FACULTY OF MEDICINE AND HEALTH SCIENCES

Role of the gut-brain axis in epilepsy

Influencing the microbiome via the vagus nerve

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Abstract

In recent years it has become evident that the gut-brain axis, an anatomical and functional connection between the gastrointestinal (GI) tract and the brain, plays an important role in neuropsychiatric disorders. Multiple studies have shown that patients with epilepsy exhibit a disturbed balance within their microbiome, which is a suggested element of epileptogenesis. Therapies with an impact on the gut microbiota – such as ketogenic diet, probiotics, antibiotics and faecal microbiota transplantation – have been shown to be efficient in some cases of epilepsy, raising the question whether the disease burden of epilepsy can be ameliorated by specifically targeting the host's microbiome. This review hypothesises that vagus nerve stimulation (VNS), typically used as therapy for epilepsy, also influences the gut microbiome composition and exhibits its effect on epilepsy through this pathway. Here, the most recent literature on both the gut-brain axis in epilepsy and the effect of VNS on the GI microbiome available on Pubmed and Embase has been collected and reviewed in order to evaluate the plausibility of this proposition. No existing articles specifically investigating this subject have been published yet, and the reported results concerning the influence of VNS on the GI microbiome composition in other diseases differ. Many interesting studies however have shown the importance of the gut-brain axis in epilepsy. Nevertheless, a definite answer to this thesis' research question cannot be provided based on the current literature so more extensive and standardised studies are recommended.

Dutch summary

In de afgelopen jaren is de appreciatie voor de darm-hersen-as, een anatomische en functionele verbinding tussen de gastro-intestinale (GI) tractus en de hersenen, in vele neuropsychiatrische aandoeningen sterk toegenomen. Verschillende studies hebben aangetoond dat patiënten met epilepsie een verstoord evenwicht vertonen in de samenstelling van hun GI microbioom. Deze zogenaamde dysbiose wordt dan ook gesuggereerd als een oorzakelijke factor in het ontstaan van epilepsie. Therapieën zoals ketogeen dieet, antibiotica, probiotica en fecale microbiota transplantatie hebben een mogelijke invloed op de samenstelling van het GI microbioom en zijn in geselecteerde gevallen effectief gebleken als behandeling voor epilepsie. Deze resultaten roepen de vraag op of de ziektelast van epilepsie kan worden verbeterd door zich specifiek te richten op het microbioom van de gastheer. Deze thesis stelt de hypothese dat nervus vagus stimulatie (NVS), een behandelingsmethode die frequent wordt toegepast als therapie voor epilepsie, eveneens de samenstelling van het GI microbioom beïnvloedt en via deze weg zijn therapeutisch effect bij epilepsie realiseert.

Om de plausibiliteit van deze stelling te evalueren, werd de meest recente literatuur over zowel de rol van de darm-hersen-as in epilepsie als het effect van NVS op het GI microbioom opgezocht op Pubmed en Embase, waarna deze werd verzameld en samengevat in deze review. Tot op heden zijn er nog geen artikelen gepubliceerd die dit specifiek onderwerp onderzoeken, en de gerapporteerde bevindingen betreffende de invloed van NVS op de samenstelling van het microbioom in andere ziekten lopen uiteen. Verschillende interessante studies hebben echter wel reeds het belang van de darm-hersen-as in epilepsie aangetoond. Desalniettemin kan een definitief antwoord op de onderzoeksvraag van deze review niet gegeven worden op basis van de huidig beschikbare literatuur. Meer uitgebreide en gestandaardiseerde studies worden dan ook aanbevolen.

1. Introduction

This review investigates the hypothesis that the mechanism of the therapeutic effect of vagus nerve stimulation (VNS) in epilepsy is mediated through its ability to affect patients' gastrointestinal (GI) microbiome.

Epilepsy is a chronic brain disorder that is characterised by the occurrence of two or more recurrent, unprovoked seizures. Seizures are short episodes of involuntary movements of (a part of) the body and/or loss of consciousness due to the sudden occurrence of abnormal hypersynchronous activity of neurons in the brain cortex (1). It affects around 50 million people worldwide. Known causes of epilepsy are stroke, brain tumours and infections, severe head injury, congenital abnormalities and certain genetic syndromes. However, in more than 50% of epilepsy patients, the cause of the disease remains unidentified. Multiple theories have been proposed in attempt to explain the pathophysiology of epileptogenesis, the process by which a previously normal brain network is functionally altered, resulting in an increased vulnerability for seizures (2). Some of these suggested mechanisms are: a disturbed balance between excitatory and inhibitory signals (3, 4); inflammation (3, 5); immunological mechanisms (6); and even an auto-immune reaction (5, 7, 8). These and other mechanisms are reviewed elsewhere (2). Anti-epileptic drugs (AED) are the first-line treatment in epilepsy. AEDs typically affect the excitability of the neurons. In about 30% of all patients AEDs are unable to sufficiently control the epileptic seizures (9). When seizures persist, even after the use of at least two adequately chosen AEDs whether or not in combination, the epilepsy is considered 'drug-resistant' (DRE) (10). For DRE, other therapeutic options such as epilepsy surgery, neurostimulation – e.g. VNS and deep brain stimulation (DBS) – or dietary measures may be considered.

The importance of modulating the vagus nerve system to control seizures has been known for years; already in the 1880s it was described how a massage of the carotid region in the neck is able to affect seizure activity (11). In 1952, Zanchetti and colleagues proved that it was indeed stimulation of the vagus nerve which ceased chemically induced seizures (12, 13). Between 1988 and 1998, the E01-E05 trials were conducted (11). In these randomized controlled clinical trials, the aim was to investigate the efficacy, safety and tolerability of VNS therapy, for which patients with DRE were implanted with a medical device able to activate vagal nerve fibres in the neck region. It was demonstrated that long-term VNS therapy led to a 50% seizure reduction in about 40% of the patients, while therapy was deemed safe and well tolerated. VNS has been approved as therapy for epilepsy by the US Food and Drug Administration (FDA) and the European Union (12). The exact mechanism of action is not fully understood. The vagus nerve, the 10^{th} cranial nerve, is an important component of the autonomous nervous system (ANS), more specifically of the parasympathetic nervous system (11, 13, 14). It is the longest cranial nerve and reaches all the way into the abdomen, providing the innervation of several organs in the thorax and abdomen, including a great part of the GI tract. The vagus nerve consists of 20% efferent and 80% afferent fibres. The afferent fibres relay information to the brainstem nucleus tractus solitarius (NTS) and project to multiple structures in the pons, the midbrain, the cerebellum and the cerebrum (12, 13). These afferent fibres and their central projections are believed to mediate the anti-epileptic effect of VNS. Crucial mechanisms of VNS involve the brainstem locus coeruleus and the norepinephrine neurotransmitter release in the brain, the raphe nucleus and its serotonin levels, changes in the thalamocortical system, desynchronization of hypersynchronized cortical activity (11, 14, 15), and changes in blood flow in various regions of the brain (11). Apart from epilepsy, VNS is also used in depression (11, 13) and is under investigation for other disorders such as migraine, Alzheimer's disease (AD), mood disorders, obesity and inflammatory bowel disease (IBD) (12).

In recent years, there is growing interest for the role of the microbiome in the pathogenesis of various neuropsychiatric disorders such as AD, multiple sclerosis (MS), Parkinson's disease (PD), autism spectrum disorder (ASD) (5), amyotrophic lateral sclerosis (ALS) (16), depression, anxiety (16) and epilepsy. Already in 1916, a role for the GI micro-organism *Bacillus epilepticus* in the pathogenesis of epilepsy was proposed (17). Today, this theory is no longer attributed to one organism, but to the whole GI microbiome. The GI microbiome comprises a population of 10^{8}-10^{11} organisms consisting of over 1000 different species of bacteria, viruses, protozoa, archaea and fungi that altogether encode 150 times as many genes as the human genome (17, 18). All these species live in symbiosis with their human

host and form a dynamic balance within the gut. A disturbance of this balance, a dysbiosis, is considered an important factor in the development of some neurological diseases (1). The influence of the GI microbiome on the brain – and vice versa – is mediated by the so-called 'microbiota-gut-brain axis' or simply 'gut-brain axis' (7, 16). This includes both neuroanatomical, immunological as well as humoral connections between the gut and the brain (5, 18).

Since the vagus nerve forms a direct nervous tissue connection between the brain and the colon, it is considered an important component of the gut-brain axis. Naturally this raises the question whether this nerve is able to influence and/or alter the GI microbiome in order to obtain a favourable effect on the pathogenesis of epilepsy. If so, it may be possible to elicit this effect with therapeutic modulation of the vagus nerve, such as VNS. In other words, we investigated whether VNS may have a beneficial effect on the development and course of epilepsy via the GI microbiome. In this review the available literature on both the role of the gut-brain axis in epilepsy and the possibility to influence the microbiome via VNS is brought together in an attempt to answer these questions.

2. Methods

2.1 Pubmed

Based on the research question of this review a search strategy was developed for three concepts: (a) epilepsy; (b) vagus nerve stimulation; and (c) gut microbiome (table 1, appendix). Synonyms were sought for every concept and where possible these were entered as Mesh-terms. Every Mesh-term and every other synonym was also included as TIAB. Subsequently, each developed concept was entered separately in the *Pubmed advanced search builder* in order to assess the number of hits. On 15/3/2020 there were 233002 results for concept (a); 140390 for (b); and 149077 for (c). Combining these three search queries with an 'AND' operator resulted in one hit. This was deemed insufficient, so the decision was made to combine the concepts in pairs, separated with the 'AND' operator. This gave the following results: 3870 hits for the combination (a) & (b); 149 for (a) & (c); and 477 for (b) & (c).

Since there is sufficient literature on the role of vagus nerve stimulation in epilepsy, some filters were used so that only reviews with a publication date between 1/1/2020 and 15/3/2020 were included. This narrowed the selection for the combination (a) & (b) down to eight results on Pubmed.

For the manuscripts on the gut-brain axis itself – the combination of concepts (b) & (c) – only the most recent insights were desired. Therefore, only manuscripts from the last five years (from 1/1/2015 to 15/3/2020) were selected. This reduced the number of results for the combination (b) & (c) to 250.

As the combination of the concepts epilepsy (a) and gut microbiome (c) was the main focus of this review, all 149 manuscripts with this combination were selected.

2.2 Embase

A similar strategy was applied in Embase; the same three concepts were used, again with all possible synonyms (table 2, appendix). Both the function *major focus* as well as *ti,ab,kw* were indicated in the search strategy. This resulted in the following number of hits on 15/3/2020: 385999 results for concept (a); 195248 for (b); and 120552 for (c). The three developed concepts were combined with an 'AND' operator, which gave 10 results of which three were relevant. In order to have a more complete overview, the concepts were also combined in pairs. This gave the following results: 12286 hits for the combination (a) & (b); 217 for (a) & (c); and 895 for (b) & (c).

For the combination (a) & (b) the filters '2020' and 'review' were selected, which reduced the number of results to 45 articles.

For the articles about the gut-brain axis itself – the combination of concepts (b) & (c) – only the most recent insights were desired. Therefore, only articles from the last five years (from 1/1/2015 to 15/3/2020) were selected. This brought the number of results for the combination (b) & (c) down to 490.

As the combination of the concepts epilepsy (a) and gut microbiome (c) was the main focus of this review, all 217 manuscripts with this combination were selected.

2.3 EndNote

For every combination, the articles retrieved from both Pubmed and Embase were brought together in three different EndNote libraries: one for the results of Pubmed and Embase for the combination (a) & (b); one for the combination (b) & (c); and one for the combination (a) & (c). In every library duplicates were sought and deleted. After reading title and abstract, all non-English articles, irrelevant manuscripts and those without abstract were also deleted.

Based on the abstract of the manuscripts for the combination (a) & (b), only four addressed a broad enough overview of the use of VNS in epilepsy, so only these were finally selected.

For the combination (b) & (c) the manuscripts had to deal with the top-down influence of electrical stimulation of neurological structures on the GI microbiome, which was the case for 17 articles.

Reasons for considering a manuscript from the combination (a) & (c) irrelevant were: no mention of epilepsy; a minor role for epilepsy; or dealing with the influence of the microbiome on AEDs. After this selection, 45 manuscripts remained.

The useful manuscripts identified on Pubmed and Embase by combining the three concepts were also included when the concepts were combined in pairs. Ultimately the full text of 66 manuscripts was sought and reviewed. For 15, the full text was not available via open access. After reading full text of the other articles, 13 more were discarded. Combining the three EndNote libraries into one resulted in two more duplicate articles, which were deleted, leaving a total of 36 useable articles that were found with the search strategy of this review (figure 1, appendix). Three more relevant manuscripts that were not included in the results of this search strategy were found after exploring the reference list of these 36 articles, thus resulting in a final total of 39 articles used for this review.

3. The GI microbiome, the gut-brain axis and their role in epilepsy

This section aims to provide evidence for the role of the GI microbiome in the aetiology and evolution of neurological disorders, more specifically of epilepsy. The pathophysiology of epilepsy is multifactorial with both genetic and environmental factors playing a role (3, 16). These factors may also have an effect on the GI microbiome, which in turn could affect the brain. Both animal and human studies have shown that gut microbiota can play an important role in neurodevelopment and behaviour by producing hormones, immune factors and metabolites (18, 19). A normal GI microbiome has been shown to lead to proper brain development and behaviour, whereas for example germ-free mice are shown to display deficits herein (16, 20).

3.1 The GI microbiome

Every part of the human body that is in contact with the outside world, is colonised with bacteria, some 100 trillion in total (18). Around 80% of these reside in the GI tract. Together with a variety of other micro-organisms such as protozoa, archaea and fungi, as well as different sorts of viruses, these bacteria form the GI microbiome. Of the more than 100 microbial species, two phylae primarily define this microbiome, namely *Bacteroidetes* and *Firmicutes*, together accounting for at least 70-75% of the total amount of bacteria in the GI

tract. Other phylae like *Proteobacteria*, *Actinobacteria*, *Fusobacterium* and *Verrucomicorbia* are relatively less common.

All these micro-organisms live in symbiosis with their human host (8, 18). By continuously stimulating the immune system, gut bacteria create a state of low degree physiological inflammation which prevents pathogenic microbial growth. They also stimulate intestinal epithelial cell regeneration and nourish the mucosa by producing short-chain fatty acids (SCFAs). Furthermore, they are important in drug and poison removal, and they are able to synthesize and metabolise certain nutrients, vitamins and hormones. Together, the different species of bacteria maintain a dynamic equilibrium (17, 18). This means that the composition of the GI microbiome is subject to changes throughout life. New-borns receive their initial microbiome from their mothers – with differences in composition between children born vaginally and by caesarean section – and after the first year of life the formation of an increasingly complex GI microbiome is initiated to reach its full complexity at an adult age. Afterwards, in adulthood, multiple factors continue to affect the microbiome such as aging, antibiotic use, drugs, infections, diet and stress.

In order to identify the different GI microbiota, researchers commonly use sequencing techniques (21). Most frequently, the 16S ribosomal RNA (rRNA) is sequenced, seeing as this is a highly conservative region in the genetic material of most prokaryotes. The 16S rRNA contains several hypervariable regions (V1-V9) on the basis of which the differentiation of the different microbial taxa can be made. Similar sequences are clustered in so-called operational taxonomic units (OTUs) and are then compared to databases in order to match OTU and taxon correctly. The cut-off value of sequence similarity within an OTU is usually 97%. With these data, the diversity of a sample can be calculated; α-diversity shows the richness of the community within a single sample, whereas β-diversity indicates compositional variations between different samples.

3.2 The gut-brain axis

The brain and the GI tract are anatomically and functionally connected through the so-called ‘gut-brain axis’ (16). The interaction between the gut-brain axis and the GI microbiome is called the ‘gut microbiota-brain axis’ (7, 18). This term encompasses the bidirectional communication between the GI tract and the brain via nervous, metabolic, endocrine, humoral and immune pathways (figure 2).

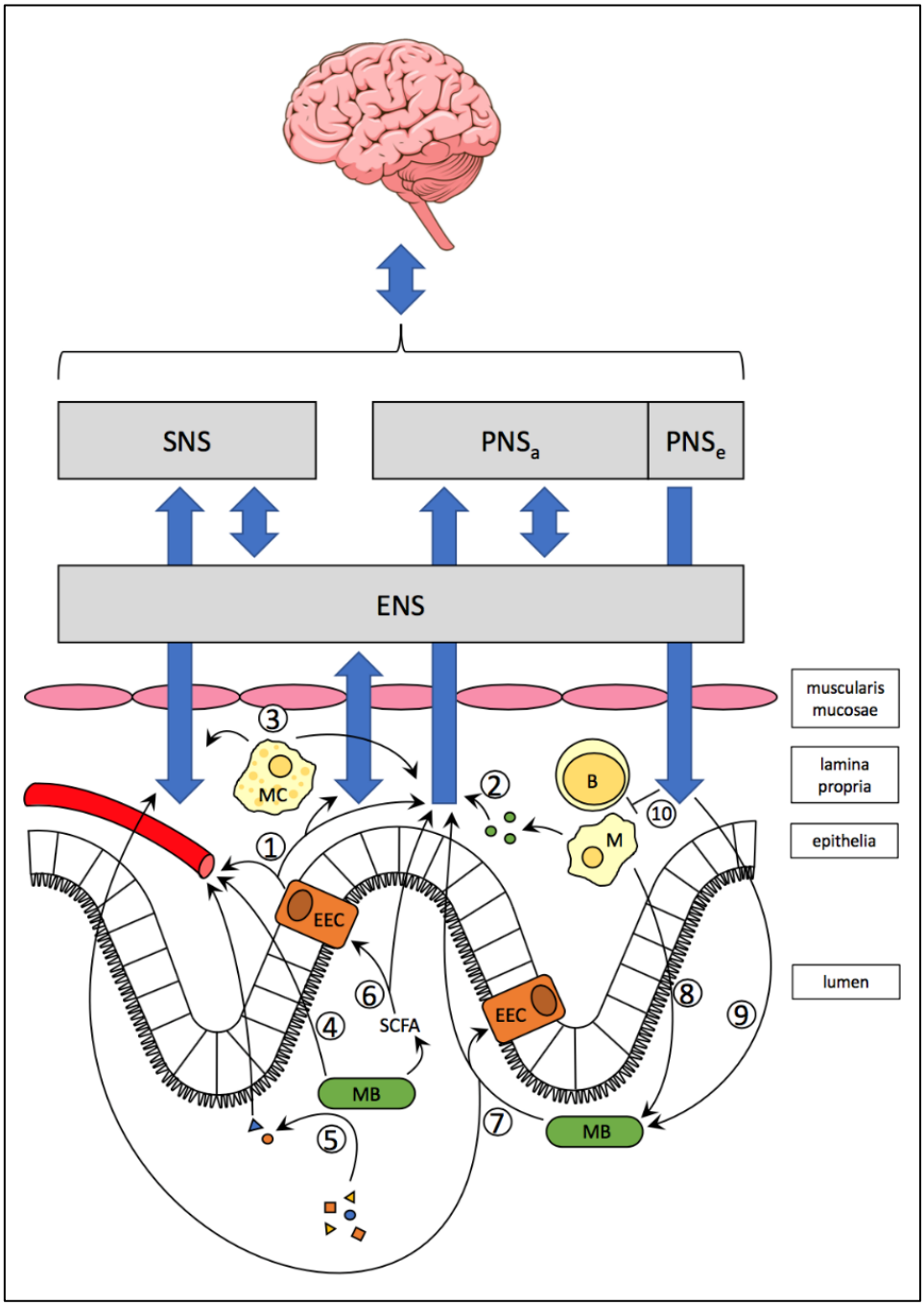

Figure 2: The gut microbiota-brain axis. *Release of hormones by EECs (1), cytokines stimulating the HPA (2), release of MC mediators (3), bacterial production of neurotransmitters (4), bacterial metabolism of neurotransmitter precursors (5), bacterial production of SCFAs (6), detection of bacterial products by TLRs (7), local immunity regulates microbiota (8), endogenous neurotransmitters bind on bacterial receptors (9), CAP inhibits inflammation (10).*

Sympathetic nervous system (SNS); parasympathetic nervous system afferents (PNS_a) & efferents (PNS_e); enteric nervous system (ENS); mast cell (MC); B-cell (B); macrophage (M); enteroendocrine cell (EEC); gut microbe (MB); short chain fatty acids (SCFA); Toll-like receptor (TLR); hypothalamic-pituitary-adrenal axis (HPA); cholinergic anti-inflammatory pathway (CAP).

3.2.1 The neuroanatomical pathway

The GI tract is innervated by the ANS, which consists of the parasympathetic nervous system – originating in both the cranial and the sacral parasympathetic nuclei –, the sympathetic nervous system and the enteric nervous system (ENS) (22). The functions of the parasympathetic and the sympathetic nervous system are essentially the opposite of one another; parasympathetic activity in the GI tract stimulates motility and secretion, whereas sympathetic activity reduces intestinal activity (23). The vagus nerve, the 10th cranial nerve, is one of the most important parasympathetic nerves. 20% of the vagal nerve fibres are efferent fibres that send signals from the brain to the intestines. The other 80% are afferent fibres, which means they conduct sensory impulses from mechano- and chemoreceptors in the gut to the brain. The afferent endings in the gut are located either on the apical side of villi (villus afferent endings), or around the base of the crypts (crypt afferent endings), but they do not cross the epithelial layer (24). The cell bodies of these afferent fibres are located in the nodose ganglion and relay their signals to the NTS in the brainstem. From here on, the vagal afferents communicate with a multitude of regions intracranially such as the parabrachial nucleus, the locus coeruleus, the paraventricular nucleus of the hypothalamus, and the amygdala (22). The efferent fibres of the vagus nerve originate in the dorsal motor nucleus of the vagus nerve (DMNV) located ventrally to the NTS. Vagal afferent and efferent fibres, as well as the sympathetic fibres, stand in strong connection with the ENS in the mucosa and muscular wall of the GI tract. The ENS consists of a myenteric plexus regulating the motility of intestines, and a submucosal plexus controlling the GI blood flow, secretion and mucosal barrier function (23). Despite its strong interaction with the vagus and other autonomous nerves, the ENS is perfectly able to function independently from the influence of the central nervous system (CNS), hence the name the ENS is sometimes given: ‘the second brain’.

3.2.2 Interaction with the nervous system

The GI tract is also connected to the brain through the release of hormones and regulatory peptides (23). These hormones are often anorexigenic such as cholecystokinin (CCK), glucagon-like peptide-1 and peptide YY, or orexigenic such as ghrelin and orexin and may reach the brain via the bloodstream (24). When released by the enteroendocrine cells (EECs), these hormones can also locally interact with specific receptors on the afferent fibres of the vagus nerve which eventually results in endocrine, autonomic, emotional and cognitive responses mediated by the brain. In preclinical research it has been demonstrated that these responses do not occur or are declined in rats after vagotomy in the case of CCK and ghrelin, which serves as an argument for the role of the vagus nerve in this pathway (23).

Activation of vagal nerve endings in the GI tract can also affect the hypothalamic-pituitary-adrenal axis (HPA) (12). Cytokines such as interleukin 1β (IL-1β) can activate the vagal afferent fibres in the intestinal wall. Through the NTS, these afferents are able to stimulate the parvicellular division of the paraventricular nucleus of the hypothalamus, which in turn secretes corticotrophin-releasing factor (CRF). CRF subsequently triggers adrenocorticotropic hormone (ACTH) release by the pituitary gland, finally resulting in the release of corticoids by the adrenal gland, which have anti-inflammatory properties.

The vagus nerve is also able to regulate peripheral inflammation via the cholinergic anti-inflammatory pathway (CAP) (12). This pathway inhibits inflammation by suppressing the production of inflammatory cytokines. The mechanism by which this occurs is via the vagal efferents, which inhibit e.g. macrophages and B lymphocytes through the secretion of acetylcholine (Ach). The Ach receptor on these cells necessary for this inhibition is the α7 subunit of the nicotinic Ach receptor (α7nAChR). The vagus nerve could modulate the intestinal microbiota via the CAP not only by modulating local immunity, but also by possibly influencing the intestinal permeability (22). Indeed, the α7nAChR has a protective effect on the tight barrier functions of the GI tract (24). Another hypothesised pathway concerning the vagal role in the intestinal barrier function involves the modulation of enteric glia via the vagus nerve, resulting in an increased expression of tight-junction proteins (12).

A final example of a functional connection between the gut and the brain is the immune pathway. Certain immune cells in the GI tract, such as the mast cells, can stimulate parasympathetic or sympathetic afferent fibres by secreting e.g. histamine, bradykinin or serotonin. This way, they can increase intestinal motility and mucosal secretion (17).

3.2.3 Gut microbiota-brain axis

Most of the pathways of the gut-brain axis described above, can be influenced by the GI microbiome, thus creating essentially a ‘gut microbiota-brain axis’ (7). Since the nerve endings of the ANS do not cross the epithelial border of the intestines, there is no direct interaction between these fibres and the gut microbiota (24). There are however different ways in which indirect communication can occur.

Many microbiota have the ability to produce neurotransmitters and neuropeptides which are almost identical to those produced by human cells (17). For example, serotonin is produced by *Enterococcus, Streptococcus* and *Escherichia*. *Escherichia* also produces noradrenaline and dopamine, whereas *Bifidobacteria* and *Lactobacillus* produce γ-aminobutyric acid (GABA). Only the latter is most likely able to influence the brain, since there are no transporters for the

other microbiota-produced neurotransmitters located on the blood-brain-barrier (BBB). These may however reach the brain through the vagus nerve or the circumventricular organs (24).

The GI microbiome is able to regulate the levels of endogenously produced neurotransmitters by altering the amounts of necessary building blocks (17). Indeed, precursors of dopamine, noradrenaline, serotonin, tryptophan and tyrosine are decreased in the brain of germ-free (GF) mice. Amino acids that are γ-glutamylated (GG-AAs) are hypothesised to be easier transportable to the brain than regular amino acids (25). Some combinations of bacteria have been shown to decrease the activity of γ-glutamyltranspeptidase, resulting in a reduction of easy available neurotransmitter building blocks.

Apart from neuro-active compounds, some bacteria also produce SCFAs by fermenting insoluble dietary fibres (17). SCFAs are necessary in the GI tract for mucus production and nourishment of the mucosa. These metabolites are able to stimulate the sympathetic nervous system (26), vagal afferent terminals and EECs (24). The latter do not only express receptors for SCFAs, but they also possess toll-like receptors (TLR) which enables them to detect bacterial products such as lipopolysaccharides (LPS). Both types of receptors can also be found on the vagal afferent fibres which means that gut microbiota should be able to directly stimulate the vagus nerve.

A striking example of the interaction between gut bacteria and the vagus nerve can be found in an *ex vivo* study where administration of *Lactobacillus johnsonii* in a jejunal segment resulted in an increased firing rate of the afferent vagal nerve fibres (24). The excitability of these fibres has also been shown to be increased by *Lactobacillus reuteri* by inhibiting the opening of calcium-dependent potassium channels (26).

The bidirectionality of the gut-brain axis is also a trait of the gut microbiota-brain axis, which means the brain also has influence on the microbiome. For example, the brain is able to regulate the intestinal permeability and local immunity via the CAP, which could have an influence on the microbiome (22). Intestinal permeability is shown to decrease after VNS (24). Furthermore, several bacteria reportedly have receptors for humane neurotransmitters; e.g. *Pseudomonas* has a high affinity for GABA and *Escherichia coli* 0157:H7 has receptors for (nor)epinephrine (26). Finally, basic GI functions such as secretion and motility are regulated by the ANS. These functions are of importance for the formation and stability of the intestinal mucus layer and biofilm in which the GI bacteria thrive.

Evidence for these bilateral interactions can be found in several studies reporting differences in stress reactivity, anxiety and depressive-like behaviours between GF mice and specific-

pathogen-free (SPF) mice (18, 23, 26), as well as studies showing a different microbiome after a long period of stress (18). Furthermore, evidence can be found for the importance of the GI microbiota in development and maturation of the CNS (26) and in the permeability of the blood-brain barrier (17).

3.3 The GI microbiome and epilepsy

A dysbiosis or disturbance of the equilibrium between the different bacterial species in the GI tract is associated with various neurological disorders such as ASD, MS, PD and AD (4). Its role in epilepsy has been investigated pre-clinically and clinically.

Besides leading to complex changes in the composition of the GI microbiome, stress also increases intestinal permeability permitting a microbiota-driven pro-inflammatory state with implications for neuroinflammation (27). Chronic stress is found to increase the susceptibility to epilepsy, probably via an altered microbiome. In a study by Medel-Matus et al. the threshold and duration of kindled seizures were compared between six groups of rats: the first consisting of sham-stressed rats with no microbiota transplant, the second of stressed rats with no microbiota transplant, the third of sham-stressed recipients transplanted with microbiota of sham-stressed donors, the fourth of sham-stressed recipients transplanted with microbiota of stressed donors, the fifth of stressed recipients transplanted with microbiota of sham-stressed donors, and the sixth of stressed recipients transplanted with microbiota of stressed donors. It was shown that chronic stress both accelerated kindling and extended the seizure duration. Interestingly, transplantation of the intestinal microbiome from stressed to the sham-stressed animals also showed accelerated kindling and increased duration of the seizures compared to before the transplant. In other words, the stressed microbiome mimicked the effects of stress itself. On the contrary, transplantation of microbiota form sham-stressed to stressed animals counteracted the pro-epileptic effects of chronic stress. Even though the microbiota of neither the sham-stressed nor the stressed group were sequenced which means a change in microbiota composition cannot be proven, the authors suggest the role of dysbiosis in mediating the effects of stress on epilepsy.

Recently Safak et al. compared the intestinal microbiome composition of 30 patients with idiopathic focal epilepsy and 10 healthy controls with 16S rDNA sequencing of the V3 and V4 region (5). At phylum level they found that the patients had lower abundances of *Firmicutes*, *Bacteroidetes* and *Actinobacteria* and an increased amount of *Proteobacteria* compared to the healthy controls. Interestingly, *Fusobacteria* were detected in the patient group whilst being absent in the control group. Significant differences were also found at genus level.

It appears that the severity of epilepsy is correlated with different changes in the GI microbiome. Peng et al. compared the composition of the GI microbiome of 42 patients with DRE, 49 patients with drug-sensitive epilepsy and 65 healthy controls who came from the patients' families to reduce the impact of diet on the GI flora (19). Sequencing of the V3 and V4 region of the 16S rDNA showed a similar community richness and evenness between the drug-sensitive and the healthy group, whereas these parameters were increased in the DRE group meaning their GI microbiome was more diverse. This was because of an increased abundance of multiple rare bacteria. Furthermore, β-diversity analysis showed that the microbiome composition of DRE patients was different from that of the drug-sensitive and healthy group. Compared to these groups – whose microbiome composition was similar – the DRE group had a GI microbiome with relatively lower abundances of *Bacteroidetes* and higher abundances of *Firmicutes*. Also noteworthy is the relative increase of various rare bacteria in the DRE group, such as *Verrucomicrobia.* Based on their findings, the authors proposed a possible mechanism by which dysbiosis of the GI microbiome could be able to induce DRE. They hypothesised that the overgrowth of rare bacteria may result in a high level of ABC transporter metabolism, leading to a decrease in the absorption of AED.

The study of Peng et al. also showed differences between patients with more than four seizures per year and those with four or less seizures per year. More specifically *Bifidobacteria* and *Lactobacillus* were found increased in patients with fewer seizures, suggesting a protective effect of these bacteria on epilepsy. Indeed, both *Bifidobacteria* and *Lactobacillus* have been shown to promote synthesis of GABA which is a known inhibitory neurotransmitter.

Another example in which the importance of the composition of the GI microbiome in the pathophysiology of epilepsy is suggested, can be seen in the case study of a 22-year old epilepsy patient with Crohn's disease (28). This girl had a 17-year history of epilepsy for which she was treated with sodium valproate. She received a faecal microbiota transplantation (FMT) as treatment for Crohn's disease, after which the use of sodium valproate was ceased. In the entire 20 months of follow-up and in the period thereafter the patient never had a recurrence of epilepsy and remained seizure-free without AED. This raises the question whether epilepsy can also be treated with FMT. A trial aiming to answer this question has been registered (NCT02889627) but no results are available yet (4).

3.4 Modulating the composition of the GI microbiome

3.4.1 Diet

3.4.1.1 Ketogenic diet

As mentioned, the diet is one of the factors which is able to change the dynamic balance within the GI microbiome (7). An example of a specific diet used in the treatment of DRE is the ketogenic diet (KD). KD is a high-fat and low-protein and carbohydrate diet with the purpose of mimicking a fasting state (29). The aim is to reach a ratio 4:1 of fat to protein and carbohydrates together, which means the meal contains four times as many grams of fat for every gram of protein and carbohydrates combined (4). The high amounts of dietary fat are metabolised in the liver into ketone bodies like acetoacetate and β-hydroxybutyrate, which in turn are used as energy substrates for several tissues including the brain.

In 1998, authors of a study on the efficacy of KD on seizures in 150 children observed that if no 50% seizure reduction was reached within the first three months of therapy, it was unlikely that improvement of the seizures would be seen afterwards (1). This means that these first three months are a crucial period for the effectiveness of KD and many studies afterwards based their intervention duration on these findings. A recent Cochrane review on the efficacy of KD in epilepsy combined 11 randomized clinical trials in which a total of 712 children and 66 adults participated. After three months of KD therapy, seizure freedom rates varied from 0 to 55% and seizure reduction rates reached up to 85%. All studies however showed adverse effects of the intervention.

The most frequently reported adverse effects are those of the GI type (30). Nausea, vomiting, diarrhoea, abdominal pain, gastro-oesophageal reflux and constipation occur in almost 50% of the children on KD, especially at the start of the therapy. 3-7% of the patients on KD suffer from kidney stones. Other, less frequent adverse effects are metabolic abnormalities such as hypoglycaemia, hypertriglyceridemia and hypercholesterolemia, cardiovascular changes such as cardiomyopathy and, even more rare, pancreatitis.

For the anti-epileptic effect of KD, multiple mechanisms of action are suggested (1, 29, 30). A first hypothesis is that the ketone bodies themselves have an anticonvulsive effect via e.g. neuron hyperpolarization and potentiation of inhibitory neurotransmitters such as GABA. Chronic ketosis increases the expression of energy metabolism genes, which improves the function of neurons and makes them more resistant to metabolic stress, increasing their chances of survival in stressful conditions. The beneficial effect on seizure activity has also been shown in patients with no increase in ketone body levels after KD, suggesting

mechanisms independent of ketosis. A possible mechanism is the anticonvulsant effect of a decreased glucose metabolism in the brain. After all, metabolism of glucose is a fast source of energy necessary for seizure activity, whereas the slower, anaerobic metabolism reduces the availability of energy. Furthermore, increased amounts of polyunsaturated fatty acids (PUFAs) are seen during KD. These PUFAs are able to influence the concentrations of pro-inflammatory cytokines such as IL-1β through the activation of peroxisome proliferator-activated receptors, and are thus able to regulate neuroinflammation.

A final suggested mechanism of the anti-epileptic effect of KD is via an alteration of the GI microbiome composition. Possible changes in the human GI microbiome caused by KD were first investigated in a pilot study from Tagliabue et al. (31). Based on the observation from animal models that high fat diets were associated with e.g. a decrease in *Bacteroidetes* and an increase in *Firmicutes* and *Proteobacteria*, the authors compared the microbiota composition of six patients with Glucose Transporter 1 Deficiency Syndrome (GLUT1-DS) before and after three months of KD treatment. Since there were no significant differences observed at phylum level for *Bacteroidetes* and *Firmicutes*, results from the pre-clinical trials could not be reproduced. In addition, seven genera of bacteria were specifically sought for in the microbiome samples because of their possible beneficial or detrimental health effects. The only significant alteration found here was an increase in *Desulfovibrio* species. Apart from the small sample size, a big disadvantage of this study was the method used; in their RT-PCR analysis, the researchers only looked at changes in nine bacterial groups (two phylae and seven genera), thus ignoring possible alterations in the abundances of the countless other organisms in the gut.

The impact of KD on the microbiome composition has also been studied in patients with epilepsy. In a first study, 14 children with DRE were put on a ketogenic diet for one week, after which three were seizure free and six had a >50% reduction in seizure frequency (32). The microbiota of the patients before and after KD as well as that of 30 healthy subjects were analysed by 16S rRNA sequencing of the V3 and V4 region. Compared to the healthy group, the DRE patients had higher amounts of *Firmicutes* and *Proteobacteria* and decreased *Bacteroidetes* and *Actinobacteria*. At genus level a high amount of *Cronobacter* was found, which was absent in the healthy group. Even after such a relatively short therapy duration, a clear rescue of the GI microbiome composition could be noted; the most striking changes after KD were the noteworthy increase in *Bacteroidetes* and decrease of *Proteobacteria.* The former resulting mostly due to an increase in the genus *Bacteroides* and the latter thanks to a sharp

decline in the genus *Cronobacter*. *Firmicutes* and *Actinobacteria* levels remained unchanged. Perhaps with a longer therapy duration changes in these phylae could be found as well.

In contrast, in a study in which the GI microbiome of 20 DRE patients was compared before and after six months of KD therapy, a significant decrease in *Firmicutes* and *Actinobacteria* was observed (33). Similar to the study of Xie et al. they also found a significant increase in *Bacteroidetes* after KD treatment, but *Proteobacteria* were not significantly changed. There was also a distinction made between so-called responders and non-responders of KD therapy, i.e. patients who respectively achieved ≥50% seizure reduction with KD (10 patients in this study, including two patients who were completely seizure free) and those who did not reach 50% seizure reduction. Compared to the responders, non-responders were found to have significantly increased abundances of *Clostridiales, Clostridia, Ruminococcaceae, Lachnospiraceae, Alistipes* and *Rikenellaceae.*

Even more diverse results were found in the study from Lindefeldt et al. (20). Faecal microbiota of 12 paediatric epilepsy patients and 11 parental controls were compared before and after three months of KD therapy using shotgun metagenomic sequencing. B-diversity analysis indicated that the microbiome composition of the patients and the controls were different, and altered again after KD. The changes that occurred after therapy were an increase in *Bacteroidetes* and *Proteobacteria* and a decrease in *Firmicutes* and *Actinobacteria*. The latter was mainly caused at genus level by the decrease in *Bifidobacteria* in epilepsy patients after KD.

As can be read above, results from studies investigating the changes in the composition of the GI microbiome caused by KD are inconsistent. Nevertheless some concordant elements can be noticed. For example, in most studies a decrease in α-diversity is reported after KD (20, 25, 33). This means that the therapy reduces the diversity of the microbiome community, probably because of a decrease of the elevated amounts of rare bacteria that may be present in the microbiome of the epilepsy patient (19). Beta diversity analysis showed that the microbiome composition is altered after KD (20, 25, 33). The most consequently reported change is the increase of the relative abundance of *Bacteroidetes* after KD (20, 32, 33).

Recently, Olson et al. delivered the evidence that the GI microbiome is in fact necessary for the anti-seizure effects of KD (25). In two seizure mouse models – a 6-Hz-induced model of DRE and a model of genetically seizure-prone mice – they measured and compared the seizure thresholds of a group SPF mice on a control diet (CD) and of three groups treated with KD: the first consisting of SPF mice (SPF-KD), the second of GF mice and the third of mice

treated with antibiotics (Abx). The protective effect of KD on the seizure thresholds that was seen in the SPF-KD group compared to the CD group was decreased in the GF and Abx group, suggesting that the GI microbiome is needed in this mechanism of seizure protection. Moreover, the anti-epileptic effect of KD returned when GF mice were transplanted with the microbiome of SPF-KD mice. By selectively enriching the microbiome of the Abx group with certain KD-associated bacteria, it was found that specifically the combination of both *Actinobacteria muciniphila* and *Parabacteroides merdae* induced the anti-seizure effect of this therapy. Both species are necessary as this effect could not be recreated with only one of them or with other combinations. The authors were able to link these microbial changes to a decrease in GG-AAs, and an increase of GABA – and to a lesser extent glutamate – in the hippocampus.

3.4.1.2 Other dietary measures

In recent years, some variants of the KD have been developed (29). A first variant is the modified atkins diet (MAD). In this diet, the fat to protein and carbohydrate ratio is 1:1 and the carbohydrate intake is limited to 10-20 g/day. Because of this lower ratio, MAD is better tolerated than KD and has fewer side effects. Its efficacy in the treatment of DRE is comparable to that of KD. A recent meta-analysis of 70 studies concluded that there was no difference in the reduction rates of seizure frequency between children on KD and children on MAD. For adults with DRE, a meta-analysis of eight studies showed a >50% seizure reduction in 20-70% of the patients, and reported seizure freedom in 7-30% of the cases. Given that MAD is better tolerated, it is suggested that patients start their dietary therapy with MAD and switch to KD when deemed necessary (1).

Another modification of the KD is the low glycaemic index diet (LGIT) in which the carbohydrates have a glycaemic index <50 and the fat to carbohydrate and protein ratio is 0,6:1. The tolerability of this diet is even better than KD and MAD. Results of the efficacy of LGIT on DRE are promising with reported seizure frequency reduction of >50% in about 50% of the cases, but there is a shortage in high-quality studies regarding this subject.

A different example in which the effect of diet on epilepsy is observed, is the case of a 10-month old infant with DRE and cow's milk protein allergy (34). The child was diagnosed with early-onset generalized epilepsy, in which two months of AED therapy did not improve the seizure frequency. However, eliminating cow milk products from the diet did stop the seizures successfully.

3.4.2 Anti- and probiotics

The use of anti- and probiotics may also modulate the composition of the GI microbiome. Its effects on epilepsy have been studied clinically and pre-clinically. Braakman et al. followed six DRE patients who had to go through a course of antibiotics and observed that five out of six patients became temporary seizure-free while the sixth showed a significant decrease in seizure frequency (35). In all patients, this effect wore off within two weeks after discontinuation of the antibiotic. The AED treatment was continued during the use of the antibiotic, which led the authors to suggest a synergistic effect between the two drugs. After all, macrolide antibiotics, which were used by two patients, are known cytochrome p450 inhibitors and could therefore have led to an increased serum level of the AED. Apart from this mechanism, the authors also hypothesize that the seizure freedom and decrease in seizure frequency was caused by the effect of the antibiotic treatment on the GI microbiota. However, since there was no microbiota sequencing this cannot be proven. Other antibiotics such as rapamycin, minocycline and gentamicin are being examined for possible beneficial effects on epilepsy (3). Contrarily, many studies have shown a correlation between the use of certain antibiotics and an increased risk for epilepsy and seizures.

Probiotics are living organisms that, when administered, are capable of interacting with the microflora in the GI tract, changing the composition of the microbiome, finally resulting in possible health benefits (36). The effect of a mixture of *Bifidobacterium infantis, Lactobacillus rhamnosus* and *Lactobacillus reuteri* on seizures induced by pentylenetetrazole (PTZ) in rats was studied by Bagheri et al. The chemical kindling with this $GABA_A$ receptor antagonist is a model which is very similar to clinical DRE. They found that probiotic supplementation before or during the process of kindling significantly reduced seizure severity. GABA concentrations in the brain were significantly elevated in the group where chemical kindling and probiotic treatment happened at the same time and an improved antioxidant/oxidant balance was found in the kindled rats. Furthermore, the study also showed a beneficial effect on cognitive performances such as learning and memory consolidation. Other studies in rodent models demonstrated that a probiotic treatment with *Lactobacillus rhamnosus* and/or *Bifidobacterium longum* regulated the expression of GABA receptors in the brain (3).

The effect of probiotic treatment has been investigated in human patients with DRE by Gomez-Eguilaz et al. (37). 45 patients were recruited and after three months of assessment a probiotic mixture of *Lactobacillus* and *Bifidobacterium* species and *Streptococcus salivarius* was administered in combination with the patients' usual AEDs for four months. 28,9% of the patients reached a seizure reduction of 50% or more, and of these patients, 76,9% maintained

a seizure reduction in the months of follow-up after stopping the probiotic. Similar as with the use of probiotics in rats as previously discussed, an increase in GABA during the treatment was observed, however with no statistical significance.

4. VNS and the GI microbiome

In the previous section, the existence and importance of the gut microbiota-brain axis has been established. However, in order to answer the question whether the effect of VNS on epilepsy is partly due to its effect on the GI microbiome, there is a need for evidence concerning the possibility of influencing this gut microbiota-brain axis in a top-down manner with neurostimulation. In this section, an attempt will be made to assemble this evidence.

4.1 Influencing the GI microbiome with electroacupuncture

One example of electric stimulation therapy used to alter the gut microbiome composition is the use of electroacupuncture (EA). In a first study, the impact of EA on the composition of the GI microbiome in obese mice was investigated (38). The researchers sequenced the faecal microbiota of 50 mice, of which 30 were obese. These obese mice were randomly divided into five groups, each one receiving EA for a different period of time, with one group receiving no EA at all. Apart from the amelioration of the obesity parameters, EA also normalized the GI microbiome of the obese group with an optimum reached after 21 days of therapy. The normalizing effect was most evident in the bacterial species that were increased in the obese group, such as *Fusobacteria, Firmicutes* and *Spirochaetes*. Bacteria that were decreased in the obese group, such as *Acidobacteria* and *Cyanobacteria*, remained at a lower level than in the healthy group, even after EA.

Another recent study compared the microbiota of rats with induced post-inflammatory irritable bowel syndrome (PI-IBS), of which a subgroup received EA, with that of healthy rats (39). In summary, the authors found an association of PI-IBS with changes in the GI microbiome, which could be reversed with EA. More specifically the amount of *Fusobacteria* significantly decreased in the PI-IBS group after EA. Other reductions of relative abundances were found for *Bdellovibionales, Fusobacteriales, Chitinophagaceae, Bilophila, Enterococcus,* and *Vampirovibrio.*

4.2 Influencing the GI microbiome with VNS

With more relevance to the subject of epilepsy is the possible effect of VNS on the GI tract. The only described effects in literature are the very rarely reported GI side effects of VNS (11,

15). To this day, there are no studies concerning the impact of VNS on the gut microbiota of animals or patients suffering from epilepsy, but it has been researched in other diseases.

Phillips Campbell et al. used VNS in guinea pigs with mechanically induced heart failure for the purpose of evaluating its impact on the gut microbiota composition (40). In the microbiome of the group with heart failure, 10 bacterial genera differed significantly compared to the healthy control group. After VNS, eight of these genera were comparable to the healthy control group, demonstrating that VNS is able to alter the composition of the microbiota in guinea pigs with heart failure. The results also showed that some of these compositional changes were dependent on which vagus nerve was stimulated; three of the genera were only altered after VNS of the right nerve, whereas three other genera were specifically altered with VNS of the left nerve (table 3).

Table 3. Microbial genera normalised after VNS in guinea pigs with induced heart failure (40)

Both left & right VNS	Right VNS only	Left VNS only
Lactobacillus	*Dehalobacterium*	*Bacteroides*
Mogibacteriaceae	*Victivallaceae*	*Ruminococcus*
	Desulfovibrio	*SHA-98*

Furthermore, it was found that the *Actinobacteria*-to-*Proteobacteria* ratio was significantly elevated in the guinea pigs with heart failure and could be reduced after VNS on both left and right vagus nerve. The results of this study appear interesting in context of the use of VNS in order to change the gut flora, but the authors rightfully notice that there is no certainty whether the alterations in the GI microbiome are a direct result of VNS rather than a result of an ameliorated heart function due to the VNS.

The possible effects of VNS on the gut microbiota have also been investigated in mice with ALS (41). In this study, mice which were prone to the development of ALS – because they carried a mutated human superoxide dismutase 1 transgene – were subjected to one hour of VNS during a surgical procedure. One week after this intervention, faecal samples were collected and compared to the faecal samples before VNS and to those of a group that received sham-stimulation. No significant differences were found in either comparison. It must be noted that the test population was presymptomatic which means the mice did not yet have ALS. Different results might have been found if the disease was already exhibited when receiving VNS. After all, no significant differences in community richness and diversity were found between the ALS-prone and the wild type mice before VNS, so a ‘normalisation’ of the microbiome was theoretically impossible. A big disadvantage of this study is the fact that VNS

only lasted one hour and was unrepeated, which makes the results difficult to extrapolate to human epilepsy patients who receive VNS treatment for longer periods of time. Furthermore, the stimulation was performed on the right vagus nerve, whereas VNS as a treatment for epilepsy in humans usually occurs on the left vagus nerve.

5. Discussion

With the data currently available, the main question of this review – whether the therapeutic effect of VNS on epilepsy is mediated through the GI microbiome – cannot be fully elucidated. Based on the findings described in the reported studies, the hypothesis that the GI microbiome plays an important role in the pathogenesis and also the therapy of epilepsy, is supported. Several studies have shown that the microbiota of patients with epilepsy differ greatly from that of healthy controls (5, 19, 20, 32). The findings of the studies are divergent (table 4). These differences between the different study results may be due to the small study population, the different countries of residence, different age groups or the different methods (4). In order to avoid conflicting results caused by the methods used, a standardisation concerning the use of primers, databases, algorithms and other variables should be developed (21). This way, different studies could be compared with less risk of misinterpreting the results.

Table 4. Relative abundance of four microbial phylae in the microbiota of epileptic cases compared to healthy controls

Author	*Firmicutes*	*Bacteroidetes*	*Actinobacteria*	*Proteobacteria*
Safak et al. (5)	Decreased	Decreased	Decreased	Increased
Peng et al. (19)	Increased	Decreased	Increased	/
Lindefeldt et al. (20)	Decreased	Increased	Decreased	Increased
Xie et al. (32)	Increased	Decreased	Decreased	Increased

The dysbiosis of microbiota in patients with epilepsy can be potentially reduced with various interventions, of which the most researched is the KD (20, 32, 33). Again, compared with each other the individual results differ. In the study of Xie et al. the microbiome of the epilepsy patients shows a normalising trend towards the same composition as that of the healthy controls (32). Lindefeldt et al. on the other hand, reported significant changes in microbiota composition after KD in children with DRE, but these changes did not result in a microbiome composition similar to the control group (20). Whether or not the changes reported by Zhang et al. approach the gut microbiome of healthy controls is unknown seeing as they did not include such a control group in their study (33). What the results of these three authors do

have in common, is the fact that KD resulted in significant changes in microbiota composition in epilepsy patients as well as a decrease in seizure frequency in about half of the participants. In other words, KD is a therapy with a clear effect on both the severity of epilepsy and the composition of the microbiome. This double effect is no coincidence; as Olson et al. have shown, the anti-seizure effect of KD is mediated through the GI microbiome (25). The authors also rightfully notice that microbiota changes after KD likely depend on the host genetics and the original microbiome composition, which could explain the differences in the findings of Xie et al., Lindefeldt et al. and Zhang et al. Other explanations for these differences could again be the small cohorts, the different countries of residence, different age-groups and the different methods used.

Influencing the GI microbiome in order to ameliorate epilepsy has also been a suggested mechanism in studies researching the effects of antibiotics (35) and probiotics (37) on DRE in human patients. Both studies have shown a temporary reduction in seizure frequency during the therapy, but neither have sequenced the GI flora in order to evaluate the effect of the therapy on the microbiome. Therefore it is uncertain whether the beneficial effect of anti- and probiotics in these studies is a result of favourable changes in the microbiota composition. A similar study, but this time with sequencing of the microbiome, could be interesting to demonstrate this as yet.

The connection between the GI microbiome and the brain has already been investigated in certain neuropsychiatric disorders besides epilepsy, such as MS, PD, AD, ASD, ALS and depression (16, 21). Interestingly, transplanting the microbiota of patients with either MS, PD or ASD into mouse models resulted in the development of symptoms of these diseases. A similar experiment with epileptic donors might be interesting in order to evaluate the causality of microbial dysbiosis in the development of epilepsy. After all, results of the study of Medel-Matus et al. appear promising in this regard, seeing as their findings already strongly suggest the causal role of dysbiosis in chronic stress-induced epilepsy (27).

The role of the gut microbiome in above-mentioned diseases intuitively suggests that a link between the intestinal flora and epilepsy would not be far-fetched. Nevertheless, a few years ago Jackson et al. analysed the association between multiple diseases and gut microbiota from more than 2700 members of the TwinsUK cohort (42). They found a great number of associations with conditions such as IBD, diabetes mellitus type 2 and food allergies, but for epilepsy few associations were found. The authors suggested that their study was underpowered to detect any associations for epilepsy, and indeed there were few cases within the cohort who suffered from epilepsy. The number of individuals with IBD and coeliac disease

was also limited however, yet associations were found here. These findings appear disappointing in the scope of this review, but there are other, more hopeful results described that link the gut microbiota and the course of epilepsy. An interesting example is the case study of a 17 year old girl who was cured of her epilepsy after a FMT as treatment of Crohn's disease (28).

In summary, KD, anti- and probiotics and FMT all have a beneficial effect on the course of epilepsy and also impact the GI microbiota. This suggests that the gut-brain axis – the neuroanatomical and functional connection between the gut and the brain (24) – plays an important role in epilepsy, as it does in other neurological diseases. Since the vagus nerve is proven to be an important element of this gut-brain axis and since stimulation of this nerve is a known therapy for epilepsy, it would be interesting to investigate whether VNS cures epilepsy through interaction with the gut microbiota. The available literature on this subject however is very limited and the available studies show contradicting results; Phillips Campbell et al. reported a partial normalisation of the intestinal microflora after VNS in guinea pigs with pressure overload (40), whereas Haney et al. could not find a significant change at all in mouse models of ALS (41). In the latter study however, VNS only lasted one hour. The authors themselves hypothesise that chronic stimulation of the vagus nerve would likely have more impact on the microbiome composition.

It would be interesting to see a study that investigates the effect of VNS on the GI microbiota in epilepsy, as to this day no such study exists. In the case of such a study, the option may be made to aim the direction of the stimulation towards the GI tract instead of the brain. It is assumed that the frequency of stimulation used in VNS can be exploited in order to specifically stimulate afferent or efferent fibres (12). High frequency stimulation (20-30 Hz) which is usually applied in the therapy of epilepsy, activates mainly afferent nerve fibres, whereas efferent fibres are believed to be activated by low frequency stimulation (1-10 Hz). The reviewed studies investigating the effect of VNS on the GI microbiota composition both use a stimulation frequency of 20 Hz (40, 41) which, according to this theory, primarily stimulates the vagus nerve in a upstream manner. Furthermore, classic VNS typically aims to primarily direct the current of the stimulation to the brain by placing the anode of the device distally on the vagus nerve. This configuration was explicitly described in the study of Haney et al. (41). Perhaps switching the position of the electrodes and consequently reversing the direction of the current in the vagus nerve could lead to a more significant effect on the gut microbiota in studies aiming to investigate the impact of VNS on the GI tract. However, when deliberately stimulating the efferent fibres of the vagus nerve – albeit by adjusting the frequency or by switching the

position of the electrodes – one must be aware that adverse effects in the other internal organs innervated by the vagus nerve may occur more frequently.

As promising as the discussed results sound, several arguments can be put forward to reject the hypothesis that the effect of VNS on epilepsy is mediated through the GI microbiota. For example, many studies suggest that the mechanisms of the anti-convulsive effect of VNS is for the greatest part attributed to the stimulation of afferent vagal fibres (13). Physically or chemically disrupting the vagus nerve just below the implanted VNS device did not alter the effect of the therapy on epilepsy, showing the importance of the upstream pathway rather than downstream stimulation. A similar conclusion can be drawn from an experiment of Zanchetti et al. where the effect on cortical activity of VNS in an *encéphale isolé* cat could not be seen after disruption of the vagus nerve proximal of the stimulation site (12). Furthermore, the therapeutic effect of VNS has been shown to occur in less than five seconds after stimulation, which intuitively seems a too short stimulation period to substantially alter the gut microbiota composition. However, the efficacy increases after long-term use, which could suggest multiple mechanisms resulting in a cumulative anti-seizure effect. Therefore, investigating whether the alteration of the microbiome composition is a complementary pathway together with the central effects of VNS could be an interesting field of research.

A final remark about this review an most studies mentioned herein is the fact that the focus mainly lays on the bacteria in the GI tract. There are however countless other organisms that can play their part in the gut-brain axis, such as viruses, fungi and archaea. Adjusting the methods used in studies sequencing faecal samples in such a manner that all these organisms are taken into account may be recommended when a broader, more complete overview is desired.

6. Conclusion

This review tried to highlight the increasing appreciation of the GI microbiome in the pathogenesis of neuropsychiatric disorders. More and more research on this topic is conducted each year, but the studies investigating the role of the microbiota in epilepsy are scarce. The majority of these studies deal with the effect of KD on the microbiome composition, but only in a few cases these changes are functionally linked to the amelioration of this disease.

Several independent studies have reported a dysbiosis in patients with epilepsy. Together with the fact that the gut-brain axis forms a well-described anatomical and functional connection between the GI tract in which this dysbiosis occurs and the brain in which the epilepsy occurs, a connection between the two disease states can be suspected. Therefore, the gut-brain axis

could be an interesting therapeutic target in the treatment of epilepsy. The vagus nerve is one of the most important elements of the gut-brain axis, and its stimulation is a generally accepted therapy for epilepsy. Mechanisms of VNS are not fully understood, but multiple pathways probably work complementary with each other. One such mechanism might be the improvement of the GI dysbiosis. Sadly, no studies as of yet have investigated this possibility. A better understanding of the effect of VNS on the gut microbiome, and of the microbiota composition on neuropsychiatric health, could lead to new insights into the physiology and pathogenesis of epilepsy. This could in turn result in more specific and effective therapy for DRE.

Multiple studies have yet to be conducted in order to prove the causality of dysbiosis in epilepsy and to investigate the possible effect of VNS on the GI microbiota. Bigger cohorts and standardized methods are recommended to make results more reliable and easier to compare.

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Appendix

Table 1. Search strategy for the MEDLINE database consulted via Pubmed

Research question: Does vagus nerve stimulation in epilepsy have an impact on the gut microbiome?

Concept	Search strategy
Concept 1: 'epilepsy'	"Epilepsy" [MeSH] OR Epileps* [TIAB] OR Epilept* [TIAB] OR "Seizures" [MeSH] OR Seizure* [TIAB] OR Aura [TIAB] OR Aurae [TIAB]
Concept 2: 'vagus nerve stimulation'	"Vagus Nerve" [MeSH] OR Vagus* [TIAB] OR Vagal* [TIAB] OR Tenth Cranial Nerve* [TIAB] OR "Cranial Nerve X" [TIAB] OR Pneumogastric Nerve* [TIAB] OR "Electric Stimulation Therapy" [MeSH] OR Electric Stimulation Therap* [TIAB] OR Nerve Stimulation* [TIAB] OR VNS [TIAB] OR tVNS [TIAB] OR Neurostimulation* [TIAB] OR Electric Stimulation* [TIAB]
Concept 3: 'gut microbiome'	"Gastrointestinal Microbiome"[Mesh] OR Microbiom* [TIAB] OR Microbiot* [TIAB] OR Microflor* [TIAB] OR Gastrointestinal Flor* [TIAB] OR Gut Flor* [TIAB] OR Gastrointestinal Microbial Communit* [TIAB] OR Enteric Bacteri* [TIAB] OR "Gut-Brain" [TIAB] OR "Brain-Gut" [TIAB] OR "Intestines/microbiology" [MeSH] OR "Feces/microbiology"[MeSH] OR "Germ-free life" [MeSH] OR "Germ-free" [TIAB]

Table 2. Search strategy for Embase

Research question: Does vagus nerve stimulation in epilepsy have an impact on the gut microbiome?

Concept	Search strategy
Concept 1: 'epilepsy'	'epilepsy'/exp OR (epileps* OR epilept*):ti,ab,kw OR 'seizure'/exp OR (seizure* OR 'comitial disease' OR 'falling sickness'):ti,ab,kw
Concept 2: 'vagus nerve stimulation'	'vagus nerve'/exp OR (vagus* OR vagal* OR vagi OR 'tenth cranial nerve*' OR 'cranial nerve X' OR 'cranial nerves X' OR 'pneumogastric nerve*'):ti,ab,kw OR 'nerve stimulation'/exp OR ('nerve stimulation*' OR neurostimulation* OR 'electric stimulation*'):ti,ab,kw OR 'implanted vagus nerve stimulator'/exp OR (VNS OR tVNS OR T-VNS):ti,ab,kw
Concept 3: 'gut microbiome'	'intestinal flora'/exp OR 'gut microbiome'/exp OR ((intestin* OR gastrointestin* OR enteric OR gut) NEAR/2 (microbiom* OR microbiot* OR microflor* OR bacteria*)):ti,ab,kw OR (microbiom* OR microbiot* OR microflor*):ti,ab,kw OR 'gut brain axis'/exp OR ('gut brain' OR 'brain gut'):ti,ab,kw

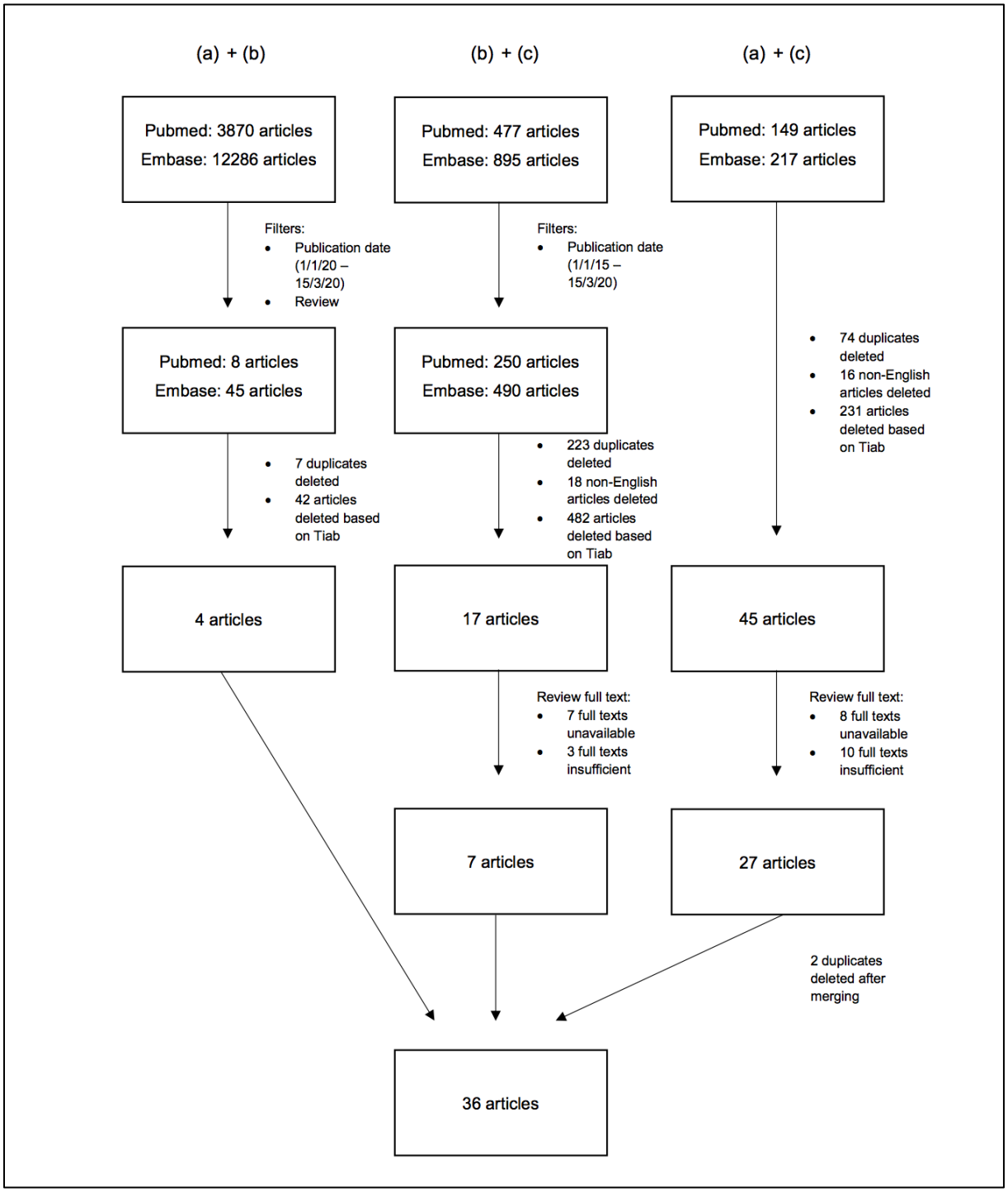

Figure 1: flowchart of the method used to select articles found by the search strategy

(a) epilepsy; (b) vagus nerve stimulation; (c) gut microbiome.