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

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**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 in utero, 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 microbiome 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 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 EDC (Rosenfeld, 2017), but also transforming EDC to affect host function (Williams et al., 2019).

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

89

90 **2. Interactions of microbiota and the endocrine axis in animals**

91 **2.1 The gastrointestinal microbiome and the regulation of feeding and satiation**

92 Upon ingestion of a meal, nutrients in the GI tract stimulate a complex set of hormones,
93 peptides, and neurotransmitters that are responsible for bidirectional signaling along the gut-
94 brain axis (GBA). Much of this bidirectional communication takes place via enteroendocrine
95 cells (EEC)–specialized cells in the GI epithelium that are responsible for excretion of important
96 signaling molecules and peptides (Sandhu et al., 2017). Among these hormones are
97 cholecystokinin (CCK) and peptide YY (PYY), which are responsible for signaling satiation
98 either through direct EEC-nerve communication or indirect paracrine mechanisms (Batterham
99 and Bloom, 2003; Butt and Volkoff, 2019; Sandhu et al., 2017). EEC can also release glucagon-
100 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 (SCFA) (Lin et al., 2012; Tolhurst et al., 2012). Among the species in the gut microbiome, members of the phylum Bacteroidetes are known to produce the SCFA acetate and propionate, whereas the Firmicutes are primarily responsible for the production of butyrate (Kau et al., 2011). While SCFA 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 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 (Figure 1). This was attributed to increased food intake and nutrient digestion, both of

126 which are under the control of enteric endocrine signaling (Ye et al., 2011). A recent review of
127 the contributions of the microbiome to livestock show similar results, as alterations in microbial
128 communities affected feeding and satiation among numerous species (O'Callaghan et al.,
129 2016).

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

143

144 **2.2. Microbiota, stress, and the hypothalamic-pituitary-adrenal (HPA) axis**

145 For several years, questions surrounding the role of the microbiome in the regulation of
146 the hypothalamic-pituitary-adrenal (HPA) axis have been proposed. It has been learned that the
147 composition of the maternal microbiome, as well as the timing and progression of initial
148 colonization, are intimately tied to childhood development and the HPA axis (de Weerth, 2017).
149 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. (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

175 microbiota. Birds were treated with corticosteroid implants, and 16S sequencing was conducted
176 on their fecal samples (Figure 1). Intriguingly, the study revealed that several potential avian
177 pathogenic taxa (e.g., *Microvigas*, *Helicobacter*, and *Pseudomonas*) were underrepresented in
178 the gut following corticosterone implants. Commensal microbiota (e.g., Firmicutes) were also
179 underrepresented in the birds with the corticosteroid implant. The study contradicted popular
180 thought that increased levels of stress hormones contributes to an increased risk of pathogenic
181 bacteria proliferation. However, chick weight was lower in implanted birds, suggesting there may
182 be a negative trade-off. These conflicting results highlight the need for further understanding of
183 the connection between the HPA axis and the microbiome. In another study in birds, broiler
184 chickens were heat stressed and growth and cortisol measured in addition to the microbiome
185 response (Shi et al., 2019). With heat stress, the levels of cortisol increased from 10-30 %,
186 which was measured on multiple days throughout the study (1, 7, 14, 28 days). The rise in
187 cortisol also correlated to a shift in the gut microbiota composition in the caecum (Shi et al.,
188 2019). Heat stress for 7 days increased the Firmicutes and the Tenericutes and decreased the
189 prevalence of the Bacteroidetes by approximately 30 %. Although it was not possible to discern
190 whether the cortisol was modifying the microbe composition directly, the study proposes an
191 interesting link between heat stress and the gut microbiota.

192 Other experiments using non-rodent models have revealed a potential role for cortisol in
193 mediating the relationship between the GBA. In a study in male Yorkshire piglets, correlative
194 relationships between serum serotonin, serum cortisol, colon volatile fatty acids, and the
195 microbiome were investigated (Mudd et al., 2017). The study revealed that the *Ruminococcus*
196 were negatively correlated with cortisol. This was significant because the authors proposed that
197 cortisol may be the candidate signal mediating the interactions between *Ruminococcus* and
198 brain N-acetylaspartate, an amino acid derivative that plays a crucial role in protecting neuronal
199 development and function from injury. Petrosus et al. (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 stress (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. (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., 2018) are

248 well established. However, it is intriguing to hypothesize how microbiota might contribute to
249 these processes, potentially through the synthesis of biomolecules with signaling potential, or
250 via other mechanisms. Investigating such relationships present a potentially rich area of future
251 research.

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

269 In terms of overall fertility, there are a few studies that have investigated associations
270 between reproductive success, alterations in endocrine function and differences in gut
271 microbiota. In humans, differences in gut microbial communities, or therapeutic treatments that
272 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) (Figure 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., 2019). 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 (Figure 2). Those include the production of metabolites and the

induction of inflammatory mediators 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 SCFA in the gut but can travel far from their production location (Macfarlane and Macfarlane, 2012; Vijay and E Morris, 2014). Butyrate and propionate can affect dopamine and noradrenaline synthesis, and propionic acid is suspected to also 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 relationship between these SCFA 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. (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 (Figure 1).

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. (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. (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 (EDC), 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 EDC and endogenously produced compounds (Colburn 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 Dianmanti-Kandarakis et al., 2009 and Gore et al., 2015 for review). Since microbiota interface with chemicals at these sites of exposure, EDC 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 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 SCFA (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.

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

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

395 Linking microbiota's role in endocrine dysfunction is challenging. By integrating 16S
396 rRNA sequencing, targeted mass spectrometry, *in vitro* estrogen receptor assays, and fertility
397 measurements, Williams et al. found that SWR females that excrete the highest levels of equol

display the highest level of fertility (Williams et al., 2019) (Figure 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., 2019), 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 direction: 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 (Figure 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.  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 (Williams et al., 2019; (Vázquez-Baeza et al., 2018).

## 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.

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**Figure captions**

**Figure 1**: Highlighted examples of known interactions between gut microbiota and the endocrine system, particularly those associated with reproduction, stress and the HPA axis, behavior, feeding and satiation [fructooligosaccharide (FOS) & mannan-oligosaccharide (MOS)], and endocrine disruption.

**Figure 2**: Gut microbiota may influence neuroendocrine function through several actions, including the production of metabolites and neurotransmitters, the induction of inflammatory meditators, and the interaction with the enteric nervous system (ENS), locally, or systemically through the vagus nerve.

**Figure 3**: The study of the microbiome-endocrine interactions requires multi-dimensional approaches, 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.

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Figure 1.

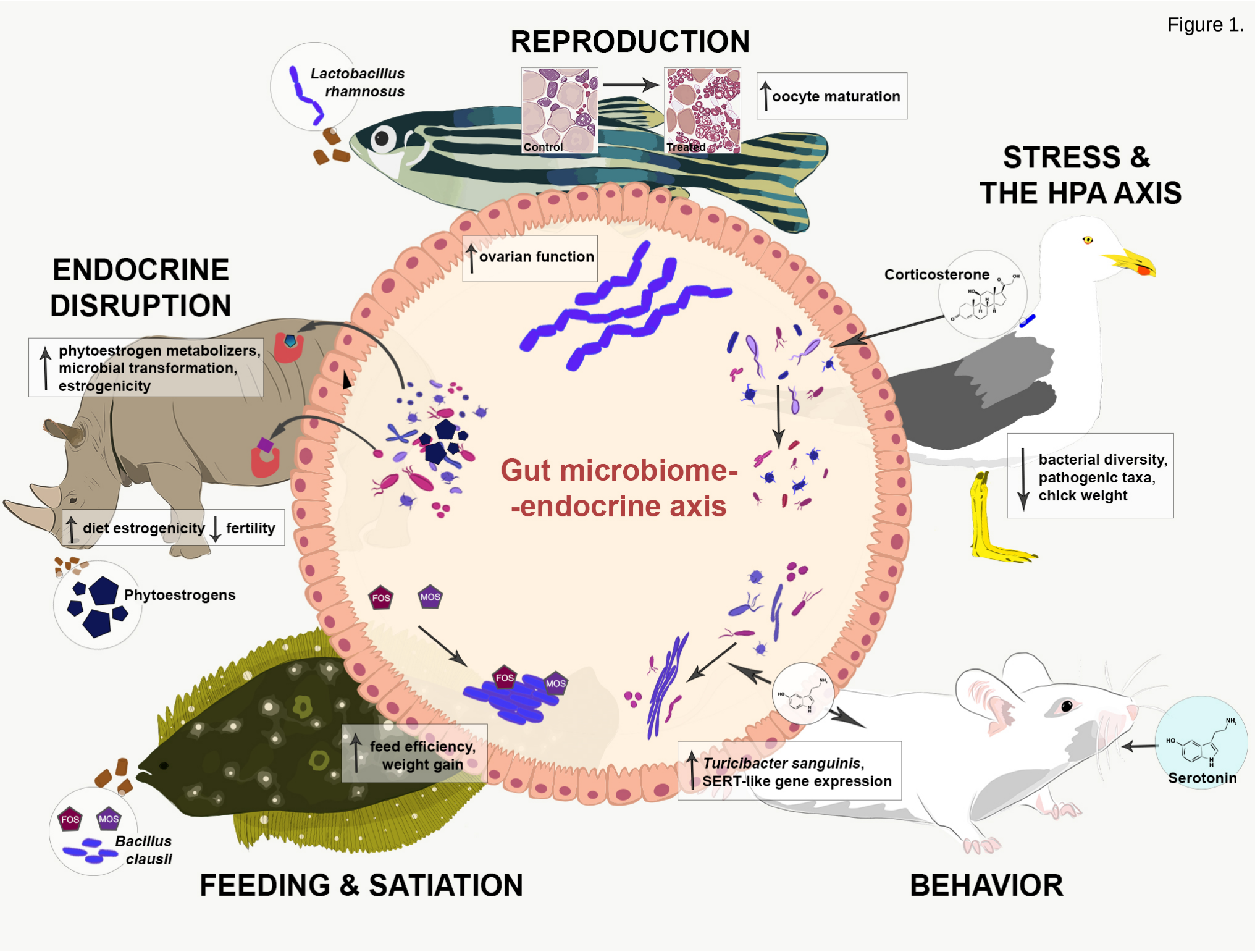

REPRODUCTION
Lactobacillus rhamnosus
Control
Treated
oocyte maturation
ovarian function
STRESS & THE HPA AXIS
Corticosterone
ENDOCRINE DISRUPTION
phytoestrogen metabolizers, microbial transformation, estrogenicity
diet estrogenicity
fertility
Phytoestrogens
Gut microbiome--endocrine axis
bacterial diversity, pathogenic taxa, chick weight
FOS
MOS
feed efficiency, weight gain
Turicibacter sanguinis, SERT-like gene expression
Serotonin
Bacillus clausii
FEEDING & SATIATION
BEHAVIOR

Figure 2.

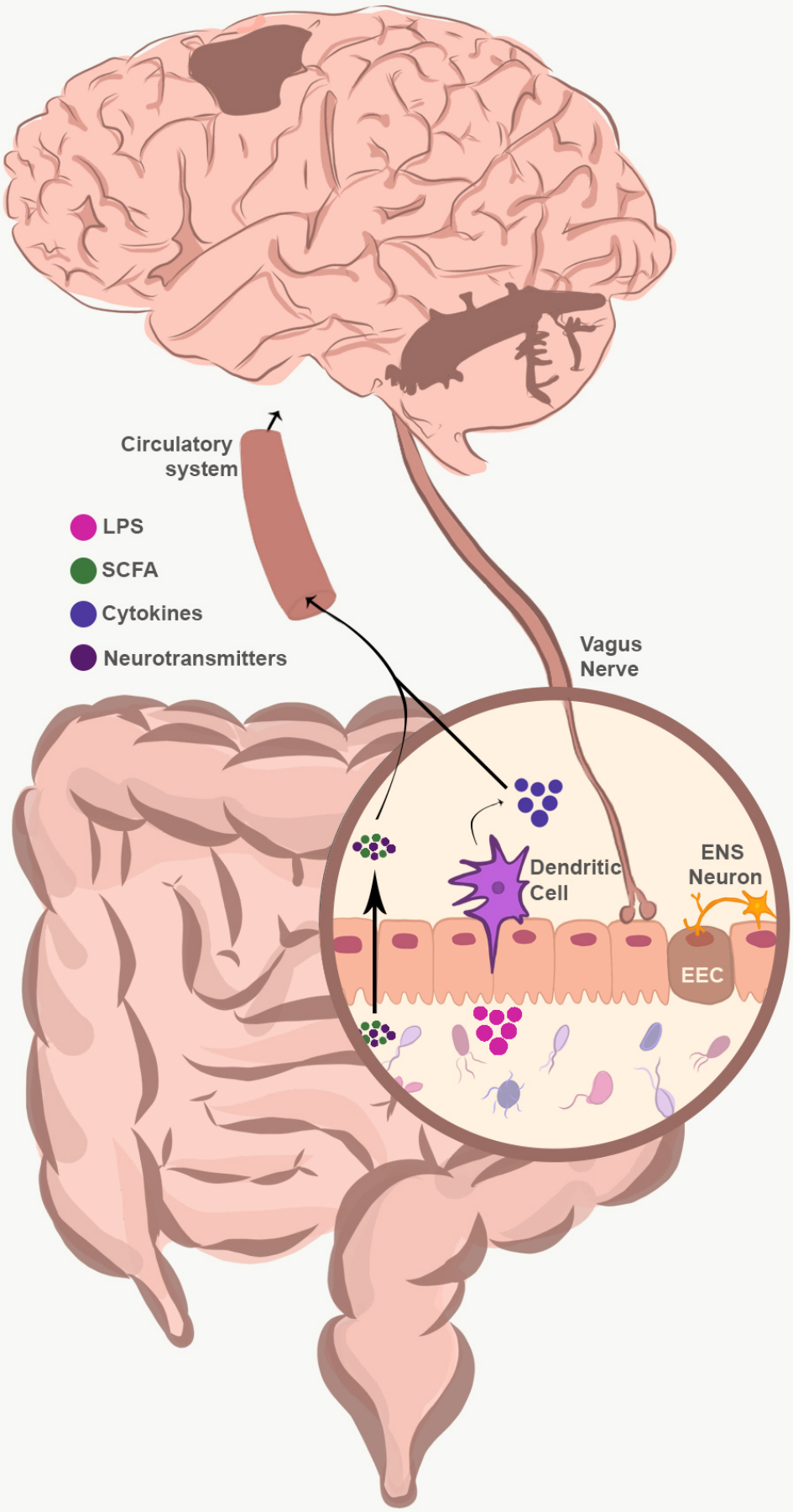
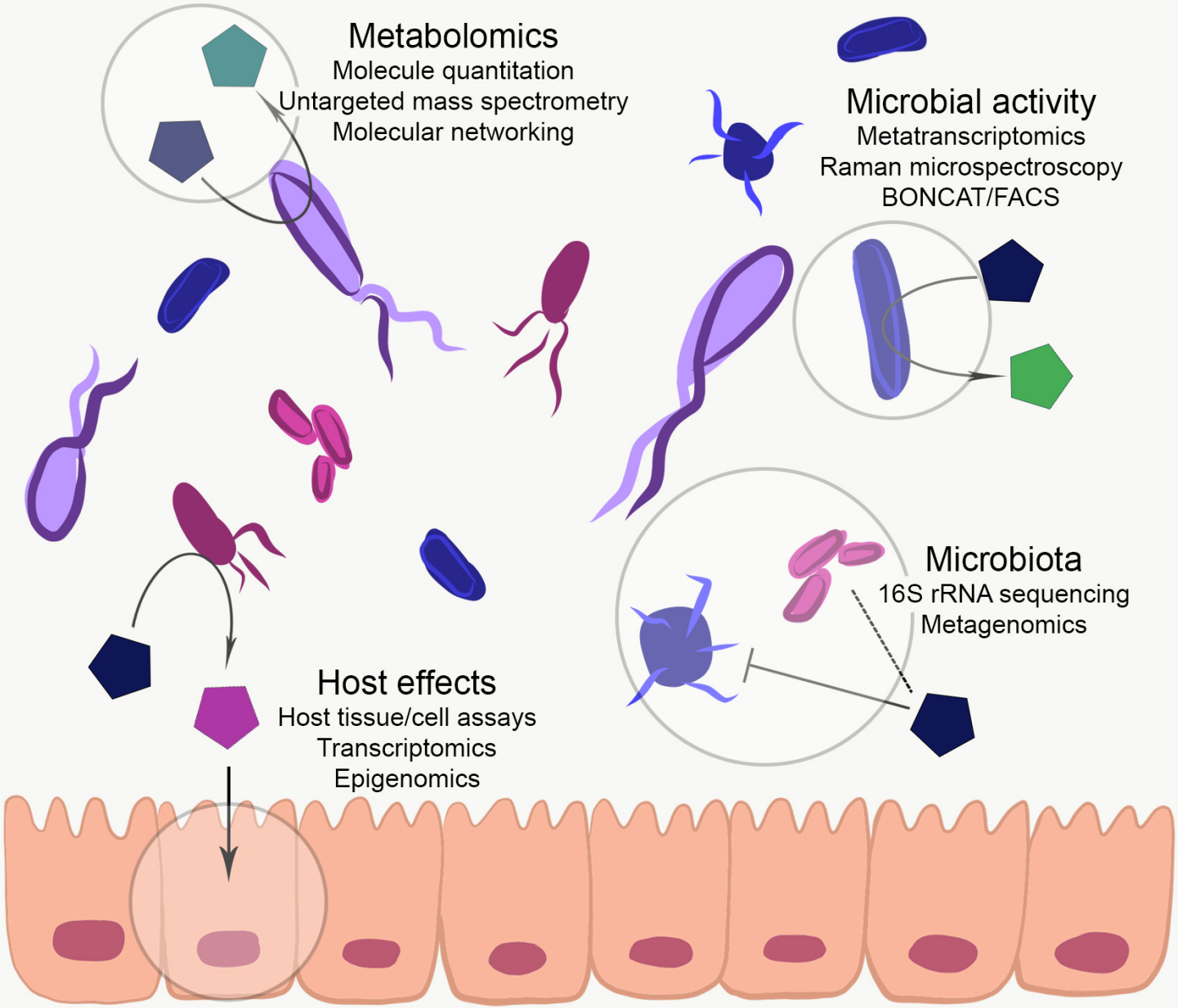

Circulatory system
LPS
SCFA
Cytokines
Neurotransmitters
Vagus Nerve
Dendritic Cell
ENS Neuron
EEC

Figure 3.


Metabolomics
Molecule quantitation
Untargeted mass spectrometry
Molecular networking
Microbial activity
Metatranscriptomics
Raman microspectroscopy
BONCAT/FACS
Microbiota
16S rRNA sequencing
Metagenomics
Host effects
Host tissue/cell assays
Transcriptomics
Epigenomics