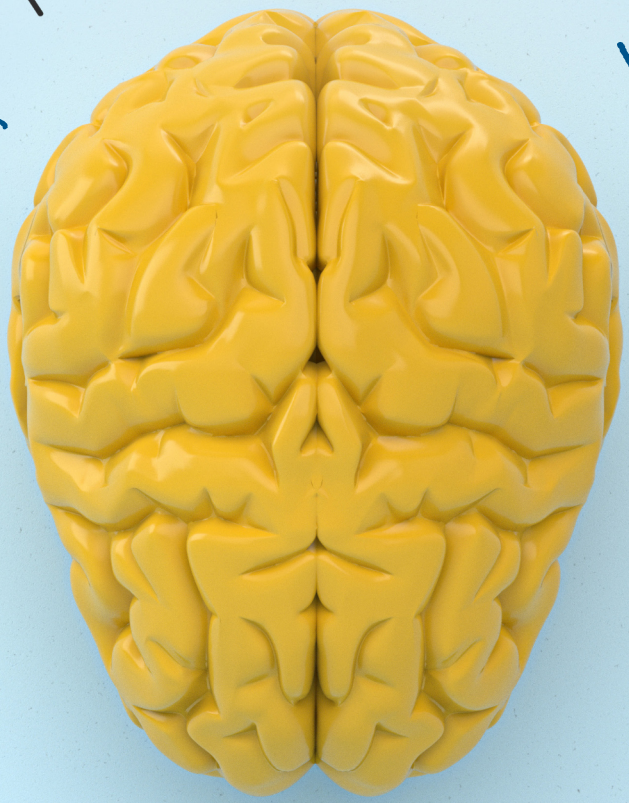
EURON PhD Days

7 & 8 February 2022 – Hasselt University

Parallel Sessions

Keynote Lectures



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EURON PhD days 2022 at Hasselt University

We are happy to welcome you to the 22nd edition of the EURON PhD Days. This year the EURON PhD days is organized at Hasselt University.

These two days will be an opportunity to share your insights, results and ideas and get acquainted with recent scientific developments outside your own university, in an international context.

The abstracts of the meeting can be found in this booklet.

We hope you will have a wonderful time at the 2022 EURON PhD days.

Best regards,

The organizing committee.

Ellis Nelissen, Chritica Lodder, Assia Tiane, Annet van de Waterweg Berends, Melissa Schepers and Ben Rombaut

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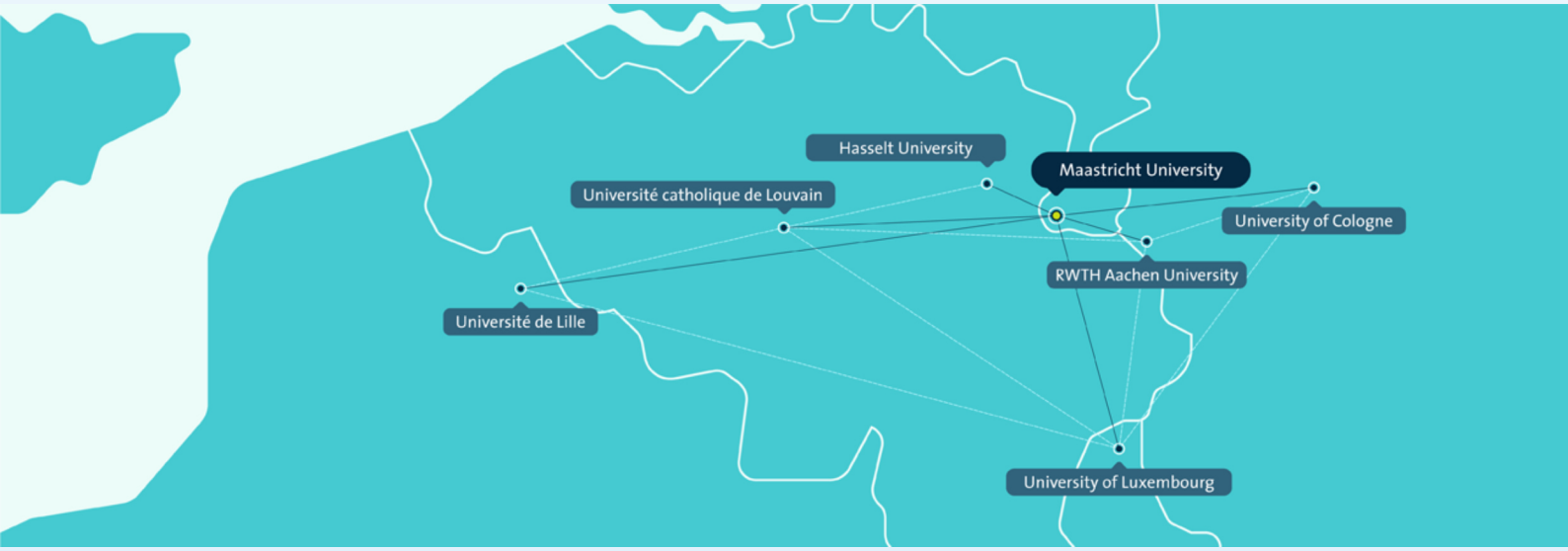
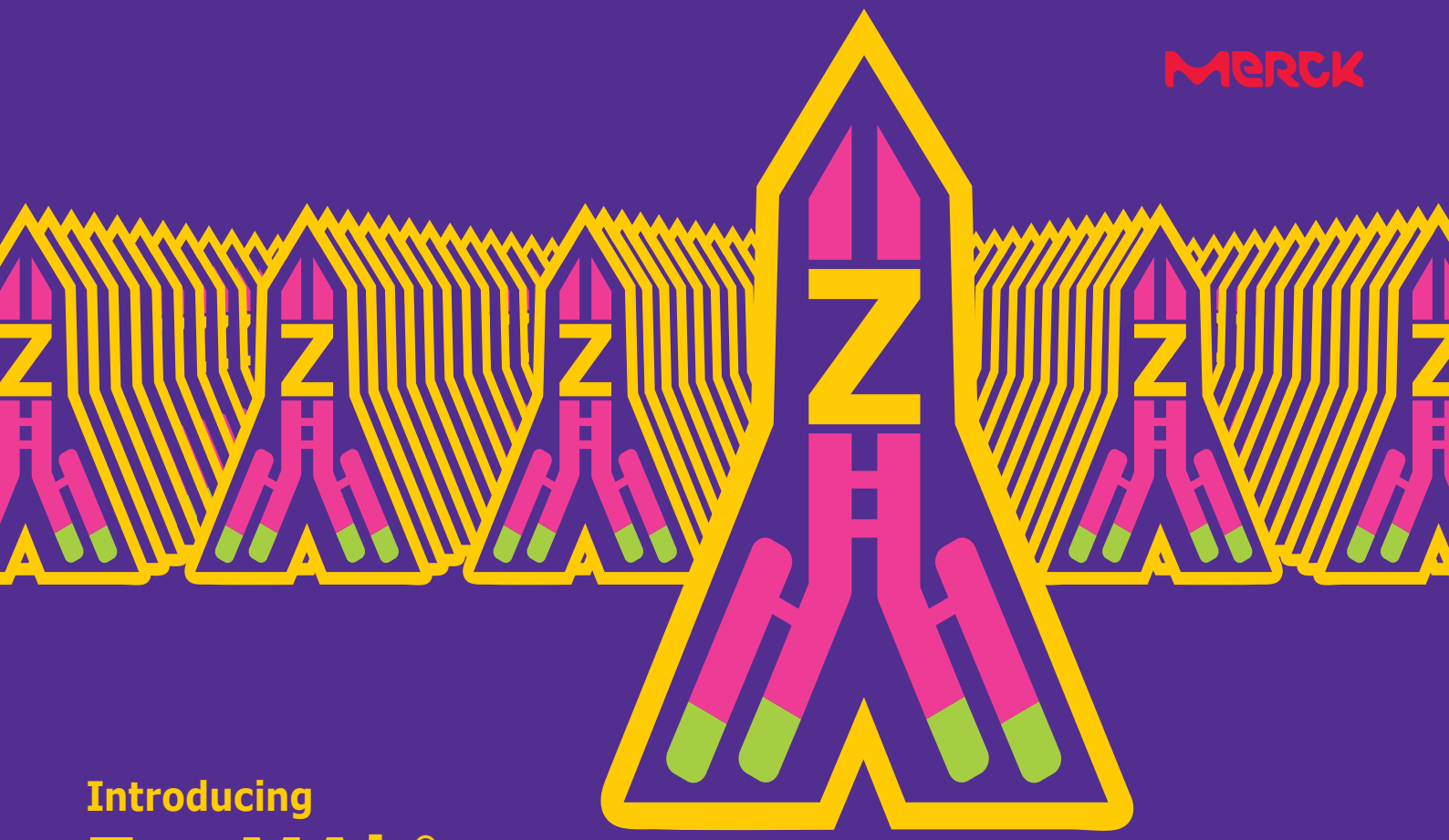


EUROPEAN GRADUATE SCHOOL OF NEUROSCIENCE

Training to reach Excellence in Neuroscience in Europe

The European Graduate School of Neuroscience (EURON) is a Graduate School consisting of institutes and departments of seven universities in Belgium (UC Louvain and University of Hasselt), Germany (RWTH Aachen University and Universität Köln), France (Université de Lille), Luxembourg (Université de Luxembourg) and the Netherlands (Maastricht University). EURON is coordinated by the School for Mental Health and Neuroscience (MHeNs) from Maastricht University. By combining the expertise of our partners, EURON forms a multidisciplinary and complementary network with a perfect background to educate and train PhD students in the field of basic and translational neuroscience.



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ABSTRACT OVERVIEW

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ABSTRACTS

Susceptibility to Psychological Trauma in Relation to Mental Ill-health and Neurobiology

Ghazi I. Al Jowf [1,2,3], Rick Reijnders[1,3], Ehsan Pishva[1,3], Lars M. T. Eijssen [1,3,4], Laurence de Nijs [1,3], Bart P.F. Rutten[1,3]

1 Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs),

Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Centre, 6200 MD Maastricht, The Netherlands.

2 Department of Public Health, College of Applied Medical Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia.

3 European Graduate School of Neuroscience, Maastricht University, 6200 MD Maastricht, The Netherlands.

4 Department of Bioinformatics—BiGCaT, School of Nutrition and Translational Research in Metabolism (NUTRIM), Faculty of Health, Medicine and Life Sciences, Maastricht University, 6200 MD Maastricht, The Netherlands.

Exposure to trauma is one of the most important and prevalent risk factors for mental and physical ill-health. Excessive or prolonged stress exposure increases the risk for a wide variety of mental and physical symptoms. However, people differ strikingly in their susceptibility to develop signs and symptoms of mental illness after traumatic stress. A mental disorder such as Post-traumatic stress disorder (PTSD) is a debilitating disease affecting approximately 8% of the world population and typically develops after exposure to a traumatic event.

About 80-90% of trauma-exposed individuals do not develop PTSD suggesting an interindividual difference in vulnerability to PTSD. The biological mechanisms underlying this differential susceptibility are unknown. Epigenetic mechanisms have been proposed to underlie the relationship between exposure to traumatic stress and the susceptibility to develop PTSD.

Epigenetics refers to environmentally sensitive modifications to DNA and RNA that regulate gene transcription without altering the genetic sequence itself. Because of the brain's central role in a person's dynamic adaptations to environmental exposures, epigenetic research is pertinent for neurosciences and mental health.

Studies led by Prof. Bart Rutten at Maastricht University have suggested that DNA methylation, an epigenetic mechanism, may regulate the impact of traumatic stress on gene expression and ultimately brain functioning. Discovering the neurobiological nature of this difference may be the key to understanding the factors that constitute PTSD and might ultimately lead to new treatment options. One of the major studies to address this, is the collaborative work done on the PRISMO cohort. Previously, results have been published on the DNA methylation profiles of a subset of 96 individuals, distinguishing those susceptible and resilient to the development of PTSD [Rutten et al., 2017].

Hypothesis: We hypothesize a critical role for epigenetic mechanisms, in particular DNA methylation, in regulating the effects of exposure to traumatic stress on the risk to develop PTSD.

Objectives:

- To identify epigenetic factors related to the risk to develop PTSD after exposure to traumatic stress.
- To identify correlations between DNA methylations and mRNA expressions profiles in blood

samples.

- To determine epigenetic factors that correlate and perhaps predict the development of PTSD/ treatment response in patients diagnosed with PTSD.

Progress: In order to determine changes in gene expression, RNA-sequencing was performed on the same subset of individuals as used in the DNA methylation study. The data have been pre-processed and statistically analysed to compare susceptible to resilient profiles 6 months after return from deployment. Follow-up analyses, including pathway and Gene Ontology mapping, are conducted to biologically interpret the observed changes. These results will be aligned with the epigenetic findings and the profiles correlated between the two studies.

IGF-2 and DES[1-6]IGF-2 as a potential new therapy for ischemic stroke.

Lotte Alders, Yörg Dillen, Greet Merckx, Laura Ponsaerts, Hannelore Kemps, and Annelies Bronckaers

UHasselt – Hasselt University, Faculty of Medicine and Life Sciences, Agoralaan, 3590 Diepenbeek, Belgium

Ischemic stroke is a devastating condition, defined by the sudden focal neurological deficit that arises after obstruction of an artery providing blood to the brain. With approximately 9.5 million new cases every year, ischemic stroke is a major cause of mortality and morbidity worldwide. 30-50% of all stroke victims are left with lifelong neurological impairments, heavily impacting their quality of life. The Insulin-like growth factor 2 or IGF-2 is an essential growth factor during growth and development, but recent data implicate a possible role in neuroregeneration. IGF-2 promotes proliferation and self-renewal of neural stem cells residing in the (subventricular and subgranular zone) brain.

The aim of our study was to investigate whether IGF-2 and its variant DES[1-6]IGF-2 were also involved in promoting neural stem cell (NSC) migration in vitro, a key step in the process of neurogenesis. Indeed, both proteins were able to induce NSC migration in a transwell assay. In the presence of IGF-binding protein 6, DES[1-6]IGF-2, was significantly more potent in maintaining the enhancement of neural stem cell migration. As NSC migration goes hand in hand with angiogenesis, also the effect of these proteins on endothelial cells was investigated. Indeed, both IGF-2 and DES[1-6]IGF-2 were able to induce migration of human microvascular endothelial cells. In vivo, IGF-2 was able to induce blood vessel formation in the chick chorioallantoic membrane assay.

In conclusion, our data show that IGF-2 is a promising new therapeutic target for ischemic stroke treatment as it is capable of stimulating both angiogenesis and neurogenesis in vitro, both essential for neuroregeneration and functional recovery. In the next step, the induction of neural stem cell migration and blood vessel formation by IGF-2 will be validated in mice subjected to ischemic stroke via the distal middle cerebral artery occlusion model. Hence, with these experiments, valuable information for the generation of an effective treatment against ischemic stroke will be obtained.

Neuropathological findings in tinnitus: a human post-mortem study

Faris Almasabi[1,2,3], Faisal Alosaimi [1,3,4], Anouk Wolters[1], Minerva Corrales Terrón[1], Jasper V. Smit1[5], Yasin Temel[1,3], Marcus L.F. Janssen[1,6] and Ali Jahanshahi[1,3]

1 School for Mental Health and Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

2 Department of Physiology, Faculty of Medicine, King Khalid University, Abha, Saudi Arabia.

3 Department of Neurosurgery, Maastricht University Medical Centre, Maastricht, The Netherlands

4 Department of Physiology, Faculty of Medicine, King Abdulaziz University, Rabigh, Saudi Arabia

5 Department of Ear Nose and Throat/Head and Neck Surgery, Zuyderland, Heerlen, The Netherlands

6 Department of Clinical Neurophysiology, Maastricht University Medical Centre, Maastricht, The Netherlands

Introduction: Tinnitus is a phantom perception of sound without an external source, which often occurs with comorbidities such as depression and anxiety. Currently, the underlying neuropathology is still not fully understood. However, compelling evidence suggests that tinnitus raises as a result of changes in the auditory network involving both cortical and subcortical auditory and limbic regions. Structural and functional changes in these regions are shown in animal models of tinnitus as well as human neuroimaging studies. Whereas there is no data on cellular and structural changes in the human auditory areas that could shed light on pathophysiology of tinnitus.

To this end, we assessed the neuroanatomical changes in human brain samples of the auditory region of the thalamus; the medial geniculate body (MGB) and the inferior colliculus (IC) as well as the dorsal raphe nucleus (DRN) in this study.

Materials and methods: Paraffin embedded specimens containing the MGB, IC and DRN (n = 8, 5, and 4, respectively) were obtained from individuals with tinnitus and corresponding healthy controls (n = 7, 5, and 4 of MGB, IC, and DRN, respectively). Nissl staining was performed to measure cell density and neuronal size. Next, anti-glial fibrillary acidic protein (GFAP) and iba-1 stainings were conducted to assess astrocyte and microglia alterations. Additionally, the cell density of serotonergic and dopaminergic neurons was investigated in the DRN by using antibodies raised against Phenylalanine hydroxylase-8 (PH8) and Tyrosine hydroxylase (TH), respectively.

Results: We found reduced cell density in the MGB and IC (p=0.01, p=0.03, respectively, DRN p=0.26). Neuronal surface-size measurement showed no differences between groups in both regions (p=0.69, p=0.84, respectively). Reduced astrocytes expression was found in the IC (p=0.04, MGB p=0.44, DRN p=0.89) whereas there was no difference in microglia expression (MGB p=0.46, IC p=0.82, DRN p=0.12). Number of serotonergic neurons were reduced in the DRN (p<0.001) of cases with tinnitus, while no difference in dopaminergic neurons was observed (p=0.39).

Discussion and conclusion: In this first human post-mortem study, we have shown a significant reduction of number of neurons in the MGB and IC in persons with tinnitus. In the IC, we also observed a decrease in the number of astrocytes. Interestingly, we found a significant reduction in the number of PH8 expressing cells in the DRN. These data may shed light on tinnitus pathophysiology and provide new insights into the relationship between tinnitus and psychological disorder.

Modulation of monoaminergic systems with stimulation of the subthalamic nucleus using magnetoelectric nanoparticles alters behaviour in mice

Faisal Alosaimi [1,2], Rick Knoben[1], David Dominguez-Paredes[1], Faris Almasabi[1,3], Sarah Hescham[1], Kristen Kozielski[4], Yasin Temel[1], Ali Jahanshahi[1]

1 Department of Neurosurgery, Maastricht University Medical Centre, Maastricht, the Netherlands 6202AZ

2 Department of Physiology, Faculty of Medicine, King Abdulaziz University, Rabigh, Saudi Arabia 25732

3 Physiology Department, Faculty of Medicine, King Khalid University, Abha, Saudi Arabia 62529

4 Department of Electrical and computer Engineering, Technical University of Munich, Munich, Germany 80333

Rationale and objectives: Deep brain stimulation (DBS) is a surgical procedure used to alleviate motor disabilities in neurological disorders. However, the technique is invasive, and the technology has remained largely stagnate during the last decades. Recently, we have shown that injectable magnetoelectric nanoparticles (MENPs) may offer an approach to circumvent these limitations. However, the proposed method is in its infancy and research is required to characterize its action before it can be considered as an alternative method for conventional DBS. Herein, we aimed to investigate the effect of magnetic stimulation with MENPs on main neurotransmitter systems that have implications in DBS and movement disorders.

Methods: Mice were injected with either MENPs or magnetostrictive nanoparticles (MSNPs, as control) in the subthalamic nucleus (STN). Mice underwent magnetic stimulation, and their motor behaviour was assessed. In addition, magnetic stimulation was applied prior to sacrifice and post-mortem brains were processed for immunohistochemistry (IHC) to assess the co-expression of c-Fos with either tyrosine hydroxylase (TH), tryptophan hydroxylase-2 (TPH2) or choline acetyltransferase (ChAT) cells.

Results: We found a significant increase in c-Fos expression in the motor cortex (MC) and paraventricular (PV) region of the thalamus after magnetic stimulation. Stimulated animals showed fewer c-Fos/TPH2 double labelled cells in the dorsal raphe nucleus (DRN), as well as c-Fos/TH double labelled cells in the ventral tegmental area (VTA), but not in the Substantia nigra (SNc). Moreover, there was no significant difference in the number of c-Fos/ChAT double labelled cells in the pedunculopontine nucleus (PPN). The stimulated animals exhibited an increased distance moved in the open field test.

Conclusion: These findings suggest that magnetic stimulation with MENPs in mice enables selective modulation of the deep brain areas and animal behaviour. These behavioural responses are associated with changes in relevant neurotransmitter systems. These changes are somewhat similar to those have been observed in conventional DBS, suggesting that magnetic DBS could replace electrical DBS.

Non-cell autonomous regulation of pre-motor interneuron development in the chicken embryonic spinal cord

Andrea Angla Navarro, Mathilde Toch, Maria Hidalgo-Figueroa, Cédric Francius and Frédéric Clotman

UCLouvain, Institute of Neuroscience, Laboratory of Neural Differentiation

The Onecut (OC) factors, namely OC-1, OC-2 and OC-3 are transcriptional activator that regulate several aspects of neural development. In the spinal cord, they play key roles during differentiation and

migration of motor neurons and of ventral or dorsal interneuron populations. In addition, their inactivation induces alterations in the development of spinal interneurons that do not express the Onecut genes in control embryos. Furthermore, conditional ablation of the 3 Onecut factors from the spinal motor neurons results in similar interneuron development defects, leading to the conclusion that Onecut factors control in motor neurons a non-cell autonomous process that regulates the development of ventral interneurons. To characterize this mechanism, a RNAseq comparison of control and of Onecut-deficient motor neurons was performed. A list of candidate genes coding for secreted factors or membrane proteins was validated using RT-qPCR and in situ hybridization experiments. To assess their impact on interneuron development, these genes were overexpressed specifically in motor neurons using chicken embryonic spinal cord electroporation and interneuron development was assessed by immunofluorescence for population-specific markers. The results of these experiments will validate a possible function of these candidate genes in the non-cell autonomous control of spinal interneuron development.

Rapamycin rescues loss-of-function in blood-brain barrier transmigrated regulatory T cells

Paulien Baeten[1,2], Ibrahim Hamad[1,3], Cindy Hoeks[1,2], Lilian Aly[4], Michael Hiltensperger[4], Thomas Korn[4,5], Markus Kleinewietfeld[1,3], Niels Hellings[1,2], Bieke Broux[1,2,6]

1 University MS Center, Campus Diepenbeek, Belgium

2 Neuro-Immune Connections and Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

3 VIB Laboratory of Translational Immunomodulation, Center for Inflammation Research (IRC), Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

4 Klinikum Rechts der Isar, Institute for Experimental Neuroimmunology, Technische Universität München, Munich, Germany

5 Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

6 Cardiovascular Research Institute Maastricht, Department of Internal Medicine, Maastricht University, Maastricht, The Netherlands

Multiple sclerosis (MS) is characterized by dysfunctional regulatory T cells (Tregs), both in the periphery and in the central nervous system (CNS). It was previously shown that in an inflammatory environment, Tregs can acquire Th1 and Th17 characteristics. We hypothesized that migration across an inflamed blood-brain barrier (BBB) induces a functional and phenotypical loss in Tregs.

Using FOXP3 reporter mice, we found that exFOXP3 Tregs accumulate in the CNS of EAE animals over time, suggesting that migration into the CNS destabilizes Tregs. To elucidate the mechanism, a human in vitro model of the BBB, using the hCMEC/D3 endothelial cell (EC) line seeded in Thincerts, was employed. Tregs of healthy donors (HD) and MS patients were allowed to migrate for 24h, and RNAseq was performed on the non-migrated and migrated fractions. We identified that pro-inflammatory pathways (i.e. Th17 signalling) are increased and suppressive molecules(i.e. STAT5, LEF1, BACH2) decreased. These results were validated on protein level by flow cytometry: chemokine receptors and IL-6R are increased after migration across BBB-ECs. Indeed, inflamed BBB-ECs produce IL-6. To identify changes on the functional level, a suppression assay was performed after migration which indicates a lost suppressive capacity of Tregs in the migrated compared to the non-migrated fraction. One of the most prominently affected suppression-related pathways due to transmigration, was the mTOR pathway. Indeed, treatment of migrated Tregs with the mTORC1 inhibitor rapamycin restores and even augments their suppressive capacity. Finally, we sought evidence for this phenotype switch in MS

patients. It is known that CD49d is expressed on inflammatory and non-suppressive Tregs. Indeed, we confirmed the presence of 2 Treg subpopulations in the peripheral blood of HD and MS patients, based on CD49d expression. Analysis of paired blood and CSF samples of MS patients at diagnosis, revealed that CD49d+ Tregs are highly enriched in the CSF.

In conclusion, Tregs undergo a pathogenic phenotypic switch and loss of suppressive function after passage over BBB-ECs. Using the clinically relevant compound rapamycin, the functional stability of migrated Tregs can be restored.

Investigating neurobiological effects of chronic cortisol in human embryonic stem cell-derived neurons at different stages of neuronal development

Katherine Bassil, Laurence De Nijs, Gunter Kenis, Bart Rutten

1 School of Mental Health and Neuroscience, Maastricht University

Dysregulated hypothalamic-pituitary adrenal axis, in particular hypo- and hyper- function, has long been implicated in stress-related disorders like post-traumatic stress disorder (PTSD). However, only a small percentage of individuals are susceptible to PTSD following trauma exposure and it is unclear whether and how exposure to traumatic stress leads to alterations in cortical neurons that contribute to PTSD susceptibility in these patients. Therefore, the aim of this study was to investigate the chronic effects of the glucocorticoid cortisol (CORT), in hESC-derived neural progenitors and neurons, as a putative in vitro model of traumatic stress. We generated forebrain neurons from hESCs of a healthy individual, and investigated the effects of chronic CORT exposure at three different neuronal developmental stages: neural progenitor cells, immature and differentiating neurons, and mature neurons. We observed changes in proliferation, survival, apoptosis, neuronal morphology and neuronal activity following chronic exposure to CORT. Genome-wide transcriptomic changes were also observed at the different developmental stages. Together these results suggest a negative effect of CORT on the neural progenitor pool, differentiation of neurons and neuronal activity of maturing neurons. Future research looking into chronic CORT effects in PTSD susceptible and resilient individuals is crucial to determine underlying molecular vulnerability mechanisms.

Regulatory T Cell-derived Extracellular Vesicles: Next Generation Biomarkers and Key Players of Remyelination in Multiple Sclerosis?

Kübra Bekar

Biomedical research institute, Hasselt University

Multiple sclerosis (MS) is the most common chronic, demyelinating, and neurodegenerative disorder of the central nervous system (CNS). More than 1 million patients are diagnosed with progressive MS (pMS), featured by the failure of repair and remyelination. Currently, there is no objective way for neurologists to predict MS progression, due to the heterogeneity and complexity of the disease. Therefore, there is an urgent medical need for reliable prognostic biomarkers with high sensitivity and specificity, which would greatly enhance therapeutic management and individual patient follow-up. As a multifactorial disease, biomarker discovery for MS is shifting towards the use of combinations of different markers. This matches perfectly with the phenotypic profile of extracellular vesicles (EVs),

which are considered “biomarker treasure chests” that contain a multitude of molecular cargoes including proteins, nucleic acids, and lipids. These vesicles are released from essentially all cell types, representing a novel liquid biopsy biomarker source.

Recently, regulatory T cells (Tregs) were discovered to be crucial mediators of remyelination via the secretion of factors such as nephroblastoma overexpressed (NOV/CCN3), leukaemia inhibitory factor (LIF), and transforming growth factor β (TGF-β). Previous (unpublished) data from our research group indicate that several Treg regulatory and remyelination-associated genes are differentially expressed between distinct stages of MS. As EVs are known to largely reflect their cells of origin, we propose that in-depth proteomic analysis of Treg-EVs from RRMS and pMS patients will yield novel prognostic biomarkers for predicting MS progression. Accordingly, we hypothesise that Treg-EVs are packed with functional proteins involved in Treg-induced remyelination that can serve as sensitive candidate biomarkers reflecting remyelination failure in MS.

In this project, we aim to, in a highly original set-up, unravel the proteomic profiles of Treg-EVs in distinct stages of MS using high-resolution state-of-the-art liquid chromatography-mass spectrometry (LC-MS) to obtain a highly selective and specific set of protein markers reflecting remyelination failure in MS. This project will yield ground-breaking insights to stimulate further research on defining the possibility of developing EV-based “liquid biopsies” to monitor the progression of MS. Up to now, Treg-EVs proteomic profiles and their differences in distinct MS stages have not yet been studied, thus making this project unique, timely, and necessary.

Study of maternal mediated mechanisms in the epigenetic programming induced by maternal stress: Transgenerational transmission and oxytocin.

Ryma Benlakehal, Sara Morley-Fletcher, Dr. Anne Harduin-Lepers

University of Lille, CNRS, UMR 8576 - UGSF - Structural and Functional Glycobiology Unit

During perinatal life, adverse environments can highly impact brain development; This process, called programming, is the consequence of complex interactions between genes and the environment. Indeed, an adverse environment during the perinatal period can lead to maladaptive responses, and developing diseases in the long term.

My Ph.D. project aims at understanding the epigenetic mechanism by which maternal stress/behavior in rat programs a transgenerational transmission in the descendants through three generations, and the correction of this misprogramming by a postpartum treatment of the mother with oxytocin. The model of study is the perinatal stress rat model (PRS rat), this model is based on the protocol described by Maccari et al in 1995 and consists of gestational stress of F0 dams (3 x 45 min/ day restrain under bright light) and reduced maternal care (postnatal stress). PRS rats are characterized by enhanced HPA axis activity, impaired glutamatergic transmission, and reduced oxytocinergic tone. The alterations in PRS offspring are predicted by the reduced maternal behavior caused by gestational stress, and enhancement of maternal behavior in the stressed dam via activation of the oxytocinergic system can reverse the PRS phenotype (Gatta et al., 2018).

My thesis is divided into three different axes. The first axis focuses on highlighting if the PRS deficits get transmitted through multiple generations (from F0 to F3, via the maternal line) in both males and females, using tests like risk-taking behavior and maternal behavior, and also at the molecular level

using PCR, WB, and ELISA in brain and blood. A preliminary finding indicates that PRS-induced reduction in gene expression of stress-related markers (such as GR, MR, and mGlu2 and mGlu3 receptors), are transmitted from F1 to F2 offspring in males and that reduced maternal behavior by stress is observed in F1 (daughters) PRS generation. The second axis is understanding the epigenetic mechanisms of PRS in the mothers and offspring (F0-F3). As PRS induces a strong gene downregulation in the hippocampus, the persistence of PRS effects could be explained by changes in DNA methylation across generations. Therefore, I will use MeDIP (to evaluate DNA methylation) and ChiP (to evaluate DNA interaction) on gene candidates involved in stress-antistress, and epigenetic regulation (DNMTs, HDACs..). As PRS epigenetic transmission occurs through maternal behavior, the third axis will focus on modulating maternal behavior to reverse the transgenerational transmission of PRS deficits. By using treatments during the first week of the post-partum period, I will target the oxytocinergic system in the F0 mother, either directly with the carbetocin agonist (i.p injections, Gatta et al. 2018) or by the probiotic Lactobacillus reuteri (according to Buffington et al., 2016 L.reuteri increases Oxytocin levels). Altogether, my study aims to investigate the epigenetic mechanisms that lie at the core of the transgenerational transmission of PRS and understand the role of the oxytocinergic activation in protecting the descendants. It is also important to 1) use approaches in the mother to prevent the effects of stress and 2) that are not necessarily pharmacological like probiotics.

Intake of methylglyoxal does not impair cerebral microcirculation nor cognitive function in healthy mice

Eline Berends[1,2], Naima Amiri[1,2], Marjo PH van de Waarenburg[1,2], Jean LJM Scheijen[1,2], Denise JHP Hermes[3,6], Philippe Vangrieken[1,2], Robert J van Oostenbrugge[2,4], Sébastien Foulquier[2,5,6*], Casper G Schalkwijk[1,2*]

1 Department of Internal Medicine, Maastricht University, Netherlands,
2 School for Cardiovascular Diseases (CARIM), Maastricht, Netherlands,
3 Department of Psychiatry and Neuropsychology, Maastricht University, Netherlands,
4 Department of Neurology, Maastricht University medical Centre, Netherlands,
5 Department of Pharmacology and Toxicology, Maastricht University, Netherlands,
6 School for Mental Health and Neuroscience, Maastricht, Netherlands

*Shared last-authorship

Background: Diabetes is associated with cerebral small vessel disease (cSVD) and cognitive decline yet the underlying mechanism is poorly understood. Methylglyoxal (MGO), a by-product of glycolysis and a major precursor in the formation of advanced glycation end products (AGEs), is increased in individuals with diabetes and is associated with microvascular dysfunction. We previously showed that MGO and MGO-derived AGEs are increased in brain tissue of diabetic rats. The aim of this study was to investigate whether increased levels of circulating MGO as observed in diabetes, can cause cerebral microvascular dysfunction and cognitive impairment.

Methods: 2-3 months old male C57Bl/6J mice were treated with MGO (50mmol/l, drinking water) or not (control) for 3 months (n=17 per group). Mouse cognition and behaviour were tested before treatment and at 6 and 13 weeks of treatment. Working memory, anxiety, short-term and long-term spatial learning and memory were tested using the Y-maze task, elevated zero maze task, object location task and Barnes maze task, respectively. After 13 weeks of treatment, cerebral blood flow (CBF) was measured transcranially by laser speckle contrast imaging. Neurovascular coupling was

measured by quantifying the change in CBF in the barrel cortex upon whisker stimulation (10Hz, 30s). After sacrifice, MGO and AGEs in plasma and brain were measured by UHPLC-MS/MS. Plasma inflammatory markers were assessed by ELISA. Cortical microvessels were isolated and used for gene expression analysis with RT-PCR.

Results: Plasma MGO was increased 2-fold ($p<0.0001$) and free plasma MGO derived hydroimidazolone-1 (MG-H1) and Nε-(1-carboxyethyl)lysine (CEL) were increased 1.2-fold ($p=0.01$) and 1.7-fold ($p=0.01$), respectively in the MGO group, while other non MGO-derived AGEs were unchanged. In brains of MGO-treated animals, free MGO-H1 and CEL were increased 1.4-fold ($p=0.02$) and a 1.1-fold ($p=0.001$) in comparison to controls. In both plasma and brain, there were no differences in protein bound AGEs between MGO treated and control group. The behaviour and cognitive function remained unchanged in the MGO group vs controls. There was no effect of MGO on baseline cortical CBF and neurovascular coupling. MGO did not change the expression of the plasma inflammatory markers IFN-γ, IL-10, IL-1β, IL-6, TNF-α and chemokine (C-X-C motif) ligand 1. In isolated cortical microvessels, expression of vascular cellular adhesion molecule 1, intercellular adhesion molecule 1, sirtuin 1 and the receptor for AGEs, were unchanged.

Conclusion: Plasma MGO and MGO-derived AGEs were increased by supplementation of MGO in drinking water to a level comparable to that in diabetes. Although this was accompanied by increased levels of free MGO-derived AGEs in the brain, this was not associated with microvascular inflammation in the cortical microvessels, neurovascular coupling, and cognitive impairment. This indicates that circulating MGO by itself does not lead to microvascular dysfunction nor cognitive decline. The endogenous formation of MGO in diabetes, rather than circulating MGO, may be of importance for cerebral microvascular dysfunction and cognitive impairment.

Temperature modulation effects on temperature sensitive ion-channels in microglia

Andreea Burlacu, Yeranddy Aguiar Alpizar, Bert Brône

Biomedical research institute, Hasselt University

Temperature increase above homeostatic values occurs in normal conditions such as in high metabolic events or after intense exercise, but as well as in pathology. In the case of the nervous system, brain injuries are caused by different pathologies and have effects such inflammation which is accompanied by an increase in temperature. This combination of factors causes phagocytes such as microglia to activate. We hypothesize that an increase in temperature will attract microglial cells to the site of the temperature change occurs at. The attraction of this cell type occurs due to migration of the cells and involves reorganization of the cytoskeleton, which is dependent on the levels of Ca2+ inside the cells. Temperature sensitive ion-channels, such as transient receptor potential vanilloid 4 (TRPV4) have been identified in microglial cells and have been proved to aid in phagocytic motility either as a primary mechanism or as a mediator for other molecular cues. The activation of this channel increases the intracellular concentration of Ca2+ and has been identified alongside proteins that are part of the cytoskeleton, such as actin and it also has been proved to be present in the lamellipodia of motile cells. Up to this moment the technology available does not allow the control of the temperature at a subcellular level. Designing and testing nanodevices that possess temperature regulating centres is still needed in order to be able to observe molecular changes in the cells. By developing and using nanoscale thermometry devices, we will be able to control the temperature at a subcellular level, which in term will allow us to study the actin reorganization of the microglial cytoskeleton in real time.

Regulation of Neuronal Cell Fate Determination by Endocytic Adaptor AP-2

Santiago Camblor-Perujo[1], Hanna Küpper[1], Roohallah Ghodrat[1], Hisham Bazzi[1], Natalia L Kononenko[1,2]

1 CECAD Research Center, University of Cologne, Joseph-Stelzmann Str. 26, 50931, Germany.
2 University Hospital of Cologne, University of Cologne, Joseph-Stelzmann Str. 26, 50931, Germany.

AP-2 is a heterotetrameric complex comprised of α, β, μ, and σ subunits that links clathrin and other endocytic proteins to sites of clathrin-mediated endocytosis. Full body knockout of AP-2(μ) in mice causes embryonic lethality before day 3.5 postcoitus (Mitsunari, T. et al., 2005). In contrast, depletion of AP-2(μ) in neurons results in postnatal neurodegeneration and defective synaptic vesicle recycling (Kononenko et al., 2014, Kononenko et al., 2017). However, it does not block plasma membrane retrieval during neuronal activity, questioning the canonical function of AP-2 in neurons and suggesting that AP-2 might perform different functions (also known as moonlight functions) in mitotic versus postmitotic cells.

Using a combination of biochemical, cell biology, and live imaging approaches, we show that AP-2 controls neuronal progenitor cells (NPCs) proliferation but is not required for neuronal differentiation. In wildtype NPCs, AP-2 can be found at the centrosomes, where it colocalizes with components of the gamma-tubulin complex. Using mass spectrometry analysis, we identified GCP1, GCP2, GCP3, and GCP4 as novel interaction partners of the AP-2 complex in neuronal cells, where the interaction between the γTuSC and AP-2 was confirmed in co-immunoprecipitation studies. Deletion of AP-2μ in NPCs leads to defects in primary cilia disassembly and centrosome formation, resulting in cell cycle arrest, abnormal mitotic spindle formation, altered microtubule dynamics, and delayed cell migration. This phenotype was not reproduced in NPCs treated with clathrin inhibitor PitStop2, suggesting that this role of AP-2 is independent of its function in clathrin-mediated endocytosis. On the other hand, it was reproduced in AP-2μ KO Mouse Embryonic Fibroblasts (MEFs), suggesting that AP-2 is a general modulator of cell cycle and microtubule dynamics. In vivo, we observed a tendency to neurogenic division in the SVZ of AP-2(μ) conditional KO mice and accumulation of Dcx+ cells, causing the disorganization of the cortical cytoarchitecture.

Our data suggest a novel moonlight function of the AP-2 complex in centrosome formation trafficking components of the gamma-tubulin complex between the primary cilia basal body and the centrosome. Trafficking of these components and a proper centrosomal maturation is crucial for cell division and hence for neurogenesis and, in postmitotic immature neurons, ensure its correct migration to the cortex. Since this phenotype is conserved in other mitotic cells, AP-2 could be vital for neurogenesis and cancer.

The Antibody Dependant Neurite Outgrowth Modulation Response (ADNM) involvement in Spinal Cord Injury

Alice Capuz[1*], Mélodie-Anne Karnoub[1*], Sylvain Osien[1*], Mélanie Rose[1], Céline Mériaux[1], Isabelle Fournier[1], Franck Rodet[1], Dasa Cizkova[1,2,3], Michel Salzet[1**]

1 Université de Lille, INSERM, U1192 - Laboratoire Protéomique, Réponse Inflammatoire et Spectrométrie de Masse (PRISM), F-59000 Lille, France
2 Institute of Neuroimmunology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 10 Bratislava, Slovakia
3 Centre for Experimental and Clinical Regenerative Medicine, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia.

Spinal cord injury (SCI) can lead, depending on the degree of severity and location of the injury, to incomplete (paraplegia) or complete (tetraplegia) paralysis (Pires and Pêgo, 2015). The main causes of injury are car accidents, falls, firearm violence and sports. Unfortunately, no real effective treatments are known to date. For this reason, it is necessary to better understand the pathophysiology of the injury and the role of inflammation. To this end, a spatiotemporal proteomic study was carried out on a rat model of spinal cord injury with inflated balloon rat model. It shows in time course from 12 hours to 10 days that the most distant segments from the lesion (rostral 2 - 3, and caudal 2 – 3) are implicated in neuritogenesis. By contrast, the once closest to the lesion, (rostral 1 and caudal 1) expressed inflammatory factors (Cizkova et al.; 2014; Devaux et al., 2016; Devaux et al., 2017; Cizkova et al., 2018). These results in line with the meta-analysis of the factors produced within the segments (rostral and caudal) highlighting, the presence of immunoglobulins popping up at 12 hours for IgM whereas IgG isotypes were identified in control (without lesion). Interestingly, this shows discrepancies between what is conventionally observed in B cells, which is not in line with conventional immune response. In this context, we tried to figure out the source of these Igs. For that purpose, we investigated and demonstrated that the origin such immunoglobulin could be neuron itself. Moreover, with a use of an inhibitor of the RHoA protein, known to promote synaptogenesis and neurites growth, an increase IgG level of expression was registered in the secretome of SCI explant at 12 hours. Similarly, the expression of these IgG-specific receptors (CD16 and CD32b) were found in neurons. The use of immunoglobulin isotype specific of these receptors in biological test have allowed to demonstrated their involvement in modulating neurite growth responses. This points out, involvement of an antibody dependent neurites outgrowth response in neurons through autocrine and paracrine pathway.

Cognitive behavioral therapy for anxiety in Parkinson's disease induces functional brain changes

Guillaume Carey[1,2,3], Renaud Lopes[2,8], Anja J.H. Moonen[1,4], Anne E.P. Mulders[1,4], Joost J.A. de Jong[1,7], Gregory Kuchcinski[2,6,8], Luc Defebvre[2,3], Mark L. Kuijf[1,5], Kathy Dujardin[2,3], Albert F.G. Leentjens[1,4]

1 School for Mental Health and Neurosciences (MHeNS), Maastricht University, Maastricht, The Netherlands

2 Univ. Lille, Inserm, CHU Lille, U1172 - LilNCog - Lille Neuroscience & Cognition, F-59000 Lille, France

3 Department of Neurology and movement disorders, Lille University Medical Centre, Lille, France

4 Department of Psychiatry, Maastricht University Medical Centre, Maastricht, The Netherlands

5 Department of Neurology, Maastricht University Medical Centre, Maastricht, The Netherlands

6 Department of Neuroradiology, Lille University Medical Centre, Lille, France

7 Department of Radiology and Nuclear Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands.

8 Univ Lille, UMS 2014 – US 41 – PLBS – Plateformes Lilloises en Biologie & Santé, F-59000, Lille, France

Background: Cognitive Behavioral Therapy (CBT) reduces situational anxiety and avoidance behavior in patients with Parkinson's disease.

Objective: To identify changes in functional connectivity in the brain after CBT for anxiety in patients with Parkinson's disease (PD).

Methods: Thirty-five patients with PD and clinically significant anxiety were randomized over two groups: CBT and clinical monitoring only (CMO). Resting-state functional brain MRI was performed at baseline and after the intervention. The Parkinson Anxiety Scale (PAS) and its subscales were used to assess anxiety. An Independent Component Analysis (ICA) was performed to extract functional networks. Comparisons and regressions were performed between structures involved in anxiety and both within and between functional networks.

Results: Compared to CMO, CBT reduced the functional connectivity (FC) between the right thalamus and the bilateral orbitofrontal cortices. After CBT, improvement of situational anxiety symptoms was associated with an increased striato-cingulate, fronto-cingluate and parieto-cingulate FC and a decreased temporo-insular FC, whereas improvement of avoidance behavior was associated with a decreased temporo-cingulate and parieto-occipital FC. Moreover, CBT increased FC within the central executive network (CEN), within the language network (LN) and between the CEN and the salience network, and reduced the FC within the sensorimotor network and between the CEN and the LN. After CBT, improvement of anxiety symptoms was associated with decreased FC within the default-mode network, within the CEN and between the dorsal-attentional network and the LN.

Conclusion: Cognitive behavioral therapy for anxiety in Parkinson's disease patients induced changes in functional connectivity in anxiety-related brain circuits as well as within and between the functional networks. The observed changes in FC are different for improvement in situational anxiety and avoidance behavior, suggesting that these symptoms are modulated through different networks.

Regulatory T cells in multiple sclerosis: from methylation to (re)myelination

Sarah Chenine[1,2,3], Assia Tiane[1,2,4], Paulien Baeten[4,5], Oliver Gerlach[3], Bieke Broux[4,5], Daniel van den Hove[2,6], Tim Vanmierlo[1,2,4]

1 Department of Neuroscience, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium.

2 Department Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands.

3 Department of Neurology, Zuyderland Medical Centre, Sittard, Netherlands.

4 University MS Center (UMSC) Hasselt – Pelt, Belgium.

5 Department of Immunology and Infection, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium.

6 Department of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Würzburg, Germany.

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Multiple sclerosis (MS) is an autoimmune-induced demyelinating disease of the central nervous system (CNS). Focal demyelination, the primary hallmark of MS, results in neurodegeneration as a result of oligodendrocyte death and loss of axonal function. In MS, regulatory T cells (Tregs), which possess regenerative functions, are compromised. In homeostatic conditions, Tregs promote remyelination through the secretion of factors such as nephroblastoma overexpressed (NOV/CCN3), leukaemia inhibitory factor (LIF), inhibitor of differentiation 2 (ID2) and transforming growth factor β (TGF-β). However, it is thought that in MS, the epigenetic imprinting of Tregs is altered during the course of the disease causing a decrease in the function of Tregs. Targeting the remyelination pathway of Tregs may have ground-breaking implications for progressive MS (pMS) patients, for whom there are currently no approved treatments to target repair. Recently, we have investigated the methylation pattern of ID2 in MS patients (RRMS and SPMS) and controls through pyrosequencing. We have shown that SPMS derived Tregs display a hypermethylated pattern of inhibitor of differentiation 2 (ID2) compared to RRMS and healthy control derived Tregs. As ID2 is a regulator of Treg function, these results are intriguing and may indicate that other Treg-associated genes are also differentially methylated between MS patients and controls. Therefore, we aim to determine which Treg-associated remyelination-inducing genes have an altered epigenetic imprinting in MS patients and whether

epigenetic editing of those genes is able to promote remyelination through the adoptive transfer of epigenetically edited Tregs into models of MS. Ultimately, we aim to investigate a potential cell-based autologous Treg therapy for remyelination in pMS.

The effect of selective spatial attention on the development of secondary hyperalgesia: a replication study

Delia Della Porta[1,2], Marie-Lynn Vilz[1], Valéry Legrain[1,2,3]

1 Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium.

2 Psychological Sciences Research Institute, Université catholique de Louvain, Louvain-la- Neuve, Belgium.

3 Louvain Bionics, Université catholique de Louvain, Louvain-la-Neuve, Belgium.

Central sensitization refers to the increased excitability of nociceptive neurons in the central nervous system after intense or sustained peripheral nociceptor activation. It causes an increased mechanical pain sensitivity at the injured location (i.e., primary hyperalgesia), but also in the surrounding area (i.e., secondary hyperalgesia), and is hypothesized to play a key role in the development of pain chronicity. There is evidence that the maintenance of neurons' heightened reactivity associated with chronic pain conditions, could involve a top-down modulation operated by cognitive factors. It was recently shown that the experimental induction of central sensitization can be modulated by selective spatial attention, making attention a promising intervention target for preventing the development of chronic pain. To assess the robustness of this mechanism we conducted a preregistered replication study in a larger independent sample.

In a double-blind, within-subject design, study we investigated the impact of selective spatial attention on the development of secondary hyperalgesia. Sixty-seven healthy volunteers performed a detection task that required focusing attention towards one forearm while secondary hyperalgesia was simultaneously induced on both forearms using high-frequency stimulation (HFS). Our results showed a significant increase in mechanical sensitivity directly (T1) and 20 minutes (T2) after HFS. Yet, in contrast to the previous study, we did not find a significant difference in the development of secondary hyperalgesia between the attended vs unattended arm.

One explanation for the non-replication could be that the bottom-up capture of attention caused by the bilateral and simultaneous delivery of HFS was too strong in comparison to the top-down modulation exerted by the detection task. To enhance the efficiency of top-down modulation, we may need to reconsider the role of goal relevance during the attentional task.

Cerebrospinal fluid proteomic profiling of non-demented persons with Alzheimer's disease classified using two different neurodegeneration biomarkers (N) in the ATN classification

Aurore Delvenne[1], Johan Gobom[2], Frans Verhey[1], Inez Ramakers[1], Anne Fagan[3], Simon Lovestone[4], Johannes Streffer[5], Frederik Barkhof[6,7], Henrik Zetterberg[2], Betty Tijms[6], Pieter Jelle Visser[1,6] and Stephanie J.B. Vos[1]

1 Maastricht University, Maastricht, the Netherlands;

2 University of Gothenburg, Mölndal, Sweden;

3 Washington University, St Louis, MO, USA;

4 University of Oxford, Oxford, United Kingdom;

5 UCB Biopharma SPRL, Belgium;
6 VU University Medical Center, Amsterdam, the Netherlands,
7 Queen Square Institute of Neurology and Centre for Medical Image Computing, University College London, London, UK.

Background: The amyloid/tau/neurodegeneration (ATN) framework is a recent biomarker classification system. Several markers have been proposed as neurodegeneration marker, e.g. neurofilament light chain (NfL) and hippocampal volume (HCV). We investigated whether abnormal N-markers NfL or HCV are associated with different pathophysiological processes using CSF proteomics in non-demented persons with underlying AD pathology (A+T+).

Method: We included persons from the European EMIF-AD MBD, St Louis Knight ADRC and Maastricht BBACL study. Based on CSF Aβ1-42 (A), p-tau (T) and NfL or HCV (N), individuals were classified as preclinical AD (A+T+ N-/N+) or prodromal AD (A+T+ N-/N+). CSF proteomic data (1337 proteins) were generated using TMT mass spectrometry and compared between groups using ANOVA adjusted for age and sex. Gene Ontology analyses were performed.

Result: In preclinical AD, A+T+NfL- (n=20) and A+T+NfL+ (n=23) individuals showed similar proteomic profiles. Compared to preclinical A+T+HCV+ (n=15), preclinical AD A+T+HCV- individuals (n=29) showed an increase in the concentration of 51 proteins, that were related to nervous system and angiogenesis. Compared to prodromal A+T+NfL+ (n=95), prodromal AD A+T+NfL- (n=24) showed, an increase in the concentration of 252 proteins, that were related to extracellular matrix, nervous system, proteins, immune system and angiogenesis. No differences were found in protein levels between prodromal AD A+T+HCV- (n=22) and A+T+HCV+ (n=78).

Conclusion: Our findings indicate that different neurodegeneration markers are associated with different proteomic profiles, which partly depend on clinical stage. This implies that NfL and HCV injury markers cannot be used interchangeably.

Effects of early movement restriction on sensorimotor developtment and motor performance in rat

Orlane Dupuis[1], Marie-Hélène Canu[1], Valérie Montel[1], Julie Dereumetz[1], Jacques-Olivier Coq[2], Bruno Bastide[1], Erwan Dupont[1]

1 Univ. Lille, Univ Artois, Univ Littoral Côte d'Opale, ULR 7369, URePSSS – Unité de Recherche Pluridisciplinaire Sport Santé Société, F–59000 Lille, France.
2 Institut des Sciences du Mouvement, UMR 7287, CNRS, Université Aix-Marseille, F-13385 Marseille

Childhood is a period of construction of the organism, during which regular physical activity favors the maturation of the neuronal networks. However, some children are hypoactive because they are bedridden (illness, accident, cerebral palsy...) or they suffer from a developmental coordination disorder. In addition to the increased risk of cardiovascular and metabolic diseases, a low level of physical activity during childhood can affect the structuring and maturation of developing nerve circuits and of musculoskeletal system as well, that may lead to cognitive and behavioral impairment. In order to better understand the emergence of neuromuscular disorders in these children, a model of sensorimotor restriction (SMR) was developed in rats. It consists in casting hindlimbs of the pups from postnatal day (PND) 1 to PND 28.

In the present study, our objective was first to characterize the effect of early SMR on sensorimotor development from PND 1 to PND 28. Our results revealed that SMR induced a delay in acquisition of the main neurodevelopmental parameters such as righting, negative geotaxis, cliff avoidance, posture, placing… Moreover, morphological parameters were affected by SMR since we observed a lower body weight and an atrophy of hindlimb postural muscles. We also observed a delay in muscle maturation reflected by persistence of neonatal isoform of MHC proteins at PND 28. The second objective was to examine long term effects of SMR, during a two months recovery period without restriction. SMR rats kept a lower body weight until PND 90. Electrophysiological approach through Hoffmann reflex analysis has shown hyperexcitability of spinal cord in SMR rats, suggesting spasticity. Furthermore, the spontaneous activity (running wheels) was progressively increased from PND 28 to PND 90, but sensorimotor tests that require a good coordination between intra- and interlimb (rotarod, locomotion) showed an enduring alteration. In conclusion, physical activity and interactions with the environment are required for the harmonious maturation of organization and functions of the central nervous system.

EEG frequency-tagging suggests that fine-grained vibrotactile contrast is (partly) implemented in S1

Giulia Esposito[1], Sylvie Nozaradan[1], Olivier Collignon[1,2,3], André Mouraux[1]

1 Institute of neuroscience (IoNS), Université Catholique de Louvain, Brussels, Belgium

2 Institute of Research In Psychological Sciences (IPSY), Université Catholique de Louvain, Louvain la Neuve, Belgium

3 School of Health Sciences, HES-SO Valais-Wallis, Sion, Switzerland

Cortical processing of natural textures has been shown to originate at least in part from primary somatosensory cortex (S1). However, whether processing of contrasts in fine-grained tactile textures is already achieved in S1, or whether it requires higher level somatosensory areas of the brain remains a matter of debate. Oddball paradigms, together with EEG frequency-tagging, can be employed to investigate responses to changes occurring within fast, continuous sequences of stimuli, and represent a useful tool to investigate somatosensory processing of changes in vibrotactile texture. Here, we performed an oddball experiment in which vibrotactile sequences consisting of standard (A) and oddball stimuli (B) were presented in an AAAAB pattern, with a base and oddball presentation rate of 8 Hz and 1.6 Hz (8/5 Hz), respectively. In a first session, A and B were pure sinus vibrations differing in frequency and intensity. The main aim of this condition was to verify and characterize EEG responses to simple vibrotactile contrasts. In a second session, A and B were two different sequences of white noise matched in terms of intensity and average frequency content, but differing in terms of their complex spectrotemporal composition. Scalp topographies of responses elicited by hand and foot stimulation were compared to assess whether they followed the somatotopical organization of S1. Preliminary results show that, for both hand and foot stimulation, both conditions elicited significant EEG peaks at the oddball frequency and its harmonics. For hand stimulation, responses were maximal at contralateral parietal and frontal electrodes, consistent with activity originating from the hand area of S1. For foot stimulation, responses were maximal at central midline electrodes, compatible with activity originating from the foot area of S1. In conclusion, we show that EEG frequency-tagging, in combination with a periodic oddball paradigm, can be used to record contrast-specific somatosensory responses to a fine-grained vibrotactile contrast, and that these responses appear to originate, at least partly, from S1.

Effects of APOE deficiency in a mouse model of Alzheimer's disease

M. Gutiérrez de Ravé, C. Lodder[1], P. Botella Lucena, S. Vanherle, I.C Stancu, I. Dewachter

Department of Neurosciences, Biomedical Research Institute, Hasselt University, Belgium

Alzheimer's disease (AD) affects 50 million of people worldwide carrying a yearly economic burden of 1 trillion dollars. Brains of AD patients are characterized by the deposition of amyloid plaques and neurofibrillary tau tangles. These pathological aggregates are invariably associated with neuroinflammation, in which microglia play a critical contribution. APOE is the major genetic risk factor of AD. Mounting evidence in clinical and preclinical studies highlights the role of APOE in AD onset and progression. However, the specific APOE contribution to the different AD processes i.e. Amyloid-Tau-Neurodegeneration (ATN) is not fully understood. Here, we investigate the effect of APOE deficiency in Aß pathology together with microglial response in 5xFAD mice. Our data show that APOE strongly modulate Aβ pathology affecting: total Aβ burden, plaque morphology and aggregation state. APOE deficient mice display higher Aß deposition which is translated into larger and more diffuse plaques which have lower Aβ fibrillar enrichment. This Aβ pathology type correlates with decreased microglial response reflected in lower total reactive microglia, lower microglia-plaque colocalization and lower microglia plaque vicinity association. For future perspectives, we will investigate the effect of APOE deletion in the context of Tau pathology and finally the combined effect of APOE deficiency in our combined ATN model (Lodder et al 2021, ANC).

Oncostatin M opens the gate for T helper 17 cells during neuroinflammation

Hermans Doryssa[1,2]*, Houben Evelien[1,2]*, Baeten Paulien[1,2], Slaets Helena[1,2], Janssens Kris[1,2], Hosseinkhani Baharak[1,2], Bormans Seppe[3], Gowing Elizabeth[4], Hoornaert Chloé[4], Thoelen Ronald[3], de Vries Helga[5], Gijs Kooij[5], Zandee Stephanie[4], Prat Alexandre[4], Hellings Niels[1,2], Broux Bieke[1,2,6]

1 University MS Center, Campus Diepenbeek, Diepenbeek, Belgium.

2 Neuro-Immune Connections and Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.

3 Institute for Materials Research (IMO), Hasselt University, Diepenbeek, Belgium.

4 Neuroimmunology Unit, Centre de recherche du CHUM (CRCHUM), Montreal, Quebec, Canada.

5 Department of Molecular Cell Biology and Immunology, Amsterdam Neuroscience, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands.

6 Cardiovascular Research Institute Maastricht, Department of Internal Medicine, Maastricht University, Maastricht, The Netherlands

* Equal contribution

Blood-brain barrier (BBB) dysfunction is an intrinsic feature of neurodegenerative and -inflammatory diseases, including multiple sclerosis (MS). Oncostatin M (OSM) cytokine levels are elevated in the blood and brain of MS patients. We previously demonstrated that OSM exerts neuroprotective and remyelination-promoting functions after central nervous system (CNS) damage, warranting its potential therapeutic use. However, OSM's role in neuroinflammation and BBB function is poorly understood. To investigate the role of OSM signalling in a neuroinflammatory setting in vivo, we induced experimental autoimmune encephalomyelitis (EAE) in wild-type and OSM receptor (OSMRβ) deficient mice. CNS immune cell infiltration and BBB leakage were analysed at different timepoints during disease. Surprisingly, OSMRβ deficient mice exhibited milder EAE symptoms and the lack of a disease peak, which was associated with diminished T helper 17 (Th17) cell infiltration into the CNS and reduced BBB leakage. Effects of OSM on inflamed BBB-endothelial cells (ECs) were further investigated

in vitro using primary mouse and human brain microvascular ECs and the human cerebral microvascular EC line (hCMEC/D3). In vitro, OSM promotes secretion of the Th17-attracting chemokine CCL20 by inflamed BBB-ECs, whereas it downregulates intercellular (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression. Using flow cytometric fluorescence resonance energy transfer (FRET) measurements, we found that OSM-induced endothelial CCL20 promotes activation of the ICAM-1 ligand, lymphocyte function-associated antigen 1 (LFA-1), characterized by a conformational change. This effect was abrogated when CCL20 was neutralized in the EC conditioned medium. Finally, we found that OSM reduced the transendothelial electrical resistance of BBB-ECs in control and inflammatory conditions by downregulating cell-cell junction expression of Claudin-5 and VE-cadherin. Together, these data show that OSM contributes significantly to BBB dysfunction in neuroinflammation by inducing permeability while recruiting Th17 cells via enhanced endothelial CCL20 secretion.

When helpers turn into enemies: How cytotoxic CD4+ T cells interact with helper and regulatory T cells

Cindy Hoeks[1,2], Marjan Vanheusden[1], Liesbet Peeters[1,2], Piet Stinissen[1,2], Bieke Broux[1,2*], Niels Hellings[1,2*]

1 Neuro Immune Connections & Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

2 Universitair MS Centrum (UMSC), Hasselt, Belgium

* equal contribution

A terminally differentiated subset of CD4 T helper cells, characterized by loss of the costimulatory molecule CD28 and gain of cytotoxic activity, arises during aging and chronic inflammation. An age-inappropriate expansion of these cytotoxic CD4 T cells (CD4 CTL) has been found in autoimmune diseases like multiple sclerosis (MS). Previously, we found that this expansion is directly linked to MS disease severity and that this holds value as a novel prognostic marker in MS. However, the mechanisms behind these findings remain unclear. To understand how CD4 CTL contribute to autoimmunity, we studied the interaction between CD4 CTL, conventional CD4 helper T cells (TH cells), and CD4 regulatory T cells (Tregs). Here, we show that while CD4 CTL are resistant to suppression by functional Tregs in vitro, they simultaneously promote the suppression of TH cell proliferation in a triple co-culture system. It remains to be determined whether this is due to enhanced Treg-mediated suppression, or that CD4 CTL themselves are able to suppress TH cells. Interestingly, we found that the secretome of CD4 CTL induced a suppressive phenotype in Tregs, shown by upregulation of IL-10, granzyme B, and CTLA-4 mRNA, while also IFN-gamma gene expression was increased. We furthermore found that CD4 CTL have a pro-inflammatory and pro-survival mRNA expression profile when compared to conventional TH cells. Taken together, our results suggest that when TH cells terminally differentiate and acquire cytotoxic properties, the persistence of these cells is favored by paralleled resistance to Treg-mediated suppression and enhanced suppression of naive TH cells, possibly contributing to memory inflation.

Specific microRNA signatures are associated with enteric glia status

Amy Marie Holland[1,2], Ana Carina Bon-Frauches[2], Fränze Progatzky[3], Daniel Keszthelyi[4], Daisy Jonkers[4], Vassilis Pachnis[3], Veerle Melotte[2,5], Werend Boesmans[1,2]

1 Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

2 Department of Pathology, GROW-School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

3 Development and Homeostasis of the Nervous System Laboratory, The Francis Crick Institute, London, UK

4 Department of Internal Medicine, Division of Gastroenterology-Hepatology, NUTRIM-School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands

5 Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

Enteric glial cells (EGCs) constitute a heterogeneous and highly plastic population of peripheral neuroglial cells embedded in the gastrointestinal tract. They are integral players in the maintenance of intestinal homeostasis and their activity also profoundly influences gut pathophysiology; yet, the molecular underpinnings that govern EGC status are incompletely understood. MicroRNA-mediated gene regulation is critical for 'tuning' cellular dynamics in many organ systems and diseases. However, to date, specific microRNA profiles have not been assigned to EGCs, and the role of microRNAs in EGC phenotype and function are unknown. Ergo, our primary purpose is to elucidate the role of microRNA-mediated regulation of EGC identity and activity during gastrointestinal homeostasis and disease.

To identify microRNAs expressed by EGCs we performed microRNA sequencing of FACS-sorted EGCs isolated from Sox10-CreERT2;nuclGFP mice. This was combined with a targeted approach in which microRNAs were selected based on their evolutionary conservation, differential expression in gastrointestinal disorders, and ability to tune dynamics of CNS glia. The expression of microRNA candidates in healthy human colonic biopsies and murine colonic and small intestinal tissues was examined using qPCR. MicroRNA expression in EGCs was confirmed using fluorescence in situ hybridization (miRNAscope), and microRNA dynamics were evaluated in both in vitro, ex vivo and in vivo models for reactive EGCs.

The selected microRNAs were found to be expressed in the sigmoid colon of healthy volunteers and in the mouse colon and small intestine, albeit differentially, with the highest microRNA levels detected in the colon. A selection of these microRNAs was also detected in isolated murine myenteric plexus preparations of the colon and small intestine, with the highest transcript quantities detected in the distal colon and duodenum. Expression of these microRNAs was confirmed in both primary and secondary EGC cultures and a time-dependent increase in the level of miR-146 was associated with upregulation of markers for enteric gliosis.

This is the first evidence for microRNA expression in EGCs. Our results allude to regional microRNA expression specificity, reveal distinct microRNA signatures that can mark EGC identity, and suggest a possible role for miR-146 in establishing the 'reactive' status of EGCs.

Exploring the molecular hug between the ER and mitochondria in Charcot-Marie-tooth disease type 1A

Hanne Jeurissen, Karen Libberecht, Tim Vangansewinkel, Esther Wolfs

Cardio & organ systems, BIOMED, Faculty of Medicine and Life Sciences, Hasselt University, Diepenbeek, Belgium.

Charcot-Marie-Tooth (CMT) disease is an inherited peripheral neuropathy, affecting 1 in 2,500 people worldwide. The most common form of the disease, CMT1A, is predominantly demyelinating and is caused by a Peripheral Myelin Protein 22 (PMP22) gene duplication. PMP22 is an aggregation-prone intrinsic membrane protein of the myelin sheath produced by Schwann cells (SC). The structure and function of myelin are disrupted in CMT1A. However, it is unknown how the overexpression of PMP22 contributes to the pathogenesis and therefore, there is no cure.

Due to the overexpression of PMP22, we believe there is an overload of misfolded PMP22 in the endoplasmic reticulum (ER). We hypothesize that this induces ER stress, leading to the activation of the unfolded protein response (UPR) and Ca^{2+} signalling pathways, mainly regulated by the mitochondria-associated membranes (MAM) where the ER connects to mitochondria. Defective MAM have been demonstrated to play a major role in several neurodegenerative disorders, but their exact function is largely unexplored. Therefore, we aim to investigate the effect of PMP22 overproduction and aggregation in the ER on the UPR response and on MAM-mediated cell signalling and function in CMT1A Schwann cells.

For this study, SC and nerve tissue were isolated from wild-type (WT) or C3-PMP22 mice, an animal model for CMT1A. ER stress was observed in C3 SC and nerve tissue via qPCR with the upregulation of ER stress-related markers. Furthermore, the ER seems to be more densely organized in C3 SC in immunofluorescence stainings. Lastly, mitochondrial changes (e.g. branching) were explored in WT and C3 SC in live-cell imaging experiments using MitoTracker Green. Furthermore, we evaluated aberrant myelination in nerve tissue of CMT1A mice using transmission electron microscopy.

We showed that ER stress and mitochondrial changes are induced in CMT1A SC. In addition, ultrastructural analysis shows that myelination in CMT1A nerves is impaired. In conclusion, our preliminary data demonstrate structural differences in CMT1A SC and tissue. Future experiments are necessary to indicate how this affects SC myelination, providing important insights into CMT1A as well as other neurodegenerative and PMP22-related diseases.

Epigenetic Editing of Oxytocin in Alzheimer's Disease

Philippos Koulousakis

Department Psychiatry and Neuropsychology, European Graduate School of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands
Department of Neuroscience, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

Alzheimer's Disease (AD) is a progressive fatal neurodegenerative disease characterized by neuronal loss, brain atrophy, and cognitive disturbances. Oxytocin is a neuropeptide that has effects on many different processes, such as appetite regulation, empathy, fear, anxiety, and prosocial behaviour. Accumulating evidence suggests that oxytocin also plays a role in memory formation, mainly by maintaining long-term potentiation (LTP). Recently a link between AD-specific DNA methylation

signatures and the oxytocin promoter was established in brains of patients suffering from AD, as well as blood from healthy controls, subsequently predicting conversion to AD. The first aim of the project is to examine the effects of oxytocin treatment on AD pathology both in vitro and in vivo. The second project aim is to investigate the epigenetic signature of the oxytocin promoter in AD and its effect on pathology by means of epigenetic editing. This will be achieved by using CRISPR-dCas9 first in vitro, using the murine hippocampal cell line HT22, as well as in vivo using APP/PS1 mice.

Neurovascular dysfunction and cognitive impairment in a model of heart failure with preserved ejection fraction

S.M.P. Lambrichts[1,2,6], L. van der Taelen[1,7], D. Majcher[1], D. Hermes[3,6], N. Bitsch[7], E.A.V. Jones[4,5,7], J. Prickaerts[3,6], R.J. van Oostenbrugge[2,6,7], S. Foulquier[1,2,6,7]

1 Department of Pharmacology and Toxicology, Maastricht University, Maastricht, The Netherlands.
2 Department of Neurology, Maastricht University Medical Center, Maastricht, The Netherlands.
3 Department of Psychiatry and Neuropsychology, Maastricht University, Maastricht, The Netherlands.
4 Centre for Molecular and Vascular Biology, Department of Cardiovascular Sciences, KU Leuven, Belgium
5 Department of Cardiology, Maastricht University, The Netherlands
6 MH&Ns - School for Mental Health and Neuroscience, Maastricht University, The Netherlands.
7 CARIM - School for Cardiovascular Diseases, Maastricht University, The Netherlands.

Background: The development of vascular cognitive impairment (VCI) and heart failure with preserved ejection fraction (HFpEF) is associated with the presence of comorbidities including obesity, diabetes, hypertension and aging. Microvascular dysfunction and subsequent rarefaction, defined by a decrease in microvessels, may be a key pathological step for the development of both HFpEF and VCI.
Aim - To study the impact of combined vascular risk factors on cerebral blood flow (CBF) and cognition in a rat model of comorbidities with HFpEF.

Methods: Cognitive function of male Lean (Ln, n=15) and Obese (Ob, n=14) ZSF1 rats was studied using a series of behavioural tasks. Body weight (BW) was measured weekly and fasting plasma glucose (FPG) every two weeks. At 33-34 weeks of age, CBF was measured over the barrel cortex via a thinned skull window using laser speckle contrast imaging (LSCI). Neurovascular coupling (NVC) was assessed by whisker stimulation (5Hz, 30sec). Animals were sacrificed at 34-35w for plasma and brain analyses.

Results: BW (Ln=417±8; Ob=616±11 g) and FPG levels (Ln=80±1; Ob=129±4 mg/dl) were higher in Ob vs Ln over time ($p_{time\ x\ group}$<0.0001). At 34-35w, plasma glucose (Ln=11.1±0.3; Ob=19.7±1.3 mmol/l), triglycerides (Ln=0.7±0.1; Ob=14.5±1.5 µmol/l) and cholesterol levels (Ln=2.0±0.1; Ob=6.0±0.4 mmol/l) were increased in Ob vs Ln. Cardiac hypertrophy was observed in Ob vs Ln (heart-to-tibia: Ln=0.03; Ob=0.04 g/mm; p<0.0001). At 31w, short-term memory was impaired in Ob vs Ln rats (p<0.01) in the novel object recognition task (p_{group}<0.01). Furthermore, spatial learning was impaired in Ob vs Ln in the Barnes maze task at 22 and 32w (p_{group}<0.05; $p_{time\ x\ group}$<0.0001), as well as long-term spatial memory in the probe trial (p_{group}<0.05). Although no difference was observed in baseline CBF, NVC was decreased in Ob vs Ln rats (Ln=23.7±1.6; Ob=18.8±1.4, p<0.05).

Conclusion: Cognitive function and NVC were impaired in our HFpEF model. Subsequently, the development of cerebral microvascular dysfunction and rarefaction and their underlying mechanisms are being assessed in a longitudinal study.

Integrative omics-based patient stratification and Alzheimer's Disease subtyping

Valentin Laroche, Ehsan Pishva

Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, The Netherlands

The project aims at developing a data-driven approach to identify molecular biomarkers that allow for the identification of different Alzheimer's disease subtypes. A subtype is defined by a molecular signature, significantly common to a certain group of samples inside a dataset. To achieve this goal, several multi-omics datasets of Late Onset Alzheimer's Disease (LOAD): The Brains for Dementia Research (BDR, n=100) generated by our colleagues in the University of Exeter, the Mount Sinai Brain Bank (MSBB, n=250) and the ROSMAP (n=450). Both the MSBB and the ROSMAP are available from the Accelerating Medicine's Partnership for AD (AMP-AD) portal. These datasets will be used as a basis for the identification of such biomarkers. Once these signatures are identified, the project will aim at refining those signatures for drug repurposing, and targeted therapy for each subtype, aiming at improving the clinical conditions of the patients. Multiple state-of-the-art integrative clustering methods, including Similarity Network Fusion (SNF), Affinity Network Fusion (ANF), a regularized latent variable model based clustering using iCluster, nonnegative matrix factorization (NMF), and Consensus Clustering (CC) are currently used to stratificate the patients. Normalized Mutual Information (NMI) algorithms evaluate the quality of clustering methods. The final members of each cluster are confirmed if they are represented together throughout multiple methods. We examine the correlations between found molecular subtypes and clinical/imaging phenotypes. AD cases will be characterized using tau and amyloid pathologies, and cognitive impairments measurements. Any other available clinical information such as diagnoses, pathologies, NeuroPsychiatric Inventories (NPI) as well as patient demographics will also be checked for correlations. In order to match the identified clusters in different studies at the molecular level, we will extract the top-ranked molecular features and biological pathways contributing to the groups formed by the various clustering algorithms and compared across different brain regions/studies. The identified molecular signatures for each approved LOAD subtype enable a variety of downstream analyses including pathway enrichment analysis. Later on, disease signatures for drug repurposing methods will be identified by testing quantitative changes in expression levels between each LOAD subtype and matched controls for all brain regions/studies. Library of Integrated Cellular Signatures (LINCS)phase I and II drug signatures will be used for downstream analyses. LINCS represents a multi-institutional project whose goal is to identify and categorize molecular signatures that occur when cells are exposed to agents that perturb their normal function (http://www.lincsproject.org/). We will use Spearman's rank correlation and connectivity scores to assess the disease-drug inverse relationship for each LOAD-subtype. Replicated drugs across independent AD cohorts will be considered as a potential therapy for AD. Functional enrichment analyses will be conducted on Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) databases to gain insight into mechanisms of nominated drugs and their targets. Chemical structure similarity of all the candidate drugs will be assessed, and drug clustering will be performed to identify potential similarities.

A preclinical model of the ATN model in Alzheimer's Disease: recapitulating amyloid facilitated tau seeding and associated neurodegeneration

Chritica Lodder[1], Pablo Botella-Lucena[1], Manuel Gutierrez de Rave[1], Sarah Vanherle[1], Hannah Vanrusselt[1], Bernard Hanseeuw[2,3], Ilie-Cosmin Stancu[1], Ilse Dewachter[1]

1 Department of Neuroscience, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium
2 Institute of Neuroscience, Catholic University of Louvain, Brussels, Belgium
3 Gordon Center for Medical Imaging, Department of Radiology, Boston USA

Brains of AD patients are characterized by the presence of amyloid pathology, Tau-pathology and neurodegeneration, referred to as A-, T- and N-pathology respectively. These stages develop in a characteristic spatio-temporal way in AD patients. Previous publications demonstrated amyloid facilitated tau seeding of endogenous tau, highlighting the relevance of amyloid facilitated tau pathology. We now focused on the development of a preclinical model that recapitulates all aspects of ATN pathology, downstream of amyloid pathology. Hereto, we analyzed the effect of amyloid pathology on Tau-seeded pathology, its propagation and subsequent neurodegeneration in crosses of TauP301S and 5xFAD mice. Tau-seeded Tau-pathology was significantly increased in the presence of amyloid pathology. Tau-pathology propagated more efficiently to functionally connected brain areas at the ipsi- and contralateral side, remote from the injection site in the presence of amyloid pathology. Most strikingly, in the presence of amyloid pathology, Tau-seeding induced significant hippocampal and cortical atrophy, correlating with the level of Tau-pathology. Finally, microgliosis significantly increased at the different stages of the ATN axis in this model. Our in vivo model displays amyloid facilitated propagation of Tau-seeded pathology and Tau-induced neurodegeneration. We here present a model robustly recapitulating the ATN pathologies, providing a tool to gain mechanistic insights in the interrelation and synergism between A, T and N pathologies to gain insight in the progressive development of ATN pathologies in AD.

Targeting lipophagy in macrophages improves repair in multiple sclerosis

Mansour Haidar[*1], Melanie Loix[*1], Sam Vanherle[1], Tess Dierckx[1], Tim Vangansewinkel[2], Pascal Gervois[2], Esther Wolfs[2], Ivo Lambrichts[2], Jeroen F.J. Bogie[1], Jerome J.A. Hendriks[1]

1 Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.
2 Department of Cardio and Organs Systems, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium
*authors contributed equally

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system in which microglia and infiltrated macrophages play a crucial role. Driving these phagocytes towards an anti-inflammatory and regenerative phenotype is considered a promising strategy to halt MS disease progression. Recent research showed that sustained intracellular accumulation of myelin-derived lipids skews these phagocytes towards a disease-promoting and more inflammatory phenotype.

Our data now demonstrates that disturbed lipophagy, a selective form of autophagy that helps with the degradation of lipid droplets (LDs), is responsible for the induction of this phenotype. Stimulating autophagy using the natural disaccharide trehalose reduced the lipid load and inflammatory phenotype of myelin-laden macrophages. Moreover, trehalose was able to boost remyelination in the ex vivo brain slice model and the in vivo cuprizone-induced demyelination model.

Obtained results provide us more insight into MS lesion development and repair, and identify lipophagy induction as a promising treatment strategy for MS.

A translational and dimensional framework to investigate the antidepressant action of psychedelics

Igor Magaraggia

Department of Psychiatry and Neuropsychology, Faculty of Health, Medicine, and Life Sciences, Maastricht University

Classical psychedelics have recently gained attention from the scientific community for their presumed antidepressant effects. A small series of randomized controlled trials in various depressive disorders showed rapid and long-lasting reductions in depressive symptoms of low mood and anhedonia after a single or double administration of the drugs. In two narrative reviews, we attempted to answer the question of how psychedelics may induce such remarkable effects. We extended previously proposed models for the antidepressant effects of psychedelics by using a dimensional Research of Domain Criteria (RDoC) approach in which we link concepts from cognitive neuroscience (e.g., negativity bias and pattern separation) and the underlying biological mechanisms of neuroplasticity that seem to be stimulated upon drug administration. Moreover, we argue about the role of the acute mystical and psychedelic experience in the antidepressant effects of psychedelics by discussing preliminary evidence for the positive effects on mood and cognition of intermittent and sub-chronic administration of low doses of psychedelics (i.e. "microdosing"). The idea is that 50% of the effective dose (ED50) at treating depression may be lower than the ED50 for the mystical and psychedelic effects. As a conclusion, we stress the importance of more thorough pharmacological studies that investigate the pharmacokinetic and pharmacodynamic relationships for the effects of psychedelics and eventually propose a framework that might aid the vertical (rodent vs. humans) and horizontal (healthy vs. disease) translation of novel findings that aim to address current gaps of knowledge. We are confident to say that such approaches will help bring advancements in a field that is currently struggling to find new solutions to long-existing problems.

The use of seaweed-derived phytosterols to defeat Alzheimer's Disease

Nikita Martens[1,2,*], Melissa Schepers[2,3,*], Na Zhan[1,4], Frank Leijten[1], Gardi Voortman[1], Assia Tiane[2,3], Ben Rombaut[2,3], Janne Poisquet[2], Nienke van de Sande[1,3], Anja Kerksiek[5], Folkert Kuipers[6], Johan W. Jonker[6], Hongbing Li[4], Dieter Lütjohann[5,*], Tim Vanmierlo[1,2,3,*] and Monique T. Mulder[1,*].

1 Department of Internal Medicine, Section Pharmacology and Vascular Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands

2 Department of Neuroscience, Biomedical Research Institute, Hasselt University, European Graduate School of Neuroscience, Hasselt, Belgium

3 Department of Psychiatry and Neuropsychology, School for Mental Health and Neurosciences, Division Translational Neuroscience, Maastricht University, Maastricht, The Netherlands

4 Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, China

5 Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany 6 Department of Pediatrics, Section of Molecular Metabolism and Nutrition, University Medical Center Groningen, Groningen, The Netherlands.

*Equally contributed

Accumulating evidence indicates a key role for a disturbed cerebral cholesterol transport in the development and progression of AD. We showed that memory of AD mice improves upon activation of brain cholesterol turnover by synthetic activators of liver X receptors (LXRα/β). However, serious side effects including hepatic steatosis render these LXRα/β activators unsuitable for patients. We found that the seaweed Sargassum fusiforme, containing preferential LXRβ-agonist 24(S)-saringosterol, prevented memory decline and reduced Aβ deposition in an AD mouse model without inducing hepatic steatosis. However, Sargassum fusiforme also contains relatively high amounts of toxic elements such as inorganic arsenic (iAs), hampering the translation to humans. The use of a pure compound could serve as an alternative. We examined the effects of purified 24(S)-saringosterol on cognition and neuropathology in AD mice. Six-month-old male APPswePS1ΔE9 mice and wild-type C57BL/6J littermates received 24(S)-saringosterol (0.5 mg/25 g body weight/day) (APPswePS1ΔE9 n=20; C57BL/6J n=19) or vehicle (APPswePS1ΔE9 n=17; C57BL/6J n=19) via oral gavage for 10 weeks. Cognition was assessed using object recognition and object location tasks. Sterols were analyzed by gas chromatography/mass spectrometry, Aβ and inflammatory markers by immunohistochemistry, and gene expression by qPCR. Hepatic lipids were quantified after Oil-Red-O staining. Administration of 24(S)-saringosterol prevented cognitive decline in APPswePS1ΔE9 mice without affecting the Aβ plaque load. 24(S)-Saringosterol prevented the increase in inflammatory marker Iba1 in the hippocampus and cortex of APPswePS1ΔE9 mice. 24(S)-Saringosterol did not affect the expression of lipid metabolism-related LXR-response genes in the hippocampus nor the hepatic neutral lipid content. Thus, administration of 24(S)-saringosterol prevented cognitive decline in APPswePS1ΔE9 mice independent of effects on Aβ load and without adverse effects on liver fat content. The anti-inflammatory effects of 24(S)-saringosterol may contribute to the prevention of cognitive decline. Currently, we are investigating the potential of a Sargassum fusiforme extract free of heavy metals generated by supercritical fluid extraction and other seaweed species containing limited amounts of these toxic elements.

The Impact of Social Support and Coping Strategies in the Development and Evolution of Persistent Post-Surgical Pain

Rinaudo C.M., Mouraux A., Steyaert A.

Institut de Neurosciences, Université Catholique de Louvain

The biopsychosocial model is one of the predominant models in medicine used to understand illnesses. According to it, biological as well as psychological and social factors impact one person's health status. However, in pain, limited research has evaluated the impact of social support, even less so on post-surgical pain. In addition to that, most studies that evaluate social support in a pain setting are cross-sectional studies. Such studies do not allow to make any conclusion on the link (except for correlations) between two variables.

This prospective questionnaire-based longitudinal study will focus on patients undergoing one of the following surgical procedures: thoracotomy, sternotomy, abdominal surgery, breast surgery, gynaecological surgery (hysterectomy), or inguinal hernia treatment. These surgical interventions were chosen because they are practiced commonly and are associated with a relatively high incidence of persistent post-operative pain.

Patients are going to be recruited before undergoing surgery. An assessment, through online questionnaires, of psychosocial factors and pain before surgery is going to be made. Follow up online

questionnaires are going to be sent at different time points, up to 6 months after surgery. This time setting will allow to assess chronification of post-surgical pain.

Questionnaires are going to be online and are sent by e-mail using the REDCap platform. This platform is widely used for clinical studies using questionnaires.

The aim of the study is to determine how social support and coping strategies impact the development and evolution of post-surgical pain.

Microglial TRPV4 deficiency improves recovery after spinal cord injury by preventing excessive microgliosis

Melanie Mertens, Yeranddy A. Alpizar, Bert Brône

Biomedical research institute, Hasselt University

Spinal cord injury (SCI) is a severe life-altering neurological condition where a cascade of inflammatory and pathological processes leads to severe tissue damage and irreversible loss of function. Acutely after injury, microglia have a pivotal role as the primary phagocytic cells to clear the area and protect the glial scar. They are crucial to contain the lesion, but excessive proliferation and their pro-inflammatory character outweigh their beneficial effects, leading to sub-optimal recovery after SCI.

Interestingly, the lack of the mechanosensory channel TRPV4 (transient receptor potential vanilloid 4) is associated with reduced microgliosis and inflammation at the lesion site, improving functional recovery. TRPV4 is a Ca2+-permeable channel implicated in several microglial functions such as morphology, motility, proliferation and phagocytosis. Of note, mechanical stress and arachidonic acid metabolites are direct activators of TRPV4 present at the lesion site. Therefore, we hypothesize that recovery after SCI is improved by preventing TRPV4-induced excessive proliferation, pro- inflammatory cytokine production and phagocytosis in microglia.

To disentangle the individual contribution of microglial TRPV4, we will create microglia-specific TRPV4-deficient bone marrow chimera and a microglia-specific Trpv4 conditional knockout model using a contusive model of SCI. Recovery after injury will be evaluated using high-resolution imaging techniques and behavioral testing. Besides, we will use freshly isolated microglia to unravel the contribution of TRPV4 signaling in microglial proliferation, cytokine and nitric oxide production, cytoskeletal dynamics and phagocytosis. Altogether, we expect to provide a comprehensive view of microglial TRPV4 contribution, underscoring the value of TRPV4 as a therapeutic target for SCI treatment.

Hippocampal subfields associations with prediabetes and type 2 diabetes: Preliminary results from The Maastricht Study

J. Monereo-Sanchez[1, 2, 3], J.F.A. Jansen[1, 3], M.P.J. van Boxtel[4], W.H. Backes[1, 3, 5], S. Köhler[1, 6], C. Stehouwer[5, 7], A.A. Kroon[2, 5, 7, 8], D.E.J. Linden[1, 2], M.T. Schram[1, 5, 7, 8]

1 School for Mental Health & Neuroscience, Maastricht, the Netherlands.

2 Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, the Netherlands.

3 Department of Radiology & Nuclear Medicine, Maastricht University Medical Center, Maastricht, the Netherlands.

4 Alzheimer Centrum Limburg, Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands
5 School for Cardiovascular Diseases, Maastricht University, Maastricht, the Netherlands.
6 Department of Psychiatry and Neuropsychology (A.F.J.G., S.K., M.T.S.), Maastricht University Medical Center (MUMC+), the Netherlands
7 Department of Internal Medicine, Maastricht University Medical Center, Maastricht, the Netherlands
8 The Netherlands Heart and Vascular Centre, Maastricht University Medical Centre+, Maastricht, The Netherlands

Background: There is extensive evidence that type 2 diabetes mellitus (T2DM) is associated with an increased risk of structural brain abnormalities. Lower hippocampal volumes are commonly detected in individuals with T2DM. Yet, whether this volume reduction already occurs in prediabetes, and which specific hippocampal subfields are affected is still unclear. Therefore, we investigated whether both T2DM and prediabetes are associated with specific hippocampal subfields atrophy.

Methods: We used data from 4724 participants (58.7±8.5 years, 51.5% women) of The Maastricht Study, a population-based cohort study with oversampling for T2DM (Schram et al., 2014). Glucose metabolism status was determined with an oral glucose tolerance test and defined according to the World Health Organization 2006 criteria (World Health Organization, 2006). Participants were classified in three groups: T2DM (n=869), prediabetes (n=671), or normal glucose metabolism (NGM, n=3184). Brain imaging was acquired with a 3T Siemens MRI scan. Images were segmented using FreeSurfer v.6.0 (Fischl, 2012; Iglesias et al., 2015). The total volume of the hippocampi, as well as 12 hippocampal subfields per hemisphere were obtained, i.e.: hippocampal tail, subiculum, Cornu Ammonis (CA) 1 to 4, hippocampal fissure, presubiculum, parasubiculum, molecular layer of the hippocampus, granule cell and molecular cell layer of the dentate gyrus, fimbria, and hippocampus-amygdala-transition-area (HATA). Quality control of the resulting segmentations was performed by exclusion of outliers based on Euler numbers (Monereo-Sánchez et al., 2021). Multiple linear regression analysis was used to assess the associations of T2DM and prediabetes with total hippocampus and hippocampal subfields volumes. NGM was used as reference group. Analyses were corrected for MRI lag time, total intracranial volume, age, sex, education level, alcohol consumption, waist circumference, high density lipoprotein, and glomerular filtration rate. Given 24 subfields are analysed and to maintain a type I error rate of 5%, Matrix Spectral Decomposition (Nyholt, 2004) was used to determine the effective number of independent variables (n=13), therefore, alpha threshold for significance was set at 0.05/13= 0.0039.

Results: T2DM was associated with smaller total hippocampal volume in both hemispheres ($\beta<-0.18$, $p<2.1\times10^{-7}$). Smaller volumes were found bilaterally in those subfields integrating the hippocampal formation, i.e. dentate gyrus ($\beta<-0.16$, $p<4.2\times10^{-6}$), subiculum ($\beta<-0.15$, $p<5.3\times10^{-5}$), and CA 1 to 4 ($\beta<-0.12$, $p<1.1\times10^{-3}$); in addition to fimbria ($\beta<-0.19$, $p<1.7\times10^{-7}$) and tail ($\beta<-0.16$, $p<2.4\times10^{-5}$). Lower volumes were also observed bilaterally in the HATA subfield ($\beta<-0.11$, $p<0.05$), although it does not survive multiple comparison correction. Prediabetes showed no significant associations with total hippocampal volume, although minimal signs of atrophy that do not survive multiple comparison correction for right CA3 ($\beta=-0.08$, $p<0.05$) and fimbria ($\beta=-0.09$, $p<0.05$) were observed.

Conclusion: There is a generalized hippocampal atrophy associated with T2DM, which is independent of demographics, cardiovascular and lifestyle risk factors. This atrophy is not yet observable in our analysis for prediabetes stages, which could give a window of action in this stage for the early prevention of brain disease.

Unravelling the effect of LF-EMS in a model of ischemia/reperfusion

Amanda Moya-Gomez[1,2], Lena Perez-Font[3], Hannelore Kemps[1], Bert Brone[1], Annelies Bronckaers[1]

1 Biomedical research institute, Hasselt University

2 Department of Biomedical Engineering, Oriente University

3 National Center of Applied Electromagnetism, Oriente University (UO)

Stroke is one of the leading causes of death and acquired disability worldwide. Ischemic stroke is the most common type of stroke and its treatment is limited nowadays. Low-frequency electromagnetic stimulation (LF-EMS) is an emerging and non-invasive therapy for stroke. Previous data from our group indicated that LF-EMS improved neurological outcome in rats subjected to permanent global cerebral ischemic stroke and decreased infarct volume in mice submitted to permanent focal ischemia. However, the effect of LF-EMS at the proposed doses in a model of ischemia/reperfusion is still unknown. Here we studied the effect of LF-EMS in a gerbils submitted to 10 min bilateral common carotid artery occlusion.

Animals were stimulated with LF-EMS (13.5mT/60Hz) for 20 min during four consecutive days or sham-treated. Brain tissues were harvested 24 hours, 3 days and 7 days after stroke. Brain sections of 7 µm were stained with NeuN, Iba-1 and GFAP to investigate neurons, microglia/macrophages and astrocytes respectively. Three different fields of the hippocampus CA1 and CA2 regions were analysed. At 7 days post-surgery, the treated group presented an increased neuronal density in the pyramidal striatum compared to sham (96.14±21.7 vs 49.36±13.14). On the other hand, GFAP+ cells were significantly decreased after LF-EMS (16.36±1.71 compared to 23.00±2.52) at this time point. Iba-1 + cells were also reduced in the treated group but this effect was already evident since day 3 and peak at day 7 (15.68±2.21 vs. 26.69±4.07). To identify if this presence of microglia/macrophages in the hippocampus was due to proliferation or to migration, we performed immunofluorescent staining with Ki67 in samples from the 3th day post ischemia with no difference between groups (p=0.4974, n=4). Proliferation was also assesed in vitro using the BV2 cell line exposed to 4h of oxygen and glucose deprivation (OGD)(5%CO2 and 2% O2). After 4h, the OGD medium was changed for standard medium (to mimic reperfusion) and cells were treated with LF-EMS for 20 min. Cell proliferation was monitored in the Incucyte system. No effect in proliferation was observed between the treated and control group. Migration was evaluated with the scratch assay (using Ibidi silicone inserts) and Transwell inserts (8 µm pores). In both assays, cells were stimulated with LPS or ATP (as chemoattractant in the transmigration experiment). One treatment with LF-EMS reduced LPS-induced BV2 migration (0.97±0.22 compared to 1.55±0.11) and transmigration (1.22± 0.08 compared to 1.49±0.11). Similar to that, LF-EMS significantly reduced ATP-induced migration (0.67±0.05 vs. 1.47±0.06) and transmigration (1.19±0.11 vs. 1.78±0.12). These findings suggest that the observed LF-EMS decrease in microglia/macrophages after stroke is related to migration, not to proliferation. In conclusion, our data show that LF-EMS treatment affects microglia/macrophages in an ischemia/reperfusion model of ischemic stroke.

Cerebral hypoperfusion induced by carotid stenosis leads to hypoxia in oligodendrocyte precursor cells

Narek Manukjan[1,2], Peter Leenders[1], Florian Caiment[3], Marcel van Herwijnen[3], Hubert Smeets[3,4], Tim Vanmierlo[4,5,6], Zubair Ahmed[2], Daniel Fulton[2], W. Matthijs Blankesteijn[1], Sébastien Foulquier[1,4,7]

1 Department of Pharmacology and Toxicology, CARIM - School for Cardiovascular Diseases, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

2 Neuroscience and Ophthalmology Research Group, Institute of Inflammation and Ageing, University of Birmingham, Birmingham B15 2TT, UK
3 Department of Toxicogenomics, GROW – School for Oncology and Developmental Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands
4 MHeNs—School for Mental Health and Neuroscience, Maastricht University, PO Box 616, Maastricht 6200 MD, The Netherlands.
5 Department of Immunology, Biomedical Research Institute, Hasselt University, Hasselt 3500, Belgium.
6 Department of Psychiatry and Neuropsychology, European Graduate School of Neuroscience, Maastricht University, PO Box 616, Maastricht 6200 MD, The Netherlands.
7 Department of Neurology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

Background: Cerebral small vessel disease (cSVD) is the most prevalent cause of vascular dementia. A common feature in cSVD patients is the progression of white matter lesions (WML). A recent clinical study demonstrated that WML correlates with decreased cerebral blood flow (CBF) in cSVD patients. We hypothesize that cerebral hypoperfusion causes local hypoxia affecting the function of oligodendrocytes (OL) and their precursor cells (OPC), thereby leading to the development of WML.

Methods: 11-week-old C57BL6/J male mice were subjected to bilateral carotid artery stenosis (BCAS, n= 10) or Sham surgery (Sham, n= 7). CBF was measured by Laser Speckle Contrast Imaging before (baseline) and after surgery (d0 and d7). At d7, mice were injected with pimonidazole (60mg/kg, IP), a hypoxic probe, prior to sacrifice. Hypoxic cells and OPC/OL densities were quantified by brain immunohistochemistry for Olig2 (identifying both OPC and OL) and adenomatous polyposis coli (APC, identifying only OL). Primary mouse OPC were exposed to hypoxic (2% O_2) or normal culturing conditions (21% O_2) for 24h. Poly(A) enriched RNA was used for library preparation (Rapid Directional RNASeq) and sequenced at a depth of 33M-44M reads/sample (Novaseq Illumina sequencer). Transcriptomic data was normalized and differentially expressed genes (DEG) were identified using DEseq2. Gene Ontology (GO) enrichment pathway and gene function analysis were performed.

Results: CBF was significantly reduced in BCAS at d0 (-14,6±5,3%, p=0.02), and at d7 (-27,2±3,6%, p<0.0001) compared to baseline. An increased number of hypoxic cells in the deep cortical region (DCR) near the corpus callosum was observed in BCAS vs Sham (12,9±1,3 vs 7,8±1,1 cells/mm^2, p=0.01). The proportion of hypoxic cells that were OPC was increased (17,9±2,3% vs 7,9±3,9%, p=0.04) in BCAS vs Sham, whereas there was no difference in OPC/OL cell densities. In vitro hypoxia led to 417 DEG in primary OPC. GO enrichment analysis showed regulation of genes involved in cell migration, differentiation, angiogenesis and Wnt signaling pathways.

Conclusion: Cerebral hypoperfusion induced hypoxia in OPCs. Several signaling pathways involved in myelination and vascularity are regulated in hypoxic OPC in vitro. The function of these signaling pathways in OPC-vessel signaling will be examined in future studies as they may be of major importance to understand the origin of WML in cSVD.

The sGC stimulator BAY-747 and activator runcaciguat can enhance memory in vivo via differential hippocampal plasticity mechanisms

Ellis Nelissen[1], Nina Possemis[1], Nick P. van Goethem[1], Melissa Schepers[2], Danielle A. J. Mulder-Jongen[1], Lisa Dietz[3], Wiebke Janssen[3], Michael Gerisch[3], Jörg Hüser[3], Peter Sandner[3,4], Tim Vanmierlo[1,2], Jos Prickaerts[1]

1 Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNS), Maastricht University, Universiteitssingel 50, 6229 ER, Maastricht, The Netherlands

2 Neuro-immune connect and repair lab, Biomedical Research Institute, Hasselt University, Hasselt 3500, Belgium.

3 Bayer AG, Pharmaceuticals R&D, Pharma Research Center, 42113 Wuppertal, Germany

4 Hannover Medical School, 30625 Hannover, Germany

Soluble guanylate cyclase (sGC) requires a heme-group bound in order to produce cGMP, a second messenger involved in memory formation, while heme-free sGC is inactive. Two compound classes can increase sGC activity: sGC stimulators acting on heme-bound sGC, and sGC activators acting on heme-free sGC. In this rodent study, we investigated the potential of the novel brain-penetrant sGC stimulator BAY-747 and sGC activator runcaciguat to enhance long-term memory and attenuate short-term memory deficits induced by the NOS-inhibitor L-NAME. Furthermore, hippocampal plasticity mechanisms were investigated. In vivo, oral administration of BAY-747 and runcaciguat to male Wistar rats enhanced memory acquisition in the object location task (OLT), while only BAY-747 reversed L-NAME induced memory impairments in the OLT. Ex vivo, both BAY-747 and runcaciguat enhanced hippocampal GluA1-containing AMPA receptor (AMPAR) trafficking in a chemical LTP model for memory acquisition using acute mouse hippocampal slices. In vivo only runcaciguat acted on the glutamatergic AMPAR system in hippocampal memory acquisition processes, while for BAY-747 the effects on the neurotrophic system were more pronounced as measured in male mice using western blot. Altogether this study shows that sGC stimulators and activators have potential as cognition enhancers, while the underlying plasticity mechanisms may determine disease-specific effectiveness.

Cerebral microbleeds are associated with amyloid positivity in an age-dependent manner: The Amyloid Biomarker Study

J. Oomens[1], W. Jansen[1], O. Janssen[1], D. Alcolea[2,3], F. Barkhof[4,5], B. van Berckel[4], M. Bottlaender[6], J. Cerman[7], W. van der Flier[8], J. Fortea[2,3], J. Hort[7], J. Lagarde[9], A. Lleó[2,3], M. Olde Rikkert[10], P. Olivieri[9], K. Rodrigue[11], M. Sarazin[9], P. Scheltens[8], C. Teunissen[8], N. Maserejian[12], F. Verhey[1], S. Vos[1], P.J. Visser[1,8]

1 Maastricht University, Alzheimer Center Limburg, Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht, Netherlands.

2 Hospital de Sant Pau, Sant Pau Memory Unit, Institut d'Investigacions Biomèdiques Sant Pau, Neurology Department, Barcelona, Spain.

3 Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, CIBERNED, Madrid, Spain.

4 Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Radiology and Nuclear Medicine, Amsterdam, Netherlands.

5 University College London, Queen Square Institute of Neurology and Centre for Medical Image Computing, Department of Medical Physics and Biomedical Engineering, London, United Kingdom.

6 Service Hospitalier Frédéric Joliot, Université Paris-Saclay, CEA, CNRS, Inserm, BioMaps, Orsay, France.

7 Charles University and Motol University Hospital, Department of Neurology, Second Faculty of Medicine, Prague, Czech Republic.

8 Amsterdam UMC, Vrije Universiteit Amsterdam, Alzheimer Centre Amsterdam, Department of Neurology, Amsterdam Neuroscience, Amsterdam, Netherlands.

9 Hôpital Sainte Anne, Paris, Université de Paris, Paris, Université Paris-Saclay, BioMaps, CEA, CNRS, Inserm, Department of Neurology of Memory and Language, GHU Paris Psychiatry and Neurosciences, Orsay, France.
10 Radboud University Medical Center, Radboudumc, Alzheimer Centre, Nijmegen, Netherlands.
11 The University of Texas, Center for Vital Longevity, School of Behavioral and Brain Sciences, Dallas, United States of America.
12 Biogen, Cambridge, MA, United States of America.

Aims: Cerebral microbleeds (CMBs) are common in persons with Alzheimer's disease (AD) and are associated with hemorrhage and cognitive decline. The aim of this study was to examine the association between CMBs and amyloid pathology in individuals with normal cognition (NC), mild cognitive impairment (MCI) and dementia.

Methods: We included 781 participants with NC, 355 participants with MCI and 230 participants with dementia from 8 cohorts included in the Amyloid Biomarker Study. Amyloid-beta positivity was determined with amyloid-PET (center-specific cutoffs) or aß42 level in CSF (data-driven cutoffs). Participants were classified as having any CMBs (≥1) yes/no. Associations of amyloid positivity, APOE-e4 carriership, cognitive status, sex and age with CMBs were assessed using generalized-estimating-equations.

Results: Forty-seven percent of participants were amyloid-beta positive, 38% were APOE-e4 carrier, 48% were female and 19% had CMBs. The mean age was 68.5 years (SD11.2). CMB prevalence increased with age ($p<0.001$) and was associated with amyloid-beta status, depending on age ($p<0.001$). In those under 75 years of age, CMBs were more common in amyloid positive than amyloid negative participants. This association was reversed at older ages, where amyloid negative participants had CMBs more often. Cognitive status, APOE-e4 carriership and sex were not associated with CMB occurrence in this sample, most of whom were memory clinic patients.

Conclusions: CMB prevalence is associated with amyloid positivity in an age-dependent manner. This sheds light on the underlying pathophysiology. Future research on the background incidence rate of CMBs considering AD biomarker status would be helpful for clinical trial design and safety evaluations of AD therapies.

PDE inhibition to counteract neuroinflammation in ischemic stroke

Laura Ponsaerts[1], Melissa Schepers[1,2], Hannelore Kemps[1], Lotte Alders[1], Annelies Bronckaers[1*], and Tim Vanmierlo[1,2,3*]

1 Biomedical Research Institute (BIOMED), Hasselt University, Diepenbeek, Belgium
2 European Graduate School of Neuroscience (EURON), Universiteitssingel 40, 6229 ER Maastricht, The Netherlands
3 Department Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, European Graduate School of Neuroscience, Maastricht University, 6200 MD Maastricht, The Netherlands
*Equally contributing senior authors

Stroke remains the 3rd leading cause of death worldwide, substantially contributing to permanent disability. Ischemic stroke is the most prevalent type of stroke accounting for about 80% of all stroke cases. This devastating neurological disorder, is caused by a thromboembolic occlusion of a cerebral artery, which leads to a disturbed blood flow and subsequent deprivation of oxygen and nutrients to the surrounding brain tissue. This disturbed blood flow triggers an acute neuroinflammatory reaction with several peripheral immune cells including neutrophils and monocytes/macrophages are attracted

towards the ischemic brain. In addition, a group of enzymes named phosphodiesterases (PDEs) becomes upregulated within the brain. PDEs can be classified into 11 different families (e.g. PDE4), with each family consisting out of several genes (e.g. PDE4A-D). In addition, every gene contains multiple isoforms. We hypothesize that specific PDE gene inhibition reduces neuroinflammation following ischemic stroke, thereby also reducing the lesion size and improving functional recovery. We found that PDE4B inhibition significantly reduced the lesion size (by 2-fold) 24 hours following stroke induction in the distal middle cerebral artery occlusion (dMCAO) mouse model. In addition, the number of infiltrated neutrophils was significantly reduced 24 hours after stroke induction while anti-inflammatory macrophages were increased upon PDE4B inhibition in the dMCAO mouse model. In future experiments, the effect of PDE4B inhibition on functional outcome as well as the exact mode of action will be evaluated. Taken together, our results already indicate that PDE4B inhibition is a promising therapeutic strategy for ischemic stroke.

Machine Learning-based Prediction of Cognitive Outcomes in de novo Parkinson's Disease

Joshua Harvey, BSc,[1†] Rick A Reijnders, MSc,[2†] Rachel Cavill, PhD,[3] Annelien Duits,[2] PhD, Sebastian Köhler, PhD,[2] Lars Eijssen, PhD,[2] Bart PF Rutten, MD, PhD,[2] Gemma Shireby PhD,[1] Ali Torkamani, PhD,[4] Byron Creese, PhD,[1] Albert FG Leentjens, MD, PhD,[2] Katie Lunnon, PhD,[1] Ehsan Pishva, MD, PhD[1,2*]

1 University of Exeter Medical School, University of Exeter, Exeter, United Kingdom

2 Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNS), Maastricht University, Maastricht, The Netherlands

3 Department of Data Science and Knowledge Engineering, FSE, Maastricht University, Maastricht, The Netherlands

4 Department of Integrative Structural and Computational Biology, Scripps Research, La Jolla, California, 92037, USA.

[†] These authors contributed equally to this work

Background: Cognitive impairment is a debilitating symptom in Parkinson's disease (PD), with high variability in onset and the course of progression.

Objectives: To establish an accurate multivariate machine learning (ML) model to predict cognitive outcome in newly diagnosed PD.

Methods: We used baseline clinical and biological measures from the Parkinson's Progression Markers Initiative (PPMI) cohort as predictive variables. Annual cognitive assessments over an eight-year time span were used to define two cognitive outcomes of i) cognitive impairment, and ii) dementia conversion. Selected variables were organized into three subsets of clinical, biofluid (CSF and serum) and genetic/epigenetic (blood) measures and tested using four different ML algorithms to predict individual cognitive function in PD.

Results: For both cognitive outcomes, irrespective of the ML algorithm used, the models consisting of the clinical subset of variables performed best, with high specificity and the largest area under the curve (AUCs) (0.88 – 0.92) and Matthews Correlation Coefficient (MCCs) (0.57 – 0.80). Notably, the cognitive impairment outcome showed better sensitivity than dementia conversion outcome (0.72 – 0.81 vs 0.29 – 0.64, respectively). We observed a marginal improvement in the prediction performance when clinical, biofluid, and epigenetic/genetic variables were all included in one model. Several CSF variables and an epigenetic marker showed high predictive weighting in multiple models only when included alongside clinical variables.

Conclusion: Within the generated models, clinical predictors appear to play a more prominent role than biological or genetic predictors. ML algorithms can accurately predict cognitive impairment and dementia in de-novo PD up to eight years before cognitive decline.

Distribution and roles of the transcription factors of the Arid3 family during the development of spinal V2 interneurons

Estelle Renaux[1], Charlotte Baudouin[1], Younès Achouri[2], Frédéric Clotman[1]

1 Neural Differentiation (NEDI) Laboratory, Institute of Neurosciences (IONS), Université catholique de Louvain (UCLouvain), Brussels, Belgium

2 Transgenic Core facility, Université catholique de Louvain (UCLouvain), Brussels, Belgium

Locomotion is regulated by Central Pattern Generators (CPG) that generate rhythmic locomotor movements. These circuits located in the ventral part of the spinal cord are composed of motor neurons (MN) and of interneurons (INs). Motor neurons innervate directly the skeletal muscles while interneurons regulate the activity of motor neurons. V2 interneurons derive from the p2 progenitor domain. This domain produces intermediate V2 precursors characterized by the transient expression of Vsx1. Then those cells diversify into at least 5 distinct subpopulations characterized by the specific expression of transcription factors and specific roles during locomotion. However, the mechanisms that control their development remain poorly understood and some V2 subpopulations are yet to be characterized. An RNA sequencing experiment allowed to compare the V2 transcriptome with the transcriptome of the other cells of the spinal cord. It enabled the identification of Arid3c, a transcription factor particularly abundant in V2 interneurons. During the beginning of my thesis, I showed that Arid3c is expressed specifically in V2 interneurons. Arid3c is present in part of the V2c interneurons but also in cells that are V2 interneurons but that don't correspond to any known V2 population. Therefore, Arid3c characterizes a new V2 subpopulation. I also showed that Arid3c can stimulate the differentiation of V2c in chicken embryos. For the rest of my thesis, I will determine the role of Arid3c in V2 IN differentiation by performing gain- and/or loss-of-function experiments in the mouse.

PDE4B as a key regulator of out of control microglia

B. Rombaut[1 2], S. Kessels[1], M. Schepers[1 2], A. Tiane[1 2], D. Paes[2], P. Koulousakis[1 2], E. Willems[1], D. van den Hove[2], J. Prickaerts[2], B. Brône[1], T. Vanmierlo[1 2]

1 Department of Neuroscience, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.

2 Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands.

Background: Microglia are responsible for excessive synaptic loss, taking place as early as the prodromal phase in the pathophysiology of Alzheimer's Disease (AD). Phosphodiesterase 4 (PDE4) has been extensively studied in the inflammatory environment witnessed in late-stage AD. However, the function of breaking down cyclic adenosine monophosphate (cAMP) influences the microglial cells' capacity for phagocytosis. We hypothesized that PDE4, and in particular PDE4B which is the most abundant in microglial cells, regulates microglia-mediated synaptic elimination in the hippocampus, resulting in cognitive deficits as witnessed in APP/PS1 mice.

Materials & Methods: Primary murine microglia derived from PDE4B knockout animals were seeded 150 000 cells/well on 96-well plates and pre-treated for 30 min or 1h with 50 or 100µg of DiI-labeled synaptosomes. Percentage and mean fluorescent intensity of cells were measured using the BD FACSAria II flow cytometer. To test phagocytic capacity of amyloid beta, purified microglia were seeded 50 000 cells/well on 96-well plates and FAM-labeled Aβ was added for 1h at a final concentration of 500nM. After aspiration, fluorescence was measured and normalized for cell numbers using DAPI.

Results: We showed that disrupted PDE4B signaling alters microglial cells' phagocytic capacity of synaptosomes and amyloid beta. In summary, we show that PDE4 signaling is an important regulator of microglial phagocytosis and poses an interesting target for early prophylactic intervention.

Myelin, PDE4B and neuroinflammation; the good, the bad, and the ugly

Melissa Schepers[1,2,3], Assia Tiane[1,2,3], Ben Rombaut[1,2,3], Laura Van der Taelen[1,3], Paulien Baeten[3,4], Bieke Broux[3,4], Niels Hellings[3,4], Jos Prickaerts[2], Tim Vanmierlo[1,2,3]

1 Department of Neuroscience, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium.

2 Department Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands.

3 University MS Center (UMSC) Hasselt – Pelt, Belgium.

4 Department of Immunology and Infection, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium.

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by focal inflammatory lesions and prominent demyelination. Pathologically, MS is defined by the massive perivenular infiltration of myelin-reactive lymphocytes that subsequently disturb the homeostatic immune balance in the brain, creating a pro-inflammatory microenvironment and subsequent demyelination. Importantly, the persistent and disturbed homeostatic balance in the CNS of MS patients skews the activation of phagocytes to fuel neuro-inflammatory responses. To cease the inflammatory response in MS, restoring the balance between cytotoxic and reparative phagocytes in the CNS can be considered powerful therapeutic option.

Recently, we have shown that the full FDA-approved PDE4 inhibitor roflumilast possesses anti-inflammatory properties as we observed a suppressed emergence of neurological symptoms in experimental autoimmune encephalomyelitis (EAE) mice, a murine model for MS, and reduced phagocyte mediated production of NO upon inflammatory stimulation. However, the therapeutic dose of full PDE4 inhibitors coincides with emetic side effects, decreasing their translational value.

In this study, we identified that specifically the PDE4B subtype inhibitor, A33, exerts anti-inflammatory effects both in vitro and in vivo. Both murine and human macrophages reduced their NO production after A33 treatment upon inflammatory cytokine stimulation and myelin loading. Furthermore, using the experimental autoimmune encephalomyelitis (EAE) animal model for MS, we found that a twice daily administration of A33 (subcutaneous, 3mg/kg) improved neurological scores during the early phases of the disease without PDE4i-related side-effects. Post mortem analysis revealed a reduced percentage of inflammatory monocytes in the CNS at disease peak, which was accompanied by reduced pathogenic and increased non-pathogenic T cell percentages.

Taken together, we found that by targeting specifically the PDE4B subtype, the observed anti-inflammatory of full PDE4 inhibitors can be preserved. Furthermore, we showed that by targeting the PDE4B gene, we can bypass the emetic side effects, rendering PDE4B gene-specific inhibitors more suitable therapeutic targets.

Tumor Phenotype Is Influenced by Enteric Neuron Density in Colorectal Cancer

Simone L. Schonkeren[1], Meike S. Thijssen[1,2], Musa Idris[1,4], Isabel van Dorland[1], Kim Wouters[1], Natasja G. Lieuwes[5], Nathalie Vaes[1], Ludwig Dubois[5], Marion J. Gijbels[1,3] Werend Boesmans[1,2], Veerle Melotte[1,4]

1 Department of Pathology, GROW–School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

2 Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

3 Departments of Pathology and Molecular Genetics, CARIM–School for Cardiovascular Diseases, Maastricht University, Maastricht, The Netherlands

4 Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

5 The M-Lab, Department of Precision Medicine, GROW–School for Oncology and Developmental Biology, Maastricht University, Maastricht, Netherlands

Background: Even though it has been described that a higher abundance of nerves in, or surrounding, the tumor is associated with a worse patient prognosis, and that enteric neurons communicate with CRC cells, the role of enteric neurons in tumorigenesis and their effect on tumor phenotype still remains a black box. In this study we aim to investigate how reduced enteric neuronal density affects CRC development, progression and phenotype.

Methods: Hand2fl/+Wnt1Cre2 (hypo-innervated) and Hand2fl/+ (wildtype) mice were subjected to a colitis-associated CRC induction protocol using azoxymethane and dextran sulfate sodium. To track tumor initiation, growth and burden, we acquired and analyzed computed tomography scans during cancer induction and histologically assessed tumor specimens after sacrifice. Isolated tumor tissues from hypo-innervated and wildtype mice were subjected to RNA sequencing, followed by a gene ontology enrichment analysis (GOrilla). In addition, gene-set enrichment analysis on differentially expressed genes was performed for hallmark of cancer genes using Fisher's exact test.

Results: Despite the confirmed reduction in enteric neurons, Hand2fl/+Wnt1Cre2 mice presented with a similar tumor burden (i.e. tumor initiation, growth and progression) as the Hand2fl/+ mice. RNA sequencing of tumors showed differentially expressed genes belonging to the cancer hallmarks 'avoiding immune destruction' and 'deregulating cellular energetics' when comparing both genotypes. Moreover, an enrichment of the gene ontology (defense) response to bacterium was found.

Conclusion and future plans: A reduced number of enteric neurons prior to tumorigenesis does not directly impact murine tumor growth, but affects tumor phenotype. Further research is needed to elucidate these phenotypic differences and corresponding mechanisms. In addition, stool samples will be analyzed for microbiome differences using 16S rRNA sequencing.

Altered gastrointestinal function in a mouse model for major mental illness

Kinga Réka Tasnády[1,2], Jan Spaas[1,3], Ana Carina Bon-Frauches[2], Nathalie Vaes[2], Akira Sawa[4], Bert Brône[1], Veerle Melotte[2,5], Werend Boesmans[1,2]

1 Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

2 Department of Pathology, GROW–School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

3 Department of Movement and Sports Sciences, Ghent University, Ghent, Belgium

4 Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, US

5 Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

Neurodevelopmental disorders such as schizophrenia and autism spectrum disorder often go hand in hand with gastrointestinal dysfunction. However, the mechanisms underlying gastrointestinal symptom generation in these diseases primarily striking the central nervous system remain obscure. Aberrant expression of Disrupted in Schizophrenia 1 (DISC1), a hub and scaffold protein that plays an essential role in neural maturation and connectivity, is a major risk factor associated with the onset of major mental illnesses. Our primary goal is to understand whether perturbation of DISC1 causes faulty enteric nervous system (ENS) wiring and therefore gut dysfunction in neurodevelopmental disorders. To this end, we combined expression analysis using qPCR with in vivo and ex vivo mouse experiments to examine gut function. Gene expression was analysed on intestinal tissue and brain samples isolated from 35-days old C57/BL6J-DISC1 locus impaired (LI), heterozygous (Het) and wild type (WT) littermates (NWT=9, NHet=10, NLI=10), while gut function essays were carried out on adult mice (NWT, Het=10, NLI=8). Although robust DISC1 expression has been detected in ENS precursors at embryonic stages, we found rather low levels of DISC1 in the juvenile mouse gut. Compared to brain samples, DISC1 mRNA levels were lower in the small intestine (p=0.0175) and colon (p=0.0373). As was previously established for the central nervous system, we confirmed knockdown of DISC1 in the gut when comparing expression between LI and WT littermates. Whole gut transit time measurements (oral gavage of 6% carmine red) revealed that LI mice present with significantly faster gastrointestinal transit (97.63±27.42 min) as compared to WT (152.8±55.32 min, p=0.0347) and Het (166.5±59.75 min, p=0.0176) littermates. LI mice also showed a reduced wet weight per stool (6.15±1.95 mg) compared to WT (11.56±4.25 mg, p=0.0086) and Het (12.09±3.77 mg, p=0.0050) littermates. No changes were observed in stool water content (LI: 49.97±13.74% per average stool, WT: 54.52±13.42%, Het: 57.09±7.26%) or intestinal permeability (oral gavage of 0.6 mg/g body weight FITC-Dextran, LI: 256.0±190.5 ng/ml FITC-Dextran, WT: 267.5±257.0 ng/ml, Het:410.4±490.3 ng/ml) between the three genotypes. Together, we demonstrate that disruption of DISC1 alters gastrointestinal motility, as shown by increased faecal output frequency and decreased faecal pellet size. Intestinal mucosal functions do not appear to be affected. While speculating an important role for DISC1 in ENS development, our current studies focus on identifying the cellular source(s) of DISC1 in the adult gut, and the effects of DISC1 disruption on ENS composition and function.

Remote telephone-based assessment and the additional clinical use of speech features in the Semantic Verbal Fluency task

Daphne B.G. ter Huurne[1], Inez H.G.B. Ramakers[1], Nina Possemis[1], Nicklas Linz[3], Duy Truong[3], Alexandra König[3], Kai Langel[5], Kay Deckers[1], Frans R.J. Verhey[1,2] and Marjolein de Vugt[1,2]

1 Alzheimer Center Limburg, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands

2 Maastricht University Medical Center, Maastricht, the Netherlands

3 ki:elements UG, Saarbrücken, Germany. [4]Natrional Institute for Research in Computer Science and Automation (INRIA), Sophia Antipolis, France. [5] Janssen Clinical Innovation, Beerse, Belgium.

Background: The COVID-19 pandemic has accelerated the transition to remote neuropsychological assessment, meaning patients do not have to visit hospitals, which reduces the burden in patients, saves time, and reduces costs. However, the accuracy of a remote telephone-based administration remains unclear. We investigated the accuracy of the Semantic Verbal Fluency (SVF) task and related speech and linguistic features in the diagnostics of cognitive disorders in a face-to-face and semi-automated telephone assessment, and we examined the user-experience of both modalities.

Method: In the DeepSpA project, 134 participants were recruited from the memory clinic of the MUMC+ (Maastricht, the Netherlands). All participants underwent a face-to-face neuropsychological assessment at baseline and a semi-automated telephone-based assessment (after 6 months), both including the SVF task (animals and groceries, 60 seconds) which was administered via the Delta application (ki:elements). The relation between speech and linguistic features, such as mean word frequency, temporal and semantic clusters etc., and clinical diagnosis, were investigated using stepwise regression analyses, corrected for age, education level and gender. Questionnaires on user-experience were administered after each assessment. The questionnaires were analysed with an independent samples t-test comparing the face-to-face with the semi-automated telephone assessment.

Results: Compared to the commonly used SVF total score (AUC=0,67), automatically derived speech and linguistic features had an additional value in the differentiation between people with and without cognitive disorders in the telephone-based assessment (AUC=0,75). The user-experience ratings of simplicity, conceivability, comfortability of the assessment and wanting to repeat the assessment were comparable for both assessment modalities.

Conclusion: Automatically derived speech and linguistic features of the SVF task have an additional value in the early diagnostics of cognitive disorders. A telephone-based assessment could be considered as an easy tool to follow patients remotely over time.

Environmental factors involved in Parkinson's disease: air pollution-derived ultrafine particles induce ferroptosis in an in vitro model of human dopaminergic neurons

Emma Theerens[1,2], Anne-Sophie Rolland[2], Céline Grare[1], Hind Bouchaoui[1,2], Aurélie Jonneaux[1], Flore Gouel[2], Laura Mahoney[2], Jean-Marc Lo Guidice[1], David Devos[2], Guillaume Garçon[1]

1 Univ. Lille, CHU Lille, Institut Pasteur de Lille, ULR 4483-IMPacts de l'Environnement Chimique sur la Santé (IMPECS), 59000 Lille, France

2 Univ. Lille, Inserm, CHU Lille, UMR-S-U1172, LilNCog (JPARC), Lille Neurosciences & Cognition, F-59000, Lille, France

Parkinson's disease (PD), the second most prevalent age-related neurodegenerative disorder, is characterized by a predominant regulated cell death (RCD) of dopaminergic neurons of the Substantia Nigra pars compacta (SNpc). Only 5 % of the disease risk can be dedicated to genetic variations, meaning that many of cases are sporadic by nature. Several key environmental causes have been demonstrated but air pollution-derived ultrafine particles (UFP) remained less studied. However, a recent meta-analysis supports weak evidence for an association between air pollution, mostly originating from traffic, and PD. While apoptosis was considered the only existing RCD to explain neurodegeneration, antiapoptotic treatments failed to induce neuroprotection. In the past decade,

several new types of RCD have been described in PD and among them, ferroptosis. First described in 2012 in cancer, this iron-dependent RCD is characterized by high levels of intracellular iron and lipid peroxidation. While several key effectors, promotors and inhibitors of ferroptosis have all been separately described in PD, our research team was the first to report all of them and demonstrate their pivotal roles in in vitro, ex vivo and in vivo PD models.

In this study, we first assessed whether UFP 1) stimulate neuronal RCD, and 2) contribute to PD phenotype, through ferroptosis in differentiated dopaminergic LUHMES cells at two concentrations (2 and 10 µg/cm²) for 24h by studying the lipid peroxidation mechanism and protection by potent ferroptosis inhibitors. We then compared the obtained phenotype to the classical in vitro PD model, e.g. 1-methyl-4-phenylpyridinium (MPP^+) intoxication. Finally, we addressed whether UFP intoxication led to other RCD reported in PD, such as apoptosis and autophagy.

We first revealed a dose-dependent lipid peroxidation rate after MPP^+ and UFP treatment, by an electrochemiluminescence assay of 4-hydroxynonenal (HNE) adducts. The antioxidant defense system was investigated through glutathione peroxidase (GPx) activity and glutathione (GSH) status. Lower levels of reduced GSH and higher activity of GPX after MPP^+ treatment reflect high level of oxidative stress. Even more, after UFP there was no significant increase of GPX activity, probably due to its partial inactivation by reactive oxygen species. In addition, liproxstatin-1 (LPX) and deferiprone (DFP), both ferroptosis inhibitors, prevented cell death after MPP^+ and UFP exposure, which reinforced these cells died by ferroptosis. We then observed, by measuring the protein expression levels of an autophagy-related protein (i.e. LC3b) using western blotting (WB) that autophagy was only involved after low exposure of MPP^+ and UFP. Moreover, with the WB assessment of active caspase-3 protein expression, apoptosis-related protein, we also reported apoptosis after low exposure of MPP^+ and UFP.

To conclude, our investigation showed that differentiated dopaminergic LUHMES cells exposed to UFP died by ferroptosis and that ferroptosis is the main RCD form compared with autophagy and apoptosis. Interestingly MPP^+-induced PD condition and the exposure of UFP both led to the same results, leading to the conclusion that UFP could contribute to the development of a PD phenotype. Further in vitro and in vivo experiments are now required to support this statement.

The Prognostic Significance of Neuronal and Glial Markers in Colorectal Cancer

Meike S. Thijssen[1,2], Maartje Massen[1], G. Rademakers[1], K.A.D. Wouters[1], J. van der Meer[1], N. Buekers[1], Matty P. Weijenberg[3], Piet A. van den Brandt[3], M. J. Gijbels[1], M. van Engeland[1], Kim M. Smits[1], Werend Boesmans[1,2], Veerle Melotte[1,4]

1 Department of Pathology, GROW – School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

2 Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

3 Department of Epidemiology, GROW - School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands.

4 Department of Clinical Genetics; Erasmus University Medical Center, Rotterdam, The Netherlands

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. Although the TNM (tumor-lymph node- metastasis) staging classification system is still regarded as one of the main prognostic factors, clinical outcome can vary considerably for patients diagnosed in similar stages. Recent findings indicate that neurons and glia within the CRC tumor stroma could affect clinical outcome in CRC patients. In this study, we aim to investigate the

prognostic value of the neuronal markers neurofilament (NF) and protein gene product 9.5 (PGP9.5) and the glial marker glial fibrillary acidic protein (GFAP).

Formalin-fixed, paraffin-embedded (FFPE) tumor tissues (study cohort NF+PGP9.5: n=487, validation cohort NF+PGP9.5 n= 266, study cohort GFAP: n=447) from the Netherlands Cohort Study on diet and cancer (NLCS) were immunohistochemically stained for NF, PGP9.5 and GFAP. Two independent observers assessed the presence of NF, PGP9.5 or GFAP staining within the tumor. Associations between NF and/or PGP9.5 or GFAP expression and multiple clinical and pathological characteristics were investigated using Pearson chi-square test, long-rank tests and Kaplan-Meier. Multivariate analyses were performed with Cox proportional hazard models, adjusted for potential confounders and known prognostic factors.

NF and PGP9.5 positive nerves and GFAP positive glial cells were found within the tumor stroma. Positive staining with NF as single marker was associated with a poorer cancer specific survival (log-rankNF p = 0.013). In the multivariate analysis, adjusted for age at diagnosis, sex, tumor location, differentiation grade and TNM/dukes stage, NF was still associated with a poorer survival (HR 2.38; 95%-CI 1.37-4.13, p = 0.002). However, these results for NF could not be directly validated. In addition to NF, PGP9.5 did not show an effect on survival in the study cohort or validation cohort. In the multivariate analysis, high-density GFAP staining was associated with a better survival compared to negative staining (HR 0.56; 95%-CI 0.33-0.95, p=0.030). Low-density GFAP staining showed the same trend, but was not statistically significant (HR 0.83; 95%-CI 0.53-1.29, p = 0.396).

NF as single marker is significantly associated with a poorer survival in CRC, however these results could not be directly validated. Therefore, the validation cohort will be enlarged to a similar size as the study cohort. High-density GFAP staining is associated with better survival in CRC in the study cohort, warranting validation of these results. These findings provide new evidence for a potential prognostic role of neural markers in CRC and could supplement on the existing evidence of the biological role of neuronal structures in CRC.

DNA methylation regulates the expression of the negative transcriptional regulators ID2 and ID4 during OPC differentiation

Assia Tiane[1,2,3], Melissa Schepers[1,2,3], Renzo Riemens[2,5], Ben Rombaut[1,2,3], Patrick Vandormael[3,4], Veerle Somers[3,4], Jos Prickaerts[2], Niels Hellings[3,4], Daniel van den Hove[2,5,*], Tim Vanmierlo[1,2,3,*]

1 Department of Neuroscience, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

2 Department Psychiatry and Neuropsychology, European Graduate School of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands

3 University MS Center (UMSC) Hasselt – Pelt, Belgium.

4 Department of Immunology and Infection, Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

5 Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany

* Equal contribution

The differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes is a well-established process, coordinated by an intricate network of transcriptional regulators. Epigenetic mechanisms, such as DNA methylation, have been suggested to control this network. The exact

mechanism by which DNA methylation influences the myelin regulatory pathway during OPC differentiation remains poorly elucidated though. Here, we identified both the DNA-binding protein inhibitors, Id2 and Id4, as the main targets of DNA methylation during the differentiation of murine OPCs into mature oligodendrocytes. We show that these inhibitory transcriptional regulators display hypermethylation, concomitant with decreased expression levels, as OPCs differentiate into oligodendrocytes in vitro. Furthermore, by making use of epigenetic editing by CRISPR/dCas9-DNMT3a, we confirm that targeted methylation of Id2/Id4 boosts OPC differentiation and Mbp, Plp and Mobp gene expression in vitro. Finally, we show that in a pathological context such as multiple sclerosis (MS), methylation as well as gene expression levels of both ID2 and ID4 are altered compared to non-neurologic control human brain samples. Taken together, our data reveal that DNA methylation regulates Id2/Id4 within the transcriptional network that drives myelination during oligodendrocyte development, which does not only reveal new insights into oligodendrocyte biology, but could also lead to a better understanding of CNS myelin disorders, such as MS.

Knockdown of *Ndrg4* induces intestinal dysmotility and changes in intestinal cell composition in zebrafish

Nathalie Vaes[1], Laura Kuil[2], Naomi Kakiailatu[2], Eric M.J. Bindels[3], Joke Peulen-Zink[3], William W. Cheng[2], Bianca M. De Graaf[2], Robert M.W. Hofstra[2], Werend Boesmans[1,4], Maria M. Alves[2], Veerle Melotte[1,2]

1 Department of Pathology, GROW–School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht; The Netherlands

2 Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

3 Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

4 Biomedical Research Institute (BIOMED), Hasselt University, Hasselt, Belgium

The N-myc downstream-regulated gene 4 (*Ndrg4*), a prominent biomarker for colorectal cancer, is specifically expressed by enteric neurons. However, the role of *Ndrg4* in enteric nervous system (ENS) development and gut functioning remains unexplored. Here we aim to determine the effects of loss of *Ndrg4* on the ENS, and other major cell determinants for intestinal functioning.

Ndrg4 mutant (*ndrg4*$^{-/-}$) zebrafish were created using CRISPR-Cas9 editing in a transgenic zebrafish (Danio Rerio) line expressing the fluorescent kaede protein in enteric neurons. To assess morphological and functional differences within the ENS of wild-type (*ndrg4*$^{+/+}$) and *ndrg4*$^{-/-}$ zebrafish larvae (5 and 7 days post fertilization (dpf)), the number of enteric neurons in the distal intestine were quantified (5dpf), and intestinal motility was assessed using spatiotemporal maps (7dpf). Furthermore, single cell RNA sequencing (scRNA-seq.) was performed on isolated, papain-dissociated intestines from *ndrg4*$^{+/+}$ and *ndrg4*$^{-/-}$ zebrafish (5dpf; >200guts), to evaluate the effects of loss of *ndrg4* on the cellular composition of the ENS and intestinal tract.

Loss of *Ndrg4* affects intestinal functioning as the travel distance of peristaltic waves was significantly shorter in 7dpf *ndrg4*$^{-/-}$ zebrafish (P=0.040). The contraction frequency, wave velocity or contraction interval were however not affected. Nevertheless, *ndrg4* knockdown is associated with a significant reduction in the number of enteric neurons in 5dpf zebrafish larvae (P=0.028). Based on the scRNA-seq. data, we confirmed a drop in the *phox2bb*$^{+}$ and *elavl3*$^{+}$ neuronal population in *ndrg4*$^{-/-}$ intestines. Interestingly, in line with the reduced muscularis externa thickness observed in Ndrg4$^{-/-}$ mice, scRNA-seq. revealed a decrease in *acta2*$^{+}$ smooth muscle cells, as well as within the *fapb2*$^{+}$ enterocyte population in *ndrg4*$^{-/-}$ guts.

Knockdown of *ndrg4* results in an enteric neuropathy characterized by significantly fewer enteric neurons and shortened peristaltic waves. Moreover, the smaller enterocyte and smooth muscle cell populations in the *ndrg4*$^{-/-}$ gut suggest a role for enteric neuronal *ndrg4* in establishing intestinal cell composition during development. Future research examining the underlying mechanisms as well as in-depth characterization of each cell cluster, are needed to address how *ndrg4* specifically impacts other intestinal cell populations and their function.

Macrophage-based delivery of interleukin-13 improves functional and histopathological outcomes following spinal cord injury

Jana Van Broeckhoven[1], Céline Erens[1], Daniela Sommer[1], Elle Scheijen[2], Selien Sanchez[1], Pia M. Vidal[3], Dearbhaile Dooley[4,5], Elise Van Breedam[6,7], Alessandra Quarta[6,7], Peter Ponsaerts[6,7], Stefanie Lemmens[1] and Sven Hendrix[1,8]

1 Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, 3500 Diepenbeek, Belgium.
2 Department of Neurosciences, Biomedical Research Institute, Hasselt University, 3500 Diepenbeek, Belgium.
3 Neuroimmunology and Regeneration of the Central Nervous System Unit, Biomedical Science Research Laboratory, Basic Sciences Department, Faculty of Medicine, Universidad Católica de la Santísima Concepción, Concepción 4090541, Chile.
4 School of Medicine, Health Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland.
5 UCD Conway Institute of Biomolecular & Biomedical Research University College Dublin, Belfield, Dublin 4, Ireland.
6 Laboratory of Experimental Hematology, University of Antwerp, 2610 Wilrijk, Belgium.
7 Vaccine and Infectious Disease Institute (Vaxinfectio), University of Antwerp, 2610 Wilrijk, Belgium.
8 Medical School Hamburg, 20457 Hamburg, Germany.

Spinal cord injury (SCI) elicits a neuroinflammatory response that impedes regeneration. Pivotal players orchestrating this response are macrophages (Mϕs) and microglia. After SCI, the lesional environment is dominated by pro-inflammatory Mϕs/microglia, which contribute to secondary cell death and prevent regeneration. Therefore, reprogramming Mϕ/microglia towards a more anti-inflammatory and potentially neuroprotective phenotype has gained substantial therapeutic interest in recent years. Interleukin-13 (IL-13) is a potent inducer of such an anti-inflammatory phenotype. In this study, we used genetically modified Mϕs as carriers to continuously secrete IL-13 (IL-13 Mϕs) at the lesion site.

Intraspinal injection of IL-13 Mϕs promoted functional recovery following SCI in mice. This improvement was accompanied by reduced lesion size and demyelinated area. Furthermore, the perilesional environment was altered upon IL-13 Mϕ treatment as the number of Arg1^{+} was increased, whereas the MHCII^{+} and Oil red O^{+} cells were reduced. This enforced anti-inflammatory shift was associated with neuroprotective effects, such as reduced neuronal cell death and less detrimental axon-phagocyte contacts, suggesting suppression of axonal dieback. The beneficial effects of the IL-13 Mϕs were abolished in IL-4Rα-deficient mice, supporting the crucial role of IL-13 as an underlying mechanism. Whereas direct neuroprotective effects of IL-13 on murine and human neuroblastoma cell lines or human neurospheroid cultures were absent, IL-13 rescued murine organotypic brain slices from cell death, probably by indirectly modulating the Mϕ/microglia responses.

Collectively, our data suggest that the IL-13-induced anti-inflammatory Mϕ/microglia phenotype can preserve neuronal tissue and ameliorate axonal dieback, thereby promoting recovery after SCI.

Investigating the impact of spatial conflict on the crossed-hands analgesia phenomenon

Amélie Van Caekenberghe[1,2], Igor Iarrobino[1,2], Valéry Legrain[1,2]

1 Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium

2 Psychological Sciences Research Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Considering the posture of a body in pain is crucial to defend it against the threatening stimulus responsible for the pain. Accordingly, nociceptive inputs are mapped according to both somatotopic and spatiotopic frames of reference, respectively taking into account the location of the stimulus on the body surface and the position of the painful body part in the external space. This co-mapping has been evidenced, among others, by applying nociceptive stimuli on the hands crossed over the body midline. This posture creates a misalignment between the two reference frames, and consequently, a conflict between the output responses (“my left hand is in the right side of space", and vice-and-versa). Hence, this phenomenon is associated with a decreased perceived intensity of nociceptive stimuli applied on the hands. To explain this effect, we tested the hypothesis following which the conflict encountered is resolved by realigning the two cortical maps. This process then requires a cognitive effort, leaving out less resources available to process other stimulus features, such as its intensity. Healthy volunteers were asked to rate their intensity perception of nociceptive radiant heat stimuli applied alternatively on both hands dorsa. Intensity ratings were compared between crossed and uncrossed hands postures. In addition, predictability regarding location of the stimuli was manipulated by applying them on one hand (predictable condition) or variably between the hands (unpredictable condition). We did not observe any crossed-hands analgesia effect, and therefore fail to replicate previous findings according to which crossing the hand affects intensity perception.

Role of both innate and adaptive immunity in the exacerbation of experimental autoimmune encephalomyelitis by Murid Herpesvirus 4; the link between EBV and multiple sclerosis

Van de Waterweg Berends A.F.[1,2,3], Loos P.[1], Maquet C.[1], Broux B.[2,3], Machiels B.[1], Hellings N.[2,3], Gillet L.[1]

1 Laboratory of Vaccinology, Faculty of Veterinary Medicine, FARAH, ULiège, Belgium

2 Neuro-Immune Connections and Repair Lab, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium

3 Universitair MS Centrum (UMSC), Hasselt, Belgium

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. While it has a high prevalence, its aetiology is still poorly understood. MS is a multifactorial disease caused by a combination of genetic, immunological and environmental factors. The best-documented and most strongly associated environmental risk factor for MS is Epstein-Barr virus (EBV). However, it is yet not known how EBV contributes to the development of MS. As EBV does not replicate in vitro and does not have any animal infection model, related animal viruses, such as Murid Herpesvirus 4 (MuHV-4), could help us to address the same question in a more accessible form. Here, we compared the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS, in mock and MuHV-4 infected mice. We showed that MuHV-4 infection prior to EAE induction leads to a significantly worse clinical outcome compared to mock infected mice. Moreover, we observed a major change in the phenotype of infiltrating monocytes after MuHV-4 infection in EAE induced mice compared to mock infected mice. These monocytes showed a higher expression of MHC-II, CD86 and Sca-1, as well as Saa3 and CXCL9 related to pathogenic phenotype in MS development. Similar phenotypic alterations were

also observed in microglia in MuHV-4 infected EAE mice, especially at the chronic stage of EAE. Besides changes in the myeloid cell compartment, we observed major changes in CNS infiltrating T cells in MuHV-4 infected mice compared to mock infected mice; more $CD8^+$ T cells, less regulatory $CD4^+CD25^+$ T cells and a higher expression of Ly6C on both $CD4^+$ and $CD8^+$ T cells. Surprisingly, the increase in clinical symptoms is not dependent on MOG_{35-55} autoantibodies or MOG_{35-55} specific $CD4^+$ T cells, since significantly less of these antibodies and $CD4^+$ T cells were present in MuHV-4 infected EAE mice compared to mock infected EAE mice. All of the observed results were dependent on latency of the virus as latency deficient strain did not lead to any exacerbation of the disease. Based on this model and on this preliminary characterization, we want to understand how EBV increases overall MS risk. In the end, this project could warrant the development of EBV target therapies for MS.

Renal and cerebral small vessel disease: an inflammatory tangle

L. Van der Taelen[1,3], S. Lambrichts[1,2,4], T. Unger[3], A. Briones[5], S. Foulquier[1,2,3,4]

1 Department of Pharmacology and Toxicology, Maastricht University, Maastricht, The Netherlands.

2 Department of Neurology, Maastricht University Medical Center, Maastricht, The Netherlands.

3 CARIM - School for Cardiovascular Diseases, Maastricht University, The Netherlands.

4 MH&Ns - School for Mental Health and Neuroscience, Maastricht University, The Netherlands.

5 Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Instituto de Investigación Hospital Universitario La Paz, Madrid, Spain.

Background: The kidney and the brain are both highly susceptible to vascular injury as a result of exposure to vascular risk factors such as hypertension, diabetes, and obesity. Patients with kidney dysfunction are known to have a higher prevalence of cerebrovascular disease (1-3). A meta-analysis of 31 studies and 23,056 participants found microalbuminuria, an early marker of kidney disease, to be associated with a higher risk of all markers of cerebral small vessel disease (3). Microvascular dysfunction and subsequent vessel rarefaction are suggested to be involved in this cerebrorenal connection. Inflammation is increasingly implicated as a key pathogenic principle of cerebrovascular disease. However, the underlying mechanisms and causative pathways are yet unknown.

Aim: To study the sequential relationship between renal and cerebral microvascular dysfunction and rarefaction induced by common risk factors in the ZSF1 rat model of comorbidities.

Preliminary study: Lean (Ln, n=15) and Obese (Ob, n=14) ZSF1 rats were sacrificed at 34-35w for plasma, urine, kidney, and brain analyses. At 34-35w, plasma glucose (Ln=11.1±0.3 vs Ob=19.7±1.3 mmol/L), triglycerides (Ln=0.7±0.1 vs Ob=14.5±1.5 µmol/L) and cholesterol levels (Ln=2.0±0.1 vs Ob=6.0±0.4 mmol/L) were increased in Ob vs Ln ($p<0.0001$). Urine glucose (Ln=0.7±0.1 vs Ob=5.3±1.1 mmol/L; $p<0.0001$), the albumin/creatinin ratio (Ln=3.5±0.5 vs Ob=11.9±4.1 g/mol; $p=0.03$) and plasma urea (Ln=6.4±0.1 vs Ob=7.1±0.2 mmol/L; $p=0.01$) were all increased in Ob vs Ln. This was furthermore associated with renal hypertrophy in Ob vs Ln (kidney-to-tibia: Ob=47±2 vs Ln=31±1 mg/mm; $p<0.0001$). These data confirm altogether the development of diabetic nephropathy in this model. In addition, our group has demonstrated the presence of cognitive dysfunction in Ob vs Ln, making the ZSF1 rat model a suitable model for the study of renal and cerebral small vessel disease.

Methods: Microvascular dysfunction and the expression of inflammatory mediators will be characterized in renal and cerebral tissues from ZSF1 rats at three different time points to detect early changes in disease development. The 3D architecture and density of the microcirculation in whole brains and kidneys will be studied using tissue clearing technique combined with 2-photon and light

sheet microscopies. Furthermore, mass spectrometry imaging with integrative molecular histology will be used to investigate the pro-inflammatory lipid mediators associated with microvascular dysfunction.

Expected results/perspectives: We expect to show that capillary rarefaction and inflammation occur conjointly in renal tissue first and in brain tissue thereafter, related to the progressive expression of a set of pro-inflammatory mediators. We propose that targeting inflammatory mediators, rather than the kidneys and brain per se, could prevent disease progression, which will be addressed in a later phase. The results of this ongoing study will contribute to identifying new inflammatory drivers which wire kidney dysfunction and cerebral vascular pathology together.

Association of sleep quality and diffusion MRI derived interstitial fluid content – insights in cerebral waste clearance

Merel M. van der Thiel[1,2], Walter H. Backes[1,2,3], Gerhard S. Drenthen[1,2], Paulien H.M. Voorter[1,2], Thorsten Feiweier[4], Inez H.G.B Ramakers[2,5], Jacobus F.A. Jansen[1,2,6]

1 Department of Radiology & Nuclear Medicine, Maastricht University Medical Center, Maastricht, the Netherlands

2 School for Mental Health & Neuroscience, Maastricht University, Maastricht, the Netherlands

3 School for Cardiovascular Disease, Maastricht University, Maastricht, the Netherlands

4 Siemens Healthcare, Erlangen, Germany

5 Department of Psychiatry & Neuropsychology, Maastricht University, Maastricht, the Netherlands

6 Department of Electrical Engineering, Eindhoven University of Technology, Eindhoven, the Netherlands

Background: IntraVoxel Incoherent Motion (IVIM) MRI can detect the diffusion of cerebral interstitial fluid (ISF) (D_{int}) and its volume fraction (f_{int}), which is suggested to relate to cerebral clearance. The ISF plays a crucial part in the cerebral clearance system, as it washes the waste products through the parenchyma.

Cerebral clearance is most active during sleep, therefore reductions in sleep quality might induce impaired cerebral clearance function. ISF expands during sleep, which facilitates the clearance of waste products from in-between the cells, while the ISF-volume reduces again in awake states. Previous diffusion studies have observed alterations in general diffusivity measures dependent on sleeping patterns. However, no previous studies have examined the effects of long-term subjective sleep deprivation on cerebral diffusivity using an ISF-specific diffusion measure.
Using IVIM, the current exploratory study investigates whether alterations in a proxy of ISF-volume (f_{int}) are related to hours of sleep or self-reported sleep quality.

Method: Participants: Twenty neurotypical elderly subjects (mean age ± standard deviation: 65.1 ± 7.7 years, 35% male) were included in this study. All subjects underwent high-field MRI (7T research system, Siemens).

Image analysis: The intermediate diffusion components (D_{int}) in the range $1.5*10^{-3}$<Diffusivity<$4.0*10^{-3}$ mm^2/s were calculated using spectral analysis. The relative signal contribution of D_{int} was quantified by the ISF-fraction (f_{int}). Median f_{int} values were extracted from the white matter (WM) and gray matter (GM).

Sleep questionnaire: Sleep quality over the last month was assessed by using the Pittsburgh Sleep Quality Index (PSQI). In addition to the clinically used total PSQI (sum of all 7 components), two specific PSQI components were examined: Subjective sleep quality (PSQI 1) and Sleep duration (PSQI 3).

Statistics: Pearson correlations were computed between the PSQI scores and f_{int} for WM and GM. To check for potential confounding influences, partial correlations were performed adjusting for age and time of MRI-acquisition.
Results: A significant negative association is found between PSQI component 1 (subjective sleep efficiency) and WM f_{int} (R = -.454, p = .045), where a worse reported sleep quality significantly relates to lower WM f_{int}. After adjusting for potential confounding influences, a trend towards significance remained (R = -.437, p = .061). No other significant associations were identified between the PSQI scores and IVIM measures.

Conclusion: The current explorative study identified a lower diffusion MRI-derived proxy of ISF-volume in the WM of subjects who reported a subjective feeling of long-term sleep deprivation. A reduction in ISF volume leaves less space for waste products to be cleared from in-between the cells. Thereby, the findings of this study are supportive of an association between sleep function and cerebral clearance. Furthermore, our findings highlight the potential of the IVIM-derived ISF-fraction as a non-invasive method to measure ISF alterations related to sleep disturbances.

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Brain Outcome After Cardiac Arrest- Single Case Experimental Design Intervention Study

Pauline van Gils[1,2,4], Caroline van Heugten[1,4,5], Jeannette Hofmeijer[2,3], Simone Sep[6], Jeanine Verbunt[6]

1 Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands
2 Clinical Neurophysiology (CNPH), TechMed Centre, University of Twente, Enschede, the Netherlands
3 Department of Neurology, Rijnstate Hospital, Arnhem, the Netherlands
4 Limburg Brain Injury Center, Maastricht University, Maastricht, the Netherlands
5 Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, the Netherlands
6 Adelante Rehabilitation Foundation, Hoensbroek, Limburg, The Netherlands; Department of Rehabilitation, University Medical Centre, CAPHRI, Maastricht, The Netherlands

Aim: The survival rate of out-of-hospital cardiac arrest (OHCA) patients has increased over the past decades. This gives rise to a growing number of patients with hypoxic-ischemic brain damage and cognitive impairment. Currently, there is a lack of knowledge regarding effectiveness of treatments to improve outcomes of patients with cognitive impairment after a cardiac arrest. The primary objective is to test effectiveness of cognitive rehabilitation therapy to improve functioning on problematic well-defined personalized behaviour that is caused by an objective cognitive impairments after OHCA.

Methods: This is a randomized multiple baseline single case experimental design (SCED) intervention study. Four to six patients who survived a cardiac arrest and with cognitive impairments will be included in this study. There will be a baseline phase, an intervention phase, and a follow-up phase. The intervention will consist of a combination of direct training of the impaired cognitive function(s) and metacognitive strategy training for 42 days (6 weeks). Direct training will be done with the computer program Rehacom to train the impaired cognitive domains, for 20 minutes 5 times a week. Metacognitive strategy training will be given on a weekly or biweekly basis (6-10 sessions) by a trained therapist as current care. The subjective personalised cognitive problem will be measured on a daily

basis via the app M-path. Next to this primary outcome variable, we will also measure objective- and subjective cognitive functioning with neuropsychological tests and questionnaires respectively. We will also perform an MRI-scan (DTI) before and after the intervention to explore the relationship between baseline structural brain integrity and cognitive recovery.

(Expected) Results: It is expected that during the baseline period, the objective and subjective problems with cognition remain stable. When the intervention has started, we expect to start seeing a decrease in the subjective daily problems. We also expect to see an improvement on the objective cognitive tests and subjective questionnaires after the intervention. We hope to see that these improvements are mainted during the follow-up period of 3 months. We expect to see a correlation between improvements in cognitive functioning in structural or functional connectivity between brain areas.

(Expected) Conclusion: We expect that the combination of direct training and metacognitive strategy training will improve cognitive functioning of patients with cognitive impairment after OHCA.

The impact of Alzheimer biomarkers and vascular factors on cognitive decline in memory clinic patients

Veerle van Gils[1], Inez Ramakers[1,2], Willemijn J. Jansen[1], Leonie Banning[1], Domantį KuĶikienį[2,3], Ana Sofia Costa[2,3], Jörg B. Schulz[2,3], Frans Verhey[1], Kathrin Reetz[2,3], Stephanie J. B. Vos[1]

1 Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht University, Maastricht, the Netherlands;

2 Department of Neurology, University Hospital RWTH Aachen, Aachen, Germany.

3 JARA Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich and RWTH Aachen University, Aachen, Germany.

Background: Alzheimer's disease (AD) biomarkers of amyloid and tau pathology have shown to be associated with cognitive decline. This study aims to explore if comorbid vascular risk or burden contributes to cognitive decline in a memory clinic population.

Methods: A total of 294 patients were included from the Maastricht and Aachen university memory clinics. AD pathology was defined as cerebrospinal fluid (CSF) amyloid beta(Aβ)42 or Aβ42/40 abnormality and/or tau (p-tau) abnormality, using local cut-offs. Vascular pathology was defined differently in separate analyses, i.e. as cerebrovascular burden and vascular risk. Presence of cerebrovascular burden was defined with a composite score ≥2 (range 0-12) of white matter hyperintensities (Fazekas), cerebral microbleeds, infarcts and haemorrhages on MRI. Presence of vascular risk was defined as having hypertension, dyslipidaemia or diabetes mellitus (type I or II) from medical history diagnosis or medication use. Participants were divided into four groups: no pathology, vascular pathology, AD pathology, and mixed pathology. Outcome measures were memory, attention, executive functioning and verbal fluency z-scores, and global cognition (MMSE) up to 5 years after baseline. Linear mixed models were used to assess differences in cognitive function and decline across groups, adjusted for age, gender, education, and site.

Results: The mixed and AD pathology groups consistently showed faster cognitive decline compared to the no pathology and vascular groups on attention, verbal fluency and MMSE. This was found for both vascular pathology definitions. When vascular pathology was defined by vascular risk, all groups

showed greater decline compared to the no pathology group on executive functioning, and the AD group showed a greater decrease in MMSE scores over time compared to the mixed group.

Conclusion: AD and mixed pathologies are similarly associated with cognitive decline in a memory clinic population, although the definition of vascular pathology may have implications for the cognitive trajectory.

All you can EAAT3: restoring myelin, the brain's favourite fat

Lieve van Veggel[1, 2, 3], Melissa Schepers [1, 2, 3], Assia Tiane[1, 2, 3], Ben Rombaut[1, 2, 3], Hanne Dilliën[5], Jos Prickaerts[3], Pablo Moya[4], Rudy Schreiber[3*], Tim Vanmierlo[1, 2, 3*]

1 BIOMED Biomedical Research Institute, Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium
2 University MS Center (UMSC), Hasselt-Pelt, Belgium
3 Department Psychiatry and Neuropsychology, Division of Translational Neuroscience, European Graduate School of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands
4 Department of Sensor Engineering, Faculty of Science and Engineering, Maastricht University, Maastricht, The Netherlands
5 Facultad de Ciencias, Instituto de Fisiología, Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Universidad de Valparaíso, Valparaíso, Chile.

Multiple sclerosis is the most common neurodegenerative disorder among adolescents, with 2.5 million people suffering worldwide. When the disease progresses, demyelination due to oligodendrocyte damage leads to neurodegeneration. The eventual inability to recover from this damage is in part due to the vulnerability of oligodendrocyte precursor cells (OPCs) to oxidative stress. No treatment is yet available to target the progression of disease. In this project we aim to protect OPCs from oxidative stress to sustain endogenous remyelination processes. We hypothesise that activation of the excitatory amino acid transporter 3 (EAAT3) in OPCs holds the key to protection from inflammation-induced oxidative stress. EAAT3 is a cysteine transporter, providing the cell with the rate limiting building block for glutathione (GSH) synthesis, the key antioxidant compromised in OPCs. We aim to confirm and elucidate the role of EAAT3 in OPC differentiation and myelination in vitro. The OliNeu cell line was used to optimize the EAAT3 modulation conditions. These included propidium Iodide (PI) and MTT assays to test cell viability and mitochondrial activity, as well as a GSSG/GSH assay and immunohistochemistry analysis looking at differentiation markers (MBP and O4). After optimisation of the stressor and EAAT3 inhibitor, the same experiments were repeated in primary mouse OPCs. Cysteine depletion decreased cell viability and mitochondrial activity in both the OliNeu cell line and primary OPCs. In addition, glucose oxidase had the same effect, due to its ability to form reactive oxygen species (ROS). Cysteine presence and uptake is crucial for the viability and normal functioning of primary OPCs in vitro. Inhibition of EAAT3, or a lack of cysteine in the extracellular space decreased the capacity of OPCs to undergo differentiation. These findings provide a rationale to investigate whether activation of EAAT3 might prove beneficial for OPC differentiation and thus remyelination.

The ApoA-I mimetic peptide 5A enhances remyelination by promoting the clearance and degradation of myelin debris

Sam Vanherle[1,2], Winde Jorissen[1,2], Tess Dierckx[1,2], Melanie Loix[1,2], Elien Grajchen[1,2], Fleur Mingneau[1,2], Pascal Gervois[3], Ivo Lambrichts[3], Jonas Dehairs[4], Johannes V. Swinnen[4], Monique T. Mulder[5], Alan T. Remaley[6], Mansour Haidar[1,2], Jerome J.A. Hendriks[1,2*], Jeroen J.F. Bogie[1,2*]

1 Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, 3590 Diepenbeek, Belgium
2 University MS Center Hasselt, 3900 Pelt, Belgium
3 Department of Cardio and Organs Systems, Biomedical Research Institute, Hasselt University, 3590 Diepenbeek, Belgium
4 Department of Oncology, Laboratory of Lipid Metabolism and Cancer, Leuven Cancer Institute, University of Leuven, 3000 Leuven, Belgium
5 Department of Internal Medicine, Erasmus University Medical Center, 3015 Rotterdam, Netherlands
6 Lipoprotein Metabolism Laboratory, Translational Vascular Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, 20892 Bethesda, MD, United States of America

* authors contributed equally

Failure of remyelination underlies the progressive nature of demyelinating diseases. Recently, we and others demonstrated that impaired remyelination ensues in part due to a dysfunctional innate immune response in the central nervous system (CNS). Sustained accumulation of myelin-derived lipids and formation of lipid droplets, combined with an inability to process and export these lipids, was found to induce a disease-promoting phagocyte phenotype. Here, we find that the apoA-I mimetic peptide 5A, a molecule well-known to promote the stabilization and activity of the lipid efflux transporter ABCA1, markedly enhances remyelination in the cerebellar brain slice and cuprizone models. Guided by immunohistochemical and lipidomics analysis, the pro-regenerative impact of peptide 5A was attributed to increased uptake of remyelination-inhibiting myelin debris through the fatty acid translocase CD36. On a transcriptional level, peptide 5A controlled CD36 expression through the ABCA1-JAK2-STAT3 signalling pathway. Collectively, our findings indicate that peptide 5A promotes the induction of a repair-permissive environment by stimulating the clearance of inhibitory myelin debris, potentially having broad implications for therapeutic strategies aimed at promoting remyelination.

Specific inhibition of phosphodiesterase 4D long isoforms improves neuronal plasticity and protects against Aβ toxicity in vitro

Dean Paes[1,2], Emily Willems[1], Melissa Schepers[1,2], Ben Rombaut[1,2], Roberta Ricciarelli[3], Chiara Brullo[4], Ernesto Fedele[5], Olga Bruno[4], Daniel van den Hove[2], Tim Vanmierlo[1,2], Jos Prickaerts[2]

1 Department of Neuroscience, Biomedical Research Institute, Hasselt University, Belgium.
2 Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, the Netherlands.
3 Department of Experimental Medicine, Section of General Pathology, School of Medical and Pharmaceutical Sciences, University of Genoa, Italy.
4 Department of Pharmacy, Section of Medicinal Chemistry, School of Medical and Pharmaceutical Sciences, University of Genoa, Italy.
5 Department of Pharmacy, Section of Pharmacology and Toxicology, School of Medical and Pharmaceutical Sciences, University of Genoa, Italy.

Cyclic adenosine monophosphate (cAMP) and its downstream PKA-CREB pathway are crucial for neuroplasticity and memory consolidation. Inhibition of cAMP-degrading phosphodiesterase 4D

(PDE4D) enzymes with drugs like Gebr32a was found to improve cognition in preclinical models of Alzheimer's disease (AD). Since non-selective PDE4D inhibition still inhibits all PDE4D isoforms, treatment efficacy and likely safety can be enhanced when selectively targeting PDE4D isoforms involved in synaptic plasticity.

First, to validate the notion that PDE4D inhibition stimulates cAMP-PKA-CREB signaling and neuronal plasticity, we showed that Gebr32a increased CREB phosphorylation in vitro and dose-dependently promoted neurite elongation in both N2a and HT22 cells. Furthermore, PDE4D protein was localized in HT22 growth cones, supporting PDE4D involvement in neurite outgrowth. Moreover, PDE4D inhibition with Gebr32a (1µM) protected against Aβ-induced (1µM) neurite length reduction in HT22 cells. As specific targeting of PDE4D isoforms may be therapeutically more effective and safer, we investigated whether specific PDE4D isoforms regulate neurite growth in HT22 cells. The CRISPR-Cas9-mediated genetic knockout of the long PDE4D3, -D5, -D7 and -D9 isoforms increased average neurite length, both in the absence and presence of Aβ (1µM).

In summary, we showed that long PDE4D isoforms regulate neuronal plasticity, and that inhibition of these isoforms can yield neuronal resilience against Aβ-induced toxicity. Hence, this study supports the potential of PDE4D and its specific isoforms as a target for cognition enhancement in AD while providing new insights into target specifications for the development of more efficacious PDE4D inhibitors.

Temperature-dependent dynamics of spontaneous and trauma-induced axon plasticity

Sarra Zaghbouni, Yeranddy Aguiar Alpizar, Bert Brône

Biomedical research institute, Hasselt University

Neural networks formation throughout development and regeneration after injury was shown to be dependent on accuracy of axonal pathfinding, which was found to be influenced by chemical and physical cues. Recently, there is growing evidence that temperature can play a crucial role in axonal guidance. However, the detailed mechanisms involved in this guidance are not completely understood. We hypothesize that spontaneous and trauma-induced axon growth is repelled by increasing nanoscale temperature gradients which leads to growth inhibition and growth cone retraction.

Transient receptor potential vanilloid 4 (TRPV4) is a thermosensitive calcium-permeable channel. Its activation leads to an increase in the intracellular concentration of Ca2+ (Ca2+i). Through a supramolecular complex containing cytoskeletal proteins and regulatory kinases, TRPV4-mediated Ca2+i changes act as a direct regulator of microtubules and actin. This makes it a target for the study of axonal actin reorganization events at the subcellular levels. By using breakthrough technologies up to nanoscale temperature manipulations, we will investigate the dynamics of spontaneous axon growth during development as well as regenerative plasticity of axonal networks after trauma on diamond-based probes and using microfluidic devices that we will fabricate according to a previously established protocol.

We will combine the measurement of local temperature gradients using diamond color centers with micro-probe structures and microfluidic devices as a powerful and highly innovative tool to study the molecular mechanisms of spontaneous and trauma-induced axon growth.

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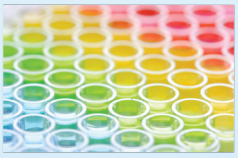

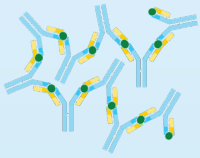

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