Mind, Mood and Microbes; Amsterdam 1 & 2 Dec 2016

CONFERENCE SUMMARY

1st international congress, on the influence of the gut microbiota at the brain
Mind, Mood and Microbes
The 1st International Conference on Microbiota-Gut-Brain Axis
Mind, Mood and Microbes; a magical connection

On December 1 and 2, 2016, hundreds of healthcare professionals and researchers from all over the world gathered in Amsterdam for ‘Mind, Mood and Microbes’, the first international conference on microbiota-gut-brain axis. This unique congress not only brought together many disciplines ranging from psychiatry to microbiology, but also gained momentum for the increasing interest and growing insights into the fascinating influence of gut microbiota on brain function.

“The enormous influence of the microbiome is crystal clear when we realize that humans are genetically > 99% microbe, and < 1% human,” Prof. Dr. John Cryan (University College Cork, Ireland) told the audience. During our lifetime, the microbiome influences various aspects of wellbeing. For some decades, it has been known that the microbiota can influence for instance certain conditions like inflammatory bowel disease (IBD), but now it appears that they are even a key factor in the development of the brain and profoundly influence brain functioning.

Influence of microbes on psychiatric disorders
Debilitating conditions such as Parkinson’s disease (PD), bipolar disorder, Alzheimer’s disease (AD), schizophrenia and traumatic brain injury might seem very different but they share similar traits and symptoms like apathy, low mood and cognitive dysfunction. Some of the underlying mechanisms of these brain disorders are also shared with a decreased connectivity between brain areas seen in almost all brain disorders. Interventions with microbiota (diet and probiotics) possess an enormous potential in the possible treatment of these disorders as microbiota is able to impact on these mechanisms. This is also supported by extensive research in rodents. It has been shown in various studies that germ-free mice exhibit deviant social behaviour and stress response compared to normal mice as well as showing different brain development (smaller brain size). Their microbiota can be altered and ‘reset’ which results in normalized behaviour. It should be emphasized though that only live bacteria have proven to be effective; heat-killed bacteria do not have the same effects.

Parkinson’s disease and multiple sclerosis
Research showed that the intestinal tract can also be considered a target for Parkinson’s Disease (PD), as Prof. Dr. Aletta D. Kranenfeld (Utrecht University, the Netherlands) explained. This is due to alterations of the intestinal barrier, changed intestinal microbiome composition as well as gut microbiota-central nervous system (CNS) communication and low grade (intestinal) inflammation associated with immune deficits. A publication in Cell the day before the symposium demonstrated that gut microbes promote α-synuclein-mediated motor deficits and brain pathology. Also, depletion of gut bacteria reduces microglia activation, short-chain fatty acids (SCFA) modulate microglia and enhance PD pathophysiology. Administration of human gut microbiota from PD patients induce enhanced motor dysfunction in mice. Other research has shown that astrocytes play important roles in the CNS during health and disease; type I interferons (IFN-I) signaling in astrocytes limits neurodegeneration through a mechanism mediated by the ligand-activated transcription factor AhR and SOCS2. In multiple sclerosis (MS) patients, AhR agonists were decreased which led to the hypothesis that decreased AhR signaling may act as a potential contributor to disease pathogenesis in MS.
Autism
Autism is another example of a disease in which microbiota play a key role. The cascade of events in early life (starting from pregnancy onwards) with regard to brain development emphasize the need to acknowledge and actively address the window of opportunities. Mouse models have demonstrated that the offspring of mice who were fed a maternal high-fat diet (MHFD) are impaired in reciprocal social interactions as well as in social conditioned place preference. They also have significant long-term dysbiosis of the gut microbiome. Faecal transplantation with ‘healthy’ bacteria normalizes their social behaviour. Moreover, it is striking that gastrointestinal (GI) problems occur frequently in children suffering from neurodevelopment disorders such as autism and attention-deficit hyperactivity disorder (ADHD) with estimations of up to 90%. These findings add to the hypothesis that the gut-brain axis is of key importance in these disorders. Research in germ-free mice shows that microbiota influence behavior, stress circuitry, stress responsively and brain structure, as Prof. Dr. Jane Foster (McMaster University, Canada) pointed out. “Host genetics and environment factors influence microbiota composition and diversity, and deciphering the molecular mechanisms involved is necessary to advancing the use of microbiota-targeted therapies for use in clinical populations.”

Food for thought
The major modulator of gut microbiome variation is diet, and food items can make specific changes. For instance, milk protein/gluten-free diet has demonstrated to improve autistic behavior. Reduced *Bifidobacterium* species were found at the age of 6 months in children later diagnosed with ADHD/Autism Spectrum Disorder (ASD). Early life supplementation with probiotics was shown to increase the number of *Bifidobacterium* species and lower incidence. Taking these findings into account, dietary counseling as part of a mental health treatment plan can be seen as a realistic step towards a more holistic view of therapy. Research from Australia suggests that altering nutrition could even treat major depression. “Bacteria which may influence the capacity to deal with stress, reducing anxiety, and perhaps positively impacting on mood are called psychobiotics,” Prof. Dr. Ted Dinan (University College Cork, Ireland) said. “Psychobiotics have potential in managing stress-related conditions and may improve cognition. We need more translational studies in this field to gain more insight.” Prebiotics and probiotics will have a role as adjunctive therapies, Dr. Phil Burnet (University of Oxford, UK) concluded, but they will certainly not replace treatment with medication. A Dutch study assessing the effect of a multispecies mixture of probiotics on cognitive reactivity to sad mood showed a reduction in vulnerability to develop depression, in particular aggressive and ruminative thoughts were improved. Further studies in high risk populations are needed to establish potentially clinical relevant effects in prevention and enhancement of well-being, but so far, the future looks promising. In years to come, much more of the mysteries surrounding mind, mood and microbes will be unraveled. Bringing the science and healthcare profession together is key to unravel the mysteries surrounding mind, mood and microbes in the future. The first steps are made during this successful conference. As initiating partner Winclove declares this will most definitely not be the last Mind, Mood & Microbes.

The content of the scientific summary is written by Constance de Koning from MedScope, a freelance medical/science writer who has worked in healthcare worldwide. www.medscope.nl

This report has been made possible by Winclove Probiotics
# CONTENT

## THURSDAY

### Keynote presentations

<table>
<thead>
<tr>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mind mood and microbes, looking across diagnostic borders</td>
<td>Prof. Dr. Iris Sommer, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands</td>
<td>7</td>
</tr>
<tr>
<td>A gut feeling about the brain: microbes as key regulators of neurodevelopment and behaviour across the lifespan</td>
<td>Prof. Dr. John F. Cryan, Department of Anatomy and Neuroscience, University College Cork, Ireland</td>
<td>8</td>
</tr>
</tbody>
</table>

### Basic and mechanistic research on the gut-brain axis

<table>
<thead>
<tr>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut feelings; molecular clues to a gut-brain axis</td>
<td>Prof. Dr. Wouter de Jonge, Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, the Netherlands</td>
<td>9</td>
</tr>
<tr>
<td>The impact of gut microbiota on adult brain plasticity</td>
<td>Dr. Grégoire Chevalier, Department of Immunology, Institut Pasteur, France</td>
<td>10</td>
</tr>
<tr>
<td>Modulation of adult neurogenesis by the microbiome; with a little help from the immune system</td>
<td>Dr. Susanne Wolf, Department of Cellular Neuroscience, Max Delbrück Center for Molecular Medicine, Germany</td>
<td>11</td>
</tr>
<tr>
<td>Neurodegenerative diseases and microbes: the gut immune brain axis in Parkinson's disease</td>
<td>Prof. Dr. Aletta D. Kraneveld, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands</td>
<td>12</td>
</tr>
<tr>
<td>Precision microbiome reconstitution reverses maternal diet-induced social behavioural and synaptic deficits in offspring</td>
<td>Dr. Mauro Costa-Mattioli, Departments of Neuroscience and Molecular &amp; Cellular Biology, Baylor College of Medicine, USA</td>
<td>13</td>
</tr>
<tr>
<td>Food, mood, drugs, and their relation with the gut microbiome</td>
<td>Dr. Alexandra Zhernakova, Department of Genetics, University Medical Center Groningen, the Netherlands</td>
<td>14</td>
</tr>
<tr>
<td>Potential new mechanisms by which microbiota affect CNS function</td>
<td>Dr. Rochellys Diaz Heijtz, Department of Neuroscience, Karolinska Institutet, Sweden</td>
<td>15</td>
</tr>
</tbody>
</table>
## Applied research in the field of the gut-brain axis

<table>
<thead>
<tr>
<th>Topic</th>
<th>Author and Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut microbiota related in Parkinson's disease</td>
<td>Dr. Filip Scheperjans, Department of Neurology, Helsinki University Hospital &amp; Department of Clinical Neurosciences, University of Helsinki, Finland.</td>
</tr>
<tr>
<td>How connections between microbiota and brain circuitry influence anxiety and depression</td>
<td>Prof. Dr. Jane Foster, Department of Psychiatry and Behavioural Neurosciences, McMaster University, Canada</td>
</tr>
<tr>
<td>Microbiota and immune system interactions in a gut-brain model of schizophrenia</td>
<td>Dr. Emily Graves Severance, Department of Pediatrics, Johns Hopkins University School of Medicine, USA</td>
</tr>
<tr>
<td>Food for thought: gut-immune-brain axis in autism</td>
<td>Prof. Dr. Aletta D. Kraneveld, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands</td>
</tr>
<tr>
<td>Gut microbiome studies in behaviour, cognition and neurodevelopment</td>
<td>Dr. Alejandro Arias Vásquez, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, the Netherlands</td>
</tr>
<tr>
<td>Role of gut microbiota and intestinal permeability in alcohol dependence</td>
<td>Dr. Sophie Leclerq, Louvain Drug Research Institute, Université catholique de Louvain, Belgium</td>
</tr>
</tbody>
</table>

## Speed presentations – selected posters

<table>
<thead>
<tr>
<th>Poster ID</th>
<th>Title</th>
<th>Author and Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4</td>
<td>Gut microbiota dysbiosis in a mouse model of Alzheimer's disease: impact on cognitive performance</td>
<td>Sylvie Claeysen, Université de Montpellier, France</td>
</tr>
<tr>
<td>P14</td>
<td>Investigation of the molecular mechanisms by which butyrate affects gut-brain axis function</td>
<td>Ashley N. Hutchinson, Örebro University, Sweden</td>
</tr>
<tr>
<td>P25</td>
<td>Relation between mood and the gut microbiota metabolite indole: results from the NutriNet-Santé study</td>
<td>Catherine Philippe, Université Paris-Saclay, France</td>
</tr>
<tr>
<td>P34</td>
<td>A new bacterium that increases blood levels of tryptophan and tyrosine produces significant alterations in the brain serotonin and dopamine systems</td>
<td>Gonzalo Torres, University of Florida</td>
</tr>
<tr>
<td>P39</td>
<td>Maternal prenatal stress is associated with the infant intestinal microbiota</td>
<td>Maartje A.C. Zijlmans, Radboud University, the Netherlands</td>
</tr>
</tbody>
</table>
## FRIDAY

**The gut/brain axis targeted interventions as novel therapeutical strategies**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Presenter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of CNS inflammation</td>
<td>Dr. Francisco Quintana, Harvard Medical School and The Broad Institute, USA</td>
<td>23</td>
</tr>
<tr>
<td>The therapeutic potential of targeting diet and the gut microbiota in brain and behaviour</td>
<td>Sarah R. Dash, IMPACT Strategic Research Centre, Deakin University, Australia</td>
<td>24</td>
</tr>
<tr>
<td>Brain-gut-microbiota axis as a novel therapeutic target</td>
<td>Prof. Dr. Ted Dinan, Department of Psychiatry and APC Microbiome Institute, University</td>
<td>25</td>
</tr>
<tr>
<td>Prebiotic modulation of emotional and cognitive function in rodents and humans</td>
<td>Dr. Phil Burnet, Department of Psychiatry, University of Oxford, UK</td>
<td>26</td>
</tr>
<tr>
<td>Pioneering probiotic clinical application in gut-brain axis: a historical perspective</td>
<td>Dr. Stéphanie-Anne Girard, Lallemand Health Solutions, Canada</td>
<td>27</td>
</tr>
<tr>
<td>Probiotics to reduce depression vulnerability: multispecies probiotics reduces cognitive reactivity to sad mood</td>
<td>Dr. Laura Steenbergen, Institute of Psychology, Leiden University, the Netherlands</td>
<td>28</td>
</tr>
</tbody>
</table>
Mind, Mood and Microbes, Looking across diagnostic borders

Brain disorders; shared complaints, shared mechanisms, shared solutions?

Prof. Dr. Iris Sommer (Brain Center Rudolf Magnus University Medical Center Utrecht, the Netherlands)

When we evaluate the complaints of patients with a brain disorder (with any brain disorder), the symptoms most bothersome are apathy, gloomy mood and cognitive difficulties. These three symptom clusters render patients isolated, bored and lonesome, yet few treatments are aimed at these three very nonspecific symptoms. As these symptoms are rather similar across brain disorders, the mechanism behind apathy, low mood and cognitive dysfunction may also be shared across disorders. Conditions such as Parkinson’s disease (PD), bipolar disorder, multiple sclerosis (MS), Alzheimer’s disease, Gilles de la Tourette, schizophrenia, Huntington’s disease, traumatic brain injury and obsessive compulsive disorder might seem very different but they share similar traits. Brain disorders are complex and involve multiple domains, such as movement, emotions cognition, and personal flexibility. The complaints start slow and remain; they can be various: fatigue, low mood, instable emotions, increased sensitivity to stress, slow and less flexible cognition. Some of the underlying mechanisms of these brain disorders are also shared. In most brain disorders, there is increased activity of the immune system, problems with glucose metabolism and many growth factors are decreased such as brain-derived neurotrophic factor (BDNF). Finally, a decreased connectivity between brain areas is seen in almost all brain disorders.

Patients with these disorders can be supported by providing mild preventive strategies in early stages before a diagnosis can be made. Also, more attention for general complaints such as fatigue and social isolation, protecting the healthy brain and develop new interventions for people with brain disorders can help. The future holds new treatment options such as deep brain stimulation, stem cell therapy, computer-brain interface, the use of monoclonal antibodies and gene therapy (for monogenetic disorders such as Huntington’s disease). Apart from these high-tech interventions, easy interventions can be considered such as food, microbiotic interventions, exercise and sports. Reducing stress and increasing relaxation is important as all these patients suffer those. Besides, reducing loneliness is key; humans are social animals and loneliness can be destructive to health. Preventing head injury as well as infections – as many brain disorders can be triggered by infections early in life – should also have priority.

KEY POINTS

- Very different brain disorders share similar complaints and possibly similar mechanisms
- Most brain disorders are characterized by increased activity of the immune system, problems with glucose metabolism, decrease of many growth factors (e.g. BDNF) and a decreased connectivity between brain areas
- Mild preventive strategies as well as more attention for general complaints, protecting the healthy brain and new interventions can be used to support patients
A gut feeling about the brain: microbes as key regulators of neurodevelopment and behaviour across the lifespan

Prof. Dr. John Cryan (University College Cork, Ireland)

Humans are genetically > 99% microbe, and < 1% human which emphasizes the enormous influence of the microbiome. Across a human’s lifespan, the microbiome continues to impact on the health and wellbeing of the individual. For instance, the microbiome in early life is hugely influenced by the mothers as vaginally born infants receive their microbes from their mother. For babies born by C-section, this is different as there is a change in microbiome in these children. Whether this persists into childhood is not quite fully understood. The Western populations have a depleted diversity of microbes from birth through childbearing years and are missing bacterial taxa present in traditional groups. In aging, the health of elderly people is directly related with the diversity of the microbiome which is associated with diet. We live in a stressful society, and the impact of stress on the brain in aging individuals adds fire to this process. Adolescence is an important period as this is the timeframe in which many brain disorders develop alongside with major changes in diet, social contact etc. The question remains: how does this work? It has been shown that the immune system plays a key role, alongside the microbiome as was demonstrated by experiments involving mice who lack microbiota. These were found to have abnormal brain development. They also showed social deficits which is similar to autism in humans. Microbiota are also involved in conditions such as irritable bowel disease (IBD). Furthermore, stress is regulating the microbiome and vice versa, which led to the concept of targeting the microbiome. The time has now come to move from research in animal models to humans.

KEY POINTS
• The microbiome has an enormous influence on humans as they are > 99% microbe
• Throughout one’s life, the microbiome continues to impact on the health and wellbeing of the individual
• Mice lacking microbiota have abnormal brain development and showed social deficits
• Stress regulates the microbiome and vice versa
• Research in humans is now needed to assess the impact of the microbiome in humans

References
Gut feelings; molecular clues to a gut-brain axis

Prof. Dr. Wouter de Jonge (Academic Medical Center, the Netherlands)

Irritable bowel disease (IBD) consists of Crohn’s disease and colitis ulcerosa (UC). The incidence of the condition in the United States (US) is 1:250 and the pathogenesis of the disease is multifactorial. It is a devastating disorder as it not only affects young people but also due to its chronic character. It is clear that inflammatory conditions are driven by the microbiome as was shown in 1958 by fecal transplantation by Eisenmann. The mucus layer in the gut is crucial to prevent colitis as it has been shown that intestinal barrier dysfunction drives colitis. Gut diversity needs to be stimulated and supported and perhaps other life forms in the gut – such as fungi of which there are many in the gut – need to be investigated as well. There seems to be a predominance of fungi which might mean that they do play a role. As approx. 50% patients have complaints of hypersensitivity but no difference in gut is visible could support this hypothesis. There is some evidence that (high percentage of C. albicans might play a role. It can be concluded that fungi trigger visceral hypersensitivity, and that an altered microbiome is present and relevant.

KEY POINTS
- Inflammatory conditions are driven by the microbiome with the intestinal barrier dysfunction driving colitis
- Fungi also play a role in the gut as does C. albicans
- In IBD, fungi trigger visceral hypersensitivity, and an altered microbiome is present and relevant in these patients

References
2. Botschuiver et al. (in revision 2016).
**The impact of gut microbiota on adult brain plasticity**

*Dr. Grégoire Chevalier (Department of Immunology, Institut Pasteur, France)*

The brain has long been considered a static organ. However, as neurogenesis takes place in the hippocampus, this is not the case. Microbiota decreases the neuronal progenitor proliferation, but it increases neurogenesis. The interaction with stress is important as stress is a very strong risk factor. Stress will eventually lead to decreased adult neurogenesis and can be restored with antidepressants. An experiment showed that chronic corticosterone treatment induces anxiety and depression-like states. It also decreases adult neurogenesis and induces dysbiosis. If microbiota from a depressed mouse is transplanted to a healthy mouse, this mouse also becomes depressed. This suggests the microbiota has a causal effect on depression, at least in rodents. New research focuses on the feedback loop of microbiota-depression-stress.

**KEY POINTS**

- The brain is not a static organ as neurogenesis takes place in the hippocampus
- Microbiota increases neurogenesis
- Stress negatively influences adult neurogenesis
- Microbiota is thought to have a causal effect on depression
Modulation of adult neurogenesis by the microbiome; with a little help from the immune system

Dr. Susanne Wolf (Max Delbrück Center for Molecular Medicine, Germany)

What happens with hippocampal neurogenesis in mice with altered microbiome? When healthy mice were given a cocktail of antibiotics for seven weeks, this led to decrease in all bacteria and a decrease in neurogenesis. Use of the running wheel is known to profoundly induce neurogenesis. This was also the case in the experiment in which the antibiotic treated mice were put on a running wheel. When the mice were given a cocktail of probiotics, this fully rescued neurogenesis in mice treated with antibiotics.

Changes in neurogenesis are accompanied by changes in memory performance as was shown by novel object recognition and dynamics in neurogenesis. Probiotic treated/normal healthy mice had higher numbers of Ly6Chi monocytes in the brain than mice treated with antibiotics. Elimination of Ly6Chi monocytes by antibody depletion or using knock-out mice led to decreased neurogenesis. Moreover, adoptive transfer of Ly6Chi monocytes rescued neurogenesis after antibiotic treatment. Thus, the Ly6Chi cells are possible messengers from the periphery to the central nervous system. It is suggested that the rescue of neurogenesis and behaviour deficits in mice treated with antibiotics by exercise and probiotics is partially mediated by Ly6Chi monocytes in the brain. Recently, the monocytes were also incorporated in reviews on potential mechanisms in the gut-brain axis. This research amplifies the importance of these cells in this communication.

KEY POINTS
- Changes in neurogenesis are accompanied by changes in memory performance
- Probiotic treated/normal healthy mice had higher numbers of Ly6Chi monocytes in the brain than antibiotic-treated mice
- Elimination of Ly6Chi monocytes led to decreased neurogenesis
- Transfer of Ly6Chi monocytes rescued neurogenesis after antibiotic treatment
- Ly6Chi cells are possible messengers from the periphery to the central nervous system
Neurodegenerative diseases and microbes: the gut immune brain axis in Parkinson’s disease

Prof. Dr. Aletta D. Kraneveld (Utrecht University, the Netherlands)

Parkinson’s disease (PD) affects 1:100 people aged > 60 years. The condition is characterized by motor symptoms (tremor, muscle rigidity, slowness of voluntary movement and postural instability) as well as non-motor dysfunction which include depression, loss of smell, dementia and intestinal dysfunction (in the early phase of the disease). The intestinal tract can be considered a target for PD; there are alterations of the intestinal barrier, changed intestinal microbiome composition as well as gut microbiota-central nervous system (CNS) communication and low grade (intestinal) inflammation associated with immune deficits. There is an increased intestinal permeability which correlates with α-Syn aggregates in intestinal tract. It could be hypothesized that PD is an inflammatory disease since these patients have higher levels of lipopolysaccharides (LPS) in the blood. LPS is a ligand for toll-like receptor 4 (TLR4) which is known to play a role in inflammation. Human data of PD in the gut showed that leaky gut is indeed a key factor alongside with enhanced TLR4+ cells. A changed microbiome, LPS exposure and subsequent TLR4-LPS association leading to leaky gut and bacterial translocation could lead to intestinal inflammation (α-Syn aggregation). Intestinal transit problems (constipation) appear, and it could be argued that a prion-like spread of α-Syn aggregates to the brain takes place, followed by TLR4-induced neuro-inflammation in the brain and dopaminergic cell loss (motor dysfunction). For instance, rotenone (a pesticide) decreased motor function and also reduced zonuline which is an indication of decreased gut-barrier function. This was also associated with reduced transit-time (constipation), which was not the case in TLR-4 knockout mice. Future studies should focus on microbiome and diet (microbiotic fibers) which improve the microbiome composition and activity, intestinal function and the L DOPA uptake, aiming at reduced neuro-inflammation.

KEY POINTS

- The intestinal tract is a target for PD due to various dynamic processes taking place in PD patients
- PD could be considered an inflammatory disease as high levels of lipopolysaccharides are present in patients’ blood
- Leaky gut and enhanced TLR4+ cells are key factors in PD

References

**Precision microbiome reconstitution reverses maternal diet-induced social behavioural and synaptic deficits in offspring**

Dr. Mauro Costa-Mattioli (Baylor College of Medicine, USA)

Autism is a group of developmental brain disorders, collectively called autism spectrum disorder (ASD). Core symptoms of ASD are social deficits, repetitive and stereotyped behaviours and communication difficulties. Potential causes include genetic factors, environmental factors (air pollution, viral infections, maternal challenge) and a combination of both. When both these factors are present, a synergistic action takes place. Over the last decades, an increase has been observed in the prevalence of both ASD and maternal obesity. Maternal obesity represents a significant mental health problem; a pre-pregnancy weight > 198 lbs (90 kg) increases ASD risk and also maternal obesity increases risk of ASD.

A mouse model showed that the offspring of mice who were fed a maternal high-fat diet (MHFD) were impaired in reciprocal social interactions as well as in social conditioned place preference. MHFD offspring also showed significant long-term dysbiosis of the gut microbiome. However, co-housing with age-matched maternal regular diet (MRD) mice normalized the social behaviour and microbiota of MHFD offspring. This positive effect is thought to be caused by ‘spontaneous feaces transplantation’, in other words: mice eating each other’s poo. Ratio between healthy and MFHD mice did not matter; one healthy mouse with 3 MFHD mice was enough for this effect to occur. Germ-free mice displayed social deficits resembling those of MHFD offspring, but when a fecal microbiota transplant from MRD - but not MHFD - donors was performed, it rescued the social behaviour by the germ-free mice. It was also shown that a single microbial treatment with live single probiotic strain (*L. reuteri*) reversed social behaviours in MHFD offspring. It is important to choose the right strain because some other Lactobacillus species were not able to induce this effect. It also seems necessary to work with live bacteria since heat-killed bacteria did not have the effect as well. Also, the reduced oxytocin levels in the MFHD hypothalamus were rescued by treatment with *L. reuteri*, and this treatment rescued social interaction-induced VTA (ventral tegmental area) plasticity in MFHD offspring. Intranasal oxytocin administration has the same effect. Oxytocin plays a role in social bonding. Thus, the working model is as follows: the offspring of mice who are fed a MHFD have altered gut microbiome, social behaviour, PVN oxytocin levels and VTA plasticity. Precision microbial restitution in MFHD offspring restores social behaviour, oxytocin levels and VTA plasticity. Ongoing questions are whether the effect of *L. reuteri* is direct, if *L. reuteri* could restore social behaviours in other ASD mouse models and by which mechanism *L. reuteri* triggers changes in the brain of MHFD offspring.

**KEY POINTS**
- Mice become less social when fed a high fat diet; their microbiota is different too
- Behaviour is normalized when microbiota is altered by cohousing with 1 normal mouse
- Only live bacteria are effective (heat-killed bacteria are not effective)
- Microbiota influences oxytocin which may explain autistic behaviour in germ-free mice

**References**
Many factors are influenced by the microbiome but other factors also influence the microbiome such as environment, diseases, medication and genetics. Thus, studying the microbiome is complex. Lifelines is a large observational follow-up study with a total of 165,000 individuals (3 generation design) and a duration of 30 years. Of these, 150 were randomly selected and extensively studied/assessed. An analysis of multiple factors in relation to microbiome was made through which 18.7% of inter-individual variation in gut microbiome could be explained. The results become increasingly interesting because when compared to a large cohort studies that runs parallel in Belgium approximately 90% of the results were in line with each other. The main finding was that diet is the major modulator of gut microbiome variation; food items can make specific changes. Proton pump inhibitors (PPI) had one of the most profound effects; they massively change the microbiota composition. PPI leads to an increase of species normally found in the oral cavities in the gut. Since PPI lower the production of gastric acid, this is in some way logical but it is not a positive sign. Antidepressants also have a strong effect on microbiota. Significant changes were seen in the abundance of 92 bacteria which was replicated in 3 cohorts. Of the diseases associated with microbiome composition, irritable bowel disease (IBS) and depression were most strongly correlated. The quality of life in IBS patients is comparable with that in patients suffering from depression and cancer. To learn optimally from these studies as well as developing interventions, it is very important to distinguish between subgroups.

**KEY POINTS**

- Large observational study assessing what influences the microbiota showed similar results as an equivalent study conducted at the same time in Belgium
- Proton pump inhibitors (PPI) had one of the most profound effects on microbiota
- Antidepressants also have a strong effect on microbiota

**References**

Potential new mechanisms by which microbiota affect CNS function

Dr. Rochellys Diaz Heijtz (Karolinska Institutet, Sweden)

Neurodevelopment disorders such as autism spectrum disorder (ASD; 1.3%) and attention-deficit hyperactivity disorder (ADHD; 5-7%) have an etiology which is poorly understood. It is remarkable that gastrointestinal (GI) problems are very common in ASD with estimations of up to 90% of patients suffering. Although there is no clear consensus, atypical gut microbiota is present. Excessive use of oral antibiotics is thought to contribute as does a genetic association between variation in the c-MET gene and autism. Microbiota-modulating based therapeutic interventions may have potential beneficial effects in these patients. Research from the Karolinska Institut in 2011 showed that gut microbes act as environmental agents that shape normal brain development and behaviour. Critical in this process is that there is a sensitive period early in life in which reverse of changes was possible. The key question remains what the mechanisms are. It can be stated that there are multiple mechanisms and that they are poorly understood. It was demonstrated that bacterial peptidoglycan (PGN) ‘motifs’ derived from the commensal gut microbiota can translocate into the brain. There seems to be an age-dependent increase in PGN levels paralleling the postnatal bacterial colonization process. Two families of pattern recognition receptors (PRRs) of the innate immune system that specifically detect PGN are highly expressed in the developing brain during specific windows of postnatal development. PGN-sensing molecules are sensitive to manipulations of the gut microbiota and absence of PGN-recognition protein 2 leads to alterations in the expression of the autism risk gene c-MET, and sex-dependent changes in social behaviour. These findings suggest that the central activation of PRRs by microbial products could be one of the signaling pathways mediating the communication between the gut microbiota and the developing brain. Moreover, given the fact that PRRs seem to play a dual role in immunity and neural development, it could be speculated that disruption of the gut microbiota may render the developing brain not only more susceptible to neurodevelopmental disorders, but also increase the risk for immune disturbances. Developing more sophisticated methods to determine the specific type of PGN fragments that are found in the developing brain remains is one of the current challenges. Bearing those findings in mind, the time has now come to move from research in animal models to research in children with neurodevelopmental disorders.

KEY POINTS
- GI symptoms are frequently seen in autism; the severity of autism depends on GI symptoms
- In adult life, this could not be reversed anymore
- Gut microbes act as environmental agents shaping brain development and behavior
- Central activation of PRRs by microbial products could be one of the signaling pathways mediating communication between gut microbiota and the developing brain

References
Gut microbiota related in Parkinson’s disease

Dr. Filip Scheperjans (Helsinki University Hospital, Finland)

Parkinson’s Disease (PD) is the second most frequent neurodegenerative disease, affecting > 1% of people over 65 years. Motor symptoms usually start between 40-70 years of age, and constipation complaints long before diagnosis are considered a risk factor as is loss of smell. The etiology is unknown but is considered to be a combination of genetic vulnerability and environmental factor(s). The concept of the disease has been changed from a neurological to a systemic disease. Signs of oxidative stress and invasion of bacteria in the colonic mucosa are seen in PD and one of the hypothesis is low grade inflammation in the gut of which the origin is unknown. This produces α-synuclein which triggers a cascade of events affecting the brain stem and spinal cord. The Helsinki study was a frequency-matched case-control study with 72 PD patients and 72 healthy controls. The main and sensitive finding was that Prevotellaceae was reduced by approx. 70% in PD, with an increase in abundance of Verrucamicrobiaceae. The correlation with symptoms of the patients was studied by comparing two groups: the tremor dominant (TD) and postural instability and gait difficulty (PGID) phenotype which showed that Enterobacteriaceae were increased in PGID group. The Chicago study – a case-control study with 38 PD patients and 34 controls – had different results. A higher abundance of inflammatory bacteria and lower abundance of Prevotellaceae was seen. A third study from Japan with 52 PD patients and 36 spouses found that hygiene possibly plays a role in the development of PD. A German study in 34 PD patients and 34 controls showed higher abundance of Enterobacteriaceae and (for the first time) short-chain fatty acids (SCFA) were assessed. It can be concluded that the 4 case-control studies are incongruent, using different recruitment criteria, different sequencing approaches and different analysis pipelines and statistics.

Very recently, a study by Sampson et al. was published in Cell which demonstrated that gut microbes promote α-synuclein-mediated motor deficits and brain pathology. It was also shown that depletion of gut bacteria reduces microglia activation, that SCFAs modulate microglia and enhance PD pathophysiology and that human gut microbiota from PD patients induce enhanced motor dysfunction in mice. In summary, an interaction between α-synuclein expression and gut microbiota is implicated in pathology, microglia activation, parkinsonian symptoms and colonic dysfunction in an α-synuclein overexpression mouse model of PD. SCFAs promote neuro-inflammation and motor deficits in the model as does transfer of PD patient microbiota, which confirms the relevance of neuro-immunological and metabolic pathways in the microbiome-PD connection. However, α-synuclein overexpression is exceedingly rare in PD patients and so far, SCFAs have found to be reduced in PD and are considered mainly beneficial for human health. Further analysis of functional aspects of microbiota and interactions with the host and diet in different stages of PD are needed.

KEY POINTS
- Patients with Parkinson’s disease (PD) have different intestinal microbiome
- Signs of oxidative stress and invasion of bacteria in the colonic mucosa are seen in Parkinson’s disease
- Published in Cell the day before this presentation:
  - gut microbes promote α-synuclein-mediated motor deficits and brain pathology
  - depletion of gut bacteria reduces microglia activation
  - SCFAs modulate microglia and enhance PD pathophysiology
  - human gut microbiota from PD patients induce enhanced motor dysfunction in mice

References
2. Keshavarzian et al. Mov Disord 2015;Sep;30(10):1351-60.
4. Scheperjans et al.
How connections between microbiota and brain circuitry influence anxiety and depression

Prof. Dr. Jane Foster (McMaster University, Canada)

Several central nervous system (CNS) genes altered in germ-free mice are known to influence anxiety-like behaviour. Findings in germ-free mice point out that microbiota influence behaviour, stress circuitry, stress responsively and brain structure.\(^1\)\(^2\) Germ-free mice brains are much smaller than those of specific pathogen-free mice with altered brain volume in key stress regions in germ-free mice. Host genetics contribute significantly to composition of the microbiome and host genetics influence behaviour. A regional-specific influence of host genetics on stress-related brain regions is suggested by research in this field. Some specific taxa are associated with different strains of mice, such as *Akkermansia* and *Lactobacilli*. Summarizing, evidence from animal models shows that microbiota influences behaviour and brain structure. Host genetics and environment factors influence microbiota composition and diversity, and deciphering the molecular mechanisms involved is necessary to advancing the use of microbiota-targeted therapies for use in clinical populations.

**KEY POINTS**

- Germ-free mice brains are much smaller than specific pathogen-free mice
- Microbiota influences behaviour and brain structure
- Host genetics and environment factors influence microbiota composition and diversity

**References**

2. Lyte et al 2006 450-3576.
Microbiota and immune system interactions in a gut-brain model of schizophrenia

Dr. Emily Graves Severance (Johns Hopkins University School of Medicine, USA)

Candida albicans, a diploid yeast, is increased in patients with schizophrenia. A study into the effects of probiotics showed that the levels of C. albicans decreased after the use of probiotics; probiotics may help to normalize C. albicans antibody levels and associated gut discomfort in schizophrenic patients. C. albicans seropositivity is associated with worse performance on Positive and Negative Syndrome Scale (PANSS) assessment of psychiatric symptoms in schizophrenia. C. albicans exposure status may be an important inclusion criterion for studies involving the microbiome and probiotics.

Toxoplasma gondii is a neurotropic Apicomplexan pathogen entering body through the gastrointestinal (GI) tract. Exposure to T. gondii is elevated in people with schizophrenia compared to controls, and is associated with elevated GI inflammation in schizophrenia. T. gondii exposure is associated with the production of N-methyl-D-aspartate receptor (NMDAR) antibodies in schizophrenia and experimental mouse models. T. gondii exposure compromises blood-gut and blood-brain barrier integrities in schizophrenia and experimental mouse models. Combined T. gondii-NMDAR antibody seropositivity negatively impacts cognition. Previously conflicting evidence for a role of pathological NMDAR antibodies in schizophrenia might be reconciled by identifying as an inclusion criterion the past exposure to T. gondii.

KEY POINTS
- Probiotics may help to normalize C. albicans antibody levels and associated gut discomfort in schizophrenic patients
- Exposure to T. gondii is elevated in schizophrenic patients compared to controls, and is associated with elevated GI inflammation in schizophrenia

References
Food for thought: gut-immune-brain axis in autism

Prof. Dr. Aletta D. Kraneveld (Utrecht University, the Netherlands)

The gut-brain axis in autism manifests itself by a changed microbiome composition, leaky gut and abdominal problems. Various studies have been conducted but the outcomes are not consistent. There is a variety of early life immune events linked with autism, such as the early life manifestation of allergic disease which is associated with deficits in neurodevelopment and behaviour. Maternal infection during pregnancy is a risk for neurodevelopmental disorders and disturbed brain maturation and neurodevelopmental impairment is found in preterm born infants with neonatal infection. Preterm gut microbiome is implicated in the pathogenesis of necrotizing enterocolitis (NEC) and NEC survivors are at higher risk for neurodevelopmental disorders. Early life antibiotics are associated with autism (including during pregnancy). The first 1,000 days of life – starting already in utero – are of utmost importance for immunity development. The critical period for autism is early life when events have a high impact on development.

Food allergy is associated with changed microbiota, and parenteral reports indicate that food allergy in autism is common. Milk protein/gluten-free diet improves autistic behaviour (cow’s milk allergic mice exhibit autistic-like behaviour). A study with early life probiotics in 75 children in which 40 received Lactobacillus rhamnosus GG and 35 received placebo < 6 months and follow-up at 13 year, showed reduced Bifidobacterium species in ADHD/ASD (at 6 months).

KEY POINTS
- The first 1,000 days of life are of utmost importance for immunity development
- Food allergy is associated with changed microbiota
- Food allergy in autism is common
Gut microbiome studies in behaviour, cognition and neurodevelopment

Dr. Alejandro Arias Vásquez (Radboud University Medical Centre, the Netherlands)

Behaviour is the common denominator for various psychiatric disorders such as schizophrenia, attention-deficit hyperactivity disorder (ADHD), major depression, bipolar disorders and autism. Human ADHD gut microbiota includes anxiety, a common comorbidity of ADHD, and alters brain structure and connectivity in regions known to be altered in human ADHD patients. Differences in gut microbiome structure exist between ADHD cases and controls. Relative abundance of a dopamine-related microbial gene that differed between ADHD cases and controls was associated with altered reward anticipation responses in the nucleus accumbens which is one of the neural hallmarks of ADHD. It can be concluded that neurodevelopment diseases such as ADHD/autism spectrum disorder (ASD) should no longer be considered brain disorders exclusively, but rather disorders that present as the effect of interactions between the microbiome, the gastro-intestinal (GI) system, diet and the brain. Investigations into how the microbiome may be used in innovative treatment strategies to improve ADHD/ASD outcome in diet sensitive patients should be initiated.

KEY POINTS
- There are differences in gut microbiome structure between ADHD cases and controls
- Neurodevelopment diseases like ADHD/ASD should be considered disorders that present as the effect of interactions between the microbiome, the GI system, diet and the brain
Role of gut microbiota and intestinal permeability in alcohol dependence

Dr. Sophie Leclerq (Université catholique de Louvain, Belgium)

Alcohol abuse has social health and economic consequences. 92% of alcohol dependent (AD) users do not receive treatment, due to stigma, lack of effectiveness of pharmacological interventions. Current treatment is limited and relapse rate is high (up to 70%). In alcohol dependence, ethanol represents approx. 40% of caloric intake. Depression, anxiety and craving are seen in AD subjects. A Belgian study assessed if intestinal permeability (IP) is induced by alcohol dependence in these subjects. The bacterial profile is altered only in AD subjects with increased IP, which supports the hypothesis that the microbial composition may influence the gut-barrier function. The IP correlates negatively with amounts of bifido’s; high permeability with less bifido’s. Indole seems to have a positive effect on gut-barrier function. Indole can be converted from tryptophan by some bacterial species.

Thus, some – but not all – patients developed leaky gut and AD subjects with high IP have altered gut microbiota. The gut barrier function is related to the psychological symptoms of alcohol dependence at the end of detoxification. So, some, but certainly not all, AD subjects develop gut leakiness (there is no direct toxicity of ethanol on intestinal epithelial cells). A trend was observed that the AD subjects tending to use more spirits had more often impaired gut-barrier function. Bacteria and/or bacterial metabolites may be involved in the regulation of the gut barrier function. AD subjects with gut dysfunction have higher scores of depression, anxiety and alcohol craving at the end of detoxification. This could possibly influence the probability of relapse through a negative reinforcement mechanism. Remarkably, the psychological symptoms did not improve in the AD subjects who had a higher intestinal permeability at the start of detoxification, in contrast to the ones with normal permeability.

KEY POINTS
- Bacterial profile is altered only in alcohol dependent subjects with increased intestinal permeability
- Some alcohol dependent subjects develop gut leakiness
- Alcohol dependent subjects with gut dysfunction have higher scores of depression, anxiety and alcohol craving at the end of detoxification
Table 1 Key posters

<table>
<thead>
<tr>
<th>Title</th>
<th>Conclusion</th>
</tr>
</thead>
</table>
| Gut microbiota dysbiosis in a mouse model of Alzheimer's disease: impact on cognitive performance (Sylvie Claeysen) | - It is suggested that the composition of the intestinal microbiota is altered in a mouse model of Alzheimer’s disease and that this dysbiosis is deleterious to the cognitive performance of the animals  
  - It has also been shown that intestinal microbiota from aged mice reduces memory function of the transplanted animals  
  - Intestinal dysbiosis may influence the progression of Alzheimer’s disease |
| Investigation of the molecular mechanisms by which butyrate affects gut-brain axis function (Ashley N. Hutchinson) | - Exposing fibroblasts to oxidative stress and pro-inflammatory cytokines – two pertubations implicated in neuropsychiatric disorders – results in decreased tryptophan transport  
  - Butyrate treatment rescues oxidative stress-induced deficits in tryptophan transport  
  - Butyrate treatment alone does not significantly impact tryptophan transport  
  - These results suggest altering tryptophan transport is a potential mechanism by which butyrate affects CNS function |
| Relation between mood and the gut microbiota metabolite indole: results from the NutriNet-Santé study (Catherine Philippe) | - Urinary indoxylsulfate concentrations were significantly higher in women with recurrent depressive symptoms  
  - These results strongly suggest that indole production by the gut microbiota may play a role in the onset of mood disorders; however, the underlying mechanisms need to be investigated |
| A new bacterium that increases blood levels of tryptophan and tyrosine produces significant alterations in the brain serotonin and dopamine systems (Gonzalo Torres) | - Germ-free mice injected with PUF1 bacteria had reduced expression of SERT and TPH2 in the prefrontal cortex  
  - Compared to PBS controls, germ-free mice injected with PUF1 bacteria had reduced expression of DAT, TH and VMAT2  
  - Serotonin levels did not change after PUF1 treatment - but serotonin turnover was increased in the prefrontal cortex  
  - In the case of dopamine, total levels were unchanged with PUF1 treatment but dopamine turnover decreased  
  - These results support the hypothesis that PUF1 injection in germ-free mice results in profound neurochemical changes in the brain dopamine and serotonin systems |
| Maternal prenatal stress is associated with the infant intestinal microbiota (Maartje A.C. Zijlman) | - There is no clear pattern in intestinal microbiome in women who experience high stress  
  - High prenatal adversity related to infant intestinal microbiota and health as follows:  
    1) increase in proteobacteria  
    2) decrease in lactic acid bacteria and Actinobacteria  
    3) increase in gastrointestinal and allergic symptoms |
Regulation of CNS inflammation

Dr. Francisco Quintana (Harvard Medical School and The Broad Institute, USA)

Astrocytes play important roles in the central nervous system (CNS) during health and disease. Identification of factors that regulate astrocyte activity may shed light on CNS physiology and guide new therapies for human neurologic disorders. Through genome-wide analyses, a transcriptional response to type I interferons (IFN-I) in astrocytes during experimental CNS autoimmunity and also in CNS lesions from multiple sclerosis (MS) patients was observed. IFN-I signaling in astrocytes limits neurodegeneration through a mechanism mediated by the ligand-activated transcription factor aryl hydrocarbon receptor (AhR) and suppressor of cytokine signaling 2 (SOCS2). Dietary tryptophan is metabolized in to AhR agonists by commensal bacteria in the gut. AhR is upregulated in astrocytes in MS lesions. Moreover, it was found that AhR agonists derived from dietary tryptophan by the metabolism of the commensal flora and the host act on astrocytes to limit CNS inflammation. AhR agonists, however, were decreased in MS patients. Decreased AhR signaling in MS patients may act as a potential contributor to disease pathogenesis. In summary, an IFN-I/AhR axis was identified that integrates immunologic, metabolic and environmental signals to regulate astrocyte activity and provides potential therapeutic targets.

KEY POINTS
- Astrocytes play important roles in the central nervous system during health and disease
- IFN-I signaling in astrocytes limits neurodegeneration through a mechanism mediated by the ligand-activated transcription factor AhR and SOCS2
- AhR agonists were decreased in MS patients
- Decreased AhR signaling may act as a potential contributor to disease pathogenesis in MS

References
The therapeutic potential of targeting diet and the gut microbiota in brain and behaviour

Sarah R. Dash (Deakin University, Australia)

The departure from traditional lifestyles and the rising disease burden of mental disorders are growing global health concerns. People are now increasingly reliant on highly processed, nutrient-poor foods, which have been linked to changes in gut microbial profiles as well as increased risk for mental disorders. Given its association with both diet and mental health, the gut microbiota has been proposed as a key mediator of this link, and experimental evidence highlights the role of microbes in regulating pathways that are key to mood and behaviour. Diet is important because the exposure is 100% (everyone eats) and it is modifiable. What you eat in the long time hugely influences the gut microbiome profile. Experiments with germ-free mice have offered evidence of the influence of microbiota. Several critical ‘windows of opportunity’ for prevention and intervention have been identified such as early life. For instance, pregnant women are more highly motivated to make nutritional changes. Also, diet and gut in childhood and adolescence are important; research in Australia showed that > 95% of Australians do not meet requirements for sufficient intake of vegetables and fruit each day. These time frames are periods of rapid development and transition that lay a foundation for future health. Adolescents have a little more to say in what they eat; they may move out and this is also a time when mental disorders generally emerge. Further, microbial dysbiosis has been associated with various mental disorders. Emerging evidence supports that dietary strategies that promote gut health may be a key aspect of psychiatric treatment. The first randomized controlled trial (RCT) which was done in food and mental health was a dietary intervention for adults with major depression, conducted in 2012. This was a trial in which a 3 month dietary intervention vs befriending control (n = 67) was compared. The results showed that the patients in the dietary intervention group significantly improved compared to the control group. Higher adherence to the diet was associated with higher symptom improvement.

More intervention studies are needed as is more insight into the mechanisms. However, a healthy diet should be promoted nevertheless (more fibres and less ‘extra’s’) as this is a low grade intervention and relatively easy to implement. The critical time points in life need to be acknowledged and collaboration between nutritionists and gastroenterologists, psychiatrists and dieticians enhanced. This can be done by dietary guidance for pregnant women, public health policy and an emphasis on food skills and nutrition education. Dietary counseling as part of a mental health treatment plan should also be taken into account.

KEY POINTS
- Gut microbiota have been proposed as a key mediator of the link between diet and mental health
- Long time consumption of what we eat hugely influences the gut microbiome profile
- Dietary strategies promoting gut health may be a key aspect of psychiatric treatment
- First study in major depression showed that dietary intervention significantly improved symptoms as did higher adherence to the diet

References
Brain-gut-microbiota axis as a novel therapeutic target

Prof. Dr. Ted Dinan (University College Cork, Ireland)

There is increasing evidence suggesting that gut microbes may be involved in neural development and function, both peripherally in the enteric nervous system and centrally in the brain. While evidence is still limited in psychiatric illnesses, there are rapidly coalescing clusters of evidence which point to the possibility that variations in the composition of gut microbes may be associated with changes in the normal functioning of the nervous system. Studies in germ-free animals indicate aberrant development of the brain monoaminergic system together with memory deficits and autistic patterns of behaviour. These deficits can be partially normalized if there is early gut colonization. There are marked differences in the gut microbiota between patients with major depression and healthy controls. There is a decrease in abundance and diversity in patients with major depression. Following a cocktail of antibiotics, a faecal microbiota transplant was performed in rats with faeces from depressed patients or healthy controls. Those rats receiving a transplant from depressed patients were anhedonic and showed anxiety-like behaviour. Also, the c-reactive proteins (CRP) and tryptophan levels in the transplanted mice became similar to depressed patients.

Bacteria may influence the capacity to deal with stress, reducing anxiety, perhaps positively impacting on mood and are now called psychobiotics. Whether, they are capable of acting like and in some circumstances replacing antidepressants remains to be seen. The mechanisms of psychobiotic action are gradually being unravelled. It has been show that Lactobacillus rhamnosus has potent anti-anxiety effects in rodents and does so by producing major changes in the expression of gamma-aminobutyric acid (GABA) receptors in the brain. The changes in these receptors are mediated by the vagus nerve, which connects the brain and gut. When this nerve is severed by vagotomy, no effect on anxiety or on GABA receptors is seen following psychobiotic treatment. A placebo-controlled crossover study of L. rhamnosus in healthy subject did not bring any effects to light. B. Longum seemed to have anti-anxiety effects and cognitive enhancing effects (improved learning) in mice. In human administration of this bacterial strain led to lower stress levels both subjective and objective (morning cortisol levels).

Communication between the brain and gut is bidirectional and complex. Increased understanding of this axis and the role of the gut microbiota may aid the development of therapies, not just for functional bowel disorders but for mood disorders also. It was concluded that the brain-gut-microbiota plays an important role in regulating stress responses. Gut microbes influence brain development and behaviour and psychobiotics (either probiotics or prebiotics) have potential in managing stress-related conditions and may improve cognition. More translational studies are needed in this field to gain more insight.

KEY POINTS
- Brain-gut-microbiota plays an important role in regulating stress responses
- B. longum seemed to have anti-anxiety effect and cognitive enhancing effects (improved learning) in mice
- L. rhamnosus has potent anti-anxiety effects in rodents
- Psychobiotics – probiotics or prebiotics – have potential in managing stress-related conditions and may improve cognition

References
Prebiotic modulation of emotional and cognitive function in rodents and humans

Dr. Phil Burnet (University of Oxford, UK)

The effects of dietary prebiotics on central glutamate neurobiology, neuroimmune responses and behaviour in rodents and humans are being investigated. Galacto-oligosaccharides (BGOS) did normalize anxious behaviour in mice with induced anxiety (by endotoxin administration) and attenuated the inflammatory response.¹ A study with prebiotics (BGOS) in humans showed that prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy subjects.² No change in subjective rating of anxiety was observed. Attention to positive stimuli is increased. Experiments with an attentional set-shifting task (medial prefrontal cortex) showed that BGOS supplemented rats demonstrate greater cognitive flexibility. Thus, bifidobacteria possess anti-inflammatory and anxiolytic properties. BGOS (not fructo-oligosaccharides; FOS) improves emotional attention perhaps as well as cognitive flexibility. More translational research is needed with more trials in (human) patients needed. Prebiotics and probiotics will have a role as adjunctive therapies but will not replace treatment with medication.

KEY POINTS
- Prebiotic use in humans has shown to reduce the waking cortisol response and alter emotional bias in healthy subjects
- Prebiotic supplemented rats demonstrated greater cognitive flexibility
- Bifidobacteria possess anti-inflammatory and anxiolytic properties
- The role of prebiotics/probiotics will be as adjunctive therapies, complementing treatment with medication

References
Pioneering probiotic clinical application in gut-brain axis: a historical perspective

Dr. Stéphanie-Anne Girard (Lallemand Health Solutions, Canada)

Probiotics have gained momentum in the media in recent years, largely due to their interactions with the gut-brain axis. The concept of brain-gut-axis dates back to the late 19th century, when William James and Carl Lange hypothesized a brain-gut bidirectional communication for the first time in the 1880s. In 1910, a publication by Philips showcased the idea that dietary intake of live beneficial bacteria could help treat melancholia. It was not until 1970 that the first indication emerged that microbiota was involved in the brain-gut dialogue by the observation that behavioural changes were linked to the absence of microbiota in piglets. During the following decades, in vivo studies were performed using challenge models with infectious microorganisms demonstrating an impact on behaviour through activation of immune-neutral mechanisms. In 1998, a direct activation of neural pathways by micro-organisms related to behaviour in particular anxiety, in the absence of an overt immunologic response was observed. From 2008-2012, the focus shifted from brain-gut axis to microbiota-gut-brain axis, with Desbonnet measuring – for the first time – a direct effect of a probiotic on the brain of stressed rats.

A study by Messaoudi et al. investigated the anxiolytic-like activity of probiotics in rats, and its possible effects on anxiety, depression, stress and coping strategies in healthy human volunteers. Rats were daily administered probiotics during 2 weeks and subsequently tested in the conditioned defensive burying test (which is a screening model for anti-anxiety agents). Also, volunteers participated in a double-blind, placebo-controlled, randomized parallel group study in which probiotics were administered for 30 days. The results showed that daily subchronic administration of probiotics significantly reduced anxiety-like behaviour in rats and alleviated psychological distress in the human volunteers. It was concluded that L. helveticus R0052 and B. longum R0175 taken in combination have shown anxiolytic-like activity in rats and beneficial psychological effects in healthy human volunteers. Since 2013, the focus has moved from everyday stress to more pathologic features such as psychological and psychiatric disorders.

KEY POINTS
• The concept of brain-gut-axis dates back to the late 19th century
• In recent years, attention shifted to the microbiota-gut-brain axis when a direct effect of a probiotic on the brain of stressed rats was measured
• Combination of L. helveticus R0052 and B. longum R0175 have shown anxiolytic-like activity in rats and beneficial psychological effects in healthy human volunteers

References
Probiotics to reduce depression vulnerability: multispecies probiotics reduces cognitive reactivity to sad mood

Dr. Laura Steenbergen (Leiden University, the Netherlands)

It has been demonstrated that probiotics can positively affect anxiety and depressive symptoms as was shown in randomized controlled trials but may they be used to prevent depression?\(^1\)\(^2\) It is known that onset, development and recurrence of depression is predicted by cognitive reactivity which is the degree to which dysfunctional thought patterns are activated.\(^3\)\(^4\) This was investigated in the first study of its kind by using a probiotic with 8 different strains. The aim of the study was to complement previous findings by assessing the possible beneficial effect of probiotics on cognitive reactivity (a vulnerability marker for depression). Forty healthy individuals without any current mood disorders were enrolled and assigned to either probiotic (n = 20) or placebo (n = 20) in a 4-week intervention. Pre- and post-intervention measurements were part of the study design. The results showed that overall, participants showed lower scores at the post-intervention measurements compared to the pre-intervention measurement. Significant interactions between sessions and group were seen for LEIDS-t total score, aggression, and rumination. These findings suggest that the intake of multispecies probiotics for a 4-week period reduced overall cognitive reactivity (vulnerability) to depression and in particular aggressive and ruminative thoughts. There were no effects on self-reported measures of anxiety and depression. It was also concluded that multispecies mixture of probiotics reduced cognitive reactivity to sad mood (vulnerability to develop depression). Further studies in high risk populations are needed to establish potentially clinical relevant effects in prevention and enhancement of well-being.

KEY POINTS
- Probiotics can positively affect anxiety and depressive symptoms
- The first study assessing the possible beneficial effect of probiotics on cognitive reactivity in humans used a probiotic with 8 different strains
- Use of multispecies probiotics for a 4-week period reduced overall cognitive reactivity (vulnerability) to depression, in particular aggressive and ruminative thoughts
- Multispecies mixture of probiotics reduced cognitive reactivity to sad mood (vulnerability to develop depression)

References
Mind, Mood and Microbes in the USA; June 2017

Following the success of Mind, Mood and Microbes Amsterdam, Winclove was invited to organize a preconference workshop at the IPA/Probiota Americas on microbiota-gut-brain axis.

Find the extended conference summary of the presentations of ‘Mind, Mood and Microbes’ at www.wincloveprobiotics.com

The content of the conference summary is written by Constance de Koning from MedScope, a medical/science writer.

Watch or share the short video, with speakers from Mind, Mood and Microbes at http://www.mindmoodmicrobes.org

Winclove Probiotics
At Winclove, we engage in probiotic research, often in collaboration with renowned research institutes and academic hospitals. This has resulted in more than 60 scientific publications with our bacterial strains or probiotic formulations over the past 12 years. We strive to provide healthcare professionals and their patients with high quality indication-specific probiotics that improve health and wellbeing and which enable people all around the world to rebalance their microbiome. We are always interested in new collaborations, both with regard to mechanistic studies in vitro as well as clinical studies with either healthy volunteers or a patient population. Besides depression, we are also focused on a range of other brain-related diseases, such as autism, attention-deficit hyperactivity disorder (ADHD), Parkinson’s disease and schizophrenia. We are able to provide input on study design, offer expertise on microbiota and probiotics and support researchers with probiotic formulations and placebo.

Interested to discuss a possible collaboration with one of our researchers? We would like to hear from you at +31 20 435 02 35 or e.pekelharing@winclove.nl, Elisabeth Pekelharing.

Winclove Probiotics
Hulstweg 11
1032 LB Amsterdam, The Netherlands
+31 (0)20-435 02 35
e.pekelharing@winclove.com
www.winclove.com