The Gut-Microbiota-Brain axis

..and its effect on Autism Spectrum Disorders

Bachelor thesis

Simon Winkel

9\textsuperscript{th} of July 2017

Supervisor

dr. R. Havekes
I. Abstract

In this review I will provide insight into the existence of the gut-microbiota-brain axis, connecting the gut, the microbiota, and the brain with one another and focus on microbiota’s prevalence in autism spectrum disorders. I will try to clarify the possible underlying mechanism resulting in autism spectrum disorders in a microbial dependent and independent way.

Humans have been a host for microorganisms for thousands of years in which both the host and the microorganisms benefit. This is called a mutualistic symbiosis which is shaped due to co-evolution and has been of major importance ever since. Disruption of this delicate interplay between the host and the microorganisms in its gut, called microbiota, may result in inflammatory bowel diseases or even neurologic disorders. Autism spectrum disorders generally known as a neurodevelopmental disorder, have been associated lately with this microbial imbalance. Microbial imbalance, dysbiosis, was known to result in intestinal diseases like inflammatory bowel disease but not in neurologic disorders. This interesting finding resulted in the hypothesis in which they described a mechanism connecting the gut with the brain and vice versa leading to this unexpected outcome. They called it the gut-microbiota-brain axis.

A study in patients, focussing on the effect of microbiota on phenotypical ASD characteristics, demonstrated microbial influence leading to a decrease in ASD characteristics when using antibiotics. This observation confirmed the gut-microbiota-brain axis hypothesis and its effect on ASDs. Follow-up studies showed differences between the microbiota of healthy controls and patients with ASDs. This study displayed a possible microbial origin of ASDs and led to the finding that the gut-microbiota-brain axis was influenced in a neuro- and enterotoxin dependent manner. This finding was not completely out of the blue as both the enteric nervous system and the brain use the same neurotransmitters and signalling molecules. However underlying mechanisms of how the gut-microbiota-brain axis functions remains unclear.

I furthermore tried to elicit the underlying origins resulting in ASDs via the gut-microbiota-brain axis. But the brain in general and the origin of ASDs are both not completely understood. Fully understanding ASDs on itself is therefore very difficult and connecting the origin of ASDs to the gut-microbiota-brain axis is even more difficult.

I will give a brief explanation of several different origins resulting in ASDs in a microbial dependent and independent way.
## II. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASDs</td>
<td>Autism spectrum disorders</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>ENS</td>
<td>Enteric nervous system</td>
</tr>
<tr>
<td>GMB-axis</td>
<td>Gut-microbiota-brain axis</td>
</tr>
<tr>
<td>HPA-axis</td>
<td>Hypothalamic pituitary adrenal axis</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
</tbody>
</table>
III. Table of Contents

I. Abstract
II. Abbreviations
III. Table of Contents

1. Introduction
   i. The gut and its microbiota
   ii. Microbiota and Autism Spectrum Disorders
   iii. The Gut-Microbiota-Brain Axis, the gut affects the brain and vice versa...
   iv. The brain and Autism Spectrum Disorders

2. Discussion

References

Appendix
Introduction

Nowadays, it is generally known that the microbiota in the gut have an effect on the host’ health, in a physical manner. However nobody could imagine that these local effects in the gut would reach as far as the brain and possibly even influence the host’ mentally state of mind... In this literature study I will focus on the gut, the microbiota, the (autistic) brain, and autism spectrum disorders (ASDs) which are disorders characterized by impaired social communicative skills, restrictive, and repetitive behaviours. I will furthermore give insight in the possible relationship between the gut, the microbiota, and the brain, called the gut-microbiota-brain axis (GMB-axis) and its effect on ASDs.

The gut and its microbiota

The gut (sometimes described as the gastrointestinal tract) of a mammal is a very important system; it is needed for the consumption, storage, and digestion of food. It is a hollow tube that belongs to the external environment of the body and ranges from mouth to anus all focused on the absorption of nutrients. However, it does not function all on its own. It is known for decades that mammals, and humans, are home to trillions of microorganisms that live in and all around our bodies. The term microorganism resembles a broad spectrum of different microbes: bacteria, archaea, protozoa, fungi and algae (and viruses). The bacteria are the most dominant form of microorganisms and trillions of these bacteria, called the microbiota, are present in our gut, this is the most dense colonized organ of the body. The microbiota exceeds the number of cells of the human body by tenfold and they fulfill for the host crucial parts in digestion, but also play a part in immune system development in the early stages of life. Usually, the microbiota and host live in harmony with each other in which the microbiota have co-evolved with their hosts and benefit from each other, this is called mutualistic symbiosis. This relationship could only be formed due to homeostasis; this term is used to describe the co-existence of the microbiota and the host’s immune system. In short, the hosts’ immune system basically recognizes self and non-self. This is a very crucial and important function because the body needs to be tolerant to body produced cells and proteins to avoid inappropriate immune responses but at the same time remain vigilant to protect the host against harmful invasive microorganisms. Due to co-evolving the immune system has learned to distinguish harmful microorganisms called pathogens from helpful microorganisms called commensals. Disruption of this delicate relationship between hosts’ immune system and the microbiota population in the gut is called dysbiosis and may lead to diseases, ranging from inflammatory bowel syndrome such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), but also metabolic diseases like obesity, allergic diseases like rhinitis and asthma, and even neurological disorders.
Microbiota and Autism Spectrum Disorders

The microbiota have been known to be important for the maintenance of good health, however studies have also shown that dysbiosis of these microbiota communities lead to chronic inflammatory intestinal disorders that comorbid with psychiatric disorders like ASDs. ASDs are neurodevelopmental disorders affecting three crucial areas of development characterized by several phenotypical appearances: impaired communicative skills, restricted social interaction, and creative or imaginative play. But, despite the fact that ASDs were known as heterogeneous neurodevelopmental disorders, many patients diagnosed with ASDs suffer from intestinal disorders such as diarrhoea, abdominal pain, constipation and gastroesophageal reflux. This has led to the hypothesis that microbiota might play a key role in the development of these ASDs. Studies focussing on the microbiota of patients with ASDs have shown interesting results of altered microbial communities when compared to healthy controls. The first evidence of the involvement of microbiota began after the publication of Ellen Bolte in 1998. This paper gave rise to the hypothesis that an infection with clostridia species might have an important role in ASDs and that treatment with the antibiotic vancomycin resulted in a reduction of her child’s autistic characteristics. This interesting result led to a follow-up study by Sandler, Finegold and Bolte et al. to determine it was not a non-recurring finding.

Short-term benefit from oral vancomycin treatment of regressive-onset autism

This study by Sandler, Finegold and Bolte, et al. was to test the following hypothesis: “Disruption of indigenous gut flora might promote colonization by one or more neurotoxin-producing bacteria, contributing, at least in part, to their autistic symptomatology”. Parents of children diagnosed with ASDs reported chronic diarrhoea, loss of language, play, and social skill followed by an onset of other autism related phenotypes after broad-spectrum antibiotic use. This parental observation led to the aforementioned hypothesis formulated by researchers Sandler, Finegold and Bolte. Repeated use of antibiotics might have led to disruption of the delicate interplay between commensals and the hosts’ immune system leading to an opportunity for more invasive pathogenic bacteria like the clostridia species which may colonize the intestinal niches. Eleven children (10 boys, 1 girl) meeting the study entry criteria (Shown in Appendix 1A) were enrolled in this study and treated with vancomycin. Children were videotaped at baseline (before treatment), during treatment and at follow-up (end of the treatment) and rated by a child psychologist using the Childhood Autism Rating Scale (Appendix 1B-C). Results of these ratings are shown in table 1.
Table 1: Summarized Results of the Childhood Autism Rating Scale of Short-Term Improvement

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age at Onset of Autism, months</th>
<th>Age at Initiation of Treatment, months</th>
<th>Painful Videotapes</th>
<th>Subjective Visual Analog Rating Scales</th>
<th>Communication*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>78</td>
<td>+</td>
<td>Baseline 1.5, Post-treatment 4.7, Baseline 1.7, Post-treatment 2.2</td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>16</td>
<td>61</td>
<td>+</td>
<td>Baseline 5.2, Post-treatment 5.6, Baseline 5.1, Post-treatment 5.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>43</td>
<td>0</td>
<td>Baseline 1.9, Post-treatment 2.1, Baseline 1.8, Post-treatment 2.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>57</td>
<td>Not available</td>
<td>Baseline 1.5, Post-treatment 3.3, Baseline 1.4, Post-treatment 1.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>47</td>
<td>+</td>
<td>Baseline 2.3, Post-treatment 4.6, Baseline 1.2, Post-treatment 4.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>70</td>
<td>0</td>
<td>Baseline 2.2, Post-treatment 6.0, Baseline 1.7, Post-treatment 5.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>84</td>
<td>+</td>
<td>Baseline 2.4, Post-treatment 5.0, Baseline 2.0, Post-treatment 6.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>63</td>
<td>+</td>
<td>Baseline 2.3, Post-treatment 7.7, Baseline 1.7, Post-treatment 5.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>44</td>
<td>0</td>
<td>Baseline 1.4, Post-treatment 6.7, Baseline 1.6, Post-treatment 8.2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>56</td>
<td>#</td>
<td>Baseline 2.2, Post-treatment 5.3, Baseline 2.2, Post-treatment 2.8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>18</td>
<td>56</td>
<td>0</td>
<td>Baseline 2.5, Post-treatment 6.5, Baseline 2.8, Post-treatment 6.6</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.7 (±3.4)</td>
<td>59.3 (±13.3)</td>
<td>Median</td>
<td>Baseline 2.2(1.5, 2.4), Post-treatment 5.0(3.3, 8.0), Baseline 1.7(1.4, 2.2), Post-treatment 4.6(2.2, 5.3)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Summarized Results of the Childhood Autism Rating Scale of Short-Term Improvement. The 11 subjects were rated by the Childhood Autism Rating Scale by a child psychologist at baseline, during treatment and at follow-up. *Behaviour was measured by rating the subjects’ compliance to request, mood, eye contact, attentiveness, activity level, and severity of stereotype ASD phenotypes. *Communication was measured by receptive and expressive language. Both behaviour and communication were measured on a scale between 1 = "worst" and 10 = "normal".9

Treatment with the antibiotic vancomycin gave rise to an overall improvement of the test subjects’ ASD phenotypes, both behaviour and communicative skills did improve during- and post-treatment. However, even though these improvements were very clear the improvement in behaviour and communicative skills did not persist. Children were tested 2 to 8 months after vancomycin treatment in the follow-up study and in all, except 1 child, ratings dropped to baseline; this is shown in figure 1 below. Permanent treatment of children with ASD might therefore be necessary to maintain the beneficial effects. Scores of the communicative skills showed similar patterns and are therefore not shown9.

Figure 1: Scale Measurement of Change in the Children’s ASD behaviour

Figure 1: Scale Measurement of Change in the Children’s ASD behaviour. This figure shows the scores of each of the eleven children at the baseline, during treatment and in the follow-up study. Positive scores on the y-axis implies improvement, negative scores implies deterioration of the children’s behaviours9.
The recurring ASD phenotypes of these children might be due to spore-forming bacteria like *Clostridia* that survive antibiotic use and are able to invade the intestinal niches once again when the antibiotic-cure was stopped. In conclusion, these results demonstrate the influence of microbiota on ASDs and led to a following study of Finegold SM et al. focussing on *Clostridia* species in the faecal flora of autistic children.

**Gastrointestinal Microflora Studies in Late-Onset Autism**

Finegold SM et al. studied the faecal flora of children with autism and healthy children as controls and found significant alterations in the gut microbiota of children with ASDs compared to the healthy controls. The aim of this study was to hopefully determine ≥ 1 species exclusively found in the microbiota of autistic children. Stool and gastric- and small-bowel specimen samples were collected and analysed with 16s rRNA gene sequencing. This gave rise to 9 *Clostridia* species found only in children with autism and this difference in microbiota may provide insight into the neurologic disorder autism. These papers, by Bolte, Sandler, Finegold and Bolte et al. and by Finegold et al. suggest that an altered microbiota can be linked to ASDs. Furthermore, they focussed on *Clostridia* species known to produce an entero- and a neurotoxin which gave rise to the hypothesis that release of (neuro-) toxins by microbiota may affect the brain and thus the presence of a more difficult underlying mechanism called the GMB-axis which will be described in more detail in the next paragraphs.

**The Gut-Microbiota-Brain Axis, the gut affects the brain and vice versa...**

It is generally known that our brain controls nearly everything we do. However it does not function all on its own, it is part of our central nervous system (CNS) which responds in a voluntary and involuntary way. It is therefore not surprising that our CNS is involved in the modulation of various gut functions; motility, secretion, blood flow, and gut-associated immune functions in response to psychological and physical stressors. The communication from the CNS to the gut is regulated via the autonomic nervous system (parasympathetic and sympathetic).

Gut to CNS signalling has been studied very broadly. However, this interaction between the gut and CNS was misunderstood for a long period of time; a breakthrough in understanding arose in the nineteenth century with the discovery of the enteric nervous system (ENS). The ENS has been introduced as the ‘second brain’ due to its enormous amount of nerves, complexity and similarity. In fact, the total enteric neurons in humans is approximately 400-600 million, which is even more than the total of all sympathetic and parasympathetic ganglia combined and equal to the number of neurons in the spinal cord. Furthermore, the ENS and the brain use similar neurotransmitters and signalling molecules.

The size and complexity of the ENS is not remarkable knowing that the ENS is exposed to a major challenge. It communicates closely with our largest body surface, the intestine, which inhabits the highest number of microbes, and with the gut-associated immune system which contains the majority of our body’s immune cells and lastly with the thousands of enteroendocrine cells which produce more than 20 known hormones. This interaction between the gut-microbiota and brain via neural, endocrine, immune and humoral signals is called the GMB-axis and is thought to be bi-
directional. The autonomic system with sympathetic and parasympathetic branches is involved in neural-signalling from the gut through enteric, spinal and vagal pathways to the CNS and the other way around from CNS to the gut\textsuperscript{14}.

Hormonal interaction between the gut and the CNS is provoked by environmental stress and pro-inflammatory substances that activate the hypothalamic pituitary adrenal (HPA) axis. This HPA-axis is part of the limbic system which is predominantly involved in memory and emotional responses and up-on activation, due to aforementioned origins; corticotrophin will be released leading to the production of the stress hormone cortisol. Subsequently, cortisol affects many organs and also the brain. Both neural and hormonal pathways are used by the CNS to influence the gut, and the gut uses these pathways in a similar way to influence the CNS\textsuperscript{16}.

Residential microbiota are also known to affect the gut, so therefore the microbiota is also involved in this bi-directional interplay. These findings suggest that this complex system does not only account for maintenance of gut homeostasis but is thought to have far more effects on for instance higher cognitive brain functions\textsuperscript{14}. The most fascinating (clinical) effect of microbiota on the higher cognitive brain functions was the finding that orally administered antibiotics could reverse encephalopathy in patients with decompensated liver diseases\textsuperscript{15}. Encephalopathy represents a broad spectrum of neuropsychological dysfunction in patients suffering from acute / chronic liver diseases which might ultimately lead to death. Even though the pathology is complex and thus not fully understood, it is known that the disease involves the overproduction of ammonia as gut-derived neurotoxin produced by microbiota\textsuperscript{16}. This finding furthermore confirms the suspected impact of microbiota on neuropsychological disorders as for instance ASD.

**The brain and Autism Spectrum Disorders**

Autism is a neurodevelopmental disorder and first characterized by Leo Kanner, who published his article in 1943 and described the disorder as an innate inability to create normal, emotional contact with others\textsuperscript{18}. Nowadays, diagnosis of autism hasn’t been changed much, it is still described as a disorder characterized by impaired social communicative skills, restrictive, and repetitive behaviours\textsuperscript{19}. Diagnosis of autism and related disorders are nowadays unified as one, called the autism spectrum disorder, ASD. This new name gave rise to two new meeting criteria which are used by the American Psychiatric Association for its diagnosis: 1) persistent deficits in social communication and social interaction (2) restricted, repetitive patterns of behaviour, interests, or activities\textsuperscript{19}. Even though, the number of people diagnosed with ASD rises, it is still poorly understood\textsuperscript{20}. It is thought that ASDs are highly heritable and thus a genetic disease; however interaction with environmental factors has not been excluded\textsuperscript{21}. The brain regions thought to be involved in ASDs were discovered through experimental animal studies or lesion studies in human patients and will be discussed later in more detail.

Having knowledge of the brain is needed to understand the regions which are thought to be crucial in figuring out the origin of ASDs. The brain consists of the cerebrum, the cerebellum and the brainstem, all of these together with the spinal cord is called the central nervous system. However, this literature study is focussed on the largest part of the brain: the cerebrum. The cerebrum is
divided in four different regions (see figure 2): 1) the frontal lobe, 2) the parietal lobe, 3) the occipital lobe and 4) the temporal lobe.

**Figure 2: The four different regions of the brain, and area’s involved in language and social behaviour**

Studies on ASDs have focussed on Magnetic Resonance Imaging (MRI) for neuroimaging to study abnormalities and pinpointed differences in total brain volume\(^2\), and disturbances in growth and maturation of the brain\(^3\). Lange, N et al observed an increased total brain volume growth in the early stages of life (~2-4 years old) in children with ASDs. However, this change in total brain volume declines over time and the pathological mechanism is unknown\(^4\). Further studies done by Amaral DG et al. summarized possible brain regions that are most impacted by the core features of ASD. The frontal lobe, the amygdala, Broca's area and Wernicke's area are one of the brain regions summarized by Amaral DG et al. and are used in this review.

**Behaviour**

The frontal lobe and the amygdala are one of the regions in which social behaviour is regulated. Regions involved in language functions are the Broca’s area and the Wernicke’s area essential for alertness and the processing of language and social attention\(^5\). These brain regions are selected because they are thought to be most impacted in the disorder leading to the ASDs characteristics.

The frontal lobes’ major function is motor action and temporal integration of behaviour\(^6\). Injury to the frontal lobe, might lead to reduced intensity of emotion. Patients suffering from this kind of trauma can no longer see consequences in things they have said or done\(^7\). Disorders or injury in this region of the brain are known to influence the behaviour of the patient and are therefore chosen for a pivotal role in ASDs, as patients with ASDs behave in a different manner than “normal”.

The first clinical known evidence of brain injury was the penetrating wound of an iron bar straight through the prefrontal cortex of – the now famous – patient Phineas Cage in 1848. He survived his injury but it did leave a mark on his mental state as behaviour changes developed. The mental changes Cage underwent after its accident were described as “fitful, irreverent and indulging at times in the grossest profanity”. Furthermore, friends and family said: “he was no longer Cage”\(^8\). This case
study was the beginning of the understanding of the pre-frontal cortex and demonstrates that disorders in the frontal lobe / prefrontal cortex lead to changes in behaviour and thus are thought to be involved in ASDs.

Nowadays, scientists use MRI to determine changes in brain regions between people suffering from ASDs compared to controls. This gave rise to the discovery of several involved brain regions; this literature study will focus on the amygdala, as core symptoms of ASDs are associated with amygdala dysfunction.

Two almond-shaped nuclei which are located within the temporal lobe called the amygdalae (see figure 2) are key structures for sensory information reception, processing, and assigning emotional significance. The amygdala is, just as the HPA-axis part of the limbic system and transfers this information to other effector regions and is involved in the so called “social brain” as it plays a crucial role in processing social signals as body motion, facial expression and eye gaze.

Schumann CM et al. performed a MRI study to better define the neuropathology of ASDs. The brains of male children between 7.5 and 18.5 years old with ASDs and age-matched controls were scanned and volumetric analysis was performed on the amygdala. This gave rise to a striking result, even though no differences were found in total cerebral volume of both groups and ages, the amygdala volume was increased in the younger group with ASDs (7.5-12.5 yo.) compared to the control group (see figure 3). However, the older group with adolescents (12.75-18.5 yo.) did not show any differences in amygdala volume when compared to the control group, data not shown.

Figure 3: The volumes in cubic centimetres of the left (A) and the right (B) amygdala of the younger patients (7.5-12.5 yo.) suffering from ASD compared to the age-matched control groups.
These results demonstrate an initially larger amygdala in children with ASDs as their amygdala reaches adult size at the age of ~8 years old but they do not undergo the age-related increase during adolescence which is seen in the typically developing age-related controls. This indicates a different development of the amygdala in children with ASD.

Another study performed by Schumann CM and Amaral DG focussed on quantitatively measurements of the number of neurons in the post-mortem amygdalae led to a major finding. The amygdala of 9 autistic males consisted of significant fewer neurons in the brains of children suffering from ASD when compared to 10 age-related controls. The outcome was unexpected as the greater volume of the amygdalae of young children with ASDs in the earlier mentioned study was thought to have a higher number of neurons due to higher levels of proliferation. This not being the case led to a hypothesis in which Schumann CM and Amaral DG formulated that the amygdala in young children has a normal or increased number of neurons and that the number of neurons declines later in time.

The amygdala is also involved in the detection of danger and the production of fear and anxiety. The amygdala’s response to fear is regulated with the hormones corticotrophin and cortisol leading to a stress response. The greater output of the amygdala the larger the stress response gets leading to anxiety. People suffering from ASDs are known to have a more active amygdala which leads to a higher stress response and thus anxiety, which is a feature of autism. A higher stress response in people suffering from ASDs could have harmful effects and thus leading to the loss of neurons. This hypothesis is not completely out of the blue as a similar finding has been done in studies focussing on depression, in which the amygdala is enlarged in the early stages of the disease and decreases after long term depression.

Communication

Nearby the frontal lobe is the expressive language area called the Broca’s area which is involved in the ‘production’ of speech. The region involved in the receptive language is called the Wernicke’s area and is located between the parietal and temporal lobe and allows us to understand spoken and written words. Between Broca’s and Wernicke’s area lays a bundle of nerve fibre which connects them with each other. This is an important link, before we can speak, words must be formed and assembled in Wernicke’s area and then send back to Broca’s area to produce sounds and thus the ‘production’ of speech. Any abnormalities in these areas might lead to language processing and social attention defects and thus the ASD phenotype. A study interested in this link between the Wernicke’s and Broca’s area by Just MA et al. examined the brain activity of 17 normal-developed controls and 17 autistic persons using MRI scans and discovered a difference in the distribution of activation in these two key language areas between autistic and normal-developed controls. More activation was found in the Wernicke’s area, and less activation in the Broca’s area of the autism group when compared to the control group. These results are shown in figure 4. Other brain regions did also show differences in activation but are disregarded in this review.
A possible interpretation of these findings might be that the increased activity of the Wernicke’s area is in line with an autistic persons’ strength in processing single words as they process each word of a sentence far more extensive than normal developed controls. Reduced activation in Broca’s area, which is involved in the “production” of speech, is coherent with their impaired ability to process the meaning of complex sentences.

Just MA et al. furthermore discovered differences in synchronization of the activation across cortical areas. Synchronization normally indicates collaboration between these functionally specialized areas, and is described as functional connectivity. Persons suffering from autism had significant lower functional connectivity between Broca’s and Wernicke’s area than the control-groups; these differences might provide a link in the theory of autism by Minshew et al. in which he describes the origin of autism as a disruption in complex information processing. These findings might suggest a loosened or underdeveloped connection between Broca’s and Wernicke’s area called “underconnectivity” as neural basis in the origin of autism.

Take note that, “to fully characterize the neural underpinnings of autism, it may be necessary to view it as a disorder of connections between brain regions rather than at the level of a single region.”
Discussion

This review gave insight in the existence of the gut-microbiota-brain axis, connecting the gut, the microbiota, and the brain with one another and focusses on microbiota’s prevalence in autism spectrum disorders.

Evolution led to collaboration between microbes and humans. This co-evolution led to major benefits for both and is therefore a mutualistic symbiosis. Disruption of this delicate interplay is named dysbiosis which may lead to many diseases. Many of these diseases are gut related, but also mental illnesses and neurological disorders affecting the brain have been attributed to dysbiosis. Interestingly, persons suffering from neurological disorders like ASDs are known to similarly have intestinal disorders. Despite the fact that ASDs have been known to be neurologic, studies show the influence of microbiota in ASDs, as changes in microbiota had an effect on a persons’ ASD-phenotype. This has led to the formation of a hypothesis which described the so called GMB-axis which is bi-directional and connects the gut with the brain and vice-versa. The effect of microbiota via this mechanism is known to be in a neuro- and enterotoxin dependent manner as both the ENS and the brain use the same neurotransmitters and signalling molecules. Clear evidence of the existence of the GMB-axis and its microbial influence on the brain came from human studies on encephalopathy. This first clinical study: “Current concepts in the pathophysiology and management of hepatic encephalopathy”\textsuperscript{16}, focussing on the effect of microbiota on the brain via this aforementioned GMB-axis, confirmed the hypothesis which was formed due to previously gathered information by animal studies.

I try to elicit the underlying origins resulting in ASDs via the GMB-axis. For a long period of time ASDs were seen as heritable and thus genetic neurological diseases. Lesions studies gave first insight in brain regions related to ASDs as they affected a person’s communicative-skills and behaviour. However, external influence in addition to genetics, for instance microbiota, is known to take part in a persons’ ASD as well. Despite having numerous of articles proving the existence of the GMB-axis and the microbiota affecting the brain resulting in ASD-phenotypes, the underlying mechanisms and pathways of both GMB-axis and the causes of ASDs stay unclear. This review does therefore not cover all origins of alterations in a patient’s brain leading to autistic phenotypes since this is still there to discover.

As described in this review, the antibiotic-usage to diminish growth of unhealthy microbes resulted in a response far beyond the local effects in the gut. The surprising results gave rise to the formation of several hypotheses in which the influence of microbes got a central role. This central role for microbes is, after reading this review, not special anymore. But, the discovery of the influential bacterial metabolic products on a human’s physical but in particular the mental state resulted in big question marks on the underlying mechanisms.

The gut-microbiota-brain axis is currently “the explanation” of the microbial effects on us as hosts; nevertheless the GMB-axis is not the answer to all our questions but is currently used to clarify the current findings. This GMB-axis is not simply a physical pathway comparable to the veins of the
circulatory system or the neurons of the CNS. However, it is acceptable to say that the GMB-axis uses these routes to reach their targets despite lacking a real physical appearance. Hormonal or neural messages using the GMB-axis are send via the circulatory system, or via neurons respectively.

The stress hormone cortisol, is a hormone worth mentioning. It is derived from corticotrophin which is the precursor of cortisol and is released from the HPA up-on activation by stress. Corticotrophin release is necessary to activate cortisol production in the adrenal gland and similarly involved in its release to the circulatory system to provoke a stress response. This stress response might be the cause of changed behaviour by having an effect on the social brain by influencing the amygdala, but the stress response is also thought to be the cause of a loss of neurons. Thus, stress might be a major cause to changes in some brain regions involved in social and communicative skills.

An extreme increase in cortisol production and release due to for instance microbial metabolites resulting in an immense stress response might therefore be able to affect the brain and give rise to social skill deficiencies due to the loss of neurons in the amygdala. Results proving that the loss of neurons in the amygdala led to a changed behaviour are discovered in studies on patients suffering from depression.

Furthermore, bacterial metabolites reaching as far as the brain, even across the blood-brain barrier resulting in neurological disorders are not imaginary. There is a bacteria species which produces ammonia that reaches the brain and is involved in the disease encephalopathy.

Neuronal degeneration due to the increase in bacterial metabolites affects more brain regions. The Wernicke’s and Brocka’s area for instance, which are involved in the production of speech and language processing. Both areas are connected with each other via a bundle of nerve fibres. Partial destruction of this bundle of neural fibres results in underconnectivity, which is a decreased connection between the areas. This lowered connection is demonstrated in patients with ASDs and is known to negatively affect the hosts’ communicative functions.

Changes in the state of the amygdala and the Wernicke’s and Brocka’s area’s neuronal bundle are possibly able to lead to (temporary) social skill deficiencies and underconnectivity. It is therefore not unlikely to develop a changed phenotype more towards ASDs.

It is important to be aware of, and to understand that the effects of the microbial metabolites on the mental state of mind are predominantly of a temporary origin since studies in patients with ASDs demonstrate temporary effects when changing the microbial community of the host. It is therefore not implausible to change the source, the microbiota, which is resulting in an increase or decrease of ASD phenotypes, to a more favourable state in a both physical as mental origin.

ASDs are in this fashion affected by microbes and their metabolites and thus connection between both is possible via the pathway or axis called the GMB-axis. However, the underlying mechanisms are still very unclear due to a lack of knowledge on the GMB-axis, the brain and neuronal disorders like ASD... How is it, for instance possible that people experiencing regular stress due to cortisol release from the adrenal gland do not change to a more ASD phenotype? Are these hormones different from the once produced by bacteria? Is there a specific enzyme attached to the bacterial produced hormone that is able to use the GMB-axis to guide it to the brain instead of using the
normal circulatory pathway in which hormones are released from the brain to the target organs? Furthermore, why are some brain regions affected and are others neglected?

The current approaches and techniques we have access to, but also the immense size of this subject simply do not result in the data needed to fully explain the underlying mechanisms of the GMB-axis, ASDs, and their effect on one another in this review. The GMB-axis and ASDs are still very difficult to fully understand despite having numerous of different articles written about them.

Even though we lack the knowledge to fully reveal and display the underlying working mechanism of the GMB-axis and the origin(s) of ASDs in this review, a hypothesis can be formulated; it seems that there are two different origins of Autism Spectrum Disorders: In a microbial dependent and a microbial independent manner.

The articles shown in this review give brief information about the existence of the GMB-axis and its effect via microbial produced metabolic products on ASDs. In my opinion, it is clear that the microbiota and its metabolic products affect the severity of a patients’ ASD-phenotype, but it is not yet possible to point out microbiota as an origin of ASDs. This is because ASDs are still known as neurological disorders. Despite having articles claiming that early life antibiotic use leading to disruption of the normal gut microbiota and thus dysbiosis is the origin ASD. We need to increase our general knowledge of the brain to better understand ASDs and its origin as both the brain and ASDs are still not fully understood. This will probably give new insights in all different kind of input the brain and the ENS receive from the body, and will lead to more understanding of the underlying mechanisms of the GMB-axis to discover its effect in several inflammatory as neurological diseases or disorders. Having more knowledge in this field of study might lead to the development of medicine to increase a persons’ daily quality of life by either fully healing, or to relieve symptoms, with for instance personalized microbiota transplantations.
References


Appendix

Appendix 1A  Study Entry Criteria
1. Meets diagnostic criteria for autism disorder (DSM-IV/299.00)
2. Other genetic and medical diagnoses have been adequately evaluated and ruled out
3. Definable rapid onset after 12 months of age
4. Antecedent antimicrobial use 2 months or less before autism symptom onset
5. Persistent loose stool history, with diarrhea onset before autism symptoms
6. Symptoms for 4 years or less
7. Child is between 2 and 8 years of age
8. No evidence of any significant medical problem that might complicate treatment, such as renal, cardiac, or pulmonary disease; severe anorexia; or chronic infections (eg, tuberculosis)
9. Clinically static for 3 months or more (no new neurologic, seizure, or other medications), with no elective changes during the study
10. No antimicrobial use for at least 2 months prior to entry into the study

Appendix 1B  Severe System for Videotapes

Observer Rating Analog Assessment Scales for Behavior, Communication, and Social Skills

Child's Name: ________________________________

If yes, in which tape does the child appear better?

Place mark on line where 10 = normal behavior and 0 = highly abnormal. Note "NR" on scale if not notable.

Tape number: ________________________________

GLOBAL IMPRESSION

BEHAVIOR SUBDOMAIN RATINGS

Global Behavior Rating

Perseveration

Noncompliance

Oppositional Behavior

Self-Stimulation Behavior

COMMUNICATION SUBDOMAIN RATINGS

Global Communication Rating

Expressive Language

Receptive Language

SOCIAL SKILLS SUBDOMAIN RATINGS

Global Social Skills Rating

Eye Contact

Approach Behavior

Play Skills

Appendix 1C  Physician Analog Rating Scales

Analog Assessment Scales for Behavior and Communication

Physician Rating

Date: ________________________________

Child's Name: ________________________________

Behavior: 1 5 10

Communication: 1 5 10

1 = Seemingly impossible, can't be any worse; 10 = Age appropriate
Behavior: Compliance to request, Mood (temper, outbursts, irritability), eye contact, attention, and alertness, Activity level, Interaction with others present in room, Stereotyped behavior (degree of severity).
Communication: Pronunciation, paralinguistic, Receptive language, Use of sign language and gestures (gestures/point gestures), Verbal language (prompted/spontaneous), Use of language (single words, two words together, sentences), Verbal perseveration.