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The Microbiota–Gut–Brain Axis in Determining Social Behaviours of Animals

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11.1 Introduction

The high prevalence of damaging behaviours resulting in physical harm and distress in farm animals poses important animal welfare, societal and economic concerns. These behavioural issues have long been thought to indicate frustration due to animals' barren environments. For example, the lack or absence of foraging opportunities for chickens and pigs in commercial farms is proposed to be the main driver of damaging behaviours such as severe feather pecking or tail biting. However, this ethological view does not fully explain all harmful behavioural interactions in farmed animals and why these behaviours become repetitive, fluctuate over time and vary among individuals exposed to similar environments. Therefore, animal welfare scientists are increasingly turning to other disciplines in an effort to elucidate the physiological mechanisms contributing to behavioural problems. The microbiota–gut–brain axis has emerged as an important contributor to behavioural modulation.

The physically, nutritionally and sensorially restricted environments that house farm animals can act as a powerful stressor. Research in humans and rodents indicates that stressors can alter the neurobiology and microbial communities of an animal in ways that are associated with

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behavioural disorders. Therefore, the role of the microbial community, microbe-mediated changes to the neurobiology and bidirectional communication of different components of the microbiota–gut–brain axis in the development of behavioural disorders is receiving increasing attention. It represents a promising avenue of research that can provide novel ways to tackle damaging behaviour in farmed animals.

As the study of the microbiota–gut–brain axis in farm animals is a relatively new field in animal science and welfare, this chapter is organized into six pillars: (i) introduction to observations linking the microbiota, the brain and behaviour; (ii) an overview of the microbiota–gut–brain axis and its application to animal welfare; (iii) techniques; (iv) pitfalls; (v) a conclusion; and (vi) suggestions for further reading. This chapter is not a comprehensive overview of microorganisms and the gut–brain interaction. Instead, it introduces the general topic of gut microorganisms, their potential role in animal welfare, and how intestinal microbiota management can keep farm animals physically and psychologically healthy.

11.1.1 A shift in focus from pathogens to beneficial microorganisms

Veterinary medicine has traditionally focused on identification of pathogens and the prevention and treatment of disease. Nevertheless, the vast majority of microorganisms inhabiting the body, collectively termed the microbiota, are innocuous or even beneficial. With this understanding, there is increasing enthusiasm to better understand their functions and benefits. The microbiota is found on external surfaces, such as the skin, or internal surfaces, such as the oral cavity's mucosal surface, the gastrointestinal tract and the urogenital tract. Composed of a combination of prokaryotes, eukaryotes and viruses, these microbial communities are proposed to impact the establishment of homeostasis by contributing to physiological processes, such as nutrient metabolism, defence against pathogens, immune system development and activation, and neurotransmitter production. Most recently, their influence has been proposed to extend to modulating behaviour (reviewed by Sekirov *et al.*, 2010; Jandhyala *et al.*, 2015; Trinh *et al.*, 2018).

11.1.2 The role of the environment in shaping animals' microbial communities

While a diverse microbiota minimizes pathogen colonization, an imbalance of the normal community structure (i.e. microbial dysbiosis) contributes to the risk of developing a pathogenic infection. The individual's genetic background, age and diet may impact the ease with which environmental microorganisms are incorporated into the microbiota (Sekirov *et al.*, 2010; Jandhyala *et al.*, 2015).

Maternal transfer of microorganisms is a contributor to the microbiota of an individual. Children born by caesarean section have distinct microbiota compared with those who underwent vaginal birth (Dominguez-Bello *et al.*, 2010). These differences have proven to confer long-lasting health consequences (Neu and Rushing, 2011). Differences in microbiota are also observed in farm animals, whereby pigs born by caesarean section and chicks reared without adult hens have distinct microbiota profiles from piglets that underwent vaginal delivery and newly hatched chicks kept in the presence of an adult hen, respectively (Kraimi *et al.*, 2019).

The built environment or man-made structures can also play an essential role in microbiota establishment. Prolonged exposure to indoor environments, coupled with reduced outdoor exposure, is associated with the development of mental health issues in humans (Hoisington *et al.*, 2019). Given that many farm animals are kept indoors for most or the entirety of their lives, the built environment is presumably a critical consideration in microbiota development (Kers *et al.*, 2018).

11.1.3 The role of nutrition and feeding behaviour in shaping animals' microbial communities

Diet is yet another important contributing factor to the establishment and subsequent shaping of the microbiota (Sekirov *et al.*, 2010; Jandhyala *et al.*, 2015). The dietary intake of the host is the primary energy source for the microbiota and influences microbial fermentation, bacterial numbers and species composition (Flint, 2012).

In the past, antibiotics were added to feed to increase feed efficiency and prevent disease; however, this strategy has led to a surge in antimicrobial resistance, rendering the treatment of infections difficult in humans and animals (Seal *et al.*, 2013). Consequently, treating and preventing disease using prebiotics or probiotics has become a promising alternative to ensure healthy and productive rearing conditions (Hill *et al.*, 2014). Recent scientific studies show that orally administered live or killed microorganisms and bacterial products promote a healthy gastrointestinal tract, thereby providing a viable and substantiated alternative to reducing antibiotic use in farmed animals (Seal *et al.*, 2013).

Foraging is an important behaviour that links the diet, the microbiota and behaviour. When animals are subject to a period of hunger, induced by feed restriction, it causes them to display increased physical activity, engaging in foraging behaviour to find food (Day *et al.*, 1995; Dixon *et al.*, 2014). Food can be a major driver for social interactions, including positive interactions due to animals foraging together or negative interactions due to competition. Microbiota composition is known to trigger aggregation or mate choice, and to modify foraging decisions and feeding preferences, further shaping the composition of the gut microbiota (reviewed

by Pasquaretta *et al.*, 2018). The mechanisms controlling the reciprocal influence on health and behaviour, however, are not yet elucidated.

11.2 Overview of the Gut–Brain Axis: Concepts and Definitions

The bridge connecting the environment and behaviour is the central nervous system (CNS). The current body of research points to a significant role of the gut microbiota in the communication pathways between the peripheral nervous system and the CNS. Given this strong modulatory potential on neurobiological function, the gut–brain axis has dominated the field of research investigating the relationship between gut microbiota and behaviour (Morais *et al.*, 2020).

Direct and indirect signalling by the microbiota can shape the nervous system (e.g. neurotransmitter regulation or interaction with the vagus nerve) and the immune system (e.g. cytokine modulation). As such, these pathways present an opportune target for developing microbe-based preventatives or treatments for a range of emotional or behavioural problems (Bravo *et al.*, 2011; Morais *et al.*, 2020). Despite significant research efforts in humans and rodents, the gut–brain axis of farm animals is far less studied. Therefore, further research is needed to determine whether microbiota-based approaches used to treat behavioural issues in rodents and humans can also be employed to improve farm animal welfare (Kraimi *et al.*, 2019).

11.2.1 Gut microbes and the enteric nervous system in gut–brain signalling

Gut microbes are essential for the normal development of the enteric nervous system (McVey Neufeld *et al.*, 2013; Collins *et al.*, 2014; McVey Neufeld *et al.*, 2015). Germ-free mice that lack a microbiome have lower nerve density and a reduced number of neurons per ganglion in the jejunum and ileum compared with conventional mice (Collins *et al.*, 2014). The gut contains two types of sensory nerves that carry signals from the gastrointestinal tract to the brain (i.e. afferents): (i) extrinsic primary afferents with somata outside the gut; and (ii) intrinsic primary afferent neurons (IPANs), characterized by somata located within the gut wall. The latter represent the majority of sensory fibres innervating the intestinal mucosa (Kunze and Furness, 1999). The absence of gut microbes decreases the excitability of myenteric IPANs (Collins *et al.*, 2014), which decreases the frequency and amplitude of muscle contractions in the intestine. In addition to regulating gut motility, the interaction between gut microbes and IPANs constitutes a microbiota–gut–brain axis component. This accounts for the ability of certain bacteria to modulate brain chemistry and

behaviour, as it allows the relay of signals from the lumen to vagal sensory ganglia (Perez-Burgos *et al.*, 2014). Gut microbes may also directly activate vagal chemoreceptors by producing substances that can be transported across the epithelial barrier to the portal circulation, namely short-chain fatty acids (Lal *et al.*, 2001). Alternatively, gut microbes may stimulate the release of paracrine mediators such as serotonin (5-hydroxytryptamine (5-HT)), histamine, cholecystokinin (CCK), adenosine triphosphate (ATP) and glucagon-like peptides from mucosal enteroendocrine cells (Li, 2007; Dockray, 2009). Importantly, enteroendocrine cells synapse with vagal neurons and rapidly transduce gut luminal signals using glutamate as a neurotransmitter (Kaelberer *et al.*, 2018).

11.2.2 The immune system in gut–brain signalling

There exists a well-established link between the immune system and behaviour. In humans, both depression and anxiety are associated with dysregulated immunological processes and an increase in markers of inflammation (Irwin and Miller, 2007; Liukkonen *et al.*, 2011). Moreover, pro-inflammatory cytokine-based treatments increase the risk of developing symptoms of depression, and this risk can be reduced by antidepressants, suggesting a causal relationship between inflammation and mood disorders (Capuron *et al.*, 2002). Similarly, in rodents, activation of the innate immune response leads to the development of symptoms associated with anxiety and depressive-like behaviour, and cognitive dysfunction (Wilson *et al.*, 2002; Godbout *et al.*, 2008; Raison and Miller, 2013). While direct afferent vagal signalling is necessary for the central effects of neuroactive mediators produced by microorganisms, recent studies indicate that the immune system also plays a critical role in mediating behavioural effects of gut microbes (Liu *et al.*, 2020). Indeed, specific peripheral immune cells have been linked with the modulation of behaviour. T-regulatory cells (Tregs) have been shown to mediate the anxiolytic and antidepressant-like effects of *Lactobacillus rhamnosus* in mice. The increase in Tregs coincides with a decrease in peripheral activated monocytes, which are also known to induce anxiety-like behaviour when they migrate to the brain (Wohleb *et al.*, 2015).

Biochemical circuits that are relevant in this context are the metabolism of aromatic amino acids and their interplay with tetrahydrobiopterin (BH_4). Besides being building blocks of proteins, tryptophan and phenylalanine are precursor molecules of indoleamine and catecholamine neurotransmitters, respectively. In humans, the strong relationship between the increased breakdown of peripheral tryptophan along the kynurenine axis and the increase in immune activation markers is well established (Geisler *et al.*, 2018). A pro-inflammatory oxidative milieu induces indoleamine 2,3-dioxygenase 1 (IDO-1), the main enzyme responsible for tryptophan catabolism along the kynurenine axis in the blood. Chronic

immune stimulation also correlates with reduced phenylalanine turnover (Neurauter *et al.*, 2008). Oxidative conditions support the depletion of tetrahydrobiopterin, a cofactor that is required for the conversion of phenylalanine to tyrosine by phenylalanine hydroxylase, as well as for tyrosine 3-monooxygenase to form the catecholamine precursor levodopa (L-DOPA). Moreover, synthesis of the 5-HT precursor 5-hydroxytryptophan depends on BH_4. Thus, in humans, an inflammation-induced shift of phenylalanine and tryptophan metabolic routes can further dysregulate dopaminergic and 5-HT metabolism, respectively. These, in turn, play an essential role in inflammation-associated behavioural and emotional changes (Geisler *et al.*, 2018).

In addition, dietary tryptophan and probably also microbial tryptophan catabolites coming from the tryptophanase–indole pathway exhibit immunomodulatory properties relevant for neuroinflammation, as was shown in a mouse model for multiple sclerosis (Sonner *et al.*, 2019). Thus, a multitude of interactions has to be considered. Certainly, there are differences, for example in tryptophan metabolism (e.g. IDO-1 is lacking in chickens: Yuasa *et al.*, 2015). Beyond this, more information is needed to fully explore the potential of gut–brain signalling of peripheral amino acids and their endogenous as well as their microbial-derived catabolites.

11.2.3 Neuroimmune interactions in gut–brain signalling

Both Tregs and intact vagal signalling are demonstrably important for bacterial communication with the brain. It is noteworthy that the vagus nerve innervates tissues involved in immune functions, such as the thymus, spleen, lung, liver and gastrointestinal tract. The functional implications of this anatomical relationship between the vagus nerve and the immune system were recognized with the identification of a neural circuit that controls the inflammatory response in a reflex-like manner (Tracey, 2007). In this reflex, vagal sensory afferent nerves detect mediators of the inflammatory response, such as cytokines, which are released by macrophages and other immune cells after stimulation with lipopolysaccharide (LPS) or other microbial-associated molecular patterns (MAMPs) (Pavlov and Tracey, 2012). This leads to vagal cholinergic efferent signalling, which, in turn, suppresses the activity of pro-inflammatory immune cells. Microbe-driven changes to behaviour are therefore hypothesized, depending on peripheral immune changes facilitated by this efferent arm of the anti-inflammatory reflex (Tracey, 2007).

More recently, hepatic vagal sensory afferent nerves were found to be responsible for indirectly sensing the gut microenvironment and relaying the sensory inputs to the nucleus tractus solitarius (NTS) of the brainstem (Teratani *et al.*, 2020). Signalling to the NTS activates vagal efferent nerves and, subsequently, enteric neurons that regulate the intestinal Treg population (Teratani *et al.*, 2020).

Taken together, the evidence suggests that the effect of gut microbes and/or MAMPs on intestinal motility may be linked to behavioural changes through a reflex loop. This involves an IPAN-to-vagus nerve synapse (Perez-Burgos *et al.*, 2014), leading to activation of the NTS. Subsequently, efferent vagal and enteric nervous system modulation of the peripheral immune system (Teratani *et al.*, 2020) regulates central neuroinflammation, stress responses and anxiety (Wohleb *et al.*, 2015; Liu *et al.*, 2020).

11.2.4 Application to farm animal welfare: a focus on feather pecking in chickens

The contribution of the microbiota–gut–brain axis to feather pecking challenges traditional ethological views and provides innovative, corrective solutions. Indeed, the role of the gastrointestinal tract, gut microbes, and the nervous and immune systems in feather pecking development has gained increased attention (Fig. 11.1). Birds that are descendants of a feather-pecking genetic line have distinct gut microbiota and short-chain fatty-acid profiles compared with birds from a non-pecking line (Meyer *et al.*, 2012, 2013). Birkl *et al.* (2018) and van der Eijk *et al.* (2019b) report a lower abundance of *Lactobacillus* species in the caecal excreta of birds genetically selected for feather-pecking behaviour. From a functional perspective, applying *L. rhamnosus* to the caecal gut lumen reduces the frequency and increases the amplitude of caecal contractions. Furthermore, the velocity and amplitude of caecal contractions were positively associated with the number of feather-pecking bouts (van Staaveren *et al.*, 2020). In addition to regulating chicken gut motility, gut microbes may also directly (e.g. via the vagus nerve) or indirectly (e.g. mediated via microbial metabolites or stimulation of enteroendocrine cells to release 5-HT) modulate brain chemistry and behaviour. Consequently, dissecting the gut–microbe network, identifying its contribution to behaviour and using this information to develop nutraceuticals are exciting avenues of research with important implications for preventative therapy development. To this end, Mindus *et al.* (2021) reported that *L. rhamnosus* ingestion prevented feather pecking in stressed chickens and limited caecal dysbiosis. The authors also showed that the probiotic bacteria lead to increased Tregs in the spleen and the caecal tonsils. Further investigations are needed to assess the potential of *Lactobacillus* species to act as a corrective therapy to established feather-pecking behaviour. Finally, a recent report by van der Eijk *et al.* (2020) is the first to investigate the effect of early-life microbiota transplantation on feather pecking, 5-HT levels and immune profiles in chickens. Interestingly, while the impact on the gut microbiota composition was minimal, there were significant age- and genotype-dependent changes to behaviour and the immune system.

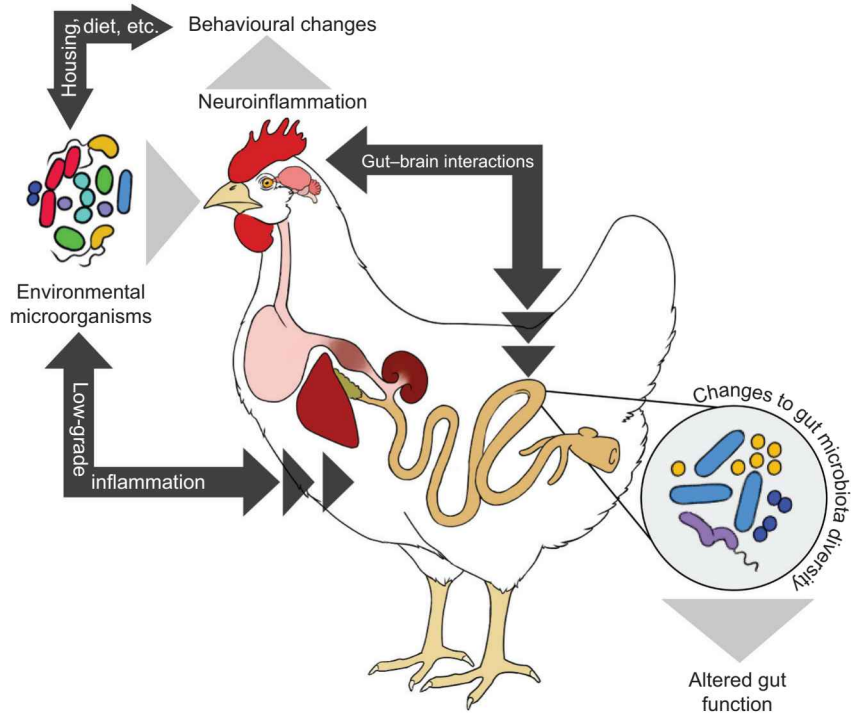

Fig. 11.1. The gut–brain axis in chickens. Environmental factors (e.g. diet, housing, microorganisms) play a key role in the immune status of chickens. Changes to the environment can produce a dysregulated gut microbiota, resulting in the secretion of microbial metabolites that are neuroactive, alter (neuro)immune signalling and can cause changes to aromatic amino acid turnover rates. The accumulation of these effects can result in behavioural changes, which in turn can influence environmental factors and microorganisms that chickens are exposed to in the future, further affecting the cycle. Figure reproduced with permission from Renée Garant.

11.3 Techniques

11.3.1 Techniques to manipulate the gut microbiota

The use of germ-free mice, mice with a defined microbiota, cross-fostering and faecal transplants are some of the tools and techniques used in rodent research to evaluate potential behavioural changes associated with gut microbiota manipulation. These techniques allow a reductionist approach to examine the influence of a single strain or simple, multi-strain treatment. Studies employing these methods generally identify whether the microbiota plays any role in a model phenotype. In farm animal research, a gut microbiota-associated phenotype of interest is modelled by comparison of antibiotic-treated and untreated animals. Following an antibiotic regimen,

it is possible to repopulate the gut with a desired microbial population via a faecal microbiota transfer. Alternatively to depletion and re-establishment of the resident microbial population, the existing gut microbiome can be manipulated using live microorganisms (probiotics), substrates utilized by potential beneficial microorganisms (prebiotics) or a combination thereof to study the association between gut microbiota and behaviour. The latter strategy using probiotics and prebiotics has the potential to treat or even prevent behavioural problems. Regardless of the technique used to manipulate the microbiota, sampling and analysis are required to assess the manipulation's effectiveness under investigation.

11.3.2 Sampling techniques to harvest microorganisms

The ideal sampling method for a live animal's gut microbiota should be non-invasive. Faecal samples or faecal swabs are often used as a proxy for the intestinal gut microbiota. It is, nevertheless, important to note that faecal microorganisms are distinct from the luminal and mucosa-associated populations. For example, Yan *et al.* (2019) found that faecal samples reasonably mirrored the phylum composition found in the intestine; however, it did not accurately represent the bacterial genera to the same degree. Direct sampling of the mucosal microbiota in live animals requires an invasive technique, as it needs biopsy/endoscopy to retrieve mucosa-associated microorganisms. While it is undeniably easier to harvest gut microbiota samples from various anatomical sites from euthanized animals, this sampling method is often only feasible within a research setting.

11.3.3 Molecular techniques to identify microorganisms

While the concept of the microbiota–gut–brain axis has been around for centuries, culture-independent microbial detection methods have enabled the identification of microbes that are highly resistant to culture-based assays. This, in turn, has spurred a boom in microbiota–gut–brain axis research. 16S ribosomal RNA (rRNA) gene sequencing for taxonomic identification is the most utilized method in the field of microbiota research. It does, however, provide little information compared with metagenomic sequencing, which randomly sequences DNA fragments from the complete genomes of viruses, prokaryotes and eukaryotes within a sample. The resulting data provides both taxonomic and functional information on the microbiota. Metagenomics can identify rare species and how microbes communicate or compete. Specialized statistical tools can use 16S rRNA sequencing data to predict metagenomics, such as PICRUSt, which uses a reconstruction algorithm to predict gene frequencies. Metabolomics identifies and characterizes the interactions between bacterial metabolites and the host. As such, this type of study prioritizes the identification of functions.

11.3.4 Techniques to measure the impact of microbiota on the immune system

Immune biomarkers can be measured in tissues or in blood; however, it can be challenging to determine the sampling site that will provide the most reliable data. Recent work found that birds' immune responses from divergently selected lines for feather pecking were distinct, thereby linking behaviour to an immune phenotype (Buitenhuis *et al.*, 2006; van der Eijk *et al.*, 2019a). Interestingly, similar work using genetic lines of pigs with varying propensities for tail biting suggests differences in immune marker levels accompanied by changes to neurotransmitter levels (Brunberg *et al.*, 2016; Nordgreen *et al.*, 2020; Veit *et al.*, 2021). Enzyme-linked immunosorbent assays (ELISAs) can quantify specific antibody titres, and fluorescence-activated cell sorting (FACS) can detect changes in immune cell populations (van der Eijk *et al.*, 2019a). In the latter, flow cytometry is used to separate cells based on cell-surface markers. Furthermore, *ex vivo* stimulation of isolated, blood-derived monocytes with MAMPs, such as LPS, can provide insight into (innate) immune competence (van der Eijk *et al.*, 2019a). To study pro-inflammatory responses, cytokine and acute-phase protein concentrations can be measured in the serum or plasma of challenged animals.

11.3.5 Techniques to elucidate gut–brain axis signalling

The response of the axis to microbiota changes can be measured using biophysical, biochemical, electrophysiological and computational modelling techniques. The integrity of vagal afferent signalling is critical to the communication between specific gut microbes and the brain. Vagotomy has been shown to block neuron activation in the brain and, concomitantly, block the anxiolytic effects and changes to social deficits that are normally observed in response to probiotics, such as *L. rhamnosus* or *Bifidobacterium longum* (Bercik *et al.*, 2011; Bravo *et al.*, 2011; Sgritta *et al.*, 2019; Bharwani *et al.*, 2020). Indeed, vagal-dependent gut–brain signalling via IPANs was first demonstrated by exposing the intestine to *L. rhamnosus*. This signalling was attenuated after nicotinic receptor or total synaptic blockade (Sgritta *et al.*, 2019). Further support for the relationship between gut microbes, IPANs and the vagus is provided by the observed absence of mesenteric afferent signalling following IPAN stimulation of germ-free mice. This signalling response is restored following conventionalization (i.e. establishing a microbiota) of the adult animals (McVey Neufeld *et al.*, 2015). Gaining a better understanding of gut–brain axis signalling necessitates investigation of the complex interactions between the vagus nerve, the immune system and the brain. Delineating these interactions requires the ability to differentiate between afferent and efferent vagal pathways involved in facilitating the effects of gut microbes on the CNS. Such techniques include selective

deafferentation using specific neurotransmitter receptor agonists conjugated to toxins (Diepenbroek *et al.*, 2017), or transgenic approaches, as have been employed in the ablation of nociceptive nerves (Talbot *et al.*, 2015). Similarly, the role of the immune system in signalling between the gut and the brain can be investigated using antibodies or transgenic animals to deplete specific cell types (Wohleb *et al.*, 2015; Liu *et al.*, 2020), or to block migration of peripheral immune cells to the brain by targeting chemotactic factors or receptors (Wohleb *et al.*, 2015). The role of distinct immune cell types in gut–brain communication can also be investigated through isolation and adoptive transfer of the cells followed by assessment of behaviour and brain function in recipient animals (Liu *et al.*, 2020).

11.4 Pitfalls

While a significant portion of the existing literature addresses interactions of the host with its bacterial inhabitants, a scant number of studies identify how intestinal inhabitants, including viruses, protozoa and fungi, affect the bacterial microbiota and influence model behaviours. While the interplay between numerous microbiota members and the host signalling pathway is admittedly complex, the rapid expansion of biomedical research and the laboratory animal infrastructure in the past decade promises that exciting revelations about health and disease are on the horizon.

The translation of small rodent or human studies to farm animal welfare research is challenging, as the techniques discussed previously are often not feasible on a large scale or in commercial settings, often due to practicality and costs. For instance, rodent studies use small numbers of inbred animals. Because farm animals are outbred, large numbers are required for studies to have meaningful and significant outcomes. Even if a sufficient farm animal population is available and the study is affordable, performing an experiment such as a probiotic trial requires careful consideration of the variables that can significantly impact the outcome. For instance, rodent studies often use germ-free models or antibiotic treatment regimens to eliminate the microbiota to investigate the effect of a single bacterium or a defined mixture of bacteria. Determining the bacterial strain to be administered in farm animals, its formulation (e.g. live or dead bacteria), delivery mode (e.g. water or feed), dosage (e.g. amend or scale-up from the rodent model; administered daily or weekly) and duration of the study can be difficult with little supporting literature. Molecular techniques can predict the metabolic impact of microbes within the host. The multi-factorial nature of behavioural disorders makes the selection of the molecular technique targeting a single pathway challenging. The close interconnectedness of the microbiota–gut–brain axis processes makes it difficult to integrate each component of the axis into a simple set of experiments. In these instances, putative underlying mechanisms, such as gut

dysmotility or visceral hypersensitivity associated with behavioural disorders, can be used to test the potential therapeutic value of a specific bacteria. For example, while *ex vivo* investigations of certain probiotics on the enteric nervous system (West *et al.*, 2017; Bharwani *et al.*, 2020; van Staaveren *et al.*, 2020) are essential from a fundamental point of view, whether these observations are replicated *in vivo* is unclear. While this remains an important step, behaviour is determined by complex interactions between the components of the gut–brain axis. Additionally, evolutionary differences in physiology and normal gut microbiota composition may render findings from human or rodent studies poorly translated to farm animal species without additional research (Birkl *et al.*, 2019). To this end, it is not always possible to make inferences about the functionality of specific microbes and their association with behaviour from previous rodent research because of the lag in understanding and characterizing farm animal gut microbiota and other physiological processes (Waite and Taylor, 2015).

The technological advances of high-throughput gene sequencing and bioinformatics analysis have expanded the study of gut microbiota. However, the variability of the methods and approaches (e.g. culture-based studies, quantitative PCR, 16S rRNA studies, use of pooled or individual samples, environmental factors), and results from different sequence centres, as well as differences in the level of detail in results reported (e.g. α- or β-diversity, phylum, class or genus level) make comparisons between studies difficult. Additionally, the number of animals used in these studies is often low, as the analysis is expensive (Stanley *et al.*, 2014; Jandhyala *et al.*, 2015). The decreasing costs of DNA sequencing have fuelled microbiome research. Nevertheless, the cost to process the sample numbers required to get reliable data needs to be assessed. Additionally, it is noteworthy that sequencing does not differentiate between dead and live microbiota, and the metabolome is not taken into account. Another limitation to the prolific and efficient use of sequencing technology is highly qualified personnel with specific expertise that can manipulate the sequencing data to extract microbial profiles, work with reference databases for taxonomic assessments, and explain potential mechanistic/physiological pathways and associate them with behaviour. Furthermore, there is a recurring emphasis on achieving a 'healthy', 'balanced', 'normal' gut microbiota, as 'dysbiosis', 'imbalance' and 'abnormal' gut microbiota are linked to disease or behavioural disorders (Sekirov *et al.*, 2010; Jandhyala *et al.*, 2015). However, a normal or ideal gut microbiota remains undefined and is likely to be variable for each individual animal.

Moving forward, the microbiota research community as a whole faces several challenges in elucidating key players of the microbiota–gut–brain axis. Many intestinal microbes remain uncultivable, which limits their characterization. Novel culture methods would be required to overcome this identification barrier, which consequently impedes elucidating their interaction with the host. Moreover, there is a need for a standardized complex

microbiota for use in germ-free models. A dialogue to determine the standardized composition and whether the microbiota of existing commercial colonies can be used for this purpose should be initiated. The maintenance of germ-free animals should also be standardized.

Another major limitation is that biochemical circuits may differ among species due to distinct enzymatic repertoires or activities, e.g. those involved in tryptophan (Yuasa *et al.*, 2015) or nitric oxide biochemistry (Schneemann and Schoedon, 2002). These are just two examples out of many that underline the relevance of the fine-tuned coordination of immunometabolic pathways in different species. Notably, differential responses to inflammatory stimuli have been demonstrated even in a comparison of inbred mouse strains (Hoover-Plow *et al.*, 2008). This implies that investigations of relevant pathways on molecular and biochemical levels should, at best, go hand in hand with genetic analysis. Nevertheless, not all responses will be predictable, and the previous discussion has already emphasized the influence of nutrition and the microbiome on immunological status.

11.5 Conclusions

We are beginning to understand the potential role of the microbiota–gut–brain axis and its ability to impact behaviour; however, the bidirectional communication between the gut, bacteria, viruses, fungi and protozoa in other body parts and the brain is mediated through the neuroimmune system and neurotransmitters, among other molecules. Dissecting this intricate network, identifying its contribution towards modifying behaviour and using this information to develop nutraceuticals to improve the welfare of farm animals are exciting avenues of research.

Further Reading

For further information we recommend the resources that are provided in bold in the reference list. In addition, we recommend the following resources:

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12

Improving Animal Welfare Using Genetic and Genomic Tools

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12.1 Introduction

12.1.1 Measuring animal welfare

The need to address and improve animal welfare has been well recognized for many decades (see Chapter 1, this volume). In the 1990s, it became clear that selection for narrowly defined breeding goals, based primarily on production traits, may result in metabolic, reproduction, health and behavioural problems in highly selected farm animals (Rauw *et al.*, 1998). Since then, the focus in animal breeding has shifted to implementation of broader breeding goals by including functional traits associated with good welfare. Animal scientists now emphasize that welfare can also be *improved* by genetic selection (e.g. Jensen *et al.*, 2008; Rauw, 2016). However, the first challenge encountered when aiming to select for improved animal welfare is deciding which traits are considered to be representative of welfare. This is a formidable task. As animal welfare has many facets, there have been many attempts to best define it (Stafleu *et al.*, 1996) and no universally endorsed definition of animal welfare has emerged (Mellor, 2016) (covered in more detail in Chapter 1, this volume). According to Broom (1991), animal welfare is a function of an animal's needs. Reduced welfare follows from a deficiency to an animal's mental and bodily stability that arouses its motivational state such that it seeks to remedy its need(s) through behavioural and physiological (i.e. coping) responses. Successful coping results in adaptation, while difficulty or failure to cope results in unpleasant

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