Effects of microbiome on vertebrate behavior

Henna Pääkkö

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Index

1. Introduction

The possible effects of the microbiome on vertebrate behavior is an emerging topic in behavioral biology, with the number of studies about this topic having greatly increased within the last five to ten years. In this thesis I will provide an overview of the main routes by which the microbiome can alter their vertebrate host's behavior. I will emphasise the gut microbiome and its function in vertebrates, but, where relevant, the possible effects of other microbiomes on animal behavior will be discussed. These other effects will be typically discussed in less detail, because of the lack of detailed research outside the vertebrate microbiome-gut-brain axis.

Currently, the information about the functional roles of microbiomes and their potential associations with vertebrate behavior is increasing every year (Figure 1) with better understanding of the bacterial effects and help of more advanced biological experimental techniques. Use of laboratory equipment and controlled experimental methods are required to confirm specific hypotheses about host-behavior-microbiome interactions, but these approaches exclude the effects that can occur in natural environments (*i.e*. processes that are not caused by human manipulation). Nonetheless, because of the lack of experiments on animals in their natural habitat, all the examples provided in this thesis are derived from human manipulations.

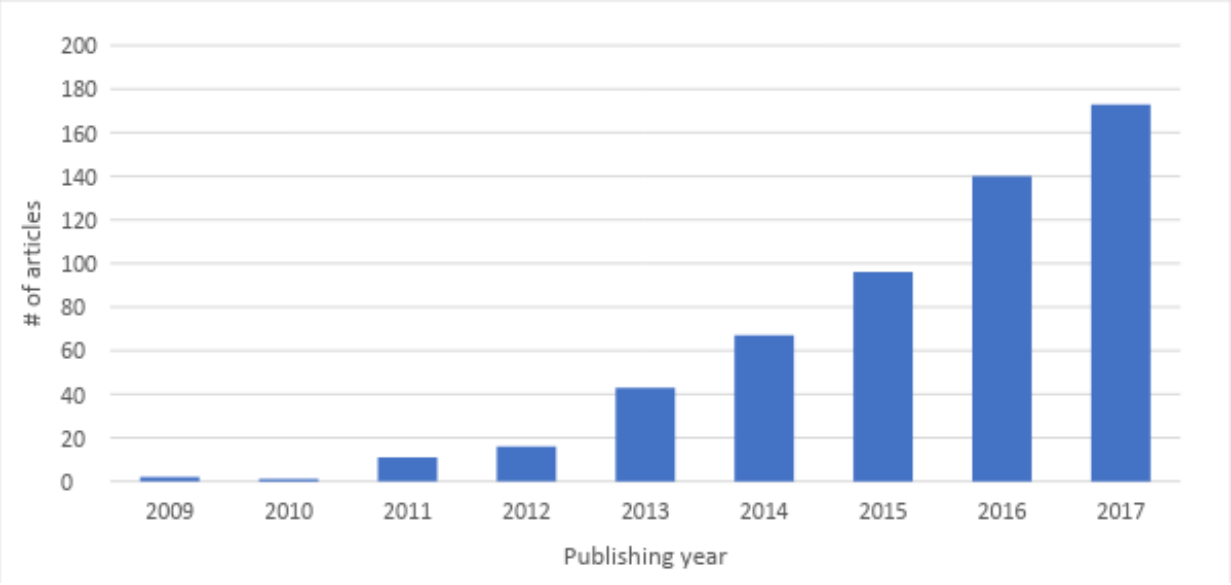

Figure 1. Number of articles by year returned by *Web of Science* (date accessed 20 June, 2018) for the search terms 'microbiome AND behavior'.

The study on the microbiome's effects on vertebrate behavior is important for diverse reasons. First, humans are vertebrates and therefore a better understanding of the effects of microbiome could improve human health, for example by preventing certain illnesses connected with microbiome function. Second, this information could help us understand evolution and diversity of certain behavioral patterns whose origins could not be explained before the knowledge on the microbiome increased. In this thesis, the main research problem I plan to address is: how can the microbiome influence vertebrate behavior? The main hypothesis is that microbiome can alter the vertebrate behavior, albeit to a certain limit. I will first provide general information about the microbiome and then discuss the gut microbiome in more detail, including its development, manipulation methods and effects on the vertebrate behavior. After that I will include some examples on the effects of the other kinds of microbiomes, to show that the gut microbiome is not the only factor in this manipulation.

2. Microbiome

Microbiomes consist of microbial (*i.e.* bacterial, fungal, protist and even viral) communities that inhabit a host individual, an animal or a plant (Vuong et al., 2017). While the fungal and viral parts of the microbiome can impact their host (Sam et al., 2017), the most numerous and perhaps best studied component of the microbiome is the bacterial community. Therefore, in this thesis I will focus on the effects of the bacterial communities and will refer to bacterial microbiome as microbiome. For example, in human bodies there is estimated to be roughly the same number of bacterial cells as human cells (Sender et al., 2016) At a molecular level, the diversity of species within a microbiome provides the host potential access to thousands of genes absent from the host's genome (i.e. novel functions); for example, some 3.3 million novel bacterial genes were identified in the human microbiome, presenting a stark contrast to the approximately 23,000 known protein-coding genes in human genome (Qin et al., 2010). The microbiome can thus be thought of as acting as a host's 'second genome', with the level of genomic diversity determining the functions potentially available to improve host health and fitness. Indeed, there is an argument to consider the host and its microbiome as a single unit - the holobiont (Richardson, 2017). It is most likely that the microbiomes have co-evolved with their hosts to increase the fitness of both the bacteria and the host species (Vuong et al., 2017).

Many microbiome bacterial communities have a commensal or mutualistic (Kopac & Klassen, 2016) relationship with their host and thus benefit the host in several ways; for example, there is now much evidence that the microbiome benefits their host by (1) helping defend against pathogens and (2) training the immune system, with the microbiota within the gastrointestinal tract also (3) supplying energy to the host by fermenting carbohydrates (Archie & Theis, 2011). In addition to these well-documented beneficial attributes of animals' microbiota, emerging studies have highlighted the impact of microbiota on host behavior (Shropshire & Bordenstein, 2016; Dinan & Cryan, 2017). The behavioral changes can be beneficial for the host and/or they are meant to improve fitness of the microbiome.

The behavioral changes can be caused by different microbial communities that are unique to each organ, which means that they have specific and diverse effects on behavior, depending on the microbiome (Perez Perez et al., 2016). However, the gut microbiome is so far, the most studied

one and has the most potential to alter the host behavior via delivering diverse metabolites (e.g. short chain fatty acids, hormones) to the host, for example directly from gut to the bloodstream.

3. Gut microbiome

Gut microbiome is an important factor in the internal manipulation of the host's behavior. Therefore, it is important to understand the basic development and the factors that affect the gut microbiome itself. Here I will review the development of gut microbiome, the ways it uses to manipulate the host and finally the actual effects on vertebrate behavior.

3.1 Development of the gut microbiome

Vertebrate microbiota communities are typically variable among individuals at birth and stabilize during development (Rodriguez et al., 2015). The knowledge on human gut microbiome has increased within the last decade. For example, the gastrointestinal tract was believed to be sterile *in utero*, but recent studies have proved this otherwise (Cresci & Bawden, 2015). However, there are multiple microorganisms in the placenta, amniotic fluid and umbilical cord, which all have effects on the fetus' developing microbiome. There are also changes in the infant microbiome throughout the pregnancy, which demonstrates that the infant gut microbiome keeps developing already in the womb (Cresci & Bawden, 2015). This gives crucial information about the importance of maternal gut microbiota health, which could be higher than previously thought, to ensure that the fetus as well as its microbiome will develop properly.

The gut microbiome community keeps developing after the birth of the infant. Even though there is contact with microorganisms already *in utero*, most of changes in an individual's gut microbiome take place postpartum. At birth, the principle microbiota are acquired from mother, depending on the method of delivery (*i.e.* vaginal or caesarean section), highlighting the fact that the infant's microbiome is affected by the one it first encounters (Cresci & Bawden, 2015). Added effects on infant's microbiome will come from the method of feeding, proved through the differenced found between breastfed and formula fed infants (Cresci & Bawden, 2015). The gut microbiome will keep developing for the first three years of one's life, after which it generally remains stable until the composition of microbiome changes in older age (Cresci & Bawden, 2015). However, there are certain factors that can affect the gut microbiome, including long-term changes in diet (Cresci & Bawden, 2015).

Host diet is one of the most important factors that determines the composition of gut microbiome (Ley et al., 2008). Humans (Carlotta De Filippo et al., 2010) and other mammals (Ley et al., 2008) that have different diets generally do not show major differences in the types of bacterial phyla that constitute the gut microbiome, rather there are major changes in the composition of phyla. The main differences in humans can be seen in the four principal bacterial phyla: Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes.

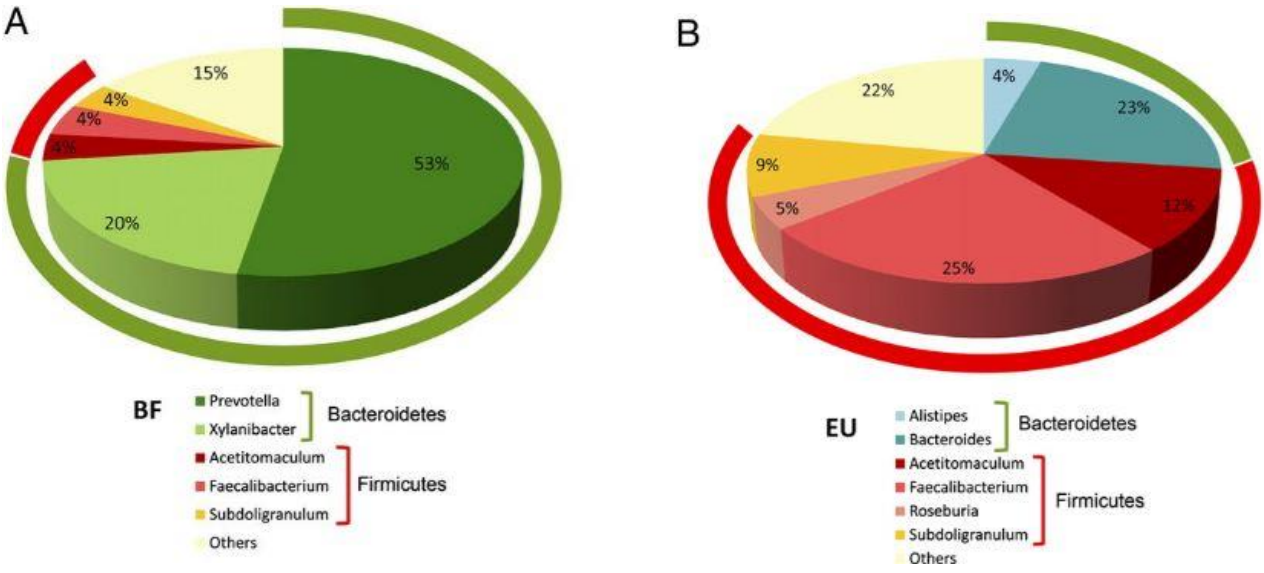

Figure 2. Gut microbiome composition in African (specifically from Burkina Faso) (A) and European (B) children communities. Adapted from De Filippo et al. (2010). Copyright (2010) National Academy of Sciences.

In the West, diets are typically high in animal protein, sugar, starch and fat, and often lack fiber, whereas diets common in African cultures are typically rich in starch, plant polysaccharides and fiber and have less animal protein or fat (Cresci & Bawden, 2015). Microbiomes in people who have Western diets are typified by an increase in abundance of Firmicutes and Proteobacteria in contrast compared with microbiomes from Africans that have greater abundance of Actinobacteria and Bacteroidetes (De Filippo et al., 2010; Cresci & Bawden, 2015; see Fig. 2). Hence, different bacteria apparently favour different types of food and utilize different substrates depending on their metabolic pathways. Similar differences can be seen in other mammalian species, where carnivores have less gut bacterial phyla than herbivores do (Ley et al., 2008). It is

important to understand the development and the factors affecting the gut microbiome, because the difference in the phyla composition could have drastic effects on the behavioral manipulation.

3.2 Mechanisms of manipulation

Diverse pathways associated with diet and the products of microbial fermentation enable the microbiome to alter host behavior. The main pathways I am going to discuss here are the hormonal, neural and receptor expression, and in addition to those some other possible pathways.

3.2.1 Hormones

One of the most studied ways of manipulation of the behavior is the microbiome's control of hormones. As mentioned in section *2. Microbiome*, the number of microbial genes, greater than the number of human genes, enables potential microbe-based manipulation of the host (Archie & Theis, 2011). For example, the mood affecting hormones in mammals, serotonin and dopamine, have been shown to have a great number of intestinal sources (Sampson & Mazmanian, 2015), where over 50 % of these hormones are produced intestinally (Alcock et al., 2014).

In addition to the mood-altering hormones, the gut microbiome can produce hormones that relate to satiety and hunger (Neuman et al., 2015). Mice (*Mus musculus*) without normal gut microbiome have been found to have a lower level of hormones that affect the hunger and food intake, for example leptin and cholecystokinin (Alcock et al., 2014). However, there is some evidence for coevolution in the host species to counter these effects, for example by the production of anti-hormone antibodies (Alcock et al., 2014). In conclusion, the gut microbiome has the potential to produce different hormones connected to vertebrate behavior, for example, by affecting the mood or hunger.

3.2.2 Neural mechanisms

One important neural pathway in communication between the gut microbiome and the brain is the vagus nerve (Alcock et al., 2014; Martin, et al., 2018). The vagus nerve works as the main communication route from the gut to the brain and *vice versa* (Alcock et al., 2014). This predisposes the vagus nerve to be used by the gut microbiome to manipulate the host. Being the main part of gut-brain axis, hijacking the vagus nerve gives the gut microbiome a straight access to the behavioral control center of the host (Alcock et al., 2014).

The vagus nerve has a major part in hunger management and changes in its functions can have an effect to weight control (Camilleri et al., 2008). For example, a blockage in the transaction between the vagus nerve and the brain has been shown to lead to weight loss through affecting the satiety and hunger of an individual (Camilleri et al., 2008). This shows that by controlling the vagus nerve, in addition to the hormonal control mentioned earlier, the microbiome can affect the eating behavior of the host.

3.2.3 Effects on neurophysiology

Brain structures responsible for behavioral control differ in germ free (GF) mice compared to the control individuals (Vuong et al., 2017). These changes could play a big part in the social deficits and behavioral changes, which are to be discussed later. The most important brain areas affected by changes in gut microbiome are amygdala, hypothalamus and hippocampus, which all showed clear differences between the GF and control individuals (Vuong et al., 2017).

In the mice amygdala there were changes in both structure and gene expression (Diaz Heijtz et al., 2011; Vuong et al., 2017). The structural changes included a significant increase in the volume of the lateral and basolateral amygdala and in the volume of central nucleus (Vuong et al., 2017). The changes in gene expression in the GF mice, revealed by RNA sequencing, included an increased expression of genes that have an important role for example in synaptic localization (Vuong et al., 2017). Also, the genes responsible for immune responses had a decreased expression in the GF mice (Vuong et al., 2017). The specific genes affected by microbial changes have been controversial between different studies, but the structural and genomic changes altogether have been acknowledged in multiple studies. These structural changes in amygdala were only partly reversible with the recolonization of the microbiome, possibly proving the importance of the microbiome in the normal development of the amygdala (Vuong et al., 2017).

The hypothalamus is another brain area which has an important role in the control of the social and stress related behavior (Vuong et al., 2017). Studies have shown that gut microbiome products have a crucial role in the function of the hypothalamus, especially in the production of certain hormones (Vuong et al., 2017). Specific bacteria in the gut microbiome can alter the production of oxytocin by regulating the oxytocin-expressing cells in the paraventricular nucleus (Vuong et al., 2017). This shows that the gut microbiome can alter the brain and the behavior simply with its metabolic products, which again proves its importance in the behavior of the host.

Finally, the changes in the hippocampus consisted of structural and neurochemical changes (Vuong et al., 2017). The structural changes included an increased volume of the CA2/3 region of the hippocampus as well as decreased numbers of branch points in the dentate granule cells (Vuong et al., 2017). The antibiotic treated mice showed a decrease in different neutrons in the hippocampal area, but these changes were reversible with postnatal treatment with probiotics (Vuong et al., 2017). However, there were also irreversible changes in the neurogenesis, which was increased in the GF mice (Vuong et al., 2017). These results show yet another crucial role of the gut microbiome in the manipulation of host behavior by affecting the structure and function of the brain.

3.2.4 Receptor expression

Different microbes in animal's gut microbiome can influence host's receptor expression (Duca et al., 2012). While the exact mechanisms of this manipulation are not completely clear, the results have proven the existing connection between the two. For example, Duca et al. (2012) showed that the germ free (GF) mice (*Mus musculus*) had divergent fat receptors compared to the mice with normal microbiome. In addition, in the study done by Swartz et al. (2012) it was shown that GF mice had higher number of sweet taste receptors compared to the control mice. These studies give a little insight to the relationship between host's receptor expression, which determines the host feeding preferences, and the gut microbiome composition.

3.2.5 Microbiome-gut-brain axis

There are other important factors that connect the brain and the gut microbiome together, which I will discuss here collectively. These routes connect the central nervous system, the neuroendocrine, the neuroimmune systems, the autonomic nervous system (both the sympathetic and the parasympathetic arms), the enteric nervous system and most importantly, the gut microbiota, creating the microbiome-gut-brain axis (Dinan et al., 2015).

One of these routes is the hypothalamic-pituitary-adrenal axis (HPA) (Dinan et al., 2015). HPA connects to the immune system, which itself isn't a direct part of the gut-brain axis, but can alter the levels of pro- and anti-inflammatory cytokines and therefore, alter the function of the brain (Dinan et al., 2015). Furthermore, the proper gut microbiome has and significant effect in the normal development of the HPA axis, in which the germ-free mice (*Mus musculus*) show higher levels of adrenocorticotrophin hormone and costerone production in stressful situations (Bravo et

al., 2012). Hence, the gut microbiome and its presence have an impact on the function of different signaling routes and can alter the development of an individual. Therefore, it has a possible role in the manipulation of the brain and behavior through these routes, which have a crucial role in the behavioral controlling.

Other bidirectional routes connecting the gut and the brain are the tryptophan metabolism and the production of short chain fatty acids (SCFAs) (Dinan et al., 2015). The role of tryptophan in the manipulation of the host behavior isn't confirmed, but it is known to have a crucial role as a precursor to multiple biologically active agents, especially serotonin (Dinan et al., 2015). Certain SCFAs have neuroactive properties, which is why they have an important role in the gut-brain axis (Dinan et al., 2015). Couple examples of these neuroactive SCFAs are n-butyrate, acetate and propionate, which can enter the bloodstream and affect the brain function through multiple receptors (Dinan et al., 2015). In conclusion, these intestinal metabolic products have a possible role in the gut-brain interaction.

Finally, microbes can produce multiple neurotransmitters, which can have serious effects on the behavioral functions of the brain (Dinan et al., 2015). The most known neurotransmitters that the bacteria can produce are GABA, norepinephrine and dopamine and serotonin (Dinan et al., 2015), previously mentioned in *3.2.1 Hormones*. These are all known to have noticeable effects on behavioral aspects and mood and therefore offer an easy way of manipulation for the microbiome (Dinan et al., 2015).

3.3 Effects on behavior

After understanding the possible methods of manipulation gut microbiome can use, it is easier to consider the actual effects gut microbiome has on vertebrate behavior. Here, I am going to go through some well-studied examples of this manipulation, including social deficits, feeding behavior, depression-like behavior and cognitive behavior.

3.3.1 Social deficits caused by changes in gut microbiome

As mentioned in *3.1 Development of the gut microbiome*, the formation of the gut microbiome begins in utero (Cresci & Bawden, 2015). The fetal microbiome impacts its health and development (Cresci & Bawden, 2015), and therefore it is important to recognize connections between the gut microbiome and the brain development to identify factors that may cause serious pathologies via an impact on microbiome. For example, a maternal high fat diet (that leads

to maternal obesity) while pregnant can increase the risk of autism spectrum disorder and other neurodevelopmental disorders and an associated social behavior in the offspring (Buffington et al., 2016). In mice, exposure to either a maternal high-fat diet (MHFD), regular diet (MRD) or were germ free (GF) had little impact on offspring weight but altered homeostasis of the gut microbiome: offspring of MHFD mice had less diversity in their gut microbiome than in the MRD offspring (Buffington et al., 2016). Moreover, diet was associated with social behavior, with the MHFD offspring being more while the MRD offspring expressed more normal behavior (i.e. choosing a familiar mouse over the empty space and showing interest to the unfamiliar mouse) (Buffington et al., 2016). These experiments raise the possibility that the gut microbiome can influence the changes in social behavior observed mediated by diet.

Microbiome community composition can change during an individual's lifetime, depending on the environment (De Filippo et al., 2010). The key timing for an impact by the microbiome on social behavior appears to be early life, with a crucial period when potential impacts on the behavior are not permanent (Buffington et al., 2016). The social deficits observed in the MHFD offspring could be reverted, for example by co-housing the MHFD offspring with normally behaving MRD offspring (Buffington et al., 2016); also, offspring of GF mice, which had similar social deficits as the MHFD offspring, displayed normal social behavior when they were colonized with gut microbiota from the MRD mice (Buffington et al., 2016) but not when they were treated with gut microbiota from MHFD mice (Buffington et al., 2016). The implication is that social behaviour is associated with a specific microbiome community.

Interestingly, the abundance of *Lactobacillus reuteri* was significantly reduced in the gut microbiome of MHFD offspring (Buffington et al., 2016). *L. reuteri* can increase the blood levels of oxytocin, which has an important role in different social behaviors, for example maternal care and social bonding (Varian et al., 2017). In addition to these, the presence of *L. reuteri* has been connected with improved learning and memory as well as longer lifespan (Varian et al., 2017). This shows that microbiome can contain bacteria species, that have a crucial part in the behavioral management of the host. Even the unbalance or absence of a single species can alter the behavior of the host so significantly, that it is not able to function normally in usual settings.

3.3.2 Feeding behavior

Even though the relationship with microbiome and their host is usually mutualistic or commensal, microbiome can alter the behavior of the host to benefit itself (Alcock et al., 2014). By

manipulating the feeding behavior of the host, the microbiome can make sure it gets the nutrients it wants and benefits the most from (Alcock et al., 2014). Therefore, the manipulation of host's feeding behavior is essential for the wellbeing of the microbiome.

The microbiome can alter the expression of host receptors, which leads to cravings for specific foods, for example sweet or savory foods (Alcock et al., 2014). Through this, the microbiome has a way of altering the feeding behavior of the host and increase the chances of the host eating the preferred nutrients (Alcock et al., 2014). Another direct route to alter the feeding behavior is through the vagus nerve, which has been discussed before. In more detail, the microbiome has ways of manipulating the traffic in the vagus nerve, therefore affecting the flow of information between the gut and the brain (Alcock et al., 2014). Disturbances in the vagus nerve have been connected to eating behaviors and changes in body weight, which shows great evidence of the role of the vagus nerve in feeding behavior manipulation (Alcock et al., 2014).

In addition to the manipulation of cravings, the microbiome can affect the hormones related with satiety and hunger (Alcock et al., 2014; Martin et al., 2018). It has already been shown that the microbiome has high resources when it comes to producing hormones, so when it comes to eating behavior, there might be even more drastic effects caused by the microbiome that previously realized. More evidence of the role of the microbiome in eating behavior is given by the fact that GF mice have been shown to have less hormones, that have a role in hunger control (Alcock et al., 2014).

Another interesting observation in the relationship between the microbiome and the feeding behavior, is the fact that microbiome compositions within a social group are like each other (Alcock et al., 2014; Archie & Tung, 2015). When it comes to feeding behavior, this would mean that the diets of individuals in the same social group are comparable with each other (Alcock et al., 2014). This could lead to bad eating behaviors spreading within the group and possibly lowering the fitness of the social group and its individuals, or alternatively healthy eating habits could increase it (Alcock et al., 2014).

The manipulation of feeding behavior will benefit the microbiome, but the effects on the host will be dependent on the composition of the microbiome (Alcock et al., 2014). A microbiome community that 'prefers' healthier nutrients will increase the health of the host, and alternatively,

a microbiome that 'prefers' unhealthier alternatives can reduce the health and therefore the fitness of the host (Alcock et al., 2014).

3.3.3 Depression-like behavior

Behavioral changes caused by the gut microbiota community are often defined as anxiety or social deficits. However, some depression-like behaviors appear to be caused by changes in gut microbiome (Gacias et al., 2016). In depression-like behavior the most common defining characteristics are social avoidance and a lack of despair-like behavior in a forced swim test (Gacias et al., 2016).

In the study done by Gacias et al. (2016), the depressive-like behavior and the connection to the changes in gut microbiome were compared between different mice genotypes (control and force-fed non-obese diabetic (NOD)). The composition of the gut microbiome is affected by alteration of the diet (e.g. by force-feeding) (Gacias et al., 2016), as mentioned in the *2.1 Development of the gut microbiome*. In the force-fed mice the Bacterioides and Firmicutes were more overall abundant than in the control mice (Gacias et al., 2016) and, in addition, NOD mice expressed more depression-like behavior (Gacias et al., 2016). These results indicate that the Bacteroidetes and Firmicutes would potentially be responsible for the behavioral changes. The role of the gut microbiome was examined further by the recolonizing of the gut of the mice genotype, that did not express depression-like behavior (Gacias et al., 2016). When recolonizing the gut with the microbiome taken from an individual expressing depression-like behavior, the mice that didn't express any, started expressing it (Gacias et al., 2016; Martin et al., 2018). This proves the fact, that the gut microbiome does have a crucial role in the expression of depressive-like behavior in mice, even when it's taken from a different individual.

3.3.4 Learning and memory

Microbiome has been noticed to influence cognitive behavior of the host, especially in learning and memory (Vuong et al., 2017). In the studies made with GF mice and rats (*Rattus norvegicus*) there were clear differences between the GF and control individuals in the spatial memory and object recognition tests (Vuong et al., 2017). In addition, control mice treated with antibiotics, to cause an imbalance in their microbiome, expressed decreased memory in the object recognition test, but not in the spatial memory test (Vuong et al., 2017). However, rats with the same kind of antibiotic treatment had a decreased spatial memory, suggesting that the effects on the cognitive

behavior is dependent on the species of the host and kind of imbalance experienced in the microbiome (Vuong et al., 2017).

There were also differences, whether the changes in cognitive behavior could be reversed back to normal behavior, which was expressed in individuals of mice and rats with normal gut microbiome (Vuong et al., 2017). The differences were again dependent on the species of the host and species of the bacteria used in the treatment (Vuong et al., 2017). It also seemed that the object recognition was easier to restore than the spatial memory deficits, but both were reversible with certain bacteria (Vuong et al., 2017). More information would be needed to understand the effects of microbiome on the cognitive behavior and the reasons behind it.

4. Behavior altered by other parts of the microbiome

Vertebrates must have ways to communicate with each other and to for example recognize their own kin from a bigger group, or to identify the social status of each other. These social behaviors can help them for example to increase the fitness of their offspring, by mating with the most compatible mate (Shropshire & Bordenstein, 2016). The role of the microbiome in these social interactions and the behavior connected to them will be discussed in more detail in the following examples.

4.1 Kin recognition

To recognize their own kin and individuals from each other, vertebrates must have different cues to do so (Vuong et al. 2017). Some vertebrates use odor cues to recognize their own kin from the others (Vuong et al. 2017). Since odor cues used by vertebrates are in certain cases produced by microbiome living in their scent organs, it has been predicted that the odor cues differ with different bacterial community structures (Archie & Theis, 2011).

Animal kin recognition and social hierarchy often relies on olfactory cues (Archie & Tung, 2015; Vuong et al., 2017). Short-chain fatty acids (SCFAs), which can be produced by the microbiome, do have a role in the production of these odor cues (Archie & Theis, 2011). Importance of microbiome for vertebrate communication is derived from experimental antibiotic removal of the bacteria from the scents glands that subsequently inhibits SCFAs production (Archie & Theis, 2011); also, bacteria harvested from scent glands continue to produce SCFAs (Archie & Theis, 2011).

One prediction of microbial odour production is that the odor cues depend upon the microbiome community structure (Archie & Theis, 2011). Interestingly, for example in chimpanzees (*Pan troglodytes*) and baboons (*Papio sp.*) (Archie & Tung, 2015), the microbiome community compositions of individuals from the same social group were more similar than the microbiome communities of individuals from more distantly-related social groups (Archie & Theis, 2011). Microbiome community composition could explain how the odor cues produced by microbiota help hosts recognize kin. For example, in badgers (*Meles meles*), odor cues are used to recognize the cubs and the adults in the group and when determining the social relationships (Vuong et al., 2017). These examples show, how important microbiome can be in order to have a functioning social group of vertebrates in multiple vertebrate species, mostly mammals.

4.2 Mate choice

Our understanding of the role of microbiota in sexual behavior and mate choice is still developing, but key discoveries also highlight an important effect of microbiome (Archie & Theis, 2011). The main ways that the microbiome appears to influence vertebrate mate choice are by affecting visual appearance (Archie & Theis, 2011) or via odor (Shropshire & Bordenstein, 2016).

Key evidence for microbiome impact on host visual appearance is derived from studies of bird species (Archie & Theis, 2011). For example, certain bacteria inhabiting feathers (e.g. feather microbiome) of house finches (*Carpodacus mexicanus*) affect the rate of degrading (Archie & Theis, 2011). Here, males with more feather-degrading bacteria have a duller appearance that makes them less appealing to females (Archie & Theis, 2011). Conversely, other feather bacteria are an important component of plumage and mate success (Archie & Theis, 2011). Male bluebirds (*Sialia* sp.) with brighter plumage, which are preferred by females, had a bigger quantity of feather bacteria than the duller individuals (Archie & Theis, 2011). Hence, the effects of feather microbiome on mate choice in birds is specific to the host and its specific microbiome.

Microbe specific volatile odors produced in the scent glands are used by many species to recognize possible mates (Shropshire & Bordenstein, 2016). These odors provide information about mate quality, for example about social/hierarchal status, age, sex and possible parasites or diseases (Shropshire & Bordenstein, 2016). These same kind of odor cues are also found in humans, especially in feet, breath, and underarm area (Shropshire & Bordenstein, 2016). The information gained from the odor cues are not used as consciously in humans as in other vertebrate species,

but women do connect certain odor cues with male attractiveness (Shropshire & Bordenstein, 2016) and that family members do recognize the scent of each other, in order to prevent inbreeding (Archie & Theis, 2011).

Another component connected to odor production and identification is major histocompatibility complex (MHC), which has a been connected to mate choice in some vertebrate species (Archie & Theis, 2011). It is predicted that microbiome is associated with the MHC-related odors in certain vertebrate species, since they cannot differentiate among individual urine samples taken from germ free (GF) individuals, for example in rats and mice (Archie & Theis, 2011). From the information gathered, it can be predicted that microbiome does have a role in mate choice, by affecting the phenotypic appearance and/or the odor cues of different vertebrate species.

5. Future directions

Studies of microbiome-vertebrate interactions have provided good evidence that the microbiome can manipulate the behavior of its host through diverse mechanisms. However, there are some aspects that need to be studied further to better understand the underlying mechanisms and effects. First, the studies are mainly focused on experiments on mice, which means that the species in both the host and the microbiome are limited. In addition, in most of the studies, the effects were noticed on GF individuals, which excludes the possibility to narrow the cause of these effects down to certain taxa of bacteria. To study the effects more broadly more studies should be done on other host species, and with more specific studies on the effects of certain taxa and/or microbiome metabolites.

One crucial aspect of the relationship between cognition and microbiome is whether influence of microbiome community structure can be reversed. Few studies have shown that it both can (Buffington et al., 2016) and cannot (Vuong et al., 2017) be reversed, depending on the effects caused by the lack of functioning microbiome. Given importance of brain development and microbiome during early life (discussed for example in section *3.3.1 Social deficits caused by changes in gut microbiome*) these studies should focus on disturbances in the microbiome during this important time of development. These studies could help us understand the possibility of treating different behavioral deficits, for example with microbiome therapy and therefore, it would be an important possibility to look into more.

Second, most of the studies are not only done with just mice and rats, but also in laboratory environment. It would be necessary to study the effects of microbiome in natural environment, with broader genetic diversity, because of the misleading results compared to the natural diversity. The animals in laboratory environment are on a certain diet decided by humans and as the microbiome is affected by the diet of the host, the microbiome could be different in the nature. Therefore, the composition of different microbes would be different and more adapted to the certain species, which could affect the effects on the behavior of the host.

Lastly, the role of the SCFAs seems to be unclear in all the studies and to draw any conclusions should be studied further. So far, the SCFAs are mentioned frequently, but the specific role or the mode of action are not discussed further. Also, the SCFAs are often mentioned as a whole, or studies use just one of them. There needs to be more focus on the way SCFAs affect the behavior (if they do), and if it is the ratio of them or the amount. Before the role of SCFAs is know better, the role of them in behavior manipulation is going to be uncertain and cannot be confirmed. This could lead to focusing on wrong aspects, when studying the connection between the vertebrate behavior and microbiome.

All this information about the effects of the microbiome on vertebrates and their behavior is important, because it helps to understand the connection between the health of the microbiome (especially in the gut) and different behavioral deficits. Especially the connection with autism spectrum disorder, depression and anxiety is notable in order to understand how to possibly prevent and treat these deficits. Even though the studies are so far done on other species, the results could give a needed insight in their function in the humans too. This is why further studies are needed to increase the knowledge and create better understanding on the mechanisms the microbiome can use and how it affects the host.

6. Conclusion

Microbiome has many ways to alter the behavior of its host, that we are only starting to understand. These manipulation methods include different hormonal, genetic and neural routes, which all offer various ways to affect the behavior. The actual effects vary depending on the species of both the host and the bacterial microbiome. In general, changes in the gut microbiome have an effect on the individual's social behavior by creating different social deficits and disturbing normal behavioral patterns. The gut microbiome is also capable of altering the feeding behavior of

the host and therefore, providing itself the nutrients it needs to reach the best possible fitness. Interestingly, the changes in gut microbiome can even cause changes in the development of host's brain structures, which again alter the behavior itself. Therefore, the health of the gut microbiome could have a greater part in the normal development of an individual than previously thought.

When it comes to the other microbiomes, for example on the skin or in the scent glands, the effects on behavior seem to be entirely directed to social behavior. Odors produced by the microbiome have a crucial part in individual recognition by providing information about the rank, health and relationships of an individual. It also plays an important role in mating behavior, by both providing actual information about the health and compatibility of the mate, but also affecting the phenotype of the mate, which can affect the mating success.

In conclusion, microbiome can influence its host's behavior in vertebrates, as predicted in my main hypothesis. The number of effects caused by the gut microbiome is larger than the ones caused by other microbiomes, but this could be simply because the gut microbiome is the most studied in this regard. It could also be, because the gut microbiome has more diverse bacterial community than the other microbiomes and has more direct ways to affect the behavior. The behavioral changes caused by the other microbiomes are more indirect, caused by interactions with other individuals. This means that the effects on the host are more dependent on these interactions and the manipulation is harder on an isolated host. The knowledge on this topic in general has increased within the last decade, which has turned out to be really helpful for the field.

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