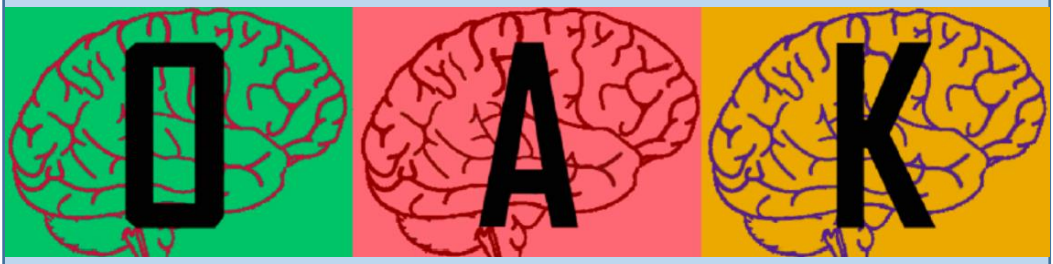


20th Annual OAK Meeting

Danish Brain Research Laboratories Meeting

Copenhagen, 16 June 2023

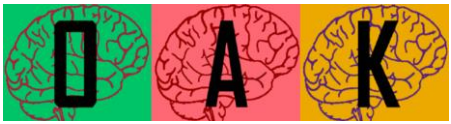
Institut for Klinisk Veterinærmedicin

Centre for Neuroscience & Stereology

20th Annual OAK Meeting

Danish Brain Research Laboratories Meeting

PROGRAM



9:30 - 10:00	Arrival & registration, coffee, breakfast
10:00 – 10:15	Welcome by **Susana Aznar** and **Hanne B. Gredal,** and presentation of Bente Pakkenberg Prize by **Arne Møller**

Session 1

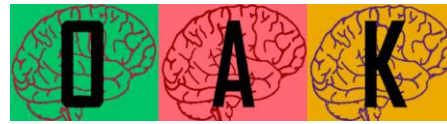
Chair: Kate Lambertsen and Kim Ryun Drasbek

10:15 – 10.30	**Simone Tonetto** Nutritional Ketosis as Treatment for Alcohol Withdrawal Symptoms.
10.30 – 10.45	**Katrine Tækker Krohn** Regulation of microglial TNF production by noncoding RNAs in multiple sclerosis lesions
10.45 – 11.00	**Jónvá Hentze** Investigating the expression of the DNAJB6 protein in clinical tissues and models of synucleinopathies
11:00 – 11:30	COFFEE BREAK (meet the sponsors)

Session 2

Chair: Gitta Wörtwein and Arne Møller

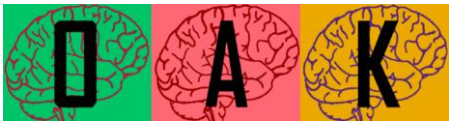
11:30 – 11:45	**Julie D. Jakobsen** The effect of siponimod on oligodendrocyte precursor cell proliferation and maturation in naive mice
11.45 – 12.00	**Helene Halkjær Jensen** Neurological consequences of human calmodulin mutations
12.00 – 12.15	**Julie Niemann Holm-Jacobsen** Targeting CSF1R in Alzheimer's disease
12.15 – 12.30	**Saint Clair Michna** Effect of inhibitor TNF on the cognitive outcomes after experimental stroke
12:30 – 12:45	**Poster pitch presentations, 5 posters, 2 min for each** (Posters will be displayed on the main scream) **Ole Borup Svendsen** SLITRK5, the genetic switch for developing OCD similar behavior? **Anchifiya Ali** Studying the Impact of Conditional Knock-Out of the Serotonin 2B Receptor on Microglial Density and Morphology



	Rebecca Best Jensen Remote ischemic conditioning; Novel treatment targeting in acute ischemic stroke **Luisa Knecht** Measuring alpha-synuclein aggregates across the human brain. **Shamica Jaiswal** Targeting Cognitive Decline in Aging: Investigating the Effects of Erythropoietin Treatment and Hypoxia Exposure.
12:45 – 13:45	LUNCH BREAK (meet the sponsors)

Session 3
Chair: Susana Aznar and Bente Finsen

13.45 – 14:00	**Katrine Tang Stenz** Remote ischemic conditioning attenuate ischemia and lead to changes in regulation of cell cycle in human brain microvascular endothelial cells
14.00 – 14.15	**Anne-Line Strange Laursen** Modelling Multiple System Atrophy-like α-synuclein pathology in mice
14.15 – 14.30	**Anna Berezovskaia** The role of M4 mAChR localized on cholinergic neurons on psychostimulant's effects on the brain and behavior.
14:30 – 14:45	**Poster pitch presentations, 4 posters, 2 min for each** (Posters will be displayed on the main scream) **Ojha, Bhavya** Modelling cortical pathology in mouse **Kata Molnár** Cortical changes in a new mouse model of amyotrophic lateral sclerosis (ALS) **Pernille L. Heidemann** MicroRNAs in inflammatory CNS diseases in dogs – Biomarkers for steroid-responsive meningitis-arteritis? **Celine Knudsen** The regulation of mGluR5 after chronic mild stress
14:45 – 15:30	COFFEE BREAK (meet the sponsors) QUESTIONS TO THE POSTER PRESENTERS



Session 4

Chair: Betina Elvfing and Jens Randel Nyengaard

15:30 – 15:45	**Camilla Termansen Erichsen** Mechanisms behind cognitive symptoms in schizophrenia and depression
15.45 – 16.00	**Tenna Remler Pedersen** Clinical phenotype and factors contributing to pain pathogenesis investigated in a nonexperimental spontaneous dog model of neuropathic pain translating to humans
16.00 – 16.15	**Rolf Fyllgraf Søkilde** Evaluation of microRNA in situ hybridization usability of long-term stored human brain bank tissue
16:15 – 16:30	BREAK (meet the sponsors)
16:30 – 16:45	Prize announcement
18:30	**DINNER** Restaurant Bistro RK, Nørrebrogade 160, 2200 Copenhagen

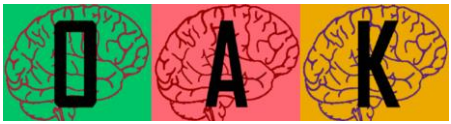
20th Annual OAK Meeting

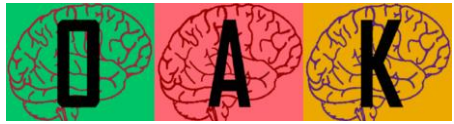
Danish Brain Research Laboratories Meeting

PROGRAM

ORAL PRESENTATIONS:

POSTER PRESENTATIONS:





Nutritional Ketosis as Treatment for Alcohol Withdrawal Symptoms

Simone Tonetto[1,2,3], Pia Weikop[4], Morgane Thomsen[1,2,3]

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[4] Center for Translational Neuromedicine, University of Copenhagen, Copenhagen, Denmark.

Aim: Upon acute and prolonged alcohol intoxication the brain undergoes a metabolic shift, associated with reduced glucose metabolism and increased acetate metabolism. This metabolic imbalance persists during abstinence, leaving the brain in a sort of energy depletion state, since the acetate metabolized from alcohol is not readily available anymore. This is thought to play a significant role in the genesis and persistency of alcohol withdrawal symptoms. This study aims at testing the efficacy of ketogenic treatments as an alternative source of acetate and thus energy, investigating their potential to rescue psychiatric and neurochemical alterations during long-term alcohol withdrawal.

Methods: Female C57BL/6JRj mice, tested in three independent cohorts (randomized block design) were intermittently exposed to alcohol vapor or air for three weeks. From the last week of alcohol exposure, mice were fed either control diet (CD, n=12), ketogenic diet (KD, n=12) or diet supplemented with ketone ester (KE, n=12). Withdrawal symptoms were assessed over a period of four weeks using a series of behavioral test, comprising alcohol self-administration, anhedonia (saccharin preference), hyperalgesia (hot-plate), anxiety-like (light-dark, zero maze) and depressive-like disturbances (tail suspension). Following the long-term abstinence, mice were re-exposed to alcohol vapor for a week to mimic a clinical relapse. Brain inflammation was measured by ELISA. Monoamines levels in the hippocampus and striatum, as well as cations content in the cerebellum, were assessed by HPLC.

Results: Alcohol-exposed mice fed CD had lower saccharin preference and saccharin consumption compared to air controls, a sign of anhedonia. Feeding mice either KD or KE rescued this symptom, up to 4 weeks into abstinence, compared to alcohol-exposed mice fed CD. KD-fed alcohol-exposed mice showed higher latency to first immobility in the tail suspension test (decreased depression-like symptoms), as well as lower plasma cholesterol levels. CD-fed alcohol-exposed mice had decreased norepinephrine levels in the hippocampus and partially in

the striatum, as well as decreased serotonin turnover (5-HIAA/5-HT ratio) in the hippocampus, compared to air controls; the latter was rescued by KE.

Conclusions: Our findings show promising effects of ketogenic treatments in ameliorating alcohol withdrawal symptoms in mice, not only in early detoxification, but also during prolonged abstinence. Some of the biochemical endpoints were also ameliorated by the ketogenic treatments, but these were measured at a single timepoint. Therefore, further investigations are needed to continuously measure and analyze specific neurochemical dynamics in freely behaving dependent mice, e.g. using fiber photometry.

Regulation of microglial TNF production by noncoding RNAs in multiple sclerosis lesions

Krohn KT[1], Davidsen LI[1], Venø MT[2], Su J[2], Villadsen B[1], Norlén T[1], Willadsen NN[1], Vollerup J[1], Clausen BH[1], Kuhlmann T[3], Nielsen HH[4], Kjems J[2], Finsen B[1].

[1]Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, [2]Interdisciplinary Nanoscience Center (iNANO), Department of Molecular Biology and Genetics, Aarhus University, [3]Institute of Neuropathology, University Hospital Münster, Germany, [4]Department of Neurology, Odense University Hospital

Multiple sclerosis (MS) is characterized by inflammatory lesions with infiltration of autoreactive T cells leading to demyelination and axonal degeneration, but also to microglial activation and upregulation of the potential, toxic cytokine tumor necrosis factor (TNF). In a murine model for MS, we have previously observed that interferon gamma (IFN-ɣ)-expressing, myelin-specific T cells exacerbate microglial activation and induce the expression of TNF protein in microglia. This study tests the hypothesis that the microglial production of TNF is regulated, in MS-like lesions in mice as well as in T cell-infiltrated MS lesions, by IFN-ɣ through specific regulatory, noncoding RNAs (ncRNAs).

Through RNA sequencing studies on RNA samples from IFN-ɣ- and vehicle-stimulated primary, murine microglia, we have selected six TNF-related ncRNAs including two potential TNF mRNA-targeting microRNAs to investigate further. Ongoing studies use in situ hybridization (ISH) and immunohistochemical stainings to detect the ncRNAs and different microglial and T cell markers, including TNF, respectively, in our murine model for MS and in autopsies from deceased MS patients with or without T cell-infiltrated lesions. Future studies using RNAscope, will enable us to detect co-localization of the ncRNAs with TNF-expressing microglia. The clinical perspective is to identify new TNF-modulating therapies for MS patients.

Keywords: Multiple sclerosis, microglia, T cells, TNF, noncoding RNA

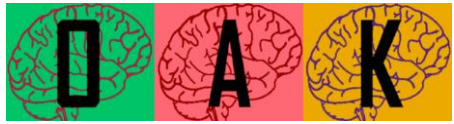
Investigating the expression of the DNAJB6 protein in clinical tissues and models of synucleinopathies

Jónvá Hentze[1], Tomasz Brudek[1], Christian Hansen[2]

1 – Centre for Neuroscience and Stereology, Bispebjerg-Frederiksberg Hospital, Copenhagen, DK
2 – University College Copenhagen, 2200 Copenhagen, DK

Background
DNAJB6 is a molecular chaperone ubiquitously expressed across several cellular compartments and all tissues in the human body, including in the brain. One of the most important functions of DNAJB6 is its ability to suppress aggregation of multiple amyloid-like proteins. α-synuclein aggregates are the pathological hallmark of diseases such as Parkinson's disease (PD) and Multiple System Atrophy (MSA). Previous studies have shown that expression of DNAJB6 is altered in synucleinopathies, and that DNAJB6 suppresses aggregation of α-synuclein. This project investigates the expression of the DNAJB6 protein in various regions of the human brain.
Methods
The samples included in this study were from post-mortem human brains from patients diagnosed with PD and MSA, and healthy controls. Expression of DNAJB6 in different brain regions was assessed using immunohistochemistry and Western Blot. Expression of DNAJB6 in the major cell types of frontal cortex from healthy human brain was investigated using immunofluorescence (IF), and relative expression levels of DNAJB6 in the different cell types were estimated based on cell counting analyses. Co-expression of DNAJB6 and α-syn in oligodendrocytes in MSA was additionally investigated using IF.
Results
The IHC stainings and WB analyses showed that DNAJB6 is widely expressed in the healthy human brain, including in PD and MSA. DNAJB6 is expressed in majority of neurons in the frontal cortex of healthy brain, while we observed expression of DNAJB6 in a little less than half of the oligodendrocytes in this region. Co-expression of α-syn and DNAJB6 was not observed in oligodendrocytes in MSA brain.
Conclusion
Based on the findings from this study, we can conclude that DNAJB6 is widely expressed in the human brain and is primarily expressed in neurons and oligodendrocytes in the healthy human brain. It remains to be determined whether DNAJB6 and α-syn co-localize in other regions in MSA brain.

Keywords: DNAJB6, Immunostaining, Synucleinopathies



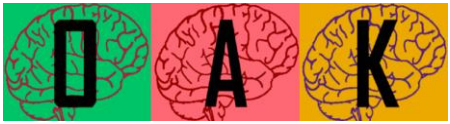
The effect of siponimod on oligodendrocyte precursor cell proliferation and maturation in naïve mice

J.D. Jakobsen[1, 2, 3], B. Finsen[1], A. F. Svenningsen[1, 2,] K. L. Lambertsen[1, 2, 3,] H. H. Nielsen[1, 2, 3]

[1]. University of Southern Denmark, Neurobiology Research, Institute of Molecular Medicine, Odense C, Denmark
[2]. University of Southern Denmark, BRIDGE - Brain Research - Inter Disciplinary Guided Excellence, Department of Clinical Research, Odense C, Denmark
[3]. Odense University Hospital, Department of Neurology, Odense C, Denmark

Siponimod has been shown to decrease disability progression and brain atrophy in secondary progressive multiple sclerosis (SMPS). Siponimod has immunosuppressive mechanisms, and expression of S1PR5 on oligodendrocyte lineage cells, indicates siponimod as a remyelinating and neuroprotective agent. We hypothetized siponimod to affect oligodendrocyte precursor cell (OPC) proliferation in naïve mice, and treated mice with 3 mg/kg oral siponimod or vehicle for 19 days. The proliferation marker BrdU was injected to track proliferation of OPCs. Flow cytometry showed a significant lymphopenia in both B- and T cell populations in animals treated with siponimod, except for double-negative T cells, which were increased and the regulatory CD25+ T cells, which were unaffected. In lymph nodes, similar observations were made, except for a significant increase in regulatory CD25+ T cells. Y-maze and open field tests were performed and showed no change in locomotor activity, willingness to explore new areas, nor memory. Immunohistochemistry with specific OPC and oligodendrocyte markers is ongoing, and will show if siponimod affects oligogenesis. These results confirm the immunomodulatory effects of siponimod and will form the basis for studies on the neuroprotective mechanisms in de- and remyelination models of MS, leading to further advantages in treatment of SPMS in the future.

Keywords: Multiple Sclerosis – Oligodendrocytes - Proliferation



Neurological consequences of human calmodulin mutations

Helene Halkjær Jensen[1], Magnus Tudsborg Frantzen[1], Malene Brohus[1], Palle Duun Rohde[2], Mette Nyegaard[2], Michael Toft Overgaard[1], Anders Olsen[1]

1. Department of Chemistry and Bioscience, Aalborg University
2. Department of Health Science and Technology, Aalborg University

Calmodulin is a ubiquitous calcium sensor, involved in processes ranging from cell division and apoptosis to muscle contraction and synaptic transmission. Mutations in calmodulin have been linked to severe cardiac arrhythmia in children. Now, several lines of evidence suggest that calmodulin mutations also impair neuronal function. In cardiac patients, approximately 1/3 also suffer from neurodevelopmental disease. Moreover, in a large cohort of exome sequenced schizophrenia patients, we find an enrichment of calmodulin mutations. Finally, in a C. elegans model, we find that human calmodulin mutations both disrupt rhythmical behavior and neurological functions such as chemosensing. Together, these observations indicate a new road for research into calmodulin mutations in neurons – either as tools to study neuronal calcium signaling, or to understand the molecular mechanisms underlying neurological disease.

Keywords: Calcium signaling, neurodevelopment, cardiac arrhythmia, C. elegans

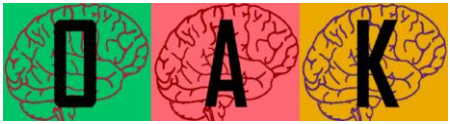
Targeting CSF1R in Alzheimer's disease

Julie Niemann Holm-Jacobsen[1], Julie Schmidt Hansen[2], Erik Nguyen Nielsen[3,4], Bente Finsen[2], Arne Møller[4], Ove Wiborg[1]

1.Department of Health Science and Technology, Aalborg University, Aalborg, Denmark
2.Department of Molecular Medicine, The University of Southern Denmark, Odense, Denmark
3.Department of Nuclear Medicine and PET, Aarhus University Hospital, Aarhus, Denmark
4.Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

The TgF344-AD rat model manifests the full spectrum of age-dependent Alzheimer's disease (AD) pathologies and cognitive disturbance (Cohen et al. 2013). In AD, microglia are gaining increased interest as an inflammatory component. The colony-stimulating factor 1 receptor (CSF1R) signaling is an important regulator of microglia, modulating their proliferation, migration, differentiation, and survival. In a mouse model of multiple sclerosis, inhibition of CSF1R has been shown to reduce microglia proliferation and modulate their phenotypes during neuroinflammation (Hagan et al. 2020). In the present study, we aim to examine the effect on the progression of AD by inhibiting the CSF1R signaling pathway. This is done by an intervention study in the TgF344-AD model with a CSF1R inhibitor. The rats are treated with the CSF1R inhibitor or placebo for three months. Behavioral phenotyping assessing spatial reference learning and memory is performed at baseline and at the end of the treatment period. Furthermore, PET scans are obtained on a subgroup of the rats with tracers specific for beta-amyloid plaques (PiB) and CSF1R (GW2580). Finally, rats are euthanized, and specimens and organs are harvested for further analyses, including histology, single-cell RNA sequencing, and microbiome analyses. The study is still in progress, and results are pending.

Keywords: Alzheimer's disease, CSF1R, microglia, behavior, PET



Effect of inhibitor TNF on the cognitive outcomes after experimental stroke

SAINT CLAIR Michna

Institut of Molecular medicine, Kate Lykke Lambersten team

Stroke is the 2nd leading cause of death in the world. After a stroke there is a neuroinflammation mediated by the upregulation of pro-inflammatory cytokine in particular TNF-α. Stroke is known to cause learning and memory impairment. Therapeutic exercise is the most common treatment strategy for the rehabilitation of stroke patients. It reduces tissue damage, improves functional recovery, and prevents recurrence of stroke. The aim was to study the effect of chronic injection of specific and non-specific TNF-α inhibitor in combination with physical activity to help for the recovery of the memory after a pMCAO. Adult male C57BL/6 mice were used to induced them a stroke in the left part of the brain and received twice a week injection of Saline, Etanercept or Xpro1595 some of these groups have access to a running wheel in their cage for 8 weeks. 3 behavior test was performed to evaluate the memory state of the mice. The Y-maze test was performed to evaluate the short-term memory, the Barnes maze to assess the spatial learning and memory and the Novel object recognition to assess the recognition memory of the mice. Chronic injection of Etanercept and Xpro1595 with physical activity don't seem to have effect on the short-term memory, exploratory behavior and recognition memory after pMCAO. Chronic injection of Etanercept and XPro1595 with PE seem affect spatial learning and memory behavior after pMCAO.

Keywords: Stroke, Etanercept, XPro1595, memory.

Remote ischemic conditioning attenuate ischemia and lead to changes in regulation of cell cycle in human brain microvascular endothelial cells.

Katrine Tang Stenz[1,2], Jesper Just[1,3], Xiu-Jie Wang[4], Kim Ryun Drasbek[1,2]

[1]Center of Functionally Integrative Neuroscience, Department of Clinical Medicine, Aarhus University, DK-
8000, Aarhus, Denmark
[2]Sino-Danish College (SDC), University of Chinese Academy Sciences, Beijing, China
[3]Department of Molecular Medicine, Aarhus University Hospital, DK-8200, Aarhus, Denmark
[4]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Identifying novel treatment options for acute ischemic stroke (AIS) is of utmost importance as AIS is one of the leading causes of death and disabilities. Current acute treatment is limited to either thrombectomy or thrombolysis, both of which must be initiated within 4.5-6 hours of symptom onset. Remote ischemic conditioning (RIC) is an interesting non-invasive treatment that is thought to activate the body's own protective mechanisms. We have studied the protective potential of RIC derived extracellular vesicles (EVs) in human brain microvascular endothelial cells (HBMECs). Utilizing an oxygen-glucose deprivation (OGD) asssay to model stroke *in vitro*, we have found that post-RIC EVs attenuate ischemia-reperfuison injury (IRI). We further identified upregulated microRNA (miRNAs) in post-RIC EVs (RIC-miRNAs) and tested their protective effect in the OGD assay. RIC-miRNA were not sufficient to attenuate IRI, they did, however, create large differences in gene expression. When examining the differentially expressed genes (DEGs), which arose after OGD in RIC-miRNA transfected HBMECs, cell cycle pathways were found to be positively regulated. During AIS, some HBMECs will be lost due to energy failure, thus, promoting endothelial cell proliferation is an important mechanism in restoring physiological conditions after AIS.

Keywords: Acute Ischemic Stroke, Remoe ischemic conditioning, Extracellular vesicles, miRNA, Endothelial cells.

Modelling Multiple System Atrophy-like α-synuclein pathology in mice

Anne-Line Strange Laursen[1,2,3,4], Mikkel Vestergaard Olesen[1,2], Tomasz Brudek[1,2], Katarina Willén[3], Karina Fog[3], Louise Torp Dalgaard[4], Florence Sotty[3], Susana Aznar[1,2]

[1]Centre for Neuroscience & Stereology, Bispebjerg and Frederiksberg Hospital, Copenhagen University Hospital, Nielsine Nielsens Vej 6B, DK-2400, Copenhagen NV, Denmark.
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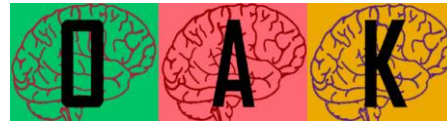
Multiple System Atrophy (MSA) is a rare, neurodegenerative disease characterized by findings of α-synuclein (α-syn) aggregates in oligodendrocytes. Our lab has established a transgenic (TG) MSA mouse model that expresses h-α-syn in oligodendrocytes. Findings suggest that α-syn in TG MSA-model brains is more soluble than in MSA brains. The aim of this study is to develop an MSA mouse model displaying insoluble α-syn aggregation in oligodendrocytes.

The TG model present with h-α-syn immunopositive oligodendrocytes, the number of which seems to increase with age, peaking at 9 to 12 months. Separation in Triton- and SDS-soluble fractions followed by Western blotting suggests that the brain α-syn is mostly soluble. In a pilot study, 6 months-old TG mice, still with relatively low h-α-syn load, were injected with h-α-syn PFFs unilaterally in striatum and survived for further 2 or 6 months. This technique is commonly used to induce insoluble α-syn aggregates in neurons in wildtype mice. Interestingly, the 8 months-old mice do display insoluble aggregation, but mainly in neurons despite the oligodendrocyte-specific overexpression of h-α-syn. We are currently waiting for the data for the 12 months-old mice, which have reached the age of high h-α-syn-load.

Keywords: Multiple System Atrophy, Mouse model, α-synuclein, pre-formed fibrils.



The role of M4 mAChR localized on cholinergic neurons on psychostimulant's effects on the brain and behavior

Anna Berezovskaia, Anders Fink-Jensen, Gitta Wörtwein

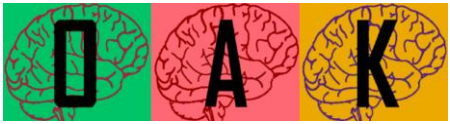
Laboratory of Neuropsychiatry, Psychiatric Center Copenhagen

Background: The balance between the striatal dopaminergic and cholinergic systems is crucial for reward-related behavior and learning. When this balance is disrupted, it can lead to neuropsychiatric disorders such as substance abuse disorder. Muscarinic receptor 4 type is widely expressed in various cell types in the striatum which has long been known as a hub for addictive effects. Studies have shown that the M4 receptor is directly involved in regulating dopamine signaling, but the specific role cholinergic interneurons (ChINs) play for various aspects of the addiction cycle are unclear and the nature of the cholinergic receptors that are critical for these is unknown.

This project aims to study the impact of deleting the M4 receptor from ChINs in the striatum on the brain and behavioral response to psychostimulants.

Methods and Results: We conducted several studies with a focus on classical and operant behavior. The mutant mice were tested for basal and drug-induced locomotor activity and there was no difference compared to wild-type mice. We also assessed the rewarding effects of cocaine using the conditioned place preference paradigm and found no difference between the knockout and wildtype animals. In addition, we evaluated operant conditioning in the mutant mice and observed no significant differences in food self-administration experiments under fixed and progressive ratio schedules.

Conclusions: These findings suggest that selective deletion of the M4 receptor from cholinergic interneurons does not affect different aspects of animal behavior as opposed to what was proposed before.



Mechanisms behind cognitive symptoms in schizophrenia and depression

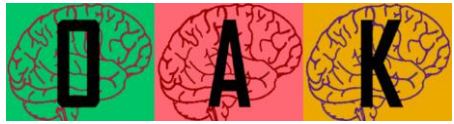
Camilla T. Erichsen[1;2], Maiken K. Bøgh[1], Kassandra Georges[1;2], Abdel-Rahman Al-Absi[1], Jytte Banner[3], Connie S. Morillo[4], Lingzhong Fan[5], Jens R. Nyengaard[1]

[1] Core Centre for Molecular Morphology, Section for Stereology and Microscopy, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[2] Sino-Danish College (SDC), University of Chinese Academy of Sciences, Beijing, China
[3] Department of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark
[4] Translational Neuropsychiatric Unit, Aarhus University, Aarhus, Denmark
[5] Brainnetome Center for National Laboratory of Pattern Recognition, Institute of Automation, Chinese Academy of Sciences, Beijing, China

Schizophrenia and depression have several underlying causes, and patients suffer from multiple symptoms that affect their life quality. It is possible to treat some of the patients' symptoms, but it is difficult to treat their cognitive symptoms. Cognitive symptoms are less studied but are equally important as they affect the patients' relationships, social abilities, and general ability to live independently. Brodmann area 46 regulates some cognitive functions, and in both disorders, this brain area is abnormally activated and has pronounced dendritic spine deficits in layer III.

This project aims to study the mechanisms behind cognitive symptoms using human Brodmann area 46 layer III biopsies from control, depressed, and schizophrenic patients. To explain some of the underlying mechanisms, quantitative immunofluorescence, western blot, autoradiography, RNA-Seq, and Nanostring will be used to look at differences in protein and gene expression between the three groups. Genetic data from schizophrenic and depressed patients will be correlated with multimodal magnetic resonance imaging data to look at changes in functional networks in Brodmann area 46. Aside from obtaining knowledge about the disorders in general, results will open a field for potential biomarkers of schizophrenia and depression and novel drug targets to treat patients' cognitive symptoms.

Keywords: Clinical neuroscience, pharmacology, psychiatry, psychology and mental health.



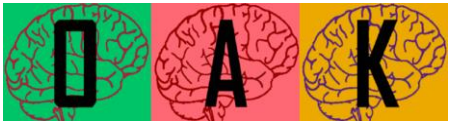
Clinical phenotype and factors contributing to pain pathogenesis investigated in a nonexperimental spontaneous dog model of neuropathic pain translating to humans

Tenna Remler Pedersen, DVM, PhD fellow; Mette Berendt, Professor, DVM; Jens Randel Nyengaard, Professor, MD, DMSc; Nanna Brix Finnerup, Professor, MD, PhD; Hanne Gredal, Associate Professor, DVM, PhD; Clare Rusbridge, Professor, DVM, PhD

Department of Veterinary Clinical Sciences, Section for Surgery, Neurology & Cardiology, University of Copenhagen
Department of Clinical Medicine - Core Centre for Molecular Morphology, Section for Stereology and Microscopy, University of Aarhus
Department of Clinical Medicine - The Danish Pain Research Center, University of Aarhus

BACKGROUND: Neuropathic pain is a debilitating public health concern due to the lack of effective treatment options. Many (50-90%) of human patients with Chiari malformation and coexisting syringomyelia suffer from neuropathic pain. A Chiari-like malformation is ubiquitous in the dog breed Cavalier King Charles Spaniel (CKCS), with concurrent syringomyelia in 25-70% of the cases. The neuropathic pain phenotype is similar to humans, suggesting a potential translational model which occurs naturally in a dog population.
OBJECTIVE: To investigate the neuropathic pain phenotype in syringomyelia using a spontaneous nonexperimental syringomyelia dog model
METHODS: Our objective is pursued through three work packages (WPs):
- WP1 (CLINIC): Detail and validate the clinical syringomyelia pain phenotype in CKCS through a retrospective clinical cohort study.
- WP2 (OMICS): Investigate and quantify potential biomarkers at a single-cell level within affected dog spinal cords, employing transcriptomic analysis using NanoString GeoMx technology.
- WP3 (HISTOPATHOLOGY): Identify cells responsible for the development of syringomyelia and neuropathic pain. Compare morphological alterations in spinal cords with syringomyelia to healthy spinal cords and correlate findings to clinical signs in dogs.
OUTCOME: We wish to contribute to translational pain research with new insights into neuropathic pain pathogenesis using a spontaneously occurring syringomyelia dog model.

Keywords: Neuropathic pain, spatial transcriptomics, quantitative immunohistochemistry, syringomyelia, spinal cord



Evaluation of microRNA in situ hybridisation usability of long-term stored human brain bank tissue

Rolf Søkilde[1], Erik Kaadt[1], Boye Schnack Nielsen[2], Betina Elfving[1]

1) Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University
2) Bioneer A/S

We have conducted a technical evaluation of long term stored archival FFPE material from the Danish Human Brain Bank. This material has been collected from patients with mental disorders during the period from 1945 to 1982. We have used commercial state-of-the-art in situ probes from ACD Bioscience targeted against microRNAs, to evaluate the quality of the material. Our technical analysis shows that microRNAs are preserved in sufficient quantities and quality to be useful for in situ hybridization against microRNAs. We therefore optimized an automated protocol for the Leica Bond Autostainer, this can then conduct chromogenic ISH stains on 30 slides a day.

In the next phase we will evaluate differentially expressed microRNAs from the Human Brain Bank, which have been identified with Nanostring NCounter technology. Three diseases have been selected and carefully curated, Schizophrenia (SZ), Bipolar Disorder (BD), and Depression (DE) with equal representation of male and females. Spatial investigation of the expression patterns of candidate microRNAs will aid the design of functional experiments. This will improve the understanding of the role of microRNAs in these neurological diseases.

Keywords: Non-coding RNA, microRNA, ISH, psychiatric diagnostics

SLITRK5, the genetic switch for developing OCD similar behavior?

Ole Borup Svendsen[1,2], Jens Midtgaard[3], Xu Ning-Long[4], Francis S. Y. Lee[5], Jens Nyengaard[1,6]

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Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition, where our understanding is limited, and today's treatment is only partially effective. The occurrence of OCD has several hypotheses, we hypothesize that a rare genetic mutation in the slitrk5 protein causes an imbalance in the excitatory and inhibitory signaling leading to a dysfunctional Cortico-Striatal Circuit (CSC). The aim is a thorough structural and functional study building upon current knowledge of this slitrk5 protein and its association with OCD. This research is based on the OCD mouse model, Slitrk5-/-, a transgenic knockout mouse. This particular mouse model has demonstrated OCD similar phenotypes, such as overgrooming behavior and increased anxiety. Previous research has detected structural changes in the orbitofrontal Cortex (OFC) and striatum, namely overactivity in OFC and reduced striatal volume. Through ultrastructural analysis and 3D reconstruction of cellular objects in the striatum, we expect to identify dissimilarities within the cortico-striatal circuit and cellular and subcellular objects between the OCD mouse model and wild-type mouse.

With comprehensive techniques incorporated, such as Serial-Block Face Scanning Electron Microscopy (SBF-SEM) we expect to detect structural alterations in the striatum contributing to a malfunctioning cortico-striatal circuit.

Keywords: OCD, SLITRK5, Electron Microscopy

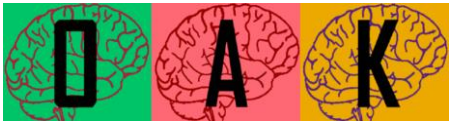
Studying the Impact of Conditional Knock-Out of the Serotonin 2B Receptor on Microglial Density and Morphology

Anchifiya Ali[1], Marco Anzalone[1], Estrid Thougaard Pedersen[1], Susanne Petersen[1], Kate Lykke Lambertsen[1], Jens D. Mikkelsen[3], Gerard Karsenty[4], Ernst-Martin Füchtbauer[5], Athanasios Metaxas[2], and Bente Finsen[1]

[1] Neurobiology Research, Department of Molecular Medicine, University of Southern Denmark, [2] School of Sciences, Department of Life Sciences, European University Cyprus, [3] Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet, [4] Department of Genetics and Development, Columbia University Medical Center, [5] Cellular Health, Intervention, and Nutrition, Department of Molecular Biology and Genetics, Aarhus University.

Alzheimer's disease (AD) is a chronic neurodegenerative disease and the most common cause of dementia. Besides the formation of intraneuronal neurofibrillary tangles and the extracellular deposition of amyloid beta (Aβ), increased microglial activity is a key feature of AD histopathology. The microglia constitute a unique type of CNS macrophages, serving homeostatic functions, and acting as a first-line defense against infection. In addition to innate immune receptors, microglia express receptors for the neurotransmitter serotonin (5-HT), which is decreased in AD. Serotonin acts as a negative regulator of microglial activation. 5-HT stimulates microglial branch motility in situ, which is important in microglial surveillance function, and acts via the 5-HT receptor 2A/B (HTR2A/B) in the exosomal release of the A-degrading enzyme insulysin. This study investigates the putative effect of HTR2B receptors on the microglial activation state, by use of mice with conditional knock-out (cKO) of the HTR2B in microglia. We have analyzed the effect of conditional HTR2B deletion on the density and morphology of Iba1+ microglia in the hippocampal stratum radiatum by use of ImageJ software. The results of this study are relevant to the understanding of 5-HT-dependent mechanisms in AD.

Keywords: Microglia, HTR2B, Conditional Knockout, Morphology, Alzheimer's disease



Remote ischemic conditioning; Novel treatment targeting in acute ischemic stroke

Elisabeth H. Lynnerup Rusholt[1,2], Bente Pakkenberg[1,2], Mikkel V. Olesen[1]

Rebecca Best Jensen[1], Maria Kjølhede[1,2], Jesper Just[1,2], Lee-Ann Clegg[4], Erik Kaadt[5], Rikke Bæk[4], Grethe Andersen[3], Birgitte Mumm[1], Betina Elfving[5], Malene Møller Jørgensen[4], Rolf Ankerlund Blauenfeldt[1,3], Kim Ryun Drasbek[1]

[1]Center of functionally Integrative Neuroscience, Aarhus University, [2]Department of Molecular Medicine, Aarhus University Hospital, [3]Department of Neurology, Aarhus University Hospital, [4]Department of Clinical medicine, Aalborg University, [5]Translational Neuropsychiatry Unit, Aarhus University

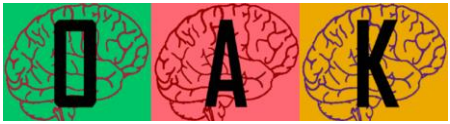
Remote ischemic conditioning (RIC) is a promising neuroprotective additive to the treatment of acute ischemic stroke patients (AIS). The mechanism behind RIC is hypothesized to be carried by extracellular vesicles (EV) and their miRNA content.

This study included 18 AIS patients and 13 non-vascular controls emitted to the hospital with stroke-like symptoms. The AIS patients were randomized to either RIC or placebo/sham treatment for 7 days. Plasma was isolated at baseline before RIC, 2h after first RIC/sham treatment, and 7 days after twice daily RIC/sham treatment.

Nanostring miRNA profiling of up to 827 unique miRNAs showed several significantly up- or downregulated miRNAs following RIC treatment. Some of these changes were sustained over the 7 days treatment period. In addition, the EV array technique revealed changes in EV surface markers following RIC at both 2h and 7 days of treatment. Finally, comparing AIS patients and non-vascular stroke mimic controls, we found several miRNAs specific for AIS.

The results of this study suggest that EVs and miRNAs plays a role in the pathophysiology of AIS while regulating the neuroprotective response of RIC. This qualifies them as potential biomarkers for novel treatment targeting in AIS.

Keywords: Acute Ischemic Stroke, Remote Ischemic conditioning, Extracellular vesicles, miRNA



Measuring alpha-synuclein aggregates across the human brain

Luisa Knecht[1], Anne-Line Strange Laursen[1], Jonas Folke[1,2], Susana Aznar[1,2]

1 – Centre for Neuroscience and Stereology, Bispebjerg-Frederiksberg Hospital, Copenhagen, DK
2 – Center for Translational Research, Bispebjerg-Frederiksberg Hospital, Copenhagen, DK

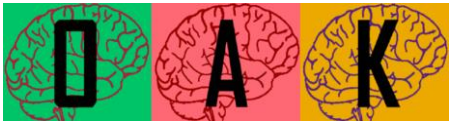
Background
Neurodegenerative diseases such as Multiple System Atrophy (MSA) and Parkinson's Disease (PD) are characterized by abnormal aggregation and accumulation of misfolded alpha-synuclein (α-syn) in the brain. However, the α-syn pathology leads to different disease trajectories and clinical manifestations.
We want to assess the α-syn aggregate load in MSA and PD brain areas to investigate in detail the disease-specific neuropathological trajectories across the brain.

Methods
Aggregated α-syn is measured in post-mortem brains including 10 MSA, 8 PD and 8 control brains and five brain regions (frontal cortex, putamen, cerebellum, substantia nigra and medulla/pons). Aggregates will be specifically measured using an assay kit (PerkinElmer) that utilizes a proximity-dependent FRET signal for the detection of aggregated α-syn species.

Results
Preliminary data shows successful detection of α-syn aggregates in the frontal cortex and substantia nigra, whereby the α-syn load is increased in MSA and PD patients compared to control brains.

Conclusion
It is hypothesized that the synucleinopathies show differences in the α-syn load across the assessed regions due to differences in the prion-like spreading of α-syn across the brain. We do further hope to investigate how individual brain pathology trajectories are associated with the clinical course by integrating clinical and proteomics data.

Keywords: Aggregated alpha-synuclein, FRET measurement, Synucleinopathies.



Targeting Cognitive Decline in Aging: Investigating the Effects of Erythropoietin Treatment and Hypoxia Exposure

Shamica Jaiswal[1], Gitta Wörtwein[1], Frederik Fussing[1], Mikkel Olesen[2]

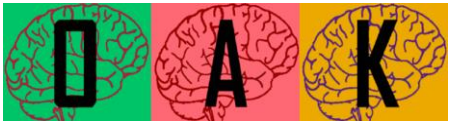
[1] Laboratory of Neuropsychiatry, Psychiatric Center Copenhagen
[2] The Research Laboratory for Stereology and Neuroscience, Department of Neurology, Bispebjerg Hospital

Background: Erythropoietin (EPO) is upregulated in response to hypoxia which in the brain plays a neuroprotective role. EPO alters synaptic connectivity and transmission of memory-related neuronal networks in hippocampal regions. We tested the hypothesis of whether EPO treatment could delay age-associated cognitive decline and if exposure to hypoxia could enhance the outcome.

Methods: With the aid of behavior tests we investigated non-spatial and spatial long-term memory which are hippocampus-dependent. Further, we quantified neuronal cell bodies in the hippocampal dentate gyrus and estimated the volumes of CA1 and CA3 regions by applying stereology.

Results: It is expected that old control rats show a decrease in the number of neuronal cell bodies in the hippocampal regions in comparison to young controls, indicative of hippocampal atrophy. It is also suspected that there will be changes in the hippocampal volume as a consequence of the EPO treatment with or without hypoxia.

Conclusions: These outcomes would support the hypothesis that exposure to hypoxia could augment the effect of EPO treatment in addition to the potential of EPO treatment as a therapeutic agent for age-related cognitive decline.



Modelling cortical pathology in mouse

Ojha, Bhavya[1], Lauridsen, Karoline[1], Khorooshi, Reza[1], Owens, Trevor[1,2]

1 Neurobiology, IMM, University of Southern Denmark,
2 Department of Neurology, Slagelse Hospital, Institute of Regional Health Research, Slagelse, Denmark.

Hypothesis: Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) in young adults. MS is believed to have an autoimmune etiology. Subpial cortical lesions and grey matter pathology are major hallmarks of progressive MS (PMS). To date, there are very few approved therapies for PMS, and they are most effective at the disease stage associated with inflammation. An intact blood-brain barrier (BBB) that restricts access to lesions is a further hindrance. The goal of our research is to establish an animal model which would enable therapeutic developments. PMS-characteristic subpial cortical pathology is not well-modelled by EAE, the most commonly-used animal model for MS. Our aim is to create meningeal inflammation, cortical inflammation with activated microglia/macrophages and understand the role of microglia in the lesion and its interaction with other immune cells involved.

Methods: Mice were fed with 0.3% cuprizone for 6 weeks. We delivered cytokines by focal injection to subarachnoid space, followed by intrathecal injection of microglia primed in a neurodegenerative environment in the 5th week. After completion of their diet plan the mice were sacrificed and the brains were processed for immunohistochemistry. As an alternative approach, we induced focal laser-induced injury for localization of cortical lesions. We surgically thinned the skull and used a two-photon laser to induce a focal burn injury in the cortex.

Results: We induced cortical inflammation by feeding the myelin toxin cuprizone to mice. With superimposition of subarachnoid injection of pro-inflammatory cytokines and intrathecal injection of primed microglia, we induced subpial cortical demyelination with intense microglial and astroglial response in the region. Similar focal subpial microglial activation was induced by laser irradiation. This was intensified by addition of a viral vector expressing IFN-γ. Preliminary results showed presence of monocytes and CD11c+ microglia in the irradiated region.

Discussion: We will further optimize and establish these animal models that mimic the hallmark pathology of PMS and investigate the underlying pathological mechanism. We are applying candidate therapeutics for the amelioration of PMS-like pathology induced in these animal models. This will improve our understanding of, and lead to better treatment of PMS.

Cortical changes in a new mouse model of amyotrophic lateral sclerosis (ALS)

Kata Molnár[1], Zhiheng Xu[2], Jens Randel Nyengaard[1], and Stine Hasselholt[1,3]

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ALS is a progressive, fatal neurodegenerative disease affecting the motor neurons of the nervous system, with limited knowledge of its cause, mechanisms, and treatment. Models investigating different mutations and their connection to ALS can help to get a better understanding of the disease. We have previously developed a mouse model to investigate a new mutation, in which we have found ALS-like clinical signs. To follow up on our findings of decreased neuron soma volume and node of Ranvier (NoR) length in motor cortex, changes in NoR and paranode length and volume were investigated using image stacks from electron microscopy, and dendritic spine density and -type changes assessed in Golgi-stained sections. Neuroinflammation has been found in ALS patients (Philips & Rothstein, 2014), therefore, microglia were stereologically quantified and their type classified based on immunohistochemical double staining and morphology. The changes observed visually from the data are as expected, e.g. a reduction in NoR and paranode volume in the ALS group compared to controls, reduced spine density and an increased number of activated microglia. These are, however, very mild, indicating that this mutation cannot explain the changes seen in ALS alone, but is rather a contributor together with other factors.

Philips, T., & Rothstein, J. (2014). Glial cells in Amyotrophic Lateral Sclerosis. Experimental Neurology, 262PB, 111–120. https://doi.org/10.1016/j.expneurol.2014.05.015

Keywords: amyotrophic lateral sclerosis, microglia, dendritic spines, electron microscopy, node of Ranvier.

MicroRNAs in inflammatory CNS diseases in dogs – Biomarkers for steroid-responsive meningitis-arteritis?

Pernille L. Heidemann[1], Emilio Mármol-Sánchez[2], Hanne Gredal[1], Susanna Cirera[3]

[1]Department of Veterinary Clinical Sciences, University of Copenhagen
[2]Department of Molecular Biosciences, Stockholm University
[3]Department of Veterinary and Animal Sciences, University of Copenhagen

Non-infectious inflammatory central nervous system (CNS) diseases in dogs, including steroid responsive meningitis-arteritis (SRMA), are presumably of autoimmune origin, although the specific disease mechanisms are not fully understood. SRMA affects young dogs that present with cervical hyperaesthesia and systemic inflammation. Prognosis is good with early diagnosis and treatment. However, reaching a diagnosis takes extensive work-up and includes analysis of the cerebrospinal fluid (CSF), which in dogs must be performed under generalised anaesthesia, comprising a risk for the dog, and cost for the owner.

The aim of this prospective case-control pilot study was to investigate microRNA-profiles in CSF to identify specific microRNAs, involved in the pathogenesis of SRMA, that could serve as biomarkers and eventually be investigated in serum, thereby offering a less invasive test for SRMA. CSF from dogs with SRMA (n=5) was compared to a group of healthy controls (n=5). Next-generation sequencing and subsequent quantitative real-time PCR verification were performed.

MiR-142-5p, miR-191-5P and miR-92a-3p were significantly upregulated in dogs with SRMA. Future studies should focus on validating these findings in a larger population and investigate their presence in serum. A control group of dogs suffering from another disease with a similar clinical presentation should be included.

Keywords: Steroid Responsive Meningitis-Arteritis, meningitis, canine, qPCR, next generation sequencing.

The regulation of mGluR5 after chronic mild stress

Celine Knudsen[1], Majken Thomsen[1], Kristoffer Højgaard[1], Ove Wiborg[2], Annie Landau[1], Betina Elfving[1]

[1]Department of Clinical Medicine, AU
[2]Department of Health Science and Technology

Major depressive disorder (MDD) is affecting millions of people worldwide, and is the leading cause of disability worldwide. The metabotropic glutamatergic receptor subtype 5 (mGluR5) may represent a promising therapeutic target for stress-related psychiatric disorders. The present study aims to investigate the availability of the metabotropic glutamate receptor 5 (mGluR5) in the medial prefrontal cortex (mPFC) of rats after chronic mild stress (CMS). The rats were treated with the atypical antidepressant, agomelatine, which showed an antidepressant-like effect in the sucrose consumption test. Using autoradiography, we showed that the levels of mGluR5 was increased in prelimbic cortex after CMS. Furthermore, treatment with agomelatine decreased the levels of mGluR5 in both prelimbic cortex and infralimbic cortex of the mPFC. Our results suggest that mGluR5 can be engaged in the symptomatology of depression.

Keywords: Depression, CMS, mGluR5, prefrontal cortex.

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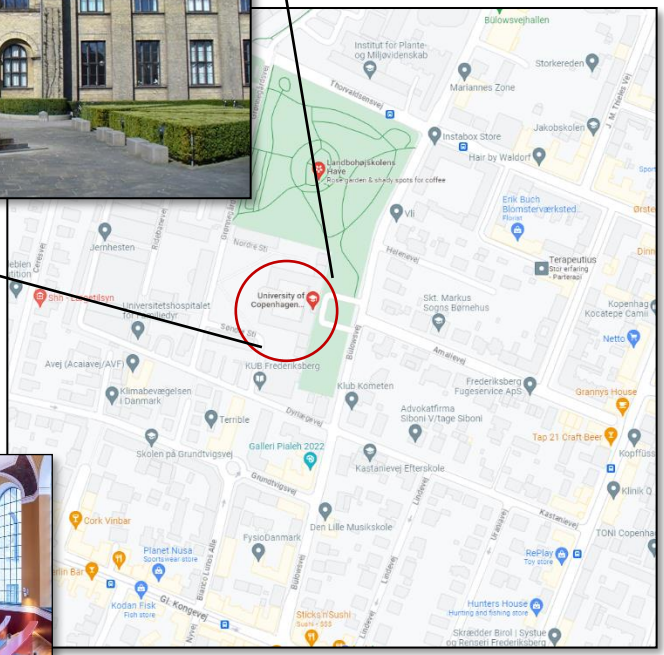
MEETING:

The meeting will take place at Festauditorium, Den gamle Landbohøjskole, Frederiksberg Campus, København Universitet

Bülowsvej 17, 1870 Frederiksberg

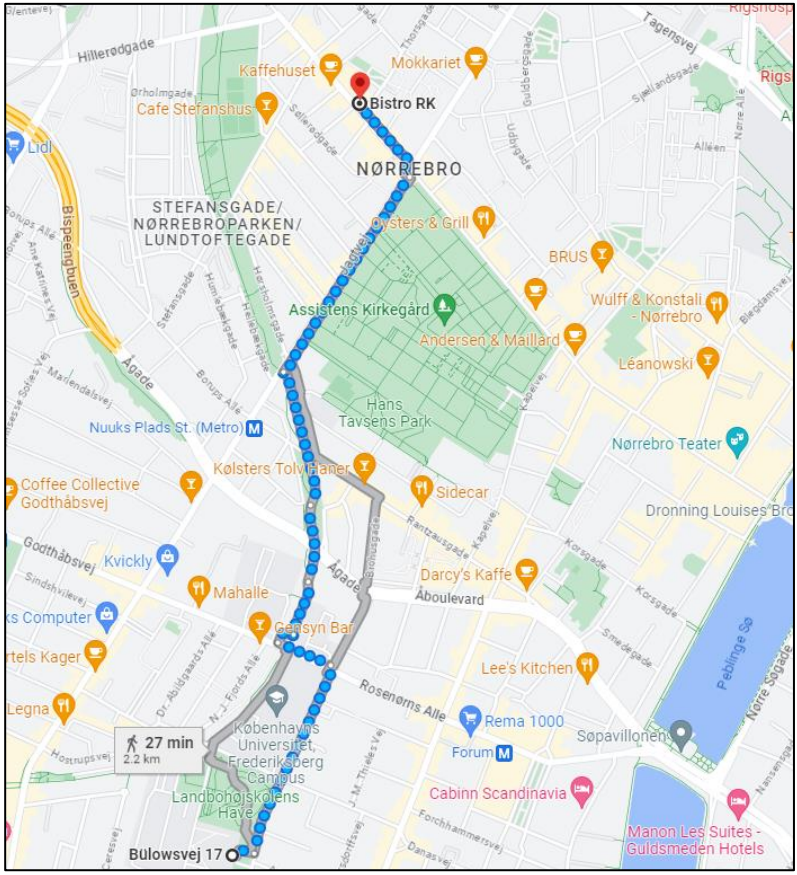


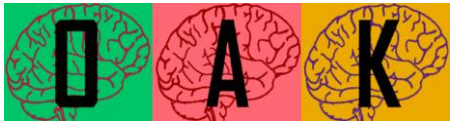
DINNER

After the meeting the OAK Meeting participants and sponsors will have a **dinner** at 18.30.

The dinner will take place at **Restaurant Bistro RK,**

Nørrebrogade 160, 2200 Copenhagen. It is a 30 min walk from Frederiksberg Campus (see map below).

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