

Diplomarbeit

**Evaluation of Psychosomatic Parameters during
the Intake of Probiotic Supplements in Bipolar
Affective Disorder**

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Preface

When I first considered the topic for my diploma thesis, I knew that it had to be in the field of psychiatry. My interest in this field was awakened after my first clinical traineeship at the Department of Psychiatry at the Medical University of Graz. I soon found out that Assoz. Prof.in Priv.-Doz.in Dr.in med. univ. et scient.med. Eva Reininghaus and her team were recruiting members and students for their studies. After a short time working as a staff member, I was recruited for the studies “ProbioBIP-one – Wirkung von Probiotika auf kognitive Funktionen bei Menschen mit bipolarer Störung in euthymer Stimmungslage” and “ProbioBIP-two - Wirkung von Probiotika auf kognitive Funktionen bei Menschen mit bipolarer Störung in depressiver Stimmungslage” and after I had collected sufficient data, I began my thesis. Already at early stages of my thesis I was fascinated by the interplay of the brain and the digestive tract, especially when I learned about all the abilities of the intestine. I was always interested in possible alternative therapies, and it was greatly compelling to have the opportunity to combine the interaction of brain and intestinal tract with probiotic supplements as an alternative therapy in my thesis.

At this point I would like to sincerely thank my two counselors Assoz. Prof.in Priv.-Doz.ⁱⁿ Dr.ⁱⁿ med. univ. et scient. med. Eva Reininghaus and Dr. med. univ. Armin Birner for their great support, especially for their time and efforts with counseling and helping me with my thesis. I am grateful for this opportunity and their trust in my capabilities, also for their warm inclusion in their team. I would also like to express my gratitude to the Medical University of Graz for the proper education. Furthermore, the whole research team at the Psychiatric Department ought to take credit for their work, especially to René Pilz, Lisa Wetzlmayr and Kerstin Klampfer, who supported me immensely.

My sincere thanks go to my parents and my grand-mother, who have supported me throughout my whole time at the university, especially during the tough times and many nervous break-downs (and thank you for the nagging, that I should carry on with my thesis – I needed it). Furthermore, I would like to thank Helena Keller for spending her time for reading and correcting my thesis. And, last but not least, I like to thank my fiancé David, for supporting me during the writing and formatting processes and in times where I’ve been about to throw my laptop out of the window, because nothing was working as expected - Thank you for being my punching bag, when I needed it!

Abstract

The microbiome can influence mood and interferes with the course of certain illnesses. Various studies have shown that the microbiome relates to psychiatric diseases, like Morbus Alzheimer and depression, but only a few, to the date, have investigated its relation to bipolar disorder.

To further elucidate the topic of microbiome and psychiatric diseases, the pilot study „Wirkung von Probiotika auf kognitive Funktionen bei Menschen mit bipolarer Störung in euthymer Stimmungslage“ was started at the Medical University of Graz under the supervision of Assoz. Prof.in Dr. Eva Reininghaus. This study seeks to explore the influence of probiotic supplements on mood, digestion and sleep. Based on this study, many examinations– including this thesis– were initiated in order to gain deeper knowledge about the relation between bipolar disorder and the microbiome.

As a result, this thesis shows that probiotic supplement treatments result in there are both subjective and quantitative, alterations in the quality of life of an individual diagnosed with bipolar disorder. RM-ANOVAS and IBM SPSS 22 were used to analyze the applied questionnaires.

Improvements of mood, digestion and sleep could be shown, including a significant reduction of manic and depressive symptoms. In detail, 56% (= 15 patients) noted an improvement in the digestion and 30% (= 8 patients) did experience an enhancement in their mood. A significant decrease of manic symptoms has been noted in the Mania Symptom Scale-questionnaire ($p=0.048$, $F(2,18)=3.621$), which was filled out by the probands as well as in the Young Mania Rating Scale-questionnaire ($p=0.023$, $F(2,17)=4.751$). Concerning the depressive symptoms, a significant decline was determined in the rumination subscale ($p=0.037$, $F(1,20)=3.988$) of the Leiden Index of Depression Sensitivity-Revised score between visit two and three.

The results of this pilot study suggest diverse effects of probiotic supplements on somatic and psychiatric symptoms in euthymic individuals with bipolar disorder and serves as an impetus for further research on this topic.

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List of Abbreviations

BD I = Bipolar Disorder I

BD II = Bipolar Disorder II

BDI-II = Beck-Depression Inventory II (Kammer, 1983)

BMI = Body Mass Index

CAMI = Critical Assessment of Metagenome Interpretation (Sczyrba, A., et al., 2017)

DSM-IV = Diagnostic and Statistical Manual of Mental Disorders

Eta² = eta square value

F = value of the F-test

GABA = Gamma-aminobutyric acid

HAM-D = Hamilton Depression Scale (Hamilton, 1960)

hsCRP = highly sensitive C-reactive Protein

IBS = Irritable Bowel Syndrome

ICD 10 = International Classification of Diseases

IL-6 = Interleukin 6

LcS = Lactobacillus casei strain Shirota

LEIDS-r = Leiden Index of Depression Sensitivity-Revised (Van der Does and Williams, 2003)

M = Means

MMSE = Mini-Mental State Examination (Folstein et al., 1975)

MSS = Mania Symptom Scale (Krüger et al., 1997)

P = Statistical Significance

SD = Standard Deviations

SNRI = Serotonin-Noradrenalin-Reuptake-Inhibitor

SSRI = Selective Serotonin-Reuptake-Inhibitor

UKU = Udvalg-for-Kliniske-Undersøgelser-side-effects-rating-scale (Lingjaerde et al., 1987)

WHOQOL = World Health Organisation Quality of Life (Angermeyer et al., 2000)

YMRS = Young Mania Rating Scale (Young et al., 1978)

Introduction

Bipolar disorder is an affective disorder and is characterized by the two opposing mood states of mania and depression (1). Due to these recurrent pathological mood alterations of the patient, the individual therapy can be challenging to find. According to the most recent research, a possible future treatment strategy could be the intake of probiotic supplements as their intake has already been associated with improvements of symptoms in illnesses such as dementia and depression (2, 3).

One of the newest studies of interest concerns the close bond between the abdominal brain and the brain. The discovery of this connection inaugurated a whole new range of therapeutic possibilities, especially when traditional medication cannot fully help.

The possible effects of such probiotic supplements on symptoms of bipolar disorder have not yet received their deserved attention. Therefore, at the Psychiatric Department of the Medical University in Graz, Austria, two pilot studies were performed to test the effects of probiotic supplements in probands with a diagnosis of bipolar disorder.

First, a general theoretical discourse on bipolar disorder is made in chapter 1, followed by a detailed description of the studies in chapter 2. The results are then shown in chapter 2.2, further discussed in chapter 3, and concluded in chapter 4.

1 Bipolar Disorder and the Abdominal Brain

1.1 Bipolar Disorder

1.1.1 Definition

Bipolar disorder is a mental illness which belongs to the affective disorders. It is characterized by two different mood states: depression and (hypo-) mania (1).

An affective disorder is an illness with extreme changes in mood, which are accompanied by disturbances in energy levels, psychomotricity, sleep, appetite as well as lust and interest in life (4).

According to the international classification of diseases (ICD-10), depression can be diagnosed if at least two of the three main symptoms (depressive mood, loss of interest or joy and loss of impetus) and at least one of the other symptoms (like loss of self-confidence, thoughts of being guilty, loss of concentration, psychomotor inhibition, sleep disorder, loss or gain of appetite and suicidal thoughts or actions) is prevalent. During a severe episode of depression, delusions like nihilistic ideas or hypochondria are possible (1). The symptoms of depression must last a minimum of two weeks, and misuse of drugs or medications – as well as organic disease – as a cause of these symptoms must be excluded (4).

In contrast, people experiencing an episode of mania are in high spirits or short-tempered for at least one week (4). The elevated mood state may not be concordant with the actual situation of the affected patients. Patients suffering from hypomania have increased energy and a reduced need for sleep. They tend to misjudge their feeling for distance to other people, are easy to distract, have many different ideas within a short time and overestimate themselves. Many of them spend a lot of money, more than they actually have. The patients have a lower urge to eat but an increase in sexual activity. Hypomania is a lighter and shorter form of mania where a patient has more control of their actions, whereas mania includes a loss of their grasp on reality. Mania can also lead to delusions of grandeur and even to criminal actions (1). Hypomania must endure for a minimum of four days while mania must endure for a minimum of one week (4).

Mixed episodes either combine symptoms of both depression and mania at the same time, or present with a rapid change of manic and depressive symptoms (4).

Bipolar disorder can also be divided into four groups (According to DSM-IV):

- 1) Bipolar-I-disorder: there is at least one manic and one depressive episode during lifetime
- 2) Bipolar-II-disorder: there is at least one hypomanic as well as one depressive episode during lifetime and there must not be any manic phase; it is often mistaken for an unipolar depression
- 3) recurring manic or hypomanic episodes
- 4) other bipolar disorders
 - a) Special forms of bipolar disorder include cyclothymia which is an instable mood change of melancholia and hypomania for at least two years
 - b) Rapid cycling is a course of disease where at least four different episodes occur within one year. It can occur in individuals with BD I as well as BD II (1, 4).

1.1.2 Course of Disease

Although most of the affective disorders have their onset between the ages of 30 and 40, in most cases of bipolar disorder the symptoms start much earlier, yet only 20% of patients are diagnosed before the age of twenty. Compared to unipolar disorders, individuals with bipolar disorder tend to have more and shorter episodes. Prevalence rates in men and women are similar (5).

It has been well-established that bipolar disorder is multifactorial. Twins research found an accordance rate of 80% of monozygotic twins compared to dizygotic twins (20%). Further proof of genetic influence is shown by the fact that if both parents are diagnosed with bipolar disorder, the risk that the child is affected is up to 50% (5). Some genetic regions which could influence the outburst of this disease are chromosome 18, 6q16-q22 or 22q11-q22, among others (6). Besides the genetic influence, somatic diseases or psychological strain like stress, divorce or abuse could lead to an outbreak of this disorder (5).

Although bipolar disorder is a chronic disease, its development can - with help of therapeutic and prophylactic medication - be influenced positively in many cases (7). One of the most important influencing factors is the compliance of the patient and the control of comorbidities, e.g. alcohol addiction. Alcohol abuse or addiction is common in about 60 to 70% (4) of the patients; misuse of cannabis, cocaine or anticonvulsants is also highly frequent. In such circumstances, the first priority of therapy is to treat the addiction, and the second is to treat the bipolar disorder. Other psychiatric comorbidities are anxiety disorders

(50% of the patients; mostly panic attacks, social phobia or agoraphobia), obsessive-compulsive disorders (about 20% of the patients) or personality disorders (about 50% of all patients with an affective disorder) (4). All of these comorbidities- including severe episodes of depression, impulsive or violent behaviour or loss of compliance- increase the risk of suicide. About 19% of the patients commit suicide (1). There is also a higher risk of somatic comorbidities like obesity, diabetes or metabolic syndrome caused by different factors such as genetics, as well as inflammatory pathways, medication or unhealthy diet and/or lack of sufficient exercise (5).

1.1.3 Therapy

Like the treatment of most of psychiatric diseases, the therapy for bipolar disorder is a combination of pharmacotherapy and psychotherapy (4).

The main goals of psychotherapy are: to lead the patient to a more structured conduct of life and to increase awareness of the first signs of depressive or manic episodes (so-called “warning symptoms”). If warning symptoms are noticed in time, strategies to prevent the episode can be applied. This form of psychotherapy, usually taught in psychoeducative group settings, is an effective and efficient approach (4).

For the pharmacotherapy there are two different methods that must be included: First, to start with a mood stabilisation therapy and second, to treat the acute symptoms of depression or mania if treatment with mood stabilizers does not sufficiently control these symptoms (1).

As bipolar disorder is a life-long disease, mood stabilizing treatment should be continued as a long-term therapy for years or even – in many cases – lifelong (1).

1.1.3.1 Mania

State-of-the-art therapy of non-psychotic mania includes the mood-stabilizing agents’ lithium, a few anticonvulsants (valproic acid, lamotrigine, carbamazepine or oxcarbazepine) and many atypical antipsychotics (aripiprazole, olanzapine, quetiapine, risperidone, ziprasidone). Haloperidol is a second-line option, but the risk-benefit analysis must always be kept in mind (7).

Generally, monotherapy is desirable. If a patient is already being treated with a mood-stabilizer, the most obvious option is to increase the dosage until the manic symptoms remit. Severe episodes, however, are immediately treated with an augmented therapy, usually a combination of mood-stabilizers and antipsychotics. Benzodiazepines are useful for treating

motoric enagements, but if there are any addictions in the patient's history, sedative antipsychotics might be preferable (7).

Due to the massive overconfidence of most patients in the state of mania, in-patient treatment is required in many cases. In case of uncooperative behaviour, the initial treatment is an injection of aripiprazole, olanzapine, haloperidol or zuclopenthixol into the patient's muscle. An additional sedation with benzodiazepines is often needed (1).

Lithium can be used to treat acute manic episodes, but under strict surveillance due to its narrow therapeutic range. If the level of the medication is above 1,2 mmol/l the risk of side effects is raised, and above 1,6 mmol/l symptoms of intoxication are possible. Other possibilities include valproic acid or carbamazepine, however in case of a lack of response to a monotherapy, a combination of lithium and valproic acid is a good option (7).

The choice of atypical neuroleptics is based on their respective side effects, especially when co-medication is used additionally (7).

If psychotic symptoms are prevalent during the manic episode, valproic acid shows better results than lithium. Therefore, in these cases an antipsychotic is commonly used, but an atypical antipsychotic is usually preferable due to its lower rate of extra pyramidal side effects (7).

1.1.3.2 Mixed episode and rapid cycling

Research has shown that individuals in a mixed episode respond better to valproic acid and carbamazepine compared to lithium. Atypical neuroleptics are also a good choice although only ziprasidone is licensed for mixed episodes. Antidepressants should be avoided (7).

While monotherapy is generally desirable, the course of rapid cycling usually requires additional treatment options. State-of-the-art treatment uses a combination of two mood stabilizers. In even more severe cases or lack of response to the usual treatment, it might be necessary to prescribe three mood stabilizers. In case of drug resistance, electroconvulsive therapy might be favoured (7).

1.1.3.3 Depression

During an acute phase of depression, antidepressants might be added to the existing mood stabilizing treatment. State-of-the-art antidepressant treatment includes selective serotonin reuptake inhibitor (SSRI), bupropion or moclobemide. Furthermore, atypical antipsychotics have shown antidepressant properties. Quetiapine can be used as monotherapy. Results have

shown (7) that tricyclic antidepressants are related to a higher risk of a switch into mania. If an antidepressant is used, it should at least be taken until remission of depressive symptoms. If withdrawn earlier, the possibility of recurrence rises (7).

Olanzapine (often combined with fluoxetine) and lamotrigine show good preventive effects in the treatment of bipolar-II-disorder. Nevertheless, data on the acute treatment of bipolar-II-disorder depression is scarce. Guidelines suggest therapy with quetiapine; second choices include lithium, lamotrigine, valproic acid, or combinations of these substances (1).

If the combined therapy of an antidepressant and a mood stabilizer does not show sufficient positive effects, a second mood stabilizer should be added. As the risk of suicide is high, electroconvulsive therapy should be kept in mind in case of the occurrence of drug resistance (7).

1.2 The Abdominal Brain

1.2.1 Definition

For many years, has been common knowledge that the brain is the most important organ in our body, while the alimentary tract is responsible for admission of food, digesting and depositing. Today the brain (with the sum of over 86 billion of neurons (8)) has still lost none of its importance (9). However, the intestinal tract beholds one of the greatest amounts of neurons, nearly as many as the spinal cord. It contains 400 till 600 million neurons and includes the same type of neurotransmitters and neurotransmitter receptors as the brain (although some of these neurotransmitters have opposite effects in the two different organs) (10). It also produces over 20 hormones like melatonin and serotonin - 95% of the endogenous serotonin is produced by the intestine - and contains the largest part of the immune system (8, 9).

The neuroplexus of the intestines can be divided into two parts (detailed drawing of structure can be seen in Figure 1): the plexus myentericus (or Auerbach's plexus) and the plexus submucosus (or Meissner's plexus). The plexus myentericus lies between the outer muscle layer and controls the muscles of the tract responsible for the transport of nutrition. The plexus submucosus lies near the inner mucous membrane and influences functions like the release of hormones and the uptake of water. The nervous system of the intestine contains three different types of receptors, each for different functions. It contains receptors for sensory perception (e.g. for pressure or chemical stimulus), interneurons for

interconnections, and motor neurons with inhibiting as well as stimulating impulses on the muscles. Together, the actions of these three receptor types influence blood circulation and immune system function. The intestinal tract contains four-fifths of all immune cells; as such, it is the largest immune organ of the whole body (10).

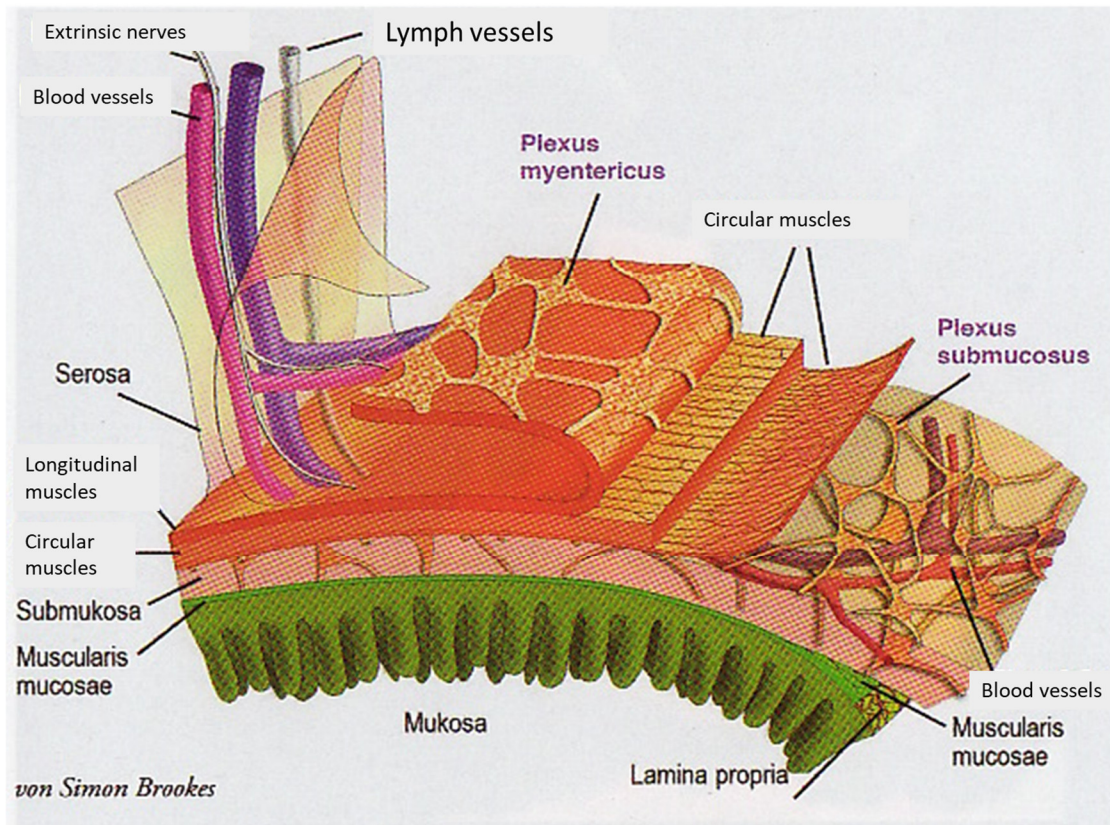


Figure 1 – The anatomy of the intestinal wall¹

Due to its large number of neurons, the bowel is the only organ which can work independently from the brain. When the entire tract is isolated, it retains the ability to ingest for days (see Figure 2). Studies have shown that if the intestinal tract of rats is separated from the body and brain, it still digests and defecates if it is contained in a nutrient fluid. And due to the peristaltic reflex, it digests always in the same direction (10).

¹ Source: See (7), page 80ff, image 6. (own translation) – in case of any publication besides the publication at the Medical University of Graz, the picture must be removed due copyright reasons (for further information see List of Figures

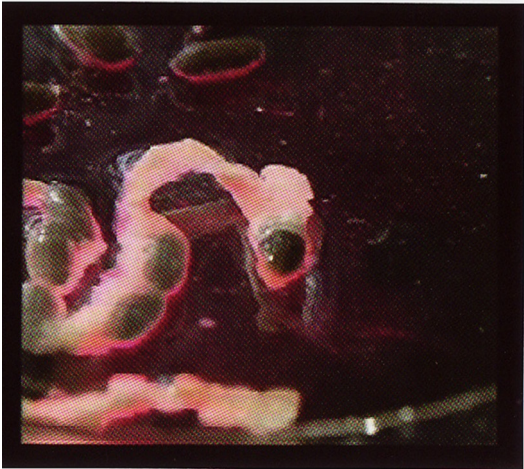
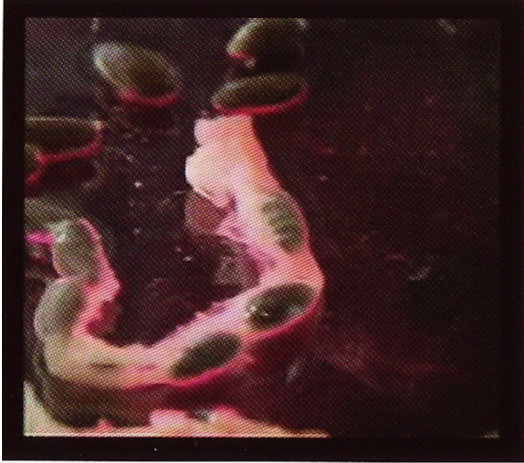


Figure 2 – Still image of the isolated- but still functional- digestive system²

In healthy conditions, the gut communicates with the brain by giving urgent information, which manifests as hunger, thirst, stress, or infections, but also by giving a positive message when everything is fine. This communication is possible due to the tight neural connection of the intestine and the brain, the immune cells produced by the gastrointestinal tract, and the neurotransmitters of the bowel, which can also influence mood (11).

This elaborate nervous system would be superfluous if the only function of our intestines were digestion. Our body maintains only what it needs, for example if someone does not use his or her muscles, they regress. If the gastrointestinal system can only digest, then a great part of this nervous system has no function and would break down. Since these complex nervous connections exist, it is reasonable to hypothesize that the alimentary tract has many more functions and duties than digestion alone (10).

² Source: See (7), page 80ff, image 7. – in case of any publication besides the publication at the Medical University of Graz, the picture must be removed due copyright reasons (for further information see List of Figures

The gut and brain are interlinked by three different nerves. The most famous nerve of this neural conjunction is the vagus nerve (10). The vagus nerve is the tenth cranial nerve, part of the parasympathetic nervous system and reaches down to the beginning of the colon (11). The second parasympathetic nerve is the nervus pelvicus and supplies the colon and anus. These two parasympathetic nerves react to physiological stimuli. The third nerve is the nervus splanchnicus, which is part of the sympathetic nervous system and is mostly activated by pathological stimuli like pain (10). Almost 90% of these tracts are afferent, which means that most of the time the bowel system sends the information to the brain. The brain only intervenes in case of danger. For example, the vomiting centre is only in the brain, so the feeling of nausea begins there, and emesis can be provoked solely by the brain (11). However, it does not mean that the brain gets the information of every eaten meal. The intestine only sends information to the brain if it seems to be important due to habituation and conditioning of its neural system. For instance, if a person eats an apple for the first time and this “event” is not associated with something unpleasant, the nervous system of the intestine can remember it when another apple is eaten again. It knows that an apple is not harmful and does not send this information – “an apple has been eaten again” - to the brain. So, the brain must not regulate the digestion, as long as the nervous system of the intestine can control it on its own. Furthermore, when the nervous system of the digestive tract sends information to the cortex, this information has to pass the thalamus, the “bouncer” of the brain (9).

Signals from the digestive system reach different regions of the brain, i.e. the limbic system, the insula, the prefrontal cortex, the anterior cingulate cortex and the hippocampus. These brain structures influence the characteristic behaviour of a person, are highly involved in the regulation of emotions, and are centrepieces of processes regarding morality, fear, memory and motivation. This connection could be one of the reasons that the intestinal tract can affect the mood (12).

Studies have shown (13) that the level of depression in mice can be shown by the length of time they swim in a basin which they cannot leave. Mice that have more depressive characteristics do not swim as long as their “healthy” mates, as they do not have the same urge to live. This is a common way to test if a new antidepressant is effective- by testing if it can lengthen the time the depressivemice are swimming (9,13). An Irish team of researchers (14) proved that a bacterium called *Lactobazillus rhamnosus* JB-1, which has a positive effect on the intestine, has a similar impact as antidepressants. Mice which were fed

with this bacterium not only swam for a longer time, they also had a lower level of stress related hormones in their blood compared to the control-fed mice (14). They even performed above their conspecifics in memory and learning tests. But when the nervus vagus was disconnected, there was no longer any difference between the control and experimental mice groups (14).

Due to the vagus nerve and the other two aforementioned neural conjunctions, the brain and the gut work together from birth on. They determine an enormous part of the basic feelings of an infant. Therefore, the infant is satisfied when it is well-fed, but can be devastated when flatulence occurs. When it grows older, it experiences the world by all senses; so, if the intestine is now bothered by flatulence, it only can interfere with the mood in a subtler way (9).

If the nervous system of the brain and the guts are compared with each other, the brain outnumbered the intestine by the number of neurons. However, both systems have the same structure. They contain the same nervous cells, the same neurotransmitters and the same other messenger materials, though they do not function the same way. The nervous system of the intestinal tract cannot communicate over longer pathways because it does not need to. Every ganglion of this system has its own inter-, motor-, and sensorneurons which can apply every action. For communication between all its different organs, the system possesses separated switch centres which are interlinked with the interneurons (for a microscopic image of the intestinal neural system, see Figure 3). The nervous cells of the brain, however, are ordered in specific functional units and are packed very tightly together. This allows for communication between different regions and organs, but leads to increased vulnerability of the brain compared to the intestine (10).

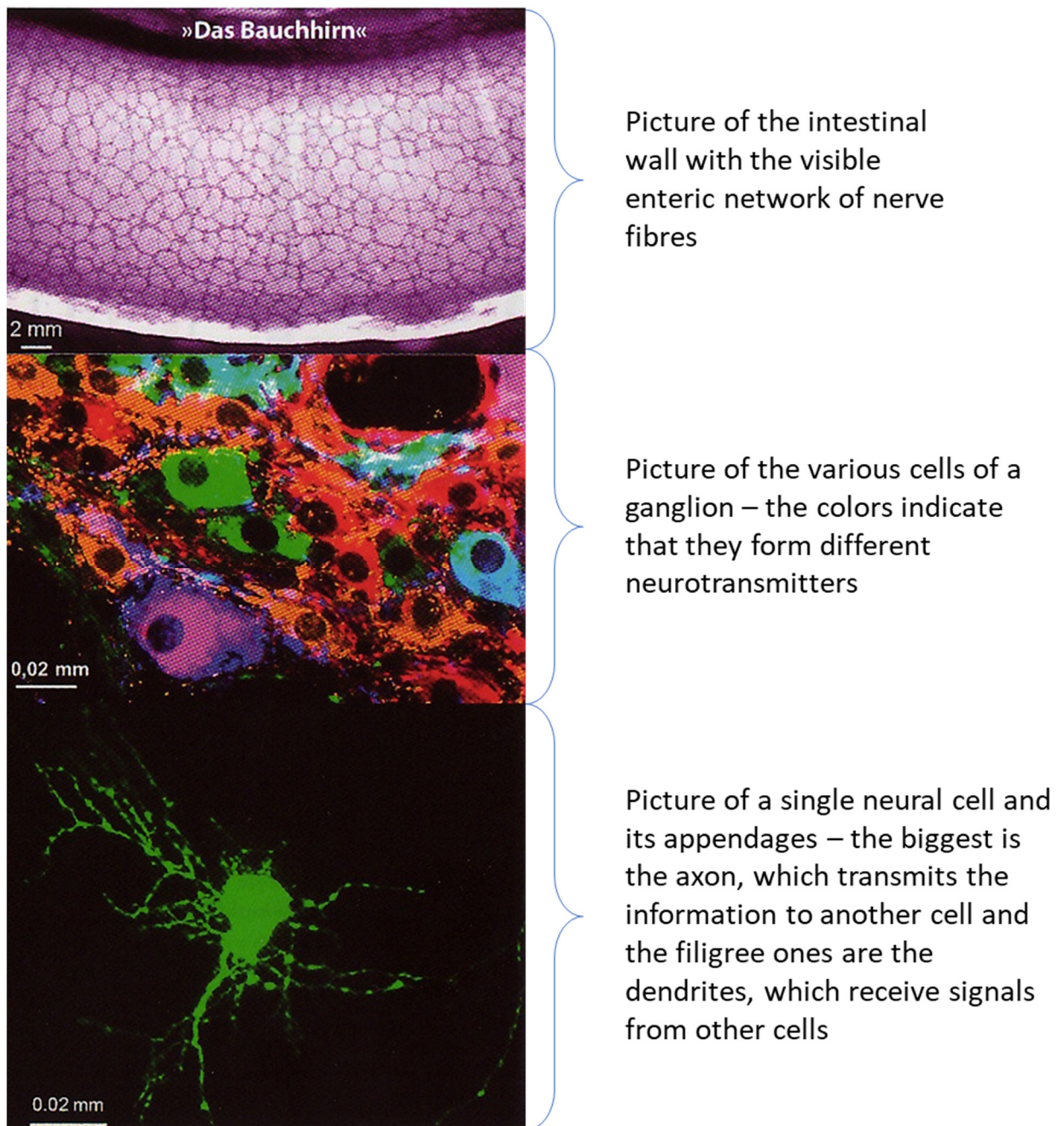


Figure 3 – “The abdominal brain (in German “das Bauchhirn”)” - microscopic images of the neural system of the intestine³

With all its different functions, the intestinal tract is often called “the second brain” or “the abdominal brain”. One of the most important questions is if the abdominal brain is able to learn. One part of learning is reacting to a stimulus; this is done by activating or inhibiting

³ Source: See (7), page 80ff, image 4. (own layout design and translation) – in case of any publication besides the publication at the Medical University of Graz, the picture must be removed due copyright reasons (for further information see List of Figures

specific neurons. The main stimuli of the digestive system are the dilation of the intestinal wall and the tangency of the mucosa. Terence Smith (15), a neurogastroenterologist, proved that these reflexes provoke the peristaltic reflex. The abdominal brain can also habituate or potentiate its reaction to a certain stimulus. It even has a long-time memory, which could explain the origin of effects such as irritable bowel syndrome (IBS); some IBS patients continue to suffer the same symptoms after the disease itself has subsided, due to the long duration hyperexcitability of the affected nerves. Classical conditioning is also possible. For example, chemotherapy patients might associate foods eaten shortly before the treatment with the side effect nausea and react to it with emesis upon eating it again, even if chemotherapy has already finished (10).

The intestine can not only learn and remember certain reactions in association with nutrition, it also is influenced by different mood states, as well as stress. Newest research has shown that the influence of stress on the abdominal brain is completely independent from the brain. A study on this subject showed the following results (16): rat babies were separated from their mothers until day fourteen after their birth. The outcome of this study proved that this stressor triggered an enormous enhancement in the permeability of the colon and bacterial colonisation in the mesenteric lymph nodes, spleen and liver. These transformations even led to macroscopic damages of the intestine (16). Another research team at the University of Amsterdam (17) showed that breast-fed baby rats which were separated from their mother for one hour daily- a huge stress moment for them - still suffer from a low stress tolerance up to effects as extreme as irritable bowel syndrome as adults. But most importantly: this low stress tolerance was transmitted over generations (17).

Further evidence for stress transmission has been given by an Irish team of scientists about maternal stress (18). In a rat model they tested the outcome of late gestational stress in the offspring. Prenatal stress can affect many physiological systems and the microbiome of the intestine, which will be mentioned later, e.g. the hypothalamic-pituitary-adrenal stress axis is moved to a hyperactive state. This impact may lead to further organic dysregulations of the offspring, including neurodevelopmental disorders such as attention deficit hyperactivity disorder and autism spectrum disorder (18).

Stress affects the digestive system in two different ways. The better-known reaction is bowel movement paralysis caused by the neurotransmitter norepinephrine. This reaction is triggered by a short-duration high stress level, e.g. when an animal or human is hunted by another. In contrast, if a longer period of intensive stress occurs, the stress hormone

corticotropine releasing factor (CRF) is secreted. CRF stimulates, among others, the immune system of the intestine and the abdominal brain, which activates the movements and secretions of the gastrointestinal tract in a stronger way than the inhabitation of the sympathetic nervous system. The resolution of this reaction is mostly diarrhea (10). This mechanism was proven by the gastroenterologist Thomas P. Almy (19) who lived during the middle of the last century. He told a healthy young man who was undergoing a colonoscopy that they had found a tumor by this inspection. During the following 20 minutes, this information led to a stronger blood circulation, secretion and motility of the patient's intestinal system (19).

Stress is one of the most important stimuli, as it results in the abdominal brain and the central nervous system working together. During a period of stress, the brain needs more energy. Therefore, the intestinal system decreases its functions and duties to provide the requisite energy. However, this is not a suitable solution for long term periods of stress. The results are mostly fatigue, loss of appetite, or diarrhea. That reaction leads to the question of whether the digestive system can help to reduce stress levels. The answer is not clear to date, but in this new field of research the latest results (13, 20) have shown that it is possible to lower the symptoms of stress by increasing tolerance against it and by the effective use of different coping mechanisms. These possibilities are feasible largely due to another part of the abdominal brain: The microbiome or microbiota (9).

1.2.2 The Microbiome

The microbiome is the sum of all genes of the bacteria in the human body, which is approximately 150 times bigger than the whole human genome. Every person holds roughly 100 billion bacteria-most of it in the colon- totaling around two kilograms. 90% of the whole "gut population" are bacteria. They can be divided into more than twenty different strains, but most of the bacteria in the intestine can be classified into these five tribes: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia*. These tribes can be divided into many different classifications, and these classifications into even more various families. Within each family, the bacteria are similar in their function. They serve a variety of purposes, such as subdividing indigestible food, providing energy for the intestine, producing vitamins, depleting medication or toxins, among many other functions (9).

This microbiome differs in species and numbers from person to person; even monozygotic twins have a different microbiota (9). In fact, there are variations over time as well: the same person will have a different microbiota as a baby versus at 3-year old - the age at which the

microbiota reaches an adult state (21). A newborn baby has a microbiota which is temporary dominated by *Enterobacteriaceae* and *Staphylococcus*, but as an older infant the major part of the gut bacteria is mostly *Bifidobacterium* and some lactic acid bacteria (22) like *Lactobacillus* or *Pediococcus* (23). This specific microbiota will change again after weaning, when solid food is presented, by the shift of the major proportion of *Bacteroides*, *Prevotella*, *Clostridium*, *Ruminococcus* and *Veillonella*. Microbiomes of children are also more stable compared to those of infants and show less variabilities among individuals (22). Preterm infants, as an example, have a completely different microbiome in comparison to full-term babies. The microbiota of full-term infants has a higher diversity and more common genera, whereas preterm babies have a higher amount of potentially pathological bacteria like *Klebsiella* or *Clostridium difficile* (21).

The initial development of the microbiome depends on an enormous number of different variables (21), even the blood type (24). Probably the most important external influence of the gut colonization is the mother, due to the nursing, feeding and intimate contact between the mother and her child. This could be proven with the fact that the microbiome of the mother and the child, when it is one month old, are functionally as well as phylogenetically very close. The mode of delivery has also a great impact on the development. Children who were vaginally born, have a different microbiota during the first years of their life compared to those who were born by cesarean section. The former profit from the bacterial flora in the mother's vagina, which could for example contain *Lactobacillus*, *Prevotella* or *Sneathia*, whereas babies who were born via cesarean section inherited the bacteria strains from the mother's skin (e.g. *Staphylococcus*, *Corynebacterium* or *Propionibacterium*). Another difference is that vaginally born children obtain a higher amount of *Bifidobacteria* than cesarean-delivered children. This could have a greater impact on the children in later life. Lower numbers of *Bifidobacteria* in early infancy (from six to twelve months of life) are associated with obesity when the child reaches the age of seven. Besides the mode of delivery, the mode of feeding influences the colonization in a great way. Compared with formula-fed babies, infants who were breast-fed show higher amounts of *Lactobacillus* and *Bifidobacteria* and lower amounts of *Staphylococcus*, *Bacteroides*, *Clostridium coccoides* and *Enterobacteria* (21).

Apart from the mother, the environment of the baby influences the development of the microbiome. For instance, a child with older siblings has a greater amount of *Bifidobacteria*, but a lower total amount of bacteria in its feces compared to an only child. The geographical

location and the cultural traditions are also an intense influence on the colonization. These differences do not only appear between continents, but also between diverse countries on the same continent. Variations of the microbiome between German and Finnish infants can be taken as an example. German babies have a higher number of *Akkermansia muciniphila*, *Clostridium histolyticum*, *Bacteroides* and *Prevotella*, but fewer numbers of *Bifidobacterium* than Finnish babies (21). Drug-based treatments of the mother or the baby have a profound effect on the colonization of the infant. Studies have shown that the use of an antibiotic therapy decreases the diversity of the microbiota and selects for drug-resistant bacteria. This could lead to a higher risk of developing allergic illnesses, like atopic diseases, asthma, or diabetes mellitus type 1 (22). In Britain, a cohort study including 11,532 children could prove that the usage of antibiotics in the first six months of the infants life will lead to a significantly greater body weight than in unexposed children (21).

Much of the research in the literature thus far has investigated anthropometric data in association with the microbiota. It has been shown that some individuals gain or lose weight more easily than others due to the prevalent types and amount of microbiota. Also, in the context of coping with stressful situations, it is assumed that people who can cope better with a stressful situation than others have a different intestinal colonization which could influence their coping mechanism (9).

There is a bidirectional communication between the brain and the microbiome. The pathway for this exchange of information can hold a wide range of physiological channels, such as from the autonomic nervous system or neuroimmune system. Even gut bacteria can generate neurotransmitters which are found in the central nervous system. For instance, the *Lactobacillus brevis* can produce gamma-aminobutyric acid (GABA). This could be proof that the brain has an impact on the variety of the microbiota, or vice versa (25).

If normal weight, germ-free mice got bacteria from overweight mice, they gained much more weight compared to others fed by the same food. Therefore, the bacteria might also influence body-weight in humans (9). Ruth Ley has shown in her review (26), that the microbiome has an impact on the inflammation, insulin resistance and obesity due to the interactions with the endocrine and epithelial cells. Therefore, a stable and healthy microbiome can help to maintain a normal weight (26). Another example are the *Bifidobacteria*: the less the amount of it in the body, the higher the risk of becoming overweight in adolescence. In addition, the mice fed with the obesity-associated microbiome developed an anxiety-like behavior due to

the substantiation of neuroinflammation. Even diseases like diabetes mellitus type two could be transferred in this way (9).

To maintain a stable health, a diverse microbiome should always be taken care of. Obesity, as an example, is related to a reduced bacterial variety. This diversity grows by time and depends not only on the afore-mentioned life events and interactions, but also on diet. A Mediterranean diet, which could be a protective factor for depression, is particularly advantageous for the microbiota. Even better for the microbiome are prebiotics. These are nutritional products which can reach the colon, where the majority of the gut bacteria is colonized, and are not resorbed prior to this point. Prebiotics contain a large amount of inulin (e.g. in garlic, onions, asparagus, artichokes, parsnip), fructooligosaccharides (e.g. tomatoes, bananas, rye, oats), resistant starch (e.g. green bananas, white, red or green beans, cooled potatoes, rice, noodles), and pectin, which is in fruits, vegetables, and lactulose (like in heated milk and other milk products). Other examples of prebiotic viands are green tea, almonds, honey, coffee, red wine, dark chocolate, and even beer (11).

An example of the importance of a balanced microbiota can be shown by examining the mechanisms which cause IBS, mentioned previously. This illness, although not life-threatening, is often associated with an altered behavior and increased prevalence of psychiatric disorders. It seems that the communication between the brain and the digestive tract is dysfunctional in patients suffering from IBS. The cells of the nervous system are much more sensitive and react more readily than normal to small stimuli caused by the weaker intestinal barrier and the following inflammatory processes (to envision the difference between an inactive and an active ganglion of the intestine see Figure 4). These processes are the reason for the bacterial imbalance: the former strains are replaced by others which could be the root cause of the mental and personal change. It is proven that IBS patients have a different microbiota compared to healthy people. Pimentel and colleagues (27) suggest treatment with an antibiotic containing the active substance rifaximin to reduce the new bacteria strains that caused IBS. This positive effect is long-lasting, even after antibiotic intake is halted. Another method is to take prebiotics or probiotics, which will be alluded to later on (11).

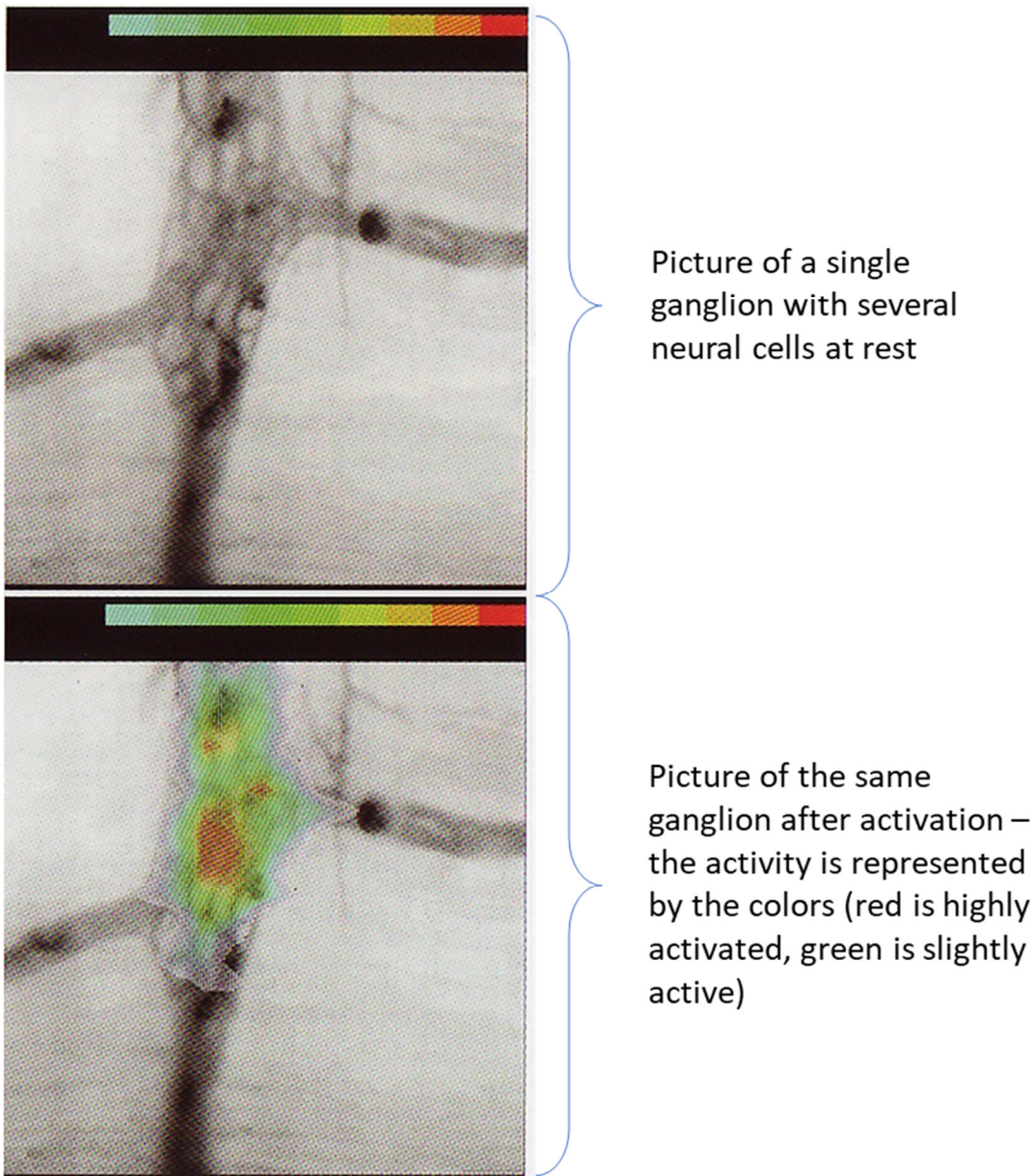


Figure 4 – Optic measurements of neural activity by electrical activation⁴

To sort the huge number of bacteria by their function, they must be classified by their families. Although an enormous quantity of bacteria-families exists, a research team in Heidelberg (28) could prove that there is an order of the more than thousands of families in the intestines. They found three different enterotypes called *Bacteroides*, *Prevotella* and *Ruminococcus*. *Bacteroides* and *Prevotella* are families of the *Bacteroidetes* and *Ruminococcus* is a family of the *Firmicutes*. In every person, one of those three families of bacteria has the largest percentage of the sum of bacteria (28). Each group has different

⁴ Source: See (7), page 80ff, image 5. (own layout design and translation) – in case of any publication besides the publication at the Medical University of Graz, the picture must be removed due copyright reasons (for further information see List of Figures)

abilities as well as disadvantages concerning digestive functions (9). *Bacteroides* are the most common group of bacteria, produce many splitting enzymes and are involved in the fission of carbohydrates and proteins by fermentation (28). Mostly they are found in the intestine of meat-lovers. They are able to gain the maximum energy of every aliment, so it is possible that this type of bacteria is one of the reasons that some people put on weight much easier than others (keeping in mind that *Bacteroides* are not the only strain which is responsible for weight gain). Another benefit - besides that they can easily gain energy - of *Bacteroides* is that they can proficiently collaborate with other bacteria. One of them is the family *Parabacteroides*, which can produce a high amount of biotin which is not only important for healthy hair, nails and skin, but also helps to produce carbohydrates (9) (by its enzyme Pyruvate carboxylase, a starter-enzyme of the gluconeogenesis (29)) and fat (9) (by its enzyme acetyl-CoA carboxylase, an important part of the lipid synthesis (29)) for the body and to decompose proteins (9). *Prevotella*, in contrast, are mostly to be found in the intestine of vegetarians (9) and it degrades proteins with the co-existing desulfovibrio mucin (28). The last one of these three species, the *Ruminococcus*, also degrades mucin with the co-occurring *Akkermansia* and is concentrated in membrane transporters (in most cases of sugars). Another function of this species is the uptake of simple sugars (28).

As mentioned before, the microbiota can generate nearly every neurotransmitter which can also be found in the brain. In this way, it is capable of interfering in behavior and central neurochemistry, which many neuroscientists called an essential paradigm shift. Here are some examples of these neurotransmitters: *Bifidobacterium infantis* can produce tryptophan, a precursor of serotonin; *Candida*, *Saccharomyces spp.*, *Streptococcus* and *Escheridia* can even secrete serotonin, and *Bacillus* is able to elevate the plasma levels of dopamine and noradrenaline. Of these neurotransmitters, serotonin is a significant key substance. It helps by the transmission of neural signals, influences the tonus of the blood vessels, plays an important role in the sleep-wake rhythm, and affects concentration and memory capacity. Furthermore, it supports cell growth and, depending on where and by what it is released, it has anti-inflammatory as well as proinflammatory functions. In addition, the influence of serotonin on behavior and mood has been proven as well. But, due to the blood-cerebral barrier, it is more difficult than expected to increase the serotonin level in the brain by the ingestion of certain aliments. For instance, chocolate – like many other viands – contains tryptophan, which (as mentioned above) is a precursor of serotonin. However, it would take a daily intake of 250 kg of dark chocolate to achieve a mood-enhancing effect. And even then, it is not certain that the whole three grams of tryptophan will reach the brain and will

not be excreted first. Therefore, the serotonin level of the abdominal brain cannot compensate for deficiencies of the serotonin concentration of the brain, but it could help to regulate it by this mechanism: increased serotonin levels of the intestine enhance signals along the gut-brain axis, which influences central nerve control. Besides, serotonin plays an important role in the maintenance of the normal gastrointestinal functions. As a consequence, serotonin eases defecation (see Figure 5) (10).

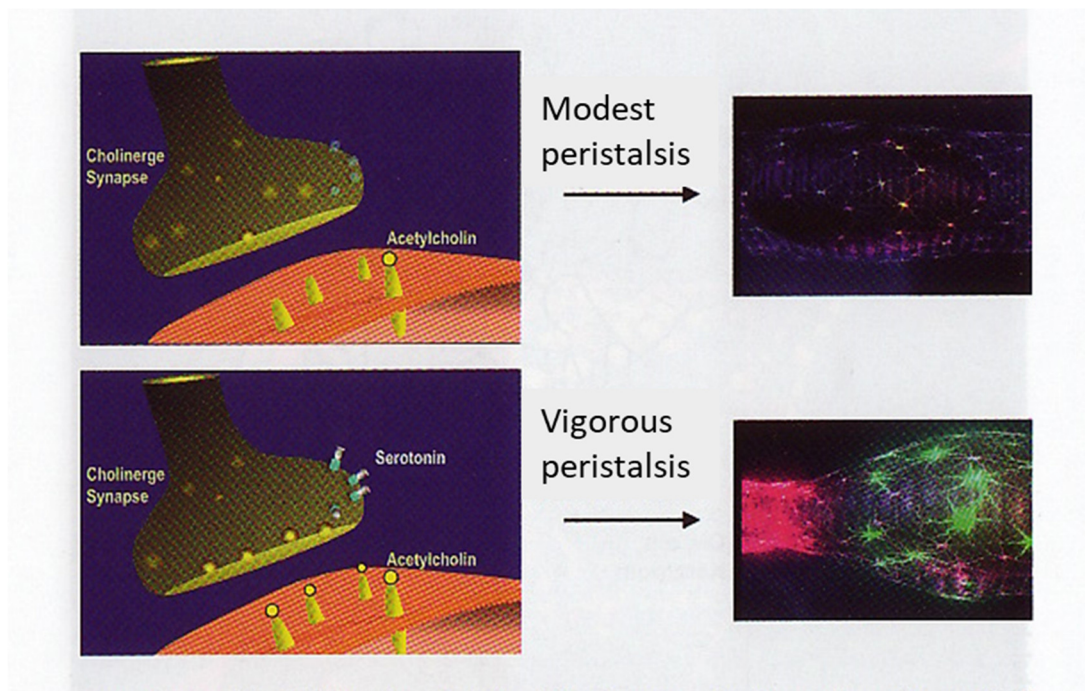


Figure 5 – The influence of serotonin on the peristalsis⁵

A dysbiosis of the microbes in the intestine has a proven implication for the brain and its functions, which could even lead to mental and neurological illnesses, for example autism, Parkinson’s disease, or amyotrophic lateral sclerosis (10, 30). This could guide towards a new way of treating these diseases, but, unfortunately, the connection of these surveillances is neither clarified nor explored enough (30). Many research teams are trying to understand the microbiome in a better way, like the Japanese team under the lead of Mai Takada (31). They tested the effects of *Lactobacillus casei* strain Shirota (LcS) in healthy medical students under stressful examinations. The probands received a LcS- fermented milk or a placebo for

⁵ Source: See (7), page 80ff, image 12. (own translation) – in case of any publication besides the publication at the Medical University of Graz, the picture must be removed due copyright reasons (for further information see List of Figures

eight weeks and their salivary cortisol levels, anxiety scores, and physical symptoms were tested. The results have shown that the LcS group had a significant lower level of salivary cortisol and fewer symptoms due to the stress compared to the placebo group, but it did not affect the subjective anxiety. The authors of the former study could prove that another bacterium, called *Bifidobacterium longum*, was also able to decrease the cortisol level (this time, the morning waking cortisol level) and additionally the subjective anxiety level of students. They even could improve their cognitive abilities (31). Bacteria like this, that have positive influence on health are called, like many others, probiotics (10).

1.2.3 Probiotics

Every day, every human swallows billions of bacteria. Most of them are not yet known, but it seems that the major part are not dangerous and the ones which could be harmful are too few to produce any damage. The minor part of these bacteria, which are well-known and investigated enough to know that they are useful and can improve health, are called probiotics (9). To improve health, 10 to 40 percent of these bacteria (e.g. *Lactobacillus rhamnosus* or *Lactobacillus casei* Shirota) have to reach the colon undigested, due to their acidotolerance. The chances of larger amounts of probiotics reaching the colon are higher when they are taken with or shortly after a meal (especially when the meal contains dairy products) because the pH-value is higher after a meal. These helpful probiotic bacteria have an enormous impact on human life (11). Even in early history, when bacteria and their benefits were unknown, people at the time had ways to use them to preserve their food. They fermented foods, such as sauerkraut, ayran, kombucha drinks, or miso soup with similar probiotic effects (9).

The benefits of probiotics can be summed up into three different groups (9):

1. They have genes which can produce fatty acids like butyrate. Their function is, among others, to maintain the intestinal villi so they can grow bigger and more stable. The bigger they are, the better they can resorb nutrients, and the more stable they become, the less they let harmful substances pass through (9).
2. They support the immune system in different ways. The probiotics can claim too much space so that pathogens cannot settle down, they can generate a few amounts of antibiotics or antibodies, or they can simply digest the nutrients the germs need (9).

3. The bacteria function as an information service for the whole body. They can show how to build mucin or defensins (bacterial antibodies) or how to react to foreign substances (9).

The digestive tract can be harmed by stress, which may weaken the intestinal barrier such that pathogens pass through it more easily. One way to rebuild this barrier, completely free from side effects (to the best of our current knowledge), is the intake of probiotics (11). Research has shown that probiotics can alleviate or even prevent diarrhea or a cold, as well as prevent or ease the symptoms of an allergy or neurodermatitis (32, 33). Other fields of research have shown promising results of the benefits of the usage of probiotics in illnesses like diabetes, obesity, and depression (11). The only disadvantage is that it is not sufficiently clear which species of bacteria can solve these problems. As of yet, the field lacks a comprehensive understanding of the effects of each bacterial species, so a “trial and error” approach is often necessary to determine which bacteria is the best for treating each respective condition (9). Two strains of probiotic bacteria are the *Lactobacilli* and the *Bifidobacteria*. These two can be digested by daily meals or dietary supplements. These two bacterial strains can be divided into many different subfamilies with different impacts on digestion. For example, the *Lactobacillus* strain contains - among many others - *Lactobacillus rhamnosus* (stables the intestinal barrier and has reassuring effects), *Lactobacillus reuteri* (supports the development of oxytocin and increases social behavior) and *Lactobacillus helveticus* (it can lower the cortisol-level and improves the intestinal barrier). *Bifidobacteria infantis* can increase the tryptophan-level in the blood and provides a better sleep due to the increase of the “sleep hormone” melatonin. *Bifidobacteria longum* has mood-enhancing effects and can reduce the symptoms of IBS. These are only a few examples of the impact of probiotics, but it shows the importance of a varied microbiome (9).

2 The Pilot Study

2.1 About the Study

Due to the many beneficial effects on different illnesses - especially the impact on treatment of depression - the research team under the guidance of Assoz. Prof.in Eva Reininghaus at the Psychiatric Department of the Medical University in Graz, Austria, investigated the influence of probiotics on people diagnosed with bipolar disorder. The main focus of the research was to identify clinical effects on psychiatric and gastrointestinal symptoms as well as cognition. In collaboration with Allergosan and BioVis, as well as support by the „Stadt Graz“, the pilot studies “ProbioBIP-one – Wirkung von Probiotika auf kognitive Funktionen bei Menschen mit bipolarer Störung in euthymer Stimmungslage” and “ProbioBIP-two - Wirkung von Probiotika auf kognitive Funktionen bei Menschen mit bipolarer Störung in depressiver Stimmungslage” were initiated. Both studies were approved by the local ethics committee of the Medical University of Graz (EK-number EK- 28-413 ex 15/16). These studies involved 42 patients which were diagnosed with bipolar disorder I or II. Men and women both received equal focus in the investigation. Study participants were divided into two different groups according to their current state of mood (euthymic or depressed) at the first study visit. Exclusion criteria were: age under eighteen, a recent manic episode, severe abuse of alcohol and/or drugs, other active severe mental illnesses or organic diseases affecting the brain, (such as epilepsy, brain tumor, a severe traumatic brain injury or an operation of the brain, other florid tumors, mental retardation, or moderate to severe dementia from MMSE 20), the intake of antibiotics in the last month, and the periodic intake of probiotics in the last year. Each participant gave informed consent and an early drop-out was possible at any time with no given reason. Study participants had to take a probiotic supplement over a time period of three months and had to do three study visits– one at the start of the study, one after one month and the last one after three months of probiotic intake. Furthermore, a stool sample as well as fasting blood was required at each visit.

For this thesis, only study participants from the ProbioBIP-one study were analyzed.

2.1.1 Hypothesis

The focus of this research was to prove the positive effects of probiotic supplements on probands with a diagnosed bipolar disorder. As hypothesis it was assumed that there would be changes in subjective perception of digestion, sleep, mood, and quality of life, as well as

in manic and depressive symptom severity in individuals with BD receiving probiotics after four and twelve weeks of continuous intake.

2.1.2 Probands and Compliance Rate

In the ProbioBIP-one study 27 euthymic individuals with bipolar disorder were included during July 2016 to July 2017. These participants had to meet the demands of four inclusion criteria: a diagnosed bipolar disorder, age over eighteen, an ongoing intake of mood stabilizers, and a euthymic mood. The last criterion was confirmed by the following two questionnaires: the Hamilton Depression Scale (HAM-D (34)) and the Young Mania Rating Scale (YMRS (35)). To outline depression or mania, the HAM-D score should not be over nine points and the YMRS should not be over fourteen points. Five of the 27 participants did not finish the study due to a stay in a psychiatric rehabilitation center or a deterioration of mood (see Figure 6).

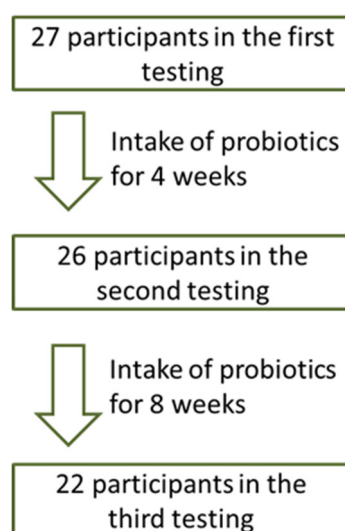


Figure 6 – Compliance during the three visits⁶

Due to low numbers of participants starting these studies during a depressive episode, this thesis will only include the euthymic attendees. So, in the following chapters, the mentioned study is the “ProbioBIP-one” study. The euthymic group included 16 men and 11 women, but there will be no gender-based separation of data depiction due to the small sample size.

Although everyone was instructed to take the probiotics daily, some probands did forget the intake for a few days (see Table 1). Between the first two visits, six participants had forgotten it once and one five times. During the last two months (between visit 2 and visit 3) eight

⁶ Source: own presentation.

patients forgot some of the intakes. The number varied per individual, with a maximum of seven forgotten sachets of probiotics. Only one indicated 21 missing sachets due to negative side effects, which will be mentioned in the results.

2.1.3 Methods

Each study visit lasted about two hours in total, depending on the proband's condition and concentration. First, it began with sampling of blood and stool, which the company BioVis analyzed in Munich. Second, the patient had to evaluate his or her current mood by answering some short questionnaires on his or her own, which included: Beck Depression Inventory II (BDI-II (36)), Mania Symptom Scale (MSS (37)), Leiden Index of Depression Sensitivity-Revised (LEIDS-r (38)) and Anhedonieskala (also known as Snaith-Hamilton Pleasure Scale (39)), lifestyle (24 hours food recall (40)), Fagerström plus (41), the short version of the "Freiburger Fragebogen" for physical activity (42), and World Health Organization Quality of Life (WHOQOL (43)). Third, the participants had to take some tests on his or her cognition: The Trail Making Test A and B (44), the Mittenecker Zeigetest (45), Zahlennachsprechtest (by Hängsen, K., D.), and the Digit Symbol Test (46)). Fourth, the examinee's mood was evaluated again, but this time by a psychiatrist or a psychologist by the following tests: Udvalg-for-Kliniske-Undersøgelser-Side-Effects-Rating-Scale (UKU (47)), YMRS and HAM-D. Fifth, anthropometric data was collected by measuring the waist and hip circumferences, and the weight and height of the probands.

Although it was desirable that the proband should take the same medication during the whole study period, changes in medication were possible and therefore recorded. The questionnaires were the same at every visit, except that a follow up screening was added during the second and the third visit. It covered some possible changes in mood, digestion and sleep of the probands over time. This questionnaire also included recent events or alterations in the proband's life, such as illnesses, stressful moments, or other life changes (e.g. a new relationship, a new diet).

2.1.4 Problems

Possible problems that can occur during the study, each followed by the proposed response, are listed below:

- The proband does not want to participate in the study anymore, but agrees that the collected data can be used, or the proband deceases during the study: previous data will be included in the results, but the test person is listed in the dropout register.

- The patient does not appear at the visit, but is willing to participate further on: another date for the visit will be identified, and he or she can pick up the needed probiotics any time.
- The test person forgets to take the probiotics too often: he or she still stays in the study, the amount of the forgotten probiotics will be noted and included in further analyses.
- The patient does not take the probiotics at all: if desired, the proband stays in the study and will be incited to take the probiotics. If this happens before the second visit, this visit will count as the first one and the proband's start will be postponed for a month. If the non-compliance happens at a later date, the proband will be excluded from the study.
- The patient did participate the for the entire length of the study, but withdraws his or her permission to use the collected data: the test person will be excluded from the study and all of his or her data will be excluded from the analysis, as well.

Although five people dropped out during this study, none of them withdrew their permission to use the already collected data. Therefore, every person's data could be included in the current analyzes.

2.1.5 The Product

The probiotic supplement used in this study was "OMNi-BiOTiC Stress Repair". It contains about 7.5 million probiotic bacteria, which should improve the barrier in the digestive system, especially against stress and its negative effects. The included bacterial strains were *Bifidobacteria bifidum* W23, *Bifidobacteria lactis* W51, *Bifidobacteria lactis* W52, *Lactobacillus acidophilus* W22, *Lactobacillus casei* W56, *Lactobacillus paracasei* W20, *Lactobacillus plantarum* W62, *Lactobacillus salivarius* W24 and *Lactobacillus lactis* W19. During the three-month study period the probands were instructed to ingest one sachet of "OmniBiotic Stress Repair" every day in the morning before the breakfast; if forgotten, it was possible to take it in the evening. The probiotic powder should be dissipated in a glass of 250 ml lukewarm water. For best results, the participant should wait ten minutes, stir again and then drink it. If a proband forgot to intake the probiotic supplement for the whole day, he/she should make a note and continue the intake the following day.

2.1.6 Statistics

The data was analyzed by the use of means (M), standard deviations (SD) and the percentage or number of the relevant variable. To track the differences between all three visit dates, analyses of variance with repeated measures design (RM-ANOVAS) were conducted. RM-ANOVAS was used by the following questionnaires: BDI (21 variables), HAMD (21 variables), YRMS (11 variables), MSS (48 variables), LEIDS-r (34 variables), WHOQOL (26 variables) and Snaith-Hamilton Pleasure Scale (14 variables). For every questionnaire only the total values were exerted. IBM SPSS 22 was used. Statistical significance was denoted by an error probability below 0.05 (=p).

The demographic statistics of all participants are listed in Table 1.

		<i>Mean</i>	<i>Standard Deviation</i>
<i>General Data</i>	Age (years)	50.7	12.2
	Females (%)	40.7	
	Fagerström Nicotine Dependence	1.2	2.1
	History of Probiotic Treatment (%)	31.3	
	<i>Psychiatric History</i>		
	Illness Duration (years)	19.3	13.3
	Number of Manic Episodes	9.0	11.5
	Number of Depressive Episodes	17.6	18.7
<i>Current Symptomatology</i>	HAM-D	2.2	2.5
	YMRS	2.4	3.6
	BDI-II	25.0	9.8
	MSS	5.62	7.51
	Leiden Total Score	43.12	15.64
<i>Anthropometric Measures</i>	BMI (kg/m ²)	29.0	5.6
	Waist/Height Ratio	52.7	17.6
	Waist/Hip Ratio	.924	.076
	WHOQOL	3.6	4.0
<i>Inflammatory Markers</i>	hsCRP (mg/L)	3.5	3.0
	IL-6 (pg/ml)	3.3	1.8

<i>Medication Intake</i>	Lithium (%)	40.7	
	SSRIs (%)	14.8	
	SNRIs (%)	33.3	
	Atypical Antipsychotics (%)	44.4	
	Anticonvulsants (%)	25.9	

Table 1 – Demographic data of the participants⁷

2.2 Results

Of the 22 participants who completed every testing, 55% never forgot the ingestion of the probiotics; the remaining percentage of forgotten intakes are listed in Table 2Table 5.

<i>Forgotten Probiotics</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>7</i>	<i>21</i>
<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>	<i>Forgotten</i>
<i>Testing 2</i>	83%	0	0	0	17%	0	0
<i>Testing 3</i>	11%	11%	11%	22%	22%	11%	11%

Table 2 – Forgotten Probiotics⁸

Only one of these probands stopped the intake of the probiotics three weeks before the last testing due to intolerable obstipation, which occurred during the testing period. The participants who dropped out of the study were not listed in this table. Although five of the 27 participants did not finish the study, all 27 probands were included in the results due to the possible and mentioned effect of the probiotics on every one of them.

22 of the 27 probands noticed some alterations in one of the three topics - digestion, mood, and sleep - which could be associated with the intake of the probiotic supplement. Two probands decided to continue to take the probiotics after the study period, one clearly stated that they will no longer ingest this kind of probiotic supplement because no improvement was noticed, and two probands did not note any differences, but did not rule out taking some in the future. Some probands did notice a change in two or all of the three topics of digestion,

⁷ Source: own presentation.

⁸ Source: own presentation.

mood and sleep, which will be listed below in detail. It should be mentioned that some probands noticed an improvement in one of these topics at the second visit, but then a deterioration at the third, and vice versa.

For a brief overview, all the changes are listed in Table 3:

<i>Alteration in Total</i>	<i>Positive Effects</i>	<i>Positive as well as Negative Effects</i>	<i>Negative Effects</i>	<i>No Changes Noticed</i>
<i>Digestion</i>	10	5	5	2
<i>Mood</i>	7	1	4	14
<i>Sleep</i>	4	0	4	10

Table 3 – Changes in digestion, mood and sleep⁹

As mentioned previously, some probands had a change of medication. Nevertheless, most of these changes were small and included a change in dosage. A third of the probands did need new medication, a higher dose, or a reduction due to mood improvement. Three of the probands who mentioned changes noted only an enhancement of at least one of the three examined topics, while three other participants mentioned improvements as well as deteriorations whereby two of them said the deteriorations outweighed the improvements. Two probands needed a change in medication due to the onset of depressive symptoms and noted only a decline in at least one of the three topics. One proband could reduce the medication, but listed deterioration in digestion. All changes in medication associated with changes in the three topics are listed in Table 4. The participants are listed in order of the changes in the three analysed topics and the number is not the same as their coded number during the assays. The last proband of the table dropped out of the study, so there is no data from the third testing.

<i>Number of Participant</i>	<i>Alteration in Medication -Visit 2</i>	<i>Alteration in the Analysed Topics – Visit 2</i>	<i>Alteration in Medication - Visit 3</i>	<i>Alteration in the Analysed Topics – Visit 3</i>
<i>1</i>	Quetiapin 200mg is added to the daily dose of 400mg Seroquel	More stable, Improvement of mood	Bupropion 150mg and Seroquel 100mg is added	Calmer

⁹ Source: own presentation.

2	Nothing changed	Less diarrhoea, optimistic mood	Milnacipran 3*25mg is stopped, Venlafaxine 75mg is added	General improvement of digestion
3	Nothing changed	General improvement of digestion, less flatulence	Lamotrigin 50mg is added	General improvement of digestion and sleep
4	Nothing changed	More frequent stool, lighter sleep, general deterioration of mood	Chlorprothixen 50mg instead of 100mg, Zyprexa 10mg instead of 15 mg	More frequent and softer stool, general improvement of digestion and sleep
5	Mirtacepine is stopped	Nothing changed	Nothing changed	Diarrhoea
6	Nothing changed	General deterioration, General improvement of sleep	Sertraline 100mg is added	More flatulence, stomach ache
7	Nothing changed	Fewer stress reactions of the intestine, obstipation, more tired	Amitriptyline-Hydrochloride 50mg instead of 25mg	Obstipation
8	Nothing changed	More dreams	Attempt: Mirtazepine 30mg but is stopped due the intolerable side effects	Diarrhoea, more dreams, general deterioration of mood, subdepressive

9	Venlafaxin 150mg and Bupropion 150mg are stopped, Olanzapine 15mg and Lorazepam 2,5mg for a month are added	Depression	Proband dropped out of the study	
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Table 4 – Alterations in medication compared to alterations in analysed topics¹⁰

2.2.1 Alterations in Participant’s Mood

According to every patient subjective opinion, 12 of 27 did note differences in their mood state; eight mentioned an improvement, five noticed a decline, and one person noted first an improvement, but in the third testing a deterioration. It should be mentioned that a severe stroke of fate happened in this participant’s life, which explained the decline. The alterations differ from participant to participant and are listed in Table 5.

<i>Mood</i>	<i>Second Visit</i>	<i>Third Visit</i>	<i>Positive or Negative Effect</i>
<i>General Improvement</i>	5	2	Positive
<i>Enhanced Drive</i>	0	1	Positive
<i>Calmer</i>	1	0	Positive
<i>More Relaxed</i>	0	1	Positive
<i>More Resilient to Stress</i>	1	1	Positive
<i>General Deterioration</i>	0	3	Negative
<i>Decreased Drive</i>	2	0	Negative
<i>More Irritated</i>	1	0	Negative

Table 5 – Alterations in the participant’s mood¹¹

It is worth mentioning that three people noted differences in more than one of these subgroups. Two individuals noted positive changes only and one participant negative changes only.

¹⁰ Source: own presentation.

¹¹ Source: own presentation.

Concerning the five patients who mentioned a worsening of mood symptoms, four of them made references to negative events in life or an episodic worsening of their mental disorder.

According to some questionnaires (MSS, YMRS and LEIDS-r) fewer alterations were measured, but this result was expected because every participant was euthymic at the time of the inclusions. A significant decline in manic symptoms was suggested by the MSS ($p=0.048$), which was filled out by the patients themselves as well as the psychiatrics-controlled YMRS ($p=0.023$) over the time period of the three tests in the multivariate analyses. It should be mentioned that the manic symptoms were already at a low point at the time of inclusion (MSS in testing 1: $M=5.2$, $SD=7.41$, in testing 2: $M=3.75$, $SD=3.99$ and in testing 3: $M=2.35$, $SD=3.39$ ($F(2,18)=3.621$, $\eta^2=0.287$); YMRS in testing 1: $M=1.47$, $SD=2.91$, in testing 2: $M=1.26$, $SD=2.08$ and in testing 3: $M=0.42$, $SD=1.84$ ($F(2,17)=4.751$, $\eta^2=0.359$)) and there was no significant difference between the individual time period between testing one and two, and testing two and three. Another significant outcome was found in the “control” subscale of the LEIDS-r. A significant decrease was determined in the rumination subscore during visit one and three ($p=0.037$) and especially between visit two and three (visit 1: $M=13.3$, $SD=5.3$, visit 2: $M=14.0$, $SD=6.0$, visit 3: $M=11.9$, $SD=5.6$; $p=0.004$, $F(1,20)=3.988$, $\eta^2=0.319$). No other significant decline or increase was detected in the other five subscales (aggression, hopelessness, acceptance, control, and risk aversion) of the LEIDS-r or in the other questionnaires (BDI, HAMD, WHOQOL and Snaith-Hamilton Pleasure Scale) (48).

2.2.2 Alterations in the Participant’s Digestion

Before the inclusion in this study, the participants had to answer a questionnaire if they experienced issues with their alimentary tract in their life previously. 54% quoted some discomfort, which is listed in Figure 7. Of these 54%, fifty percent did mention gastrointestinal problems caused by stress.

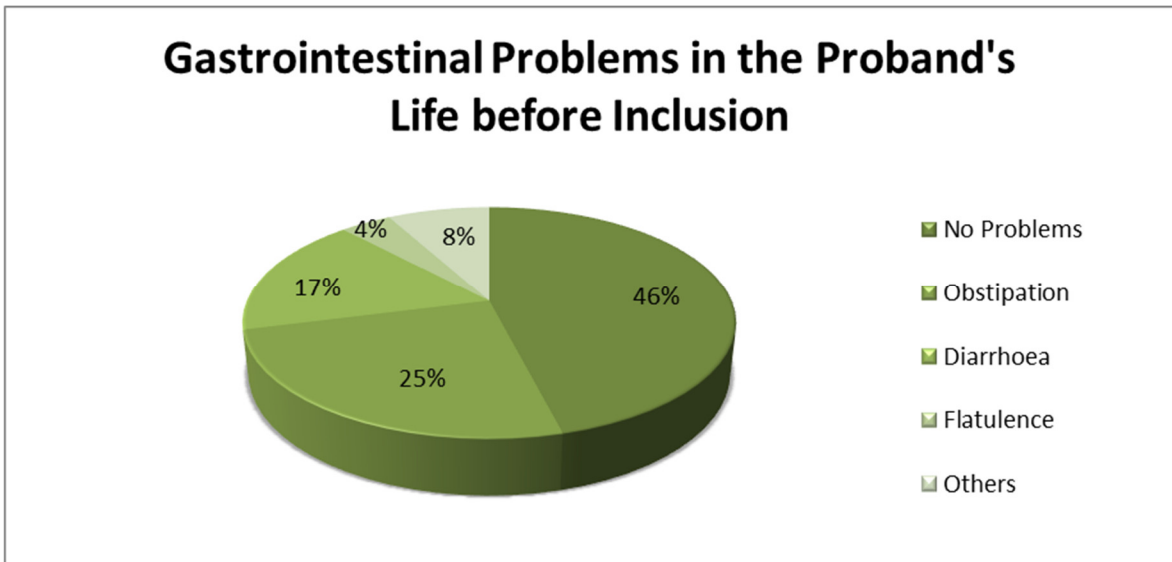


Figure 7 – Gastrointestinal problems in the Proband’s Life before inclusion¹²

At the end of the study period, 15 patients (=56%) noticed an improvement in their digestion, 10 (=37%) mentioned deterioration, and 5 (=20%) stated an enhancement as well as a decline. Specific changes varied widely and are presented in Table 6. One of the five patients, who mentioned a change in both directions, noted an improvement during the first and the second visit, but a worsening between the second and third visit, probably caused by a stressful psychosocial event. The other four people did notice an improvement and deterioration at the same time. One of them even stopped the intake of the probiotics caused by intolerable obstruction.

¹² Source: own presentation.

<i>Digestion</i>	<i>Second Visit</i>	<i>Third Visit</i>	<i>Positive or Negative Effect</i>
<i>General Improvement</i>	1	5	Positive
<i>Less Flatulence</i>	2	1	Positive
<i>More Frequent Stool</i>	3	2	Positive
<i>Harder Stool</i>	3	2	Positive
<i>Softer Stool</i>	3	2	Positive
<i>More Often Stool</i>	1	0	Positive
<i>Loss of Weight</i>	1	2	Positive
<i>Fewer Stress-Reactions</i>	1	0	Positive
<i>General Deterioration</i>	1	0	Negative
<i>Stomach Ache</i>	0	1	Negative
<i>Diarrhoea</i>	1	2	Negative
<i>Obstipation</i>	2	2	Negative
<i>Gain of Weight</i>	0	2	Negative
<i>More Flatulence</i>	1	2	Negative

Table 6 – Alterations in the participant’s digestion¹³

2.2.3 Alterations in the Participant’s Sleep

Some participants noted changes affecting the sleep. Four probands conveyed an improvement, another four quoted a decline, whereby one of them claimed that the sleep was getting worse but acknowledged that the depressive symptoms enhanced at the same time throughout the duration of the testing time as well. Two people (one of them throughout the whole testing period) experienced a higher frequency of dreams, but could not evaluate if this had a positive or negative effect on their lives. The range of alterations is listed in Table 7.

<i>Sleep</i>	<i>Second Visit</i>	<i>Third Visit</i>	<i>Positive or Negative Effect</i>
<i>General Improvement</i>	1	1	Positive
<i>Longer Sleep Duration</i>	1	0	Positive
<i>Less Tired</i>	0	1	Positive
<i>More Dreams</i>	2	1	Neither Nor
<i>More Tired</i>	2	1	Negative
<i>Less Restful</i>	0	1	Negative
<i>Lighter Sleep</i>	1	0	Negative

Table 7 – Alterations in the participant’s sleep¹⁴

¹³ Source: own presentation.

¹⁴ Source: own presentation.

2.2.4 Alterations in more than one Topic

Fifteen participants experienced alterations in more than one domain. Two quoted a change in all three topics. The changes were rated from only positive to only negative effects; some noted both, which is depicted in Table 8.

<i>Alterations</i>	<i>Only Positive Effects</i>	<i>Only Negative Effects</i>	<i>Positive as well as Negative Effects</i>
<i>In All Three Topics</i>	1	2	1
<i>Mood and Digestion</i>	4	2	2
<i>Sleep and Digestion</i>	0	0	4
<i>Mood and Sleep</i>	0	1	0

Table 8 – Number of alterations in more than one topic¹⁵

¹⁵ Source: own presentation.

3 Discussion

The aim of the current thesis was to explore the clinical utility of probiotic supplements in people who are diagnosed with bipolar disorder. A wide range of studies have demonstrated favourable effects of the probiotic supplements on many other diseases (2, 3, 20, 32, 33), but there are only a few studies concerning bipolar disorder (49, 50, 51). A research team in Michigan, USA, - for instance - suggests that an increase of the *Faecalibacterium* in people with bipolar disease could reduce certain symptoms of this illness (49). *Faecalibacterium* has anti-inflammatory properties and a sufficient amount of it is associated with a healthier physical state. To verify the positive effects on bipolar disorder, another study must take place with an appropriate study design (49).

We found a significant reduction of ruminative thoughts as well as a significant reduction of manic symptoms. Other changes were not significant. According to the results of this study, the major part of individuals with bipolar disorder in a currently euthymic state reported benefits on mood, digestion, and sleep during the intake of probiotic supplement. Although the improvement of mood was not as promising as in other studies (3, 26, 32, 33), significant positive outcomes were evaluated. The main enhancement was found in the change of digestion, which was expected because of the vast quantity of digestive functional problems at the time of inclusion.

Nevertheless, the cause of the lack of significant effects in some parts of the study could be due to the low number of participants and the fact that the impact of probiotics is subject to the trial and error principle. As mentioned previously, each person has a different microbiome with different species of bacteria and many different variables can influence this microbiome. A research team could prove that the intake of atypical antipsychotics is associated with a decreased amount of bacterial species in the body (50). This fact and the varied amount and species of bacterial strains could influence the individual results. Therefore, the few changes can be explained by many different reasons, including the product itself. It is possible that OMNiBiOTiC® stress repair did not offer the requested bacteria for every proband. This product contains strains of bacteria which can improve the intestinal barrier and prevent damages of the digestive system caused by stress (52). To mention some of the benefits: *Lactobacillus casei* can lower a high cortisol level (31), *Lactobacillus acidophilus* can be advantageous in the treatment and prevention of intestinal infections (53), *Lactobacillus paracasei* can inhibit the activity of E. coli (54), *Lactobacillus*

salivarius and *Lactobacillus plantarum* can improve flatulence (55). *Lactococcus lactis* can hinder the motility of pathological bacteria like Salmonella (56). *Bifidobacteria lactis* can improve the unpleasing symptoms of IBS by reducing the abdominal girth and treating distension (57), and *Bifidobacteria bifidum* can prevent neonatal necrotizing enterocolitis (58). These effects depend on a regular intake and on a sufficient amount of the bacteria reaching the colon, both of which could be easily prevented by, e.g. uncompliant intake or slow digestion, respectively (9).

The usage of probiotic supplements is still based on trial and error. For better accuracy, a compilation of probiotics according to the individual's microbiome would be desirable. Therefore, analysis of the microbiome has become an interesting subject of research. Due to the enormous pace of research, a large quantity of software and methods for analysing the microbiome has been released after just a few tests of their functionality. Therefore, an international research team from the University of Vienna developed a method to verify future analysis of the bacterial genome via computer simulation (51). In addition, it can interpret the data and – in this way – help to close gaps in data analysis. This method is called “Critical Assessment of Metagenome Interpretation” (CAMI) and concept is that the software simulates the data of artificial microbiomes, mostly of unknown bacteria strains, in the typical way like the bacteria reacts and lives. So, other available methods of data-analysis can use these simulated data and the results will be compared under real circumstances. Using methods such as these, it is possible that in near future that the still unknown functions and benefits of bacterial families can be resolved (51).

Due to the recent focus on the microbiome and the brain-gut-axis, an ancient treatment, which was recommended in ancient China, undergoes its renaissance. The stool transplantation developed over the last few years thanks to a new trend in the United States of America, usually without medical supervision- which is urgently advised against. The method is simple – an extract from a stool sample is intermingled with a saline solution and is injected in the clean and empty colon. However, some severe side effects are possible. Some highly contagious germs can be transmitted this way; even diseases can be transferred by this method. A slim mother got a stool sample from her overweight daughter due to an intermittent infection of clostridia, and gained over sixteen kilograms in the seventeen months after the procedure (10). Researchers are trying to create a synthetic stool sample, which will be free from damaging substances (9). But two unsolved problems are standing in the way of developing a new and safe treatment. First, it is possible, same as by the intake

of probiotics, that the same stool sample has different effects on every individual person and – as of yet – it is not known how a healthy stool is composed (10). So far, the only indication of this treatment is an untreatable severe infection of clostridia difficile (9). Even then, this impact only lasts for six weeks, so until this time, the intestinal tract must regenerate as otherwise the infection can reoccur in a short period (10). To date, studies analysing the effect of a stool transplantation on patients with IBS do not show promising results (10).

The transplantation of probiotics, however, has had potential in treating illnesses for a long period of time. Some studies show positive effects in the therapy of diabetes mellitus (59, 60, 61). Ongoing research is even trying to prevent the onset of diabetes mellitus type one (62). These studies underline that probiotics do not only influence the alimentary tract, but also the immune system and metabolic processes. Unfortunately, about 60% of the intestinal bacteria are still widely unexplored. One of the reasons is that for a substantial number, the right nutrient medium has not been discovered. Many of the bacteria die and are dismantled before further investigation. Therefore, the exploration of possible probiotics in the whole microbiome is still time-consuming and strenuous (9, 21).

3.1 Limitations

One of the greatest limitations is the low number of participants and the missing placebo-controlled group, as well as the lack of diversity of the probands. These problems were known before and mostly underlie the cause that this study is a pilot study and should be an impetus for further researches and studies. Another limitation is the ongoing euthymic mood of the participants. More alterations could be possible in a manic or depressive state, which will be evaluated in the second study that includes only participants in depressive mood.

Furthermore, changes of lifestyle, such as a new diet or sport, have not been recorded in detail because they were noted only based on the memory of the participants. Alterations in these subjects could lead to changes in the discussed three topics (mood, digestion and sleep).

Another limitation is the follow up questionnaire on the changes of these three topics. Although this questionnaire is well thought-out, it is challenging to differentiate the cause of the changes. Some of them could be caused by the probiotic supplement, but changes of medication and mood due to the bipolar disorder, life events and even coincidences because of the closer self-inspection of the probands should be considered.

The study also might be limited by the time period of three months, which might be too short for observation of possible changes. Lastly, lack of compliance of some probands could be the reason for the limited changes.

4 Conclusion

In sum, many studies have shown a positive effect of probiotics on numerous diseases like Morbus Alzheimer and diabetes mellitus. This study is one of the first which tested the effects on individuals with bipolar disorder in a currently euthymic state. The results were not as promising as desired, but many participants noted an improvement in one or even in all of these three topics. Therefore, another study, including a placebo group, is taking place at the Psychiatric Department of the Medical University of Graz to confirm these results by a larger amount and a better compliance of the participants.

This investigation is a pilot study in a rather new scientific field. Further studies with a higher number of participants or other probiotic products with different compilations of bacterial species are highly recommended. Nevertheless, this study could show improvements in the three topics of mood, digestion, and sleep, and motivated some participants for further intakes of probiotic supplements.

Research in the field of probiotics and the microbiome is mostly still at early stages, but may yield new therapies for a wide range of medical issues with hardly any side effects. Although it is doubtful that probiotics could replace common medication while the knowledge about the possible range of benefits is still at its beginning, the results are showing promising data for further investigation.

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