 527, 59.
Quinn, R.A., Nothias, L.-F., Vining, O., Meehan, M., Esquenazi, E., Dorrestein, P.C., 2017. Molecular networking as a drug discovery, drug metabolism, and precision medicine strategy. Trends Pharmacol. Sci. 38, 143–154.
Richards, A.L., Muehlbauer, A.L., Alazizi, A., Burns, M.B., Findley, A., Messina, F., et al., 2019. Gut microbiota has a widespread and modifiable effect on host gene regulation. mSystems 4, e00323–00318.

Ridlon, J.M., Ikegawa, S., Alves, J.M., Zhou, B., Kobayashi, A., Iida, T., et al., 2013. Clostridium scindens: a human gut microbe with a high potential to convert glucocorticoids into androgens. J. Lipid. Res. 54, 2437–2449.
Rosenfeld, C.S., 2017. Gut dysbiosis in animals due to environmental chemical exposures. Front. Cell. Infect. Microbiol. 7, 396.
Samuel, B.S., Shaito, A., Motoike, T., Rey, F.E., Backhed, F., Manchester, J.K., et al., 2008. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding g protein-coupled receptor, gpr41. Proc. Natl. Acad. Sci. U.S.A. 105, 16767–16772.
Sandhu, K.V., Sherwin, E., Schellekens, H., Stanton, C., Dinan, T.G., Cryan, J.F., 2017. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. Transl. Res. 179, 223–244.
Sheng, Y., Ren, H., Limbu, S.M., Sun, Y., Qiao, F., Zhai, W., et al., 2018. The presence or absence of intestinal microbiota affects lipid deposition and related genes expression in zebrafish (danio rerio). Front. Microbiol. 9, 1124.
Shi, D., Bai, L., Qu, Q., Zhou, S., Yang, M., Guo, S., et al., 2019. Impact of gut microbiota structure in heat-stressed broilers. Poultry Sci. 98, 2405–2413.
Shimizu, K., Muranaka, Y., Fujimura, R., Ishida, H., Tazume, S., Shimamura, T., 1998. Normalization of reproductive function in germfree mice following bacterial contamination. Exp. Anim. 47, 151–158.
Singer, E., Wagner, M., Woyke, T., 2017. Capturing the genetic makeup of the active microbiome in situ. ISME J. 11, 1949.
Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., et al., 2004. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. J. Physiol. 558, 263–275.
Sorbara, M.T., Pamer, E.G., 2019. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. Mucosal. Immunol. 12, 1–9.
Steen, A.D., Crits-Christoph, A., Carini, P., DeAngelis, K.M., Fierer, N., Lloyd, K.G., et al., 2019. High proportions of bacteria and archaea across most biomes remain uncultured. ISME J.
Stilling, R.M., van de Wouw, M., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2016. The neuropharmacology of butyrate: the bread and butter of the microbiota-gut-brain axis? Neurochem. Int. 99, 110–132.
Strandwitz, P., 2018. Neurotransmitter modulation by the gut microbiota. Brain Res. 1693, 128–133.
Tan, W., Pang, Y., Tubbs, C., Thomas, P., 2019. Induction of sperm hypermotility through membrane progestin receptor alpha (mPRα): a teleost model of rapid, multifaceted, nongenomic progestin signaling. Gen. Comp. Endocrinol. 279, 60–66.
Tillisch, K., 2014. The effects of gut microbiota on cns function in humans. Gut Microbes 5, 404–410.
Tolhurst, G., Heffron, H., Lam, Y.S., Parker, H.E., Habib, A.M., Diakogiannaki, E., et al., 2012. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the g-protein–coupled receptor ffar2. Diabetes 61, 364–371.
Trosvik, P., de Muinck, E.J., Rueness, E.K., Fashing, P.J., Beierschmitt, E.C., Callingham, K.R., et al., 2018. Multilevel social structure and diet shape the gut microbiota of the gelada monkey, the only grazing primate. Microbiome 6, 84.
Tubbs, C., Hartig, P., Cardon, M., Varga, N., Milnes, M., 2012. Activation of southern white rhinoceros (*Ceratotherium simum simum*) estrogen receptors by phytoestrogens: potential role in the reproductive failure of captive-born females? Endocrinology 153, 1444–1452.
Tubbs, C.W., Moley, L.A., Ivy, J.A., Metrione, L.C., LaClaire, S., Felton, R.G., et al., 2016. Estrogenicity of captive southern white rhinoceros diets and their association with fertility. Gen. Comp. Endocrinol. 238, 32–38.
Vázquez-Baeza, Y., Callewaert, C., Debelius, J., Hyde, E., Marotz, C., Morton, J.T., et al., 2018. Impacts of the human gut microbiome on therapeutics. Ann. Rev. Pharmacol Toxicol. 58, 253–270.
Velmurugan, G., Ramprasath, T., Gilles, M., Swaminathan, K., Ramasamy, S., 2017. Gut microbiota, endocrine-disrupting chemicals, and the diabetes epidemic. Trends Endocrinol. Metab. 28 (8), 612–625.
Vijay, N., Morris, M.E., 2014. Role of monocarboxylate transporters in drug delivery to the brain. Curr. Pharm. Des. 20, 1487–1498.
Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y., et al., 2016. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat. Biotechnol. 34, 828.
Williams, C.L., Ybarra, A.R., Meredith, A.N., Durrant, B.S., Tubbs, C.W., 2019a. Gut microbiota and phytoestrogen-associated infertility in southern white rhinoceros. mBio 10, e00311–00319.
Williams, C.L., Caraballo-Rodríguez, A.M., Allaband, C., Zarrinpar, A., Knight, R., Gauglitz, J.M., 2019b. Wildlife-microbiome interactions and disease: exploring opportunities for disease mitigation across ecological scales. Drug Discov. Today Dis. Models. https://doi.org/10.1016/j.ddmod.2019.08.012.
Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., et al., 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161, 264–276.
Ye, J.D., Wang, K., Li, F.D., Sun, Y.Z., 2011. Single or combined effects of fructo-and mannan oligosaccharide supplements and bacillus clausii on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the japanese flounder *Paralichthys Olivaceus*. Aquac. Nutr. 17, e902–e911.
Yurkovetskiy, L., Burrows, M., Khan, A.A., Graham, L., Volchkov, P., Becker, L., Antonopoulos, D., Umesaki, Y., Chervonsky, A.V., 2013. Gender bias in autoimmunity is influenced by microbiota. Immunity 39 (2), 400–412.