DANDRITE Symposium 2025

The Adaptive Brain

Gene Expression and Regulation for Plasticity

Abstract Book

DANDRITE
Danish Research Institute of Translational Neuroscience
Nordic EMBL Partnership for Molecular Medicine

LUNDBECKFONDEN

Welcome to the Poster Sessions

We are delighted to present the poster contributions for **the DANDRITE Symposium 2025 – The Adaptive Brain**. The posters reflect a wide range of exciting research and innovation, with representation from both junior and senior researchers, and we encourage all attendees to engage with the presenters during the sessions.

Each poster has been assigned a number, which you can find in this abstract book. Please note the following practical details:

- Posters with **even numbers** will be presented on **20 August**
  Posters with **odd numbers** will be presented on **21 August**

- A poster prize will be awarded to the **Best Poster**, as voted by attendees on site. The winner will be announced during the **closing session on 21 August**.

Eva Dorothea Kaulich, Postdoc
Max Planck Institute for Brain Research

Understanding synaptic activity-states through their transcriptomic profiles

 # 1

Authors:

Kaulich, E., Fürst, N., Mosbacher, J., Ray, R. D., Waselenchuk, Q., Tushev, G., Langer., J. and Schuman, E. M.

Synaptic plasticity involves dynamic remodeling of the local transcriptome, shaping synaptic transmission and adaptation. We employ two complementary approaches to investigate activity-dependent transcriptome dynamics at synapses. First, we elicit synaptic plasticity in hippocampal slices using protein synthesis-dependent plasticity paradigms and isolate fluorescently labeled synapses via Fluorescence-Activated Synaptosome Sorting (FASS, (Biesemann et al., 2014; Hafner et al., 2019; van Oostrum et al., 2023; Kaulich,Waselenchuk et al., 2024) for RNA-seq and LC-MS/MS analysis. Our results reveal plasticity-driven shifts in the global synaptic transcriptome, with a rapid initial enrichment of specific transcripts during plasticity that is not sustained. This local increase in transcripts precedes major changes in the excitatory synaptic proteome. Our results suggest that some of the basal and plasticity-induced local transcriptome may itself be sensitive to protein synthesis inhibition. Second, we use CaMPARI, a calcium-sensitive photoactivatable fluorescent protein, to isolate synapses of rat cortical neurons based on their activity state. Transcriptomic analysis reveals molecular profiles associated with different activity states, suggesting that synaptic activity reorganizes the transcriptome. Our ongoing work aims to better understand activitydriven transcriptome remodeling at synapses and to identify the regulatory mechanisms underlying activity-dependent mRNA dynamics.

Sara Sejer Sørensen, Research Assistant
Aarhus University, Department of Biomedicine, DANDRITE

EZ-HCR: Whole-Tissue Spatial Imaging of RNA and Proteins

 # 2

Authors: Sara Sejer, Noor De Jong and Dong Won Thomas Kim

Traditionally, visualizing and locating RNA and proteins in the brain has relied on slicing and staining tissue sections. However, this approach is labor-intensive and disrupts the native spatial context, which is particularly problematic when investigating rare or unknown targets. The slicing process may destroy or miss regions containing low-abundance or region-specific molecules, making comprehensive analysis difficult.

To address these limitations, we have developed EZ-HCR: a whole-tissue staining and imaging protocol that enables high-resolution spatial mapping of RNA and proteins in intact brain tissue within 10 days, from tissue harvest to imaging.

The protocol begins with tissue clearing, allowing for whole-tissue imaging using light-sheet microscopes. We then apply hybridization chain reaction staining, which utilizes small probes that efficiently penetrate the tissue, enabling detection at sub-cellular resolution for both RNA and proteins. Importantly, the probes can be removed and reapplied, allowing multiple rounds of staining for different targets, overcoming limitations imposed by microscope laser lines and filter sets. The workflow is automated, with most steps consisting of incubation phases that significantly reduce hands-on time. While this protocol has been optimized for mouse brains, both the clearing and staining steps have shown versatility across a range of tissue types,

In summary, EZ-HCR enables the generation of whole-brain, high-resolution spatial datasets that allow direct comparisons of RNA and protein expression across brain regions within the same tissue. The method's flexibility and scalability make it a powerful tool for spatial biology across diverse research areas.

Anna Duncan, Assistant Professor & Group Leader
Aarhus University, Department of Chemistry

Molecular modelling of complex neural membranes

 # 3

In the biomodelling group we build *in silico* molecular-scale models of complex and crowded membranes of the neural system in order to understand how these membranes with myriad components are dynamically organised. In particular, we have recently built a model of the synaptic vesicle (SV) of glutaminergic neurons, containing 8 of the most populous SV proteins and 4 lipid species. Using this model we will run molecular dynamics simulationsnto give molecular insight into, for instance, interplay between synapsin-1 and clustering of SVs.

Anna M. Zamorano, Assistant Professor
Aarhus University, Department of Clinical Medicine, Center for Music in the Brain

Experience-dependent plasticity shapes pain processing

 # 4

Authors: Anna M. Zamorano[1, 2], Boris Kleber[1], Chuwen Chen[2], Samantha Millard[2], Enrico de Martino[2], Ainhoa Insausti-Delgado[3], Federico Arguissain[2], Shellie Boudreau[2], Peter Vuust[2], Herta Flor[2,4], Thomas Graven-Nielsen[2.]

(1. Center for Music in the Brain, Dept. of Clinical Medicine, Aarhus University & The Royal Academy of Music Aarhus/Aalborg, Denmark. 2. Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark. 3. TECNALIA, Basque Research and Technology Alliance (BRTA), Donostia-San Sebastián, Spain. 4. Institute of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany)

Repetitive sensory, cognitive, and motor training play a crucial role in the structural and functional reorganization of brain circuits. Even brief sessions of simple, repetitive movements can trigger neuroplastic changes in sensory and motor neural pathways, influencing how sensory inputs are processed. Given the essential role of experience in shaping visual, tactile, and auditory perception, we hypothesized that sensorimotor experience could also modu-late both behavioral and neural responses to pain.

To test this, we recruited 20 healthy musicians, one of the best models for studying the effects of experience-dependent plasticity in humans, and 20 non-musicians. All participants took part in three laboratory sessions (Day1, Day3, and Day8). Persistent hand pain lasting up to 14 days was induced on Day1 via intramuscular injection of nerve growth factor (NGF). During each session, participants underwent subjective pain rating assessments and quantitative sensory testing, including pressure pain thresholds and electrical de-tection thresholds (EDT). Neurophysiological measures included corticomotor excitability, event-related responses, and cortical dynamics recorded using transcranial magnetic stimulation and electroencephalography (EEG).

Compared to non-musicians, musicians showed greater sensitivity (lower EDT) and increased event related responses to sharp electrical pain. Howev-er, in response to prolonged muscle pain, they showed a resilient profile—reporting lower pain ratings, maintaining stable corticomotor excitability and EEG dynamics, and demonstrating increased left frontal alpha asymmetry, a neural marker associated with approach-related coping.

These findings indicate that experience-dependent plasticity can modulate both phasic and tonic pain responses, offering a mechanistic explanation for individual differences in pain sensitivity and vulnerability/resilience to chronic pain. Clinically, this suggests a promising direction for enhancing pain resili-ence through targeted training and developing non-pharmacological strate-gies for pain management.

Annika Ahtiainen, Postdoc
Tampere University, Faculty of Medicine and Health Technology

Electric field temporal interference stimulation of neurons in vitro

 # 5

Authors: Annika Ahtiainen[1], Narayan Puthanmadam Subramaniyam[1], Jarno Tanskanen[1], Lilly Leydolph[2], Alexander Hunold[2,3], Jens Haueisen[2], Jari Hyttinen[1]

(1. Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland. 2. Technische Universität Ilmenau, Institute of Biomedical Engineering and Informatics, Ilmenau, Germany. 3 neuroConn GmbH, Ilmenau, Germany)

Electrical stimulation (ES) techniques, such as deep brain and transcranial electrical stimulation, have shown promise in alleviating symptoms of depression and other neurological disorders in vivo. A novel, noninvasive ES method known as temporal interference stimulation (TIS) has recently gained attention for its ability to steer stimulation and selectively activate distinct brain regions [1]. However, the electrophysiological effects of TIS have yet to be demonstrated in vitro. To address this gap, we developed an in vitro "TIS-on-a-chip" system by integrating microelectrode arrays (MEAs) with a current stimulator. The stimulation was delivered via four platinum electrodes submerged in the cell medium through a custom 3D-printed cap. Rat cortical neuron cultures at 28 days in vitro were exposed to three stimulation protocols: (1) TIS using two channels at 653 Hz and 643 Hz to produce a 10 Hz envelope; (2) low-frequency stimulation (LFS) at 10 Hz; and (3) high-frequency stimulation (HFS) at 653 Hz. Unstimulated cultures were used as controls.

Our setup successfully demonstrated a novel platform for noninvasive ES in vitro, confirming spatial steerability in the "TIS on a chip" system [2]. Distinct differences in electric field strengths were observed across the TIS, HFS, and LFS conditions. As hypothesized, HFS and LFS did not enhance neuronal electrophysiological activity. In contrast, TIS elicited clear neuronal responses, most notably 24 hours post-stimulation. Interestingly, when cultures with a higher astrocyte presence were subjected to TIS, their responses differed from those with fewer astrocytes. This suggests that astrocytes may modulate neuronal sensitivity to TIS. Altogether, this approach opens new avenues for investigating the cellular mechanisms and ultimately potential clinical applications of TIS in treating neurological disorders.

Valentina Khalil, Postdoc
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Synapse-specific investigation of the single-cell gene regulatory dynamics to reveal the molecular basis of plasticity in aversive memory formation

 # 6

Authors: V. Khalil[1,2], K. Ito[1,2,] I. Faress[1,2], S. Nabavi[1,2] and T. Kitazawa[1,2]

(1. DANDRITE, The Danish Research Institute of Translational Neuroscience, Aarhus University, Aarhus, Denmark. 2. Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark)

It is widely accepted that experience-dependent plasticity is required for memory formation. However, underlying transcriptional and epigenetic changes are poorly understood. To tackle this, we are investigating the gene regulatory dynamics underpinning memory formation and how it is orchestrated with synaptic plasticity. We examined how synapse-specific plasticity manipulations could elucidate the molecular and behavioral dynamics of associative learning.

We combine synapse-specific manipulations with single-cell Multiome (i.e., RNA-seq and ATAC-seq) to understand how gene regulation is coupled with synaptic plasticity.

First, we established that optical high-frequency stimulation (HFS) of the thalamic inputs to the amygdala applied immediately after unpaired tone conditioning enhances the behavioral response to a level comparable to paired conditioning. In a separate cohort, we collected brain tissue after the conditioning session and plasticity manipulations to perform a single-cell Multiome to identify the learning—and plasticity-specific genes.

Next, we aim to manipulate target genes that emerged from our genomics analysis specifically to provide causal links between gene regulatory changes and synaptic plasticity in aversive memory formation. Our multidisciplinary approach, combining single-cell genomics and optogenetic synapse-specific manipulation, will expand our understanding of how memory is formed by orchestrating gene regulation and synaptic plasticity.

Meet Jariwala, Postdoc
University of Copenhagen, Biotech Research and Innovation Center (BRIC)

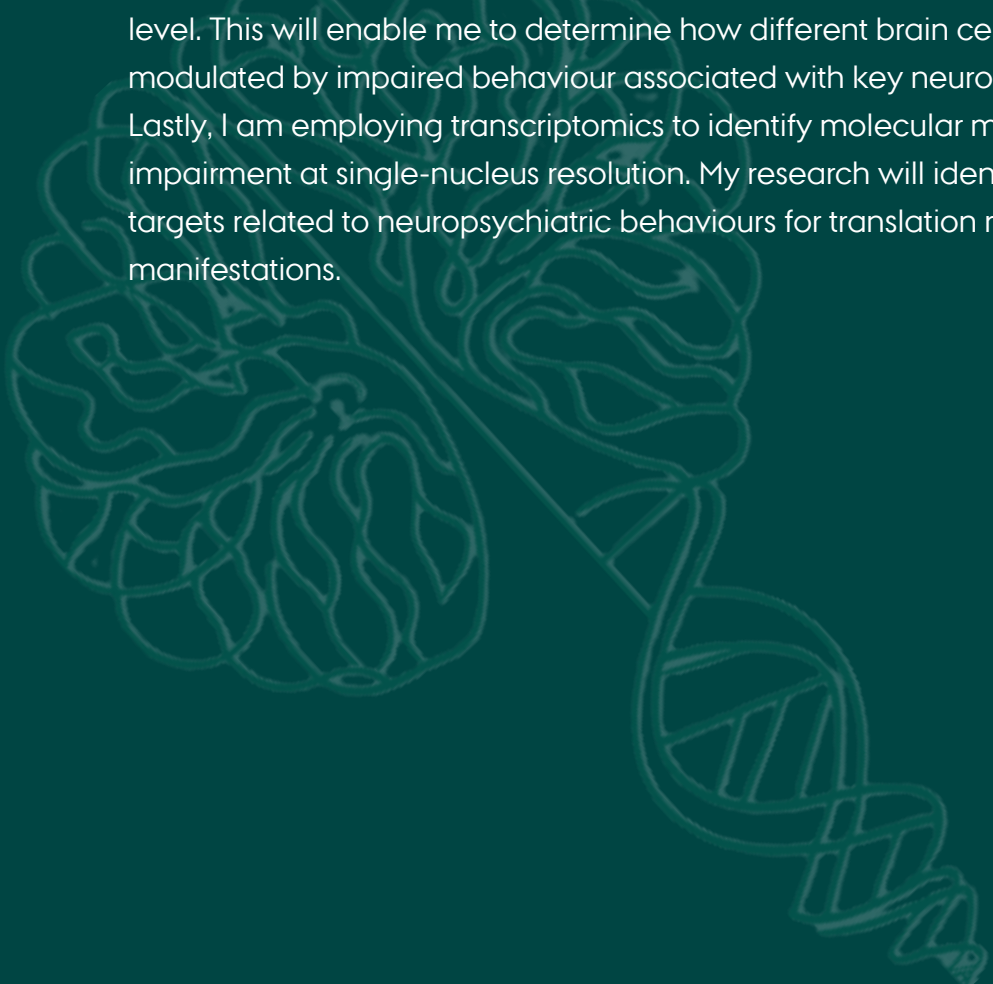
Decoding neural circuits in behavior: Using a novel technique to understand the psychiatric brain

 # 7

Neuropsychiatric disorders, such as schizophrenia and autism, affect a substantial portion of the global population, 1 in 8 individuals. Genetic risk factors have the major contribution to neuropsychiatric disorders; however, there is limited knowledge of how risk factors lead to brain circuit impairment and neuropsychiatric phenotypes.

Most affected individuals have limited medication options and exhibit social, cognitive, and adaptive challenges, hindering societal and economic integration. This is due to a lack of understanding of brain circuits and molecular mechanisms contributing to neuropsychiatric manifestations. Understanding how risk factors impact brain circuitry on a whole brain level at cellular resolution and how these circuitries are associated to specific behaviours linked to neuropsychiatric phenotypes should help develop more specific drug targets and therapeutics targeting precisely affected cell types and circuits.

I employ a clinically relevant mouse model of one of the major neuropsychiatric genetic risk factors – microdeletion in the 15q13.3 locus. Using the model, I am developing a novel technique to label psychiatric behaviour-associated circuits at whole brain level, at cellular level. This will enable me to determine how different brain cell-types and circuits are modulated by impaired behaviour associated with key neuropsychiatric manifestations. Lastly, I am employing transcriptomics to identify molecular mechanisms of circuit impairment at single-nucleus resolution. My research will identify cellular and molecular targets related to neuropsychiatric behaviours for translation research targeting specific manifestations.



Morad Kamand, Postdoc
Stockholm University, Department of Biochemistry and Biophysics

Beyond the genes: Deciphering the epigenetic landscape underlying autism spectrum disorder (ASD)

 # 8

Chromosome 16p11.2 copy number variation (CNV) is one of the most penetrant genetic contributors to autism spectrum disorder (ASD), accounting for approximately 1% of cases. Clinically, duplication of this region is associated with microcephaly and psychotic symptoms, whereas deletion correlates with macrocephaly and autistic traits. While numerous studies have explored transcriptional alterations linked to 16p11.2 CNV, the epigenetic changes—particularly how they impact gene regulatory networks at single-cell resolution—remain largely uncharacterized.

Furthermore, most studies on 16p11.2 CNV have focused on neuropathological phenotypes within the context of cortical brain development, while the potential impact on other brain regions has been largely overlooked. However, a growing body of evidence highlights the cerebellum as one of the most consistently affected structures in autism spectrum disorder (ASD) and related neurodevelopmental conditions. This underscores the critical need for a multiregional, brain-like tissue model to more comprehensively investigate the etiology of ASD.

To address these gaps, we propose a comprehensive, region-specific, and dosage-sensitive investigation of epigenetic dynamics across brain regions. We will employ isogenic induced pluripotent stem cell (iPSC) models of both 16p11.2 deletion and duplication, provided by the James F. Gusella Lab, to eliminate inter-individual genetic variability. Leveraging the nano-CUT&Tag method (Bartosovic et al., Nat. Biotech, 2023), we will map multiple chromatin marks simultaneously on the single cell level, enabling high-resolution analysis of the epigenomic landscape across key developmental timepoints and brain regions—including cerebellar and cortical lineages.

Comprehensive integrative analysis, including cell identity mapping and regulatory network reconstruction, will reveal how CNV-driven epigenetic dysregulation intersects with early brain development. These insights will advance our understanding of shared molecular pathways underlying diverse psychiatric conditions and may guide the development of targeted epigenetic therapies.

Kaho Ito, PhD student
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Epigenetic and Transcriptional Insights into Memory Engram Cells: Uncovering Cell-Type Specific Learning-Associated Genes in Different Brain Regions

 # 9

Authors: Kaho Ito, Valentina Khalil, Islam Faress, Taro Kitazawa

Memory encoding involves neuronal activity in memory engram cells, which triggers the upregulation of immediate early genes (IEGs) like *Fos*. IEGs often act as transcription factors to regulate epigenetic chromatin states of downstream genes called Secondary Response Genes (SRGs), thereby influencing neuronal plasticity-related genes. However, brain-region/cell-type specific epigenetic and transcriptional regulation of "neuronal activity-IEG-SRG cascade" in engram cell formation has been barely characterized. The fact that IEGs are activated by a variety of external stimuli, not just memory, complicates this issue.

We addressed this by currying out of memory-associated brain regions. We performed aversive conditioning in mice and analyzed the basolateral amygdala, medial prefrontal cortex, and hippocampus using sc-Multiome and sc-nanoCUT&Tag (nanoCT). scMultiome simultaneously maps chromatin accessibility (ATAC-seq) and transcriptome, while nanoCT simultaneous maps chromatin accessibility, active (H3K27ac) and repressive (H3K27me3) histone marks, respectively.

From these datasets, we identified "engram cells" as activated cells after fear conditioning, and "preconfigured cells" as randomly activated cells under the basal condition (i.e., home cage control). Next, we identified genes highly expressed in engram cells and compared their expression levels with preconfigured cells. We identified genes that were more robustly expressed in engram cells, suggesting that they were not activated by random baseline activity but were specifically associated with memory processes.

Moreover, the nanoCT analyses indicated that the epigenetic modifications potentially regulating these genes differ across cell types. These findings highlight the possibility of distinct transcriptional and regulatory mechanisms underlying memory-related gene expression in different cell types and brain regions.

Lilian Kisiswa, Associate Professor
Aarhus University, Department of Molecular Biology and Genetics

Impaired astrocyte-neuron communication in intellectual disability.

 # 10

Authors: Lilian Kisiswa[1,2*], Steffen Stokbæk[1,2], Florence Authier[1,2], Daan van Aalten[1,2*]

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Intellectual disability (ID) is a heterogenous neurodevelopmental disorder with severe social and economic impact. ID is characterized by reduced intellectual and adaptive functions that affect everyday living. ID can manifest on its own (non-syndromic) or together with other disabilities including Down's, Fragile X syndromes, attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD) and O-GlcNAc transferase-mediated congenital disorder of glycosylation (OGT-CGD). The intricate molecular heterogeneity characteristic of ID suggests that ID pathology encompasses more cells than just neurons. Indeed, emerging evidence underscores the significant involvement of astrocytes in ID pathogenesis. Using a system approach, the aim of this study is to investigate the dynamic interplay between astrocytes and neurons in ID. To this end, we use a newly developed O-GlcNAc transferase-mediated ID mouse model (*OGT*^{C921Y} mouse) together with primary astrocytes cultures, astrocyte-neuronal co-cultures and brain slices as experimental models. We detected negligible OGT expression in astrocytes, thereby ensuring that the OGT mutation primarily exerts non-cell-autonomous effects on astrocytes and making our model suitable for studying astrocyte-neuron communication.

Bulk RNA sequencing shows significant changes in astrocytic transcripts in *OGT*^{C921Y} hippocampal astrocytes. Both cortical and hippocampal astrocyte morphology is altered in *OGT*^{C921Y} brains, and these astrocytes exhibit increased levels of connexin 30, a key gap junction protein, indicating an increased functional syncytium. Furthermore, we find alterations in astrocytic functional markers (S100b, EAAT2, and Kir4.1), indicating impaired astrocytic function. Pharmacological and genetic inhibition of OGT activity in neurons impairs astrocyte morphogenesis. Taken together, these data suggest compromised astrocyte-neuron communication in ID, leading to exacerbated synaptic dysfunction. Our ongoing studies are aiming to restore this communication in an attempt to ameliorate ID symptoms.

Yumiko Kitazawa, Postdoc
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Whole-genome single-cell multimodal history tracing reveals cell identity transition

 # 11

Authors: Yumiko Kitazawa-Kawamura,[1,2,3] Valentina Khalil,[1,2,3] and Taro Kitazawa[1,2]

(1 Danish Research Institute of Translational Neuroscience-DANTDITE, Nordic-EMBL Partnership for Molecular Medicine. 2 Aarhus University, Department of Molecular Biology and Genetics. 3 These authors contributed equally to this work)

Cellular differentiation during development involves coordinated changes in transcriptional and epigenetic states. While single-cell sequencing has advanced our understanding of cell identity, it provides only static snapshots, limiting our ability to reconstruct past molecular events in the same cell. To overcome this, we developed HisTrac-seq—a whole-genome history-tracing platform based on DamID technique that enzymatically labels genomic DNA to “bookmark” regulatory states. HisTrac-seq captured both pre- and post-maturation molecular profiles within the same cell in in vitro neurodifferentiation model, and also enabled capturing both current and past molecular states of in vivo neurons, even two months after initial labeling. We also established single-cell HisTrac-seq (scHisTrac-seq), which enabled retrospective, multimodal profiling of transcriptional and epigenetic features at single-cell resolution. This revealed unexpected large-scale shifts in cell identity—termed identity jumps—which conventional methods fail to detect. Further analysis linked these jumps to premature activation of differentiation programs, highlighting a hidden layer of developmental plasticity. HisTrac-seq thus provides a powerful "temporal omics" approach for studying dynamic processes in development, plasticity, aging, and disease.

Vili Lampinen, Postdoc
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Design of Protein Binders to Probe Neurotrophin Signaling in Neuronal Connectivity and Memory

 # 12

The formation of memories and learning is based on accurate regulation of the connection between neurons. This is mainly orchestrated by neurotrophin receptors that sit atop neurons and transmit neurotrophin signaling. We aim to develop genetically encodable protein binders to study and manipulate the most elusive of these neuroreceptors, p75NTR. We use the machine learning tools RFDiffusion, ProteinMPNN, and BindCraft to design binders targeting both the extra- and intracellular domains of p75NTR. We screen for successful binders and measure their affinities towards their target with biolayer interferometry and flow induced dispersion analysis.

Finally, the binders will be used in cell culture assays to activate or block p75NTR. This can lead to activation of apoptosis, differentiation, or survival signals, which we can measure in vitro. Protein binders are genetically encodable, so they provide a whole new kind of tool set for studying intracellular neuron signaling.

Fiona Müllner, Group Leader
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

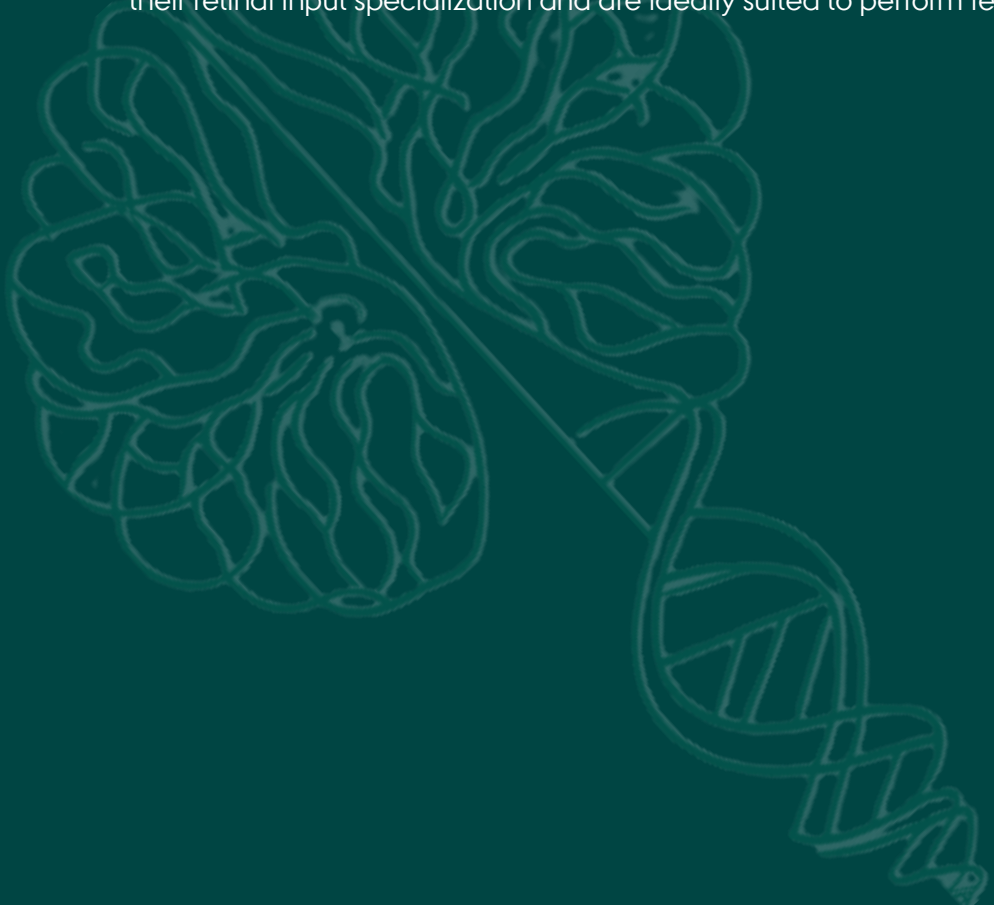
Individual inhibitory interneurons in the dLGN are functionally specialized towards distinct visual features

 # 13

Authors: Fiona E. Müllner, Botond Roska

Institute of Molecular and Clinical Ophthalmology Basel (IOB), Switzerland

Inhibitory interneurons in the dorsolateral geniculate nucleus (dLGN) are situated at the first central synapse of the image-forming visual pathway, but little is known about their function. Given their anatomy, they are expected to be multiplexors, integrating many different retinal channels along their dendrites. Using targeted single-cell-initiated rabies tracing, we found that mouse dLGN interneurons exhibit a degree of retinal input specialization similar to thalamocortical neurons. Some are anatomically highly specialized, for example, toward motion-selective information. Two-photon calcium imaging performed in vivo revealed that interneurons are also functionally specialized. In mice lacking retinal horizontal direction selectivity, horizontal direction selectivity is reduced in interneurons, suggesting a causal link between input and functional specialization. Functional specialization is not only present at interneuron somata but also extends into their dendrites. Altogether, inhibitory interneurons globally display distinct visual features which reflect their retinal input specialization and are ideally suited to perform feature-selective inhibition.



Lia Parada Iglesias, Postdoc
Aarhus University, Department of Clinical Medicine, Translational Neuropsychiatry Unit

Chemogenetic modulation of hippocampal circuits reveals a link between fear memory and depressive-like behaviour

 # 14

Authors: Lia Parada Iglesias[1], Michel Christian van den Oever[2], Gregers Wegener[1], Samia Joca[1,3].

Classic PTSD symptoms have been linked to maladaptive fear memory processing, involving fear generalization and impaired extinction learning. In contrast, cognitive and mood symptoms remain understudied, and their link to fear-related mechanisms is unclear. Here, we investigate the mechanistic link between maladaptive fear, memory impairments and depressive-like behaviour. Nine-week-old male C57BL/6J mice received bilateral dorsal dentate gyrus (dDG) injections of AAV-Fos::CreERT2 and Cre-dependent DREADDs (hM4Di or hM3Dq) or mCherry. After 3-weeks, mice underwent contextual fear conditioning with low (3×0.45 mA) or high (3×0.8 mA) shocks. Two hours later, 4-hydroxytamoxifen (25 mg/kg, i.p.) was given to label active engram cells. Mice were later tested for fear retrieval, generalization, novel object recognition (NOR), and forced swim test (FST). Clozapine-N-oxide (5 mg/kg, i.p.) was administered 30 min before each test. A subgroup underwent extinction training. DREADD expression and FOS activation were assessed via immunofluorescence. Data were analyzed using t-tests or ANOVA with Tukey post-hoc.

First, we confirm that high- but not low-intensity-conditioning induced generalization and impaired extinction. Low-intensity did not induce memory deficits; inhibition of the engram had no effect on the NOR and FST, while its activation induced memory impairments. High-intensity- conditioning induced memory impairments that were prevented by inhibition of the engram which also led to reduced immobility in the FST. This was accompanied by increased CA1/CA3 activity. Inhibition of the engram in high-intensity-conditioned animals reduced generalization; following extinction, memory impairments were not observed, and engram inhibition had no further effect. The inhibition of a dDG random engram (pre-conditioning) failed to rescue memory deficits and induced an increase in immobility in the FST. Maladaptive, but not adaptive fear memory, induces generalization, memory impairments and mediates depressive-like behaviours. The inhibition of this engram rescues these effects likely by restoring hippocampal activity. Our data suggest a circuit-level mechanism linking maladaptive fear with cognitive and affective dysfunctions.

Laís Pedrosa, PhD student
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Multi-level analysis of brain mechanisms underlying epigenetic inheritance of complex learning capabilities

 # 15

Authors: Laís Pedrosa[1], Noemie Mermet-Joret[1], Milad Nazari[1], Sanaz Ansarifar[1], Sadegh Nabavi[1]

(1. Department of Molecular Biology and Genetics, Aarhus University)

The development of intellectual skills in future generations has traditionally been attributed to heredity (nature) and environment (nurture). However, recent research in epigenetics reveals that experiences before conception can influence gene expression patterns that are inheritable by offspring. This project aims to investigate cellular and network mechanisms underlying the inheritance of learning in the hippocampus (CA1). Water-deprived mice (F0) and their offspring (F1) will be trained in an odor-based discrimination task, in which one odor predicts reward and another predicts no reward. To monitor neuronal activity throughout the learning process, large-scale calcium imaging will be conducted in freely moving animals using miniaturized microscopes. Neuronal activity patterns in CA1 will be examined in both F0 and F1 mice before, during, and after learning.

Our preliminary data indicate that, as mice learn the odor-reward-association, the pattern of functional connectivity among neurons—a proxy for task-relevant synaptic plasticity—gradually shifts toward an expert configuration. These findings are expected to deepen our understanding of the neurobiology of associative learning and how its underlying mechanisms may be inherited and expressed across generations.

Caroline C Real, Assistant Professor
Aarhus University, Department of Clinical Medicine

High-Intensity Aerobic Exercise Preserves Synaptic SV2A Density and Improves Symptoms in Parkinson's Disease: A PET Study

 # 16

Authors: Caroline C Real; Martin Langeskov-Christensen, Frederik Bonde Jensen; Anne M Landau, Nicola Pavese; Per Borghammer, Ulrik Dalgas, David J Brooks.

Introduction:
Physical exercise is a non-pharmacological intervention with beneficial effects on both motor and non-motor symptoms of Parkinson's disease (PD). However, the mechanisms behind these benefits remain unclear. We tested the hypothesis that high-intensity aerobic exercise improves symptoms and synaptic density in people with Parkinson's (PwP). This study aims to determine whether long-term exercise induces synaptic changes and supports its use as a therapeutic strategy.

Methods:
Forty PwP were enrolled and evaluated at baseline and after 24 weeks. Participants were randomized into an exercise group (PD_EX, n=20) or a control group maintaining usual activity (PD_Control, n=20). The PD_EX group underwent supervised high-intensity aerobic training (five sessions every 14 days, combining continuous and interval training) over 24 weeks. Clinical assessments included motor and non-motor questionnaires. Synaptic density was measured using [^{11}C]UCB-J PET, a marker of synaptic vesicle glycoprotein 2A (SV2A). Data distribution was assessed using the Shapiro-Wilk test. Mixed-effects models were applied to normally distributed data, and generalized estimating equations (GEE) to non-normal data, with Bonferroni correction for multiple comparisons.

Results: Exercise significantly improved non-motor symptoms as measured by UPDRS Part I ($P = 0.002$), with notable improvement in fatigue ($P = 0.013$). Depression scores (BDI-II) were significantly lower in the PD_EX group post-intervention ($P < 0.0001$). A trend toward improved mobility (Timed Up and Go) was also observed ($P = 0.074$). PET imaging showed a reduction in SUVR-1 (indicating decreased synaptic density) in the frontal ($P = 0.031$) and precentral cortices ($P = 0.02$) of the more affected hemisphere, and in the putamen of the less affected hemisphere ($P = 0.06$) in the PD_Control group—changes not observed in the PD_EX group.

Conclusions: Preliminary findings suggest that high-intensity aerobic exercise may help preserve synaptic integrity and improve non-motor symptoms in PD. These results support the potential of structured exercise programs as a disease-modifying strategy in Parkinson's disease.

Lucas Rodrigues Ribeiro, PhD student
Federal University of Minas Gerais, Department of Physiology and Biophysics, Nanobifar

MrgD Receptor Signaling Regulates Neurotransmission in the Nigrostriatal Pathway

 # 17

Authors: Lucas Rodrigues-Ribeiro1,2, Bruna da Silva Oliveira3, Kivia Soares Barretos Santos3, Arkadiusz Nawrocki2, Maria José Campagnole dos Santos1, Aline Silva de Miranda3, Cristina Guatimosim3, Martin Røssel Larsen2, Robson Augusto Souza dos Santos1, Thiago Verano-Braga1.

([1]National Institute of Science and Technology in Nano Biopharmaceutics, Department of Physiology and Biophysics, Federal University of Minas Gerais, Brazil. [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark (SDU), Denmark. [3]Department of Morphology, Federal University of Minas Gerais, Brazil.)

The renin angiotensin system is composed by a proteolytic cascade of angiotensinogen leading to the formation of several bioactive peptides, including alamandine, which acts as an endogenous agonist of the MAS related G protein coupled receptor D (MrgD). Notably, this receptor is expressed in brain regions associated with the control of reward, cognition, and motor functions, including the nigrostriatal pathway. This study aimed to assess the proteome, phosphoproteome, and N-glycoproteome of the nigrostriatal pathway in C57Bl6/J (WT) and Mrgprd-deficient mice (KO) to uncover the molecular role of MrgD in this pathway. Thus, substantia nigra (SN) and striatum (ST) regions were collected for proteomics (n=4). Tryptic peptides were labeled with TMT-16plex and submitted to simultaneous enrichment of phosphorylated and N-glycopeptides. Samples were analyzed using Orbitrap Exploris 480 and raw data was deconvoluted using Maxquant. An extensive functional validation was done. A total of 5,878 unique proteins, 19,209 class 1 phosphosites, and 5,301 N-glycosylated sites were identified, with 468 proteins, 198 phosphosites, and 67 N-glycosylated sites regulated ($p<0.05$, FC±1.2)

The MrgD deficiency resulted in significant alterations in the proteome and post-translational modifications of proteins involved in synaptic vesicle exocytosis. Quantification of exocytosis rates revealed reduced synaptic vesicle release in KO mice. Pharmacological experiments demonstrated that alamandine, but not β-alanine, significantly increased synaptic vesicle release in a MrgD-dependent manner. Behaviorally, MrgD knockout mice exhibited motor hyperactivity and increased compulsive-like. In conclusion, the MrgD signaling has an important role in neurotransmission in the nigrostriatal system, highlighting its relevance to neuropsychiatric disorders involving motor control and impulsivity.

Julia Soh, PhD student
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Investigating bacterial quorum sensing and its role in IBD

 # 18

Authors: J. Soh, G. Vanwalleghem

One of the ways by which bacteria communicate is via quorum sensing, where bacteria produce chemical messengers that regulate gene expression in a population dependent manner. We hypothesize that this system is involved in direct or indirect communication with the human gut. In particular, dysregulation of the microbiome is observed in IBD (inflammatory bowel disease) patients. One quorum sensing system involves the use of diffusible signal factors (DSF), cis-2 unsaturated fatty acids of various lengths that can induce a signal transduction through receptor proteins. This project aims to identify how DSF signals may play a role in modulating the gut microbiome in IBD.

Emilio Sopprani, Student
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Altered synaptic proteostasis drives cortical hyperexcitability in mouse model of O-GlcNAc transferase-driven intellectual disability.

 # 19

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O-GlcNAcylation, a crucial post-translational modification, is catalyzed by the enzyme OGlcNAc transferase (OGT). Mutations in the OGT gene result in OGT-mediated congenital disorder of glycosylation (OGT-CDG) characterized by intellectual disability, developmental delay and seizures. Given the symptomatic overlap between OGT-CDG and other neurodevelopmental disorders, such as autism, Down's syndrome, and Fragile X syndrome, all of which involve known synaptic impairments, we hypothesize that OGT-CDG is a synaptopathy. To test this hypothesis, we utilized a mouse model (OGTC921Y) harboring a cysteine-to-tyrosine mutation at position 921 within the catalytic domain of the OGT gene. This model recapitulates several behavioral phenotypes observed in human patients, including hyperactivity and autistic-like behaviors. Our initial findings demonstrate that OGT is ubiquitously expressed in neurons. However, its synaptic localization is restricted to glutamatergic synapses. Proteomic analysis of OGTC921Y mice revealed significant alterations in synaptic proteostasis. Furthermore, while inhibitory synapses remained unaffected, we observed an increase in both the size and number of glutamatergic synapses, accompanied by an increase in dendritic spine density. Electrophysiological recordings from OGTC921Y neurons showed comparable firing rates and spike amplitudes to those of wild-type neurons. However, mutant neurons exhibited an increased burst frequency, suggesting increased network activity. Upon stimulation, OGTC921Y neurons displayed an elevated number of spikes and evoked activity peaks, indicating enhanced excitatory activity. Collectively, these data demonstrate altered glutamatergic transmission in the OGT-CDG mouse model. This synaptic dysfunction provides a compelling explanation for the behavioral phenotypes observed in both the mouse model and human patients with OGT-CDG.

Silvia Turchetto, Postdoc
Aarhus University, Department of Molecular Biology and Genetics, DANDRITE

Synaptic Signals for Protein Degradation

 # 20

Authors: Silvia Turchetto, Valentina Villani, Wen-Hsien Hou, Vyacheslav Akimov, Blagoy Blagoev, Chao Sun

Protein degradation is an important mechanism to modify and eliminate synapses. This process requires a major cellular signal for protein degradation, ubiquitin. Despite its crucial role, we do not understand how ubiquitin regulates the turnover of diverse synaptic proteins at a wide range of suitable rates. To elucidate the synaptic regulation of ubiquitin signaling, we have obtained, by far, the most comprehensive protein ubiquitylome of synaptoneurosomes that are dissociated from the mouse cortex, containing >7000 lysine sites carried by over 2000 proteins. A subset of synaptic proteins exhibits significant subcellular preferences for ubiquitin signaling in or outside synapses.

For example, many proteins involved in neurotransmitter release and detection exhibit suppressed ubiquitin signalling outside synapses, including components of the SNARE complexes, and specific subunits of voltage-gated Ca2+ channels and AMPA receptors, etc. These proteins are often membrane-associated and primarily synthesized in the cell soma. These findings suggest that the degradation signals for key synaptic proteins are regulated in a subcellular-specific manner to ensure efficient, long-distance delivery to synapses.

Victoria Twiddy, PhD student
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Design and Validation of Functional, Internally-tagged VPS10p-Domain Receptors

 # 21

Authors: Victoria Twiddy,[1,2,3] Lilian Kissiswa,[1,2] & Magnus Kjærgaard[1,2,3]

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Synaptic plasticity, the alteration of synaptic strength in response to novel information, is widely considered the molecular mechanism underlying memory formation and is driven by changes to the arrangement and structure of the post-synaptic density. Uncovering the mechanisms by which proteins traffic molecules to and from the post-synaptic density is, therefore, a necessary step in advancing our understanding of memory.

SorCS1-3, members of the mammalian VPS10p-D receptor family, are crucial to the proper functioning and survival of neurons. They are highly prevalent in the central nervous system, have known functions in protein trafficking, and are associated with various neurological disorders including Alzheimer's disease. While SorCS1-3 share structural elements, they harbour unique cytoplasmic tail regions. Studies have shown that the tail region of the related yeast protein VPS10p is indispensable for its protein transportation function and that the tails of other mammalian VPS10p-D family members are instrumental in driving Golgi-endosomal transport. Collectively, these findings support roles for the SorCS1-3 tails in protein trafficking.

This PhD project aims to combine proteomic studies and functional assays in neurons to characterise the interactomes of the SorCS1-3 tails and to uncover the contributions of these regions to the protein trafficking functions of SorCS1-3.

Valentina Villani, Research Assistant
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Local regulators of synaptic protein stability

 # 22

As a major cellular signal for protein degradation, ubiquitin signaling is regulated by enzymes that can edit ubiquitin modifications on proteins. As potent regulators of proteostasis, these enzymes form a high-risk gene network in major brain disorders. So far, we do not even know which enzymes are expressed at synapses. To elucidate the local regulators of protein stability at synapses, I have curated a list of candidate synaptic deubiquitylases from existing proteomic data. Integrating confocal microscopy, DNA-PAINT-based single-molecule localization, and expansion microscopy, we noticed that these deubiquitylases exhibit unique patterns of subcellular expression in neurons. Our ongoing work is beginning elucidate how these ubiquitin enzymes conspire to realize subcellular-specific ubiquitin signaling and protein turnover.

Jens Andersen, Research Assistant
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Enkephalinergic Neurons in the Lateral Septum Bidirectionally Control Affective Responding

 # 23

The role of the Lateral Septum (LS) in affective and motivational regulation has remained largely unexplored since the 1960's, when Robert G. Heath performed deep brain stimulations (DBS) revealing the LS as a potential site that evokes pleasure. Subsequently, a handful of human DBS studies have further linked the LS to positive affective subjective experiences. However, the specific roles of LS neuron subpopulations in regulating affective state have not been fully elucidated.

In the present study, we found that optogenetic stimulation of LS neurons in mice leads to positive affective responding, with increased hedonic responding to Nutella and Social interaction to an opposite gender mouse - mirroring the affective profile observed in human DBS studies. To identify the neurocircuits underlying this phenotype, we employed a retrograde AAV-based viral strategy and revealed that LS neurons innervate the nucleus accumbens (NAc), a key reward-processing brain region. LS neurons projecting to the medial shell of the NAc are predominantly enkephalinergic. Surprisingly, optogenetic stimulation of LS Enkephalinergic neurons, using a Penk-Cre-dependent AAV-DIO-strategy, cause significantly reduced hedonic responding to Nutella, as well as reduced social interaction and self-care (without changing novel object exploration, locomotion or anxiety levels); while inhibiting these neurons using Gi-DREADDs causes the opposite phenotype. As, the general stimulation of LS neurons leads to positive affect, we considered if the Enkephalinergic neurons are regulated by local GABAergic interneurons. To investigate synaptic input to LS Enkephalinergic neurons, we used a modified Rabies virus strategy. We found that LS-interneurons strongly innervate enkephalinergic projection neurons. Collectively, our preliminary data indicate that LS is an affective hub which can bidirectionally regulate affective state through distinct synaptic mechanisms involving enkephalinergic signaling.

Malene Bach Jensen, Student
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Primary familial brain calcification caused by phosphate transporter PiT2 variants: Does high phosphate in the brain fluid counteract the effects of reduced expression of functional PiT2 on neuronal morphology?

 # 24

Authors: Malene Bach Jensen, Sara Sejer, Julie Gerup and Lene Pedersen

The neurodegenerative disorder primary familial brain calcification (PFBC) is characterized by calcium-phosphate deposits in various brain regions. At early clinical onset patients may present with symptoms such as mania, bipolar disorder, anxiety, ADHD, schizophrenia, and/or depression, while, eg, cognitive impairment and movement disorders present later. The minimal disease prevalence is estimated to be 2.1-6.6 per 1000. Variations in the *SLC20A2* gene, encoding the sodium-dependent inorganic phosphate (Pi) cotransporter 2 (PiT2), are associated with 61% of PFBC cases. The majority are heterozygous variants. Patients with *SLC20A2* homozygous variants have also reported; they present with more severe symptoms than heterozygous family members. Elevated Pi levels in cere brospinal fluid (CSF), but not in blood, are seen in PFBC patients.

A PiT2 knockout (KO) mouse model has been established and is a reliable model for PFBC, since the mice exhibit brain calcifications and show behavioral symptoms related to those seen in PFBC patients. PiT2-KO mice also present with elevated Pi levels in CSF, but not in blood. Our preliminary data from neuronal cultures derived from PiT2-KO and wildtype (WT) mice suggest that the absence of PiT2 affects the neuronal morphology. As mentioned, there is a higher Pi level in CSF of PiT2-KO mice and PFBC patients. To gain further insight into the role of Pi and PiT2 in neurons, we investigate whether high Pi levels can counteract the effects of lack of the Pi importer PiT2 on neuron morphology. This is done by performing experiments with neurons cultured with high Pi. Our current results will be presented.

Eva Sofie Bovbjerg, Research Assistant
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The effects of cannabidiol on neurotrophic tyrosine kinase receptor 2 localization and trafficking

 # 25

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Cannabidiol (CBD) induces a fast and sustained antidepressant elect in animal models of depression; an elect associated with changes in the TrkB signaling pathway. While recent studies suggest that antidepressants, as wells various psychedelic drugs, directly bind the TrkB receptor, the exact influence of CBD on this pathway remains elusive. This receptor is known to reside in lipid rafts, and the association to these rafts help modulate signaling. Due to the lipid-like nature of the CBD compound, and its ability to influence cholesterol metabolism, we set out to explore the influence of CBD on the lipid raft translocation and mobility of the TrkB receptor. To establish protocols for investigation of the elects of CBD on TrkB subcellular localization and mobility, with the ultimate goal of uncovering mechanisms in which CBD influences neuroplasticity.

We investigate the translocation of TrkB into lipid rafts, by isolation of detergent resistant membranes with sucrose gradient membrane fractionation. Moreover, assessment of the mobility of the receptor is studied with fluorescence recovery after photobleaching (FRAP). Lastly, the possibility of a direct interaction between CBD and TrkB is explored using microscale thermophoresis (MST).

The data confirm successful isolation of flotillin-enriched, detergent-resistant membranes, with TrkB localized to these membrane regions. Preliminary findings indicate that CBD and BDNF have opposing elects on TrkB translocation to lipid rafts. Furthermore, FRAP analysis suggests that CBD counteracts BDNF-induced increases in TrkB lateral mobility. We present two complementary methods for studying the mobility and subcellular localization of a membrane bound receptor, focusing on TrkB. Our preliminary findings show a trend of CBD influencing the BDNF induced translocation of TrkB into lipid rafts as well as general mobility in the lipid membrane.

Lars Boye Brandt, Student
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Subcellular Prediction of Neuronal Protein Localization Using Protein Language Models

 # 26

Authors: Lars Boye Brandt, Chao Sun

In silico prediction of subcellular protein localization is a powerful tool for accelerating our understanding of neuronal gene express in space, a unique feature that imparts complexity to the neuronal network. However, existing prediction models often fail to reflect the unique compartmental complexity of neurons. In this project, I extend the deep learning framework of ProtGPS—a localization prediction tool based on ESM2 protein language models—by retraining it on a curated dataset of neuron-specific proteins. Currently our model can predict over 12 compartments, with an average AUC-ROC of ~0.8 across compartments, indicating the model is good at distinguishing correct protein locations from incorrect ones. By fine-tuning the models training parameters and integrating proteins with neuron-specific compartment information, ongoing work will improve the performance of protein localization predictions across diverse neuronal compartments.

Christian F. Christensen, Postdoc

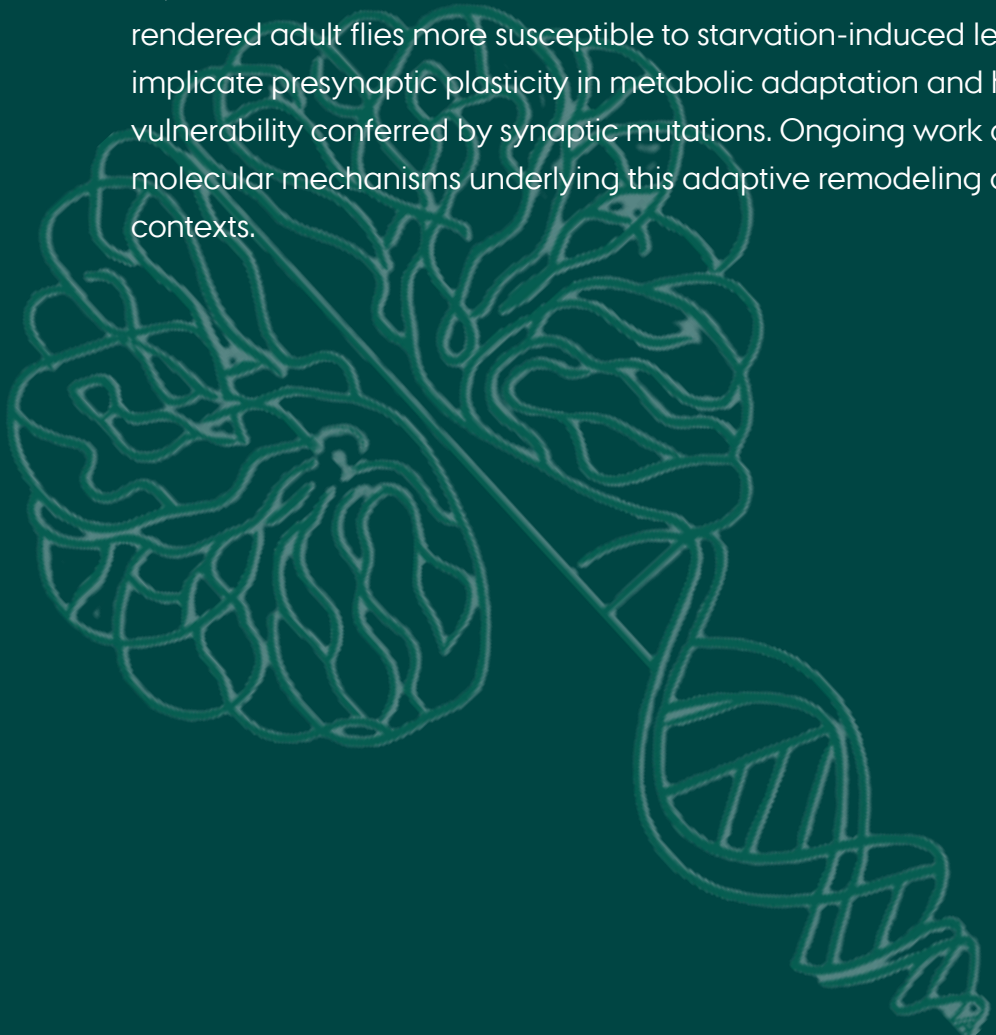
University of Copenhagen, Department of Neoruscience

Presynaptic active zones tune to nutrient availability

 # 27

Synaptic plasticity, the change of the connection strength between nerve cells, enables an organism to adapt to environmental changes. While its roles in learning and memory are well appreciated, its involvement in metabolic adaptation remains poorly understood. Using quantitative imaging of endogenously tagged synaptic proteins in the genetically tractable model organism; Drosophila melanogaster, we found that brain-wide levels of key targets for presynaptic plasticity are shifted upon metabolic challenge in adult flies. We observed that nutrient deprivation selectively alters the abundance of presynaptic components, including the voltage-gated Ca2+ channel Cacophony and the essential release-factor Unc-13.

In contrast, levels of the core active zone scaffold Bruchpilot (mammalian ELKS family) and the postsynaptic marker Dlg1 (mammalian PSD95) remain stable, suggesting a targeted remodeling of presynaptic active zones in response to shifts in metabolic state. Furthermore, introduction of a human disease-associated mutation in unc-13 (Lipstein et al., 2017) enhanced neurotransmitter release at the larval neuromuscular junction but rendered adult flies more susceptible to starvation-induced lethality. These findings implicate presynaptic plasticity in metabolic adaptation and highlight a potential vulnerability conferred by synaptic mutations. Ongoing work aims to elucidate the molecular mechanisms underlying this adaptive remodeling and its disruption in disease contexts.



Markus Ørnsvig Christensen, Student
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Synaptic Localization of Intracellular Calcium Pumps

 # 28

The sarco/endoplasmic reticulum calcium ATPases (SERCA) is the major intracellular calcium pump at synapses. Localized on the ER membrane, SERCA regulates the uptake of Ca^{2}+ into the ER lumen and is important for synaptic function. Here we apply expansion microscopy to investigate the synaptic expression of SERCA in mouse and patient-derived brain tissues. We found that SERCA appears to form clusters at synapses. These synaptic SERCA are predominately localized at the presynaptic compartment. Our findings suggest that SERCA is enriched at neuronal synapses to regulate Ca^{2}+, a major synaptic signal that orchestrate synaptic transmission and plasticity.

Line Mathilde Brostrup Hansen, Postdoc
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A single-cell resolution spatial profiling of non-neuronal cell responses to ischemic stroke in female mice

 # 29

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Acute ischemic stroke (AIS) is a global health burden, with approximately 7.63 million new incidences annually. Despite recanalization improvements, complications such as no-reflow and futile reperfusion often limit functional recovery. The mechanisms driving these phenomena remain unclear. Notably, sex differences play a significant role in AIS outcomes, with females having a greater proportion of stroke-related deaths and worse functional recovery. However, most preclinical models continue to prioritize male subjects, leaving a critical gap in understanding female-specific stroke responses. Therefore, we aim to investigate the gene expression changes in the acute phase following AIS of female mice.

To investigate mechanisms of futile reperfusion, we employed a transient middle cerebral artery occlusion model in six aged (24–26 weeks) female C57BL/6J mice. Following one hour of awake photothrombotic occlusion and 24 hours of reperfusion, coronal brain sections were analyzed using CosMx spatial molecular imaging (NanoString Technologies) and referenced to the Allen Brain Atlas for single-cell prediction.

A total of 46,957 non-neuronal cells were identified, including 18,266 vascular cells, i.e., endothelial and smooth muscle, pericytes, and vascular leptomeningeal cells. Gene ontology analysis of biological processes revealed, among others, a downregulation of calcium ion transport pathways, notably the Na^+/Ca^{2+} exchanger (Slc8a1) in endothelial cells and Inositol 1,4,5-Trisphosphate Receptor Type 1 (Itpr1) in smooth muscle cells and pericytes.

Downregulation of Slc8a1 and Itpr1 highlights disrupted vascular function despite restored blood flow. Given the critical role of calcium signaling for vascular tone, permeability, and neurovascular signaling, these alterations may disrupt brain microcirculation and impair tissue recovery after successful recanalization in females.

Josephine Dannersø, Postdoc and Sean Hansen, PhD Student
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Elucidating the Ultrastructure of the Axon Initial Segment by cryo-Electron Microscopy and Expansion Microscopy

 # 30

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Neuronal signaling is crucial to function of the vast network of neurons that are found in the brain. Essential to this is both ultrastructural compartmentalization of the single cell into e.g. somatodendritic vs axonal compartments, and on a smaller scale, microenvironments in e.g. the plasma membrane allowing for tightly controlled ion-flow. With the aim of studying neuronal function and action potential initiation and regulation, the work presented here focuses on the ultrastructure of the Axon Initial Segment (AIS). The AIS is an approx. 20-60 um long compartment of the axon localized between the soma and distal axon and is crucial to action potential initiation and regulation. Interestingly, an overall tight organization with a periodicity of ~190 nm of the cytoskeleton and numerous related scaffolding- and membrane- proteins has been found in the AIS, and this is of great importance for the specialized function of the AIS.

This project employs two leading imaging methods for elucidating cellular ultrastructure, combining cryo-electron tomography (cryo-ET) and expansion microscopy (ExM). Primary hippocampal neuronal culture was cultured directly on EM-grids and antibody labeling enabled localization the AIS. Cryo-ET data of labeled AIS was collected, and analysis showed great potential for future focused studies on different aspects of the AIS ultrastructure. The 3D information from labelled cryo-ET data in combination with immunofluorescent expansion microscopy studies showed the presence of two ankyrin G (AnkG) molecules pr. 190 nm in the organized AIS scaffold, and the results furthermore highlight the possibilities of combining different imaging methods in studies of a complex ultrastructure as that of the AIS. Preliminary cryo-CLEM and cryo-FIB-milling studies have furthermore been initiated, and a protocol for obtaining high quality cryo-ET data for future structural analysis of ion channels and pumps of the plasma membrane has been established.

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Altered synaptic properties and cogntive behavioural deficits in a novel knock-in model with a human schizophrenia-associated mutation in the postsynaptic protein stargazin

 # 32

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Disturbances to neuronal function at the level of the synapse are crucial features of neuropsychiatric disorders, such as schizophrenia. Synaptic dysfunction in the prefrontal cortex, and its consequences on adaptability in neuronal circuitry and behaviour, have been extensively linked to the disease. In this study, we are characterising the functional consequences of a recently discovered, schizophrenia-associated variant in the CACNG2 gene, coding for the postsynaptic protein stargazin (StgSCZ). The aim of our study is to uncover how dysfunction at the level of the synapse in the prefrontal cortex contributes to disease phenotypes.

Previous *in vitro* characterisation showed that the StgSCZ variant has a damaging impact on protein function, leading us to generate a knock-in mouse model carrying this variant. StgSCZ knock-in animals show impairments in cognitive function, and subtle structural changes in neuronal morphology, while functional analysis revealed impairments in AMPAR function and synaptic transmission, as well as some changes in neuronal excitability. Interestingly, we have also observed concurrent alterations in bioenergetic demands, with subtle alterations in mitochondrial function and morphology, as well as potential changes to mitochondrial Ca2+ dynamics. Taken together, our results indicate impairments to synaptic function at several levels in prefrontal circuitry. We propose that this knock-in model carrying a disease-associated variant of stargazin has significant construct validity for schizophrenia and provides a comprehensive basis for investigating disease mechanisms at the synaptic and network level.

Meheli Banerjee, PhD student
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Developing an in vitro chemoconvulsant induced platform to model TBI-associated pathways

 # 33

Traumatic brain injury (TBI) affects ~69M people annually and can lead to morbidities like posttraumatic epilepsy. Due to the complexity of *in vivo* TBI models, *in vitro* systems present an alternative approach to study specific molecular responses. We hypothesized that comparing chemoconvulsant and TBI-induced transcriptomic profiles will reveal overlap and relevant injury-related pathways. Rat primary cortical cultures were treated with 11 chemoconvulsants (4-aminopyridine, amoxapine, bicuculline, chlorpromazine, donepezil, kainic acid, pentylenetetrazol, picrotoxin, pilocarpine HCl, SNC80, strychnine HCl) for 24 h. To identify differentially expressed (DE) genes in cell cultures, RNA sequencing was performed, followed by differential expression analysis using DESeq2. Microarray data from rats with lateral fluid-percussion TBI (BioProject PRJNA437082) were analyzed using Limma to identify DE genes in the perilesional cortex at 1 d, 2 wk, 3 months, 6 months and 1 y post-TBI. Principal component analysis (PCA) evaluated the similarity between chemoconvulsants and post-TBI transcriptomic profiles and visualized sources of variation across datasets. DE genes from each chemoconvulsant and TBI time point were compared to identify similarly regulated genes. Reactome pathway analysis identified enriched pathways among the shared genes. PCA revealed bicuculline, donepezil, and chlorpromazine were closest to post-TBI time points. Number of chemoconvulsant-induced DE genes (padj<0.05) ranged from 2659 (amoxapine) to 21 (pilocarpine). TBI-induced DE genes (pval<0.05) declined, from 1125 at 1-d to 212 at 365-d. Five chemoconvulsants (amoxapine, SNC80, bicuculline, pentylenetetrazol, chlorpromazine, donepezil) showed >20 similarly regulated DE genes with TBI at 1-d and 14-d, with amoxapine showing this also at 90-d time point. Shared genes between amoxapine and TBI were enriched in pathways such as peptide ligand binding, GPCR signaling, and Class A/1 Rhodopsin-like receptors. Bicuculline overlap revealed similar pathways, along with fibrin clot dissolution. In contrast, chlorpromazine-associated genes were linked to integrin signaling and extracellular matrix organization. Our data demonstrates the potential of modeling post-TBI transcriptomic changes in vitro by chemoconvulsants.

Vivek Sanjay Belapurkar, Postdoc
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Activity-Dependent Protein Damage in Neuronal Synapses

 # 34

As the primary contacts for information transfer and storage, synapses between neurons relay information at high frequencies. This high activity depends on active local energy metabolism, which creates a hostile oxidative environment for local proteins at the synapses.

Currently, we do not know which synaptic proteins are susceptible to oxidative damage, especially during synaptic activity. To address this question, we are developing a chemogenetic tool to induce oxidative stress in a synapse-specific manner with temporal control. To visualize the oxidized synaptic proteins *in situ*, we tagged oxidized cysteine residues, a major cellular biomarker for protein oxidative modifications, for immunofluorescence microscopy. By integrating these molecular tools, synaptic activity manipulations, and synaptosome redox proteomics, we aim to elucidate the protein targets of oxidative damage at synapses and their dependence on synaptic activity.

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Isoform imbalance of autism risk gene SORCS2 disrupts cerebellar circuitry and behaviour in mice

 # 35

Pia Boxy[1,2], Sarah Desmettre[1,2], Dragos Niculescu[1,2], Lilian Kisiswa[1,2*] and Anders Nykjær[1,2*]

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**Shared corresponding authorship*

Autism spectrum disorder (ASD) is a neurodevelopmental condition that affects approximately 1 in 100 children worldwide. It is characterized by a myriad of social, cognitive and motor impairments that contribute to a reduced quality of life. While ASD is a complex disorder caused by genetic, pre- and perinatal, as well as environmental factors, it is often characterized by loss of cerebellar Purkinje cells (PCs) and decreased PC excitability. Increasing evidence highlights the cerebellum as a key hub in neurodevelopmental disorders, contributing not only to motor function but also to cognition and emotion. Its extended postnatal development renders it especially vulnerable to disruption but also presents a promising window for therapeutic intervention. We focus on **autism risk gene SorCS2**, a member of the sortilin receptor family and neurotrophic co-receptor, that is abundantly expressed within the cerebellar cortex. The receptor undergoes proteolytic cleavage resulting in a single-chain receptor which can be further processed into a two-chain isoform, each with distinct expression patterns and biological functions. By utilizing a mutagenic mouse model that solely expresses the single-chain isoform, we show that the cellular morphology, synaptic composition and excitability of Purkinje cells is impaired. Additionally, receptor isoform imbalance contributes to ASD-related behavioural changes. Our findings suggest that proper spatiotemporal regulation of the SorCS2 isoforms is critical for cerebellar circuit development and cognitive function, highlighting its relevance to ASD pathophysiology.

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Cross-Hemispheric Amygdala Synchrony: A Key to Memory and Aging Dynamics?

 # 36

Memory shapes our behavior and aids adaptation which is crucial for mental and physical health. Dysfunctional memory is linked to neuropsychiatric and neurodegenerative disorders. The amygdala's extensive neural connections regulate emotional states in cognitive function. It plays a key role in episodic memory, particularly via strengthened synapses during associative learning. Recent studies suggested that cross-hemispheric amygdala connectivity, found to be weaker in dementia, may influence memory formation. However, the underlying mechanisms are unknown.

Learning and memory rely on experience-driven changes in brain connections, with stronger synaptic connections leading to longer-lasting memories. These procedures also involve epigenetic chromatin and transcriptional changes in the nucleus. However, little is known about how structural synapse formation and gene regulation in the nucleus are orchestrated in memory formation.

In this project, we address these issues through the synergistic approaches of molecular genomics and synaptic physiology. We aim to unravel the mechanism of cross-hemisp heric amygdala in episodic memory formation and putative dysfunction in aging. Specifically, we will test the following hypotheses: 1) Cross-hemispheric synchronous amygdala activity is essential and enhanced by episodic memory, 2) The synchrony is cholinergically mediated and reduced with age, 3) Reversing structural synaptic plasticity desynchronizes the amygdalae, erasing memory, 4) Epigenetic and transcriptional changes correlate with synchrony strength, and 5) Gene regulation and synaptic plasticity are orchestrated by specific gene modules. The investigation of the hypotheses will advance our understanding of memory function at the levels of neural circuits, synaptic structure, and gene regulation. This will provide a preliminary platform for developing targeted therapies that aim at enhancing synchronous cross-hemispheric amygdala activity in dementia.

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Neuroimmune Memory: Mapping and Manipulating Inflammation in the Brain-Body Axis

 # 37

The brain constantly monitors the internal state of the body to ensure it functions within expected parameters. Among those internal signals, inflammation is an essential defence mechanism to protect us against tissue damage and infection. Recent work suggests the existence of immune memories in the brain that can remember the inflammatory state of organs even once the inflammation is gone. However, it is difficult, in most animal models, to image the subcortical brain regions through which the body signals are processed. Access to the internal processes of the body for causal manipulation is also limited. These obstacles have limited our understanding on how the brain can encode and control inflammation.
In our studies, we will use a transparent fish model to image the activity of the brain at cellular resolution to study how inflammation is represented. Which neurons are involved, and where in the brain? We will also be able to test if a topographic map of inflammatory processes in the body exists in the brain. Finally, we will manipulate the neurons we identify to manipulate inflammation, either to trigger inflammation without any infection or to lower the inflammation in the presence of an inflammatory stimulus. We expect to prove if neurons can indeed encode and recall inflammation, and whether a body map of inflammation exists in the brain.
Understanding these immune memories would open the way to new ways to manage inflammatory disease, especially chronic inflammation.

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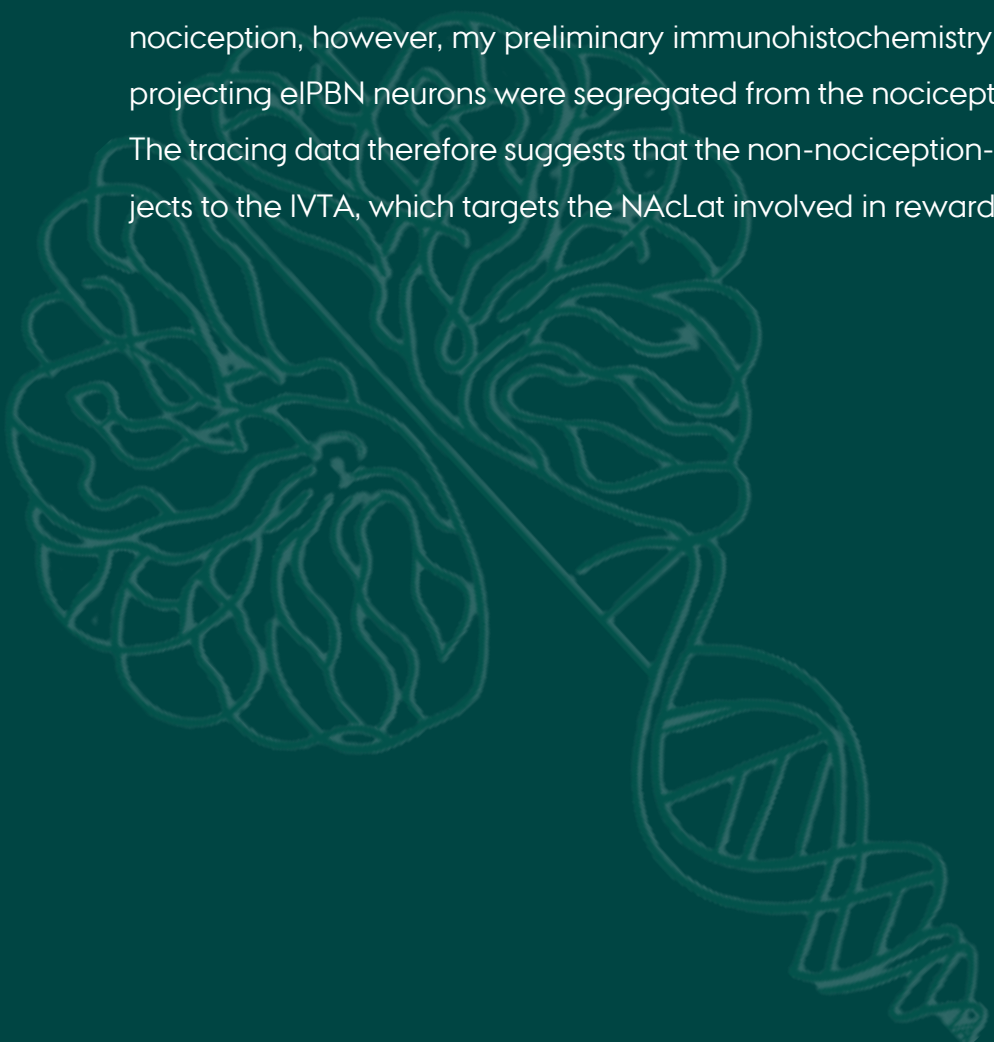
Investigating the circuit of affective touch and reward

 # 38

Early life stress has been shown to affect many functions of the adult brain, including the reward system. How neglect and the absence of affective touch may play a role in critical periods of neurodevelopment, however, remains obscured. In this project, I aim to elucidate what role affective touch plays in maturation of the developing reward system in mice. The reward system promotes social behaviour through reward-learning and reinforcement by the release of dopamine (DA) into the nucleus accumbens (NAc) from the ventral tegmental area (VTA), however, how touch generates reward during social interactions is unknown. It has recently been demonstrated that stimulation of specific sensory neurons responding to gentle, warm touch can evoke DA release in the NAc via the lateral parabrachial nucleus (lPBN) in the brainstem. To investigate this circuit furtherly, pseudorabies virus was injected into either the lateral NAc (NAcLat) or the lateral VTA (lVTA) to retrogradely trace the putative pathway from the lPBN. Interestingly, the data suggested a regionalized projection to the lVTA from the external lPBN (elPBN). Notably, the elPBN has mainly been associated with nociception, however, my preliminary immunohistochemistry data indicated that the lVTA-projecting elPBN neurons were segregated from the nociception-related elPBN population. The tracing data therefore suggests that the non-nociception-related elPBN population projects to the lVTA, which targets the NAcLat involved in reward signal processing.



Hadrien Glibert, PhD student
UCLouvain

HOXA5 importance in the precerebellar system: behavioral impact of postnatal inactivation and cerebellar projections mapping

 # 39

HOX transcription factors act as key regulators of embryonic patterning and development. HOXA5 is no exception to the rule and is involved in developmental programs across the embryo. Focusing on the central nervous system, HOXA5 is notably required in phrenic motoneurons for the proper innervation of the diaphragm and subsequent respiratory function, as well as in neurons from the lower rhombic lip for orderly migration towards their final location. *Hoxa5* expression is retained in the hindbrain after birth, mainly in subsets of neurons within the precerebellar nuclei of the pons and medulla oblongata. However, few data are available regarding its postnatal functions, at a stage corresponding to the establishment of the precerebellar projections onto the cerebellum. Based on a transcriptomic analysis of mouse brainstem following postnatal *Hoxa5* loss-of-function, we hypothesized that HOXA5 act as a regulator of synapse formation, maturation and plasticity in the precerebellar system, and as such might play a role in cerebellum-associated phenotypes or pathologies.

To investigate this hypothesis, we used the Tamoxifen inducible Cre/LoxP system to inactivate *Hoxa5* at birth in mice. Behaviors of these animals were then evaluated through a wide range of tests, from motor learning to social impairment, with the aim of assessing cerebellar functions and autism spectrum disorder-related phenotypes. Indeed, several target genes identified in the transcriptomic analysis were directly associated with autistic behavior in mice and/or humans. While motor behaviors appear unaffected by the loss of HOXA5, our results suggest a potential impact of *Hoxa5* postnatal inactivation on social and repetitive behaviors.

As an additional tool towards a better understanding of the involvement of HOXA5 in the precerebellar circuit, we next generated a *Hoxa5-tdTomato* reporter mouse line, effectively labelling *Hoxa5*-expressing (Hoxa5+) neurons. Mapping of *Hoxa5+* projections across the cerebellum in regard of the functional topography of the cerebellum described in the literature will allow to progress in understanding the link between HOXA5 and cerebellar functions and the impact of its deregulation in relation to cerebellar pathologies.

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Sertm2 is a Conserved Micropeptide that Promotes GDNF-mediated Motor Neuron Subtype Specification

 # 40

Small open reading frame-encoded micropeptides within long non-coding RNAs (lncRNAs) are often overlooked due to their small size and low abundance. However, emerging evidence links these micropeptides to various biological pathways, though their roles in neural development and neurodegeneration remain unclear. Here, we investigate the function of murine micropeptide Sertm2, encoded by the lncRNA *A730046J19Rik*, during spinal motor neuron (MN) development. Sertm2 is predicted to be a conserved transmembrane protein found in both mouse and human, with subcellular analysis revealing that it is enriched in the cytoplasm and neurites. By generating C terminally Flag-tagged Sertm2 and expressing it from the *A730046J19Rik* locus, we demonstrate that the Sertm2 micropeptide localizes in spinal MNs in mice. The GDNF signaling-induced Etv4+ motor pool is impaired in *Sertm2* knockout mice, which display motor nerve arborization defects that culminate in impaired motor coordination and muscle weakness. Similarly, human *SERTM2* knockout iPSC-derived MNs also display reduced ETV4+ motor pools, highlighting that Sertm2 is a novel, evolutionarily-conserved micropeptide essential for maintaining GDNF-induced MN subtype identity.

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DREADD inhibition of monocytes causes acute heart failure in mice – A model for Pulseless Electrical Activity?

 # 41

In recent years, the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in neuroscience has become a household strategy for manipulating specific cell populations, such as neurons or immune cells like microglia. Here, we used the fractalkine receptor, Cx3cr1, commonly used to drive expression in microglia, as a promoter for the inhibitory Gi-DREADD. This approach revealed an unexpected phenotype.

To our surprise, we found a single i.p. injection of CNO to be fatal within 5-30 min. in Cx3cr1Cre-hM4Gi mice, while healthy controls remain unaffected. 'Injection stress' from i.p. saline administration did not cause fatality.

IBA1+ fluorescent immunohistochemistry showed that the average soma size of IBA1-expressing microglia was decreased in hypothalamic regions of the Cx3cr1Cre-hM4Gi mice compared to CNO-injected controls. We are currently exploring heart IBA1+ macrophages. O-link analysis of cytokines in blood plasma from Cx3cr1Cre-hM4Gi mice revealed increased levels of anti-inflammatory cytokines and decreased pro-inflammatory cytokines. These results support monocyte inhibition.

Ultrasound measurements revealed decreased cardiac output and ejection fraction in cx3cr1-hM4Gi mice compared to baseline (before Gi activation), suggesting heart failure to be the cause of death. However, baseline ejection fraction was also found to be lower compared to controls, implying the presence of constitutive activity of the hM4Gi receptor in Cx3cr1Cre-mice. Interestingly, most hM4Gi-Cx3cr1Cre had stable heart EKGs despite loss of pump-activity, indicative of decoupling between electrical activity and heart contraction, corresponding to the clinical phenomena Pulseless Electrical Activity (PEA).

These results highlight an essential role of monocytes in heart failure caused by PEA. Moreover, they warrant caution when utilizing the *Cx3cr1* promoter to drive systemic expression of the hM4Di DREADD for exploring innate immunity.

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Expression and function of circRNAs in neural development and disease

 # 42

Circular RNAs (circRNAs) are key regulators of cellular processes and are particularly abundant in the nervous system. Some circRNAs are implicated in neural differentiation, but their precise roles in brain development remain unclear. To address this, we investigated alternatively spliced circRNAs in developing mouse and porcine brains during fetal development.

Due to overlap with linear transcripts, identifying internal exons in multi-exon circRNAs is challenging. To resolve this, we performed long-read nanopore sequencing of both polyadenylated RNAs and circRNAs from fetal tissues. We found circRNAs exhibit more complex splicing than their linear counterparts, including novel exons and intron retention events that change dynamically during development.

To spatially map circRNA splicing, we used spatial transcriptomics (2D CosMX and 3D Stellaomics) on mouse brain sections with single back-splice junction probes. Probe specificity was confirmed using a circRNA knockout mouse. This revealed distinct, cell-type-specific circRNA expression and subcellular localization. We propose that a comprehensive map of circRNA isoforms and their cytoplasmic-nuclear distribution will illuminate their roles in brain development.

To further examine circRNA function, we differentiated human embryonic stem cells into neural progenitor cells (NPCs). CircRNA levels rose during differentiation, including ciRS-7, circRMST, and circFAT3. Depleting circFAT3 altered expression of genes linked to insulin resistance—a pathway relevant to neurodevelopment. Single-cell RNA-seq of circFAT3-deficient cerebral organoids (30 and 90 days) showed loss of telencephalic radial glia and mature cortical neurons. Non-telencephalic NPCs displayed altered expression of genes like FAT4, ERBB4, UNC5C, and DCC. In vivo circFat3 knockdown by lentiviral in uteru electroploratioin in fetal mouse prefrontal cortex disrupted neocortical cell positioning.

These results suggest circFAT3 has a conserved, functional role in neural development, especially in anterior brain formation, neuronal differentiation, and migration.

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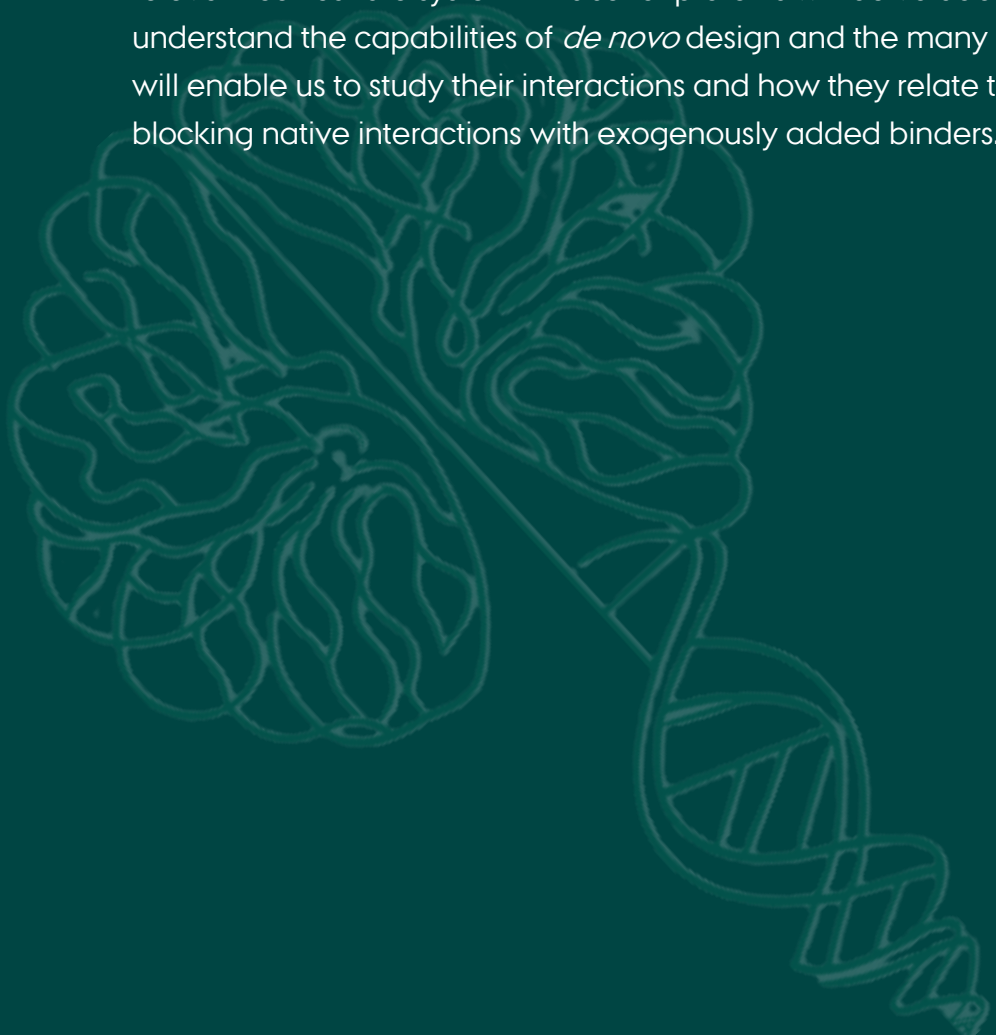
Using *de novo* protein design to study neuronal receptor interactions

 # 43

Neurotrophins and their receptors are crucial to nervous system development and in maintaining its function and plasticity. One group of neurotrophin receptors is the Vacuolar Protein Sorting 10 Protein (VPS10P) related receptors. The mammalian protein family comprises Sortilin, SorLA and SorCS1-3. The SorCS receptors have 40-60% sequence identity between themselves and though only SorCS2 has a published, high-resolution structure they are expected to adopt very similar folds. These receptors act as coreceptors to the primary neurotrophin and proneurotrophin receptors and participate in their trafficking and signalling. Genetic variants found in the genes of these receptors have been linked to several neuropsychiatric disorders among others autism spectrum disorder and Alzheimer's disease. In this work we design novel protein binders of these receptors using artificial intelligence tools for *de novo* protein design including RFdiffusion, ProteinMPNN, AlphaFold and BindCraft. The aim is to develop a set of orthogonal binders to VPS10P receptors that bind with high affinity both in vitro and in a more physiologically relevant cell culture system. This set of proteins will be valuable will help us both understand the capabilities of *de novo* design and the many functions of these receptors. It will enable us to study their interactions and how they relate to downstream signalling by blocking native interactions with exogenously added binders.



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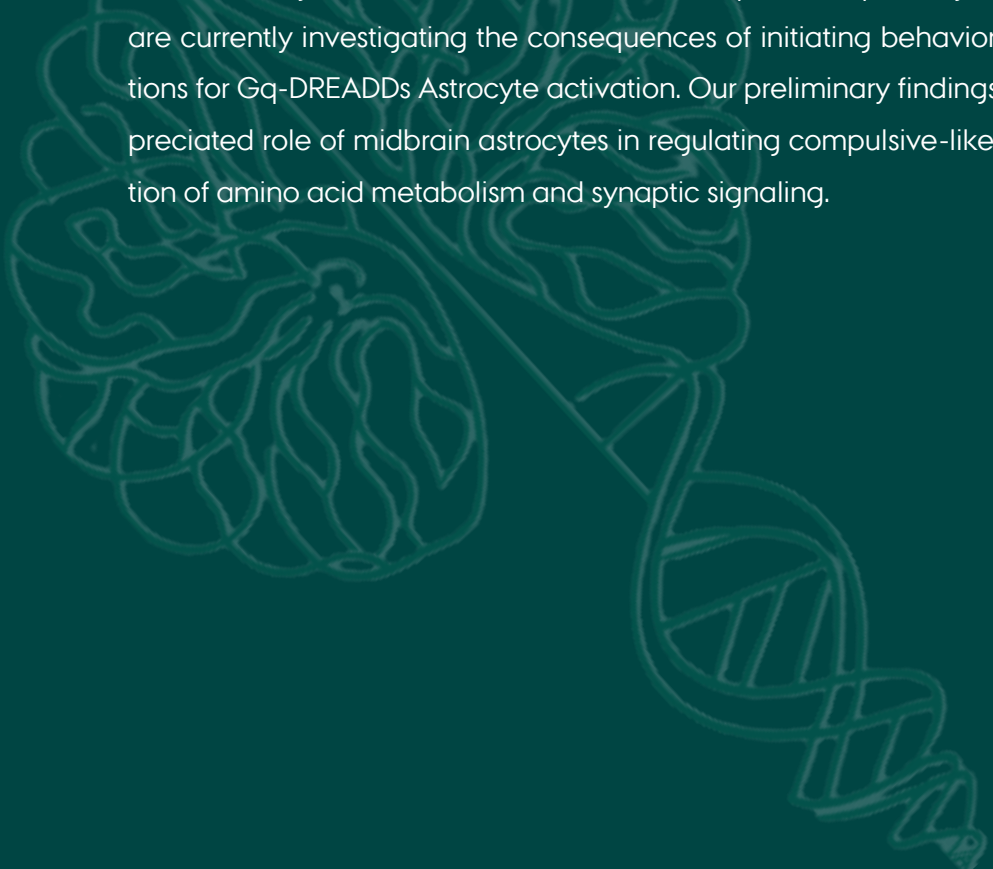
Midbrain astrocyte activation reduces taurine and serine levels and leads to decreased compulsive behavior

 # 44

Rasmussen FH., Frandsen SL., Chiper T., Pfannenmueller E., Thomsen B, Nielsen SW., Aldana BI., Klawonn AM

Astrocytes are increasingly recognized as active regulators of neuronal activity, synaptic function, and behavior. Here, we investigated their role in the midbrain - a key region involved in motor control and reward processing. Using GFAPCre transgenic mice, we selectively expressed Gq-coupled Designer Receptors Exclusively Activated by Designer Drugs (hM3Dq-DREADDs) in midbrain astrocytes via Cre-dependent AAV delivery. Chemogenetic activation of astrocytes was followed by behavioral and biochemical analyses. 45 minutes after CNO-injection for astrocyte activation, repetitive behaviors, such as digging and grooming, and increased locomotor activity in an open field arena were significantly reduced. These changes occurred without impairments in motor coordination, as assessed by the rotarod and pole tests, and did not affect anxiety-like or hedonic behaviors. High-performance liquid chromatography (HPLC) revealed decreased levels of serine and taurine in the midbrain following astrocyte activation, while glutamate, glutamine, and GABA remained unchanged. In line with this, we observed significant differences in c-fos expression of DREADDs-astrocyte-neighboring neurons, 60-90 minutes after CNO injection - indicating that behavioral effects are due to astrocyte-neuron communication. To explore temporal dynamics of Astrocyte activation, we are currently investigating the consequences of initiating behavioral tests 10 min after CNO injections for Gq-DREADDs Astrocyte activation. Our preliminary findings highlight a previously underappreciated role of midbrain astrocytes in regulating compulsive-like behaviors, possibly via modulation of amino acid metabolism and synaptic signaling.



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Candidate pathways and biomarkers in OGT-CDG - a novel intellectual disability syndrome

 # 45

O-GlcNAc Transferase Congenital Disorder of Glycosylation (OGT-CDG) is a recently discovered intellectual disability (ID) syndrome caused by mutations in the OGT gene. OGT catalyses O-GlcNAcylation, a dynamic post-translational modification affecting thousands of proteins. OGT-CDG patients present with extensive phenotypic heterogeneity, including ID, developmental delay, hypotonia, and dysmorphic features. Despite some overlap, the clinical presentation is highly variable, complicating diagnosis and impeding identification of underlying mechanisms.

To address this, we enrolled a large cohort of OGT-CDG patients and family members to systematically map OGT-CDG heterogeneity. Standardised clinical data collection and genetic screening of OGT variants were used to cluster patients based on shared and distinct symptom profiles. Blood samples from patients, healthy family members, and OGT-CDG mouse models carrying patient mutations were analysed for biomarker discovery and validation.

Preliminary data from three OGT-CDG mouse models showed upregulated Ogt and downregulated Oga mRNA levels compared to wild type mice in whole brain, leukocytes, and whole blood, respectively, suggesting that altered O-GlcNAc homeostasis may contribute to disease pathophysiology.

By integrating clinical, genetic, and biomarker data, this study aims to comprehensively characterise OGT-CDG. Defining clinical profiles and biological signatures will offer insights into disease mechanisms and guide improved diagnostics and therapies.

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Sortilin in Excitatory and Inhibitory Neurons

 # 46

A proper balance between excitatory and inhibitory neuronal activity is essential for information processing and emotional regulation. When this balance is disrupted, circuit hyperactivity or instability may occur, potentially leading to psychiatric conditions, including mood disorders. We have found that mice lacking sortilin - a member of the VPS10P domain receptor family - display manic-like traits, whereas overexpression is associated with anxiety and depression. Accordingly, bipolar patients show reduced sortilin expression, while individuals suffering from anxiety and major depression exhibit increased levels of sortilin (manuscript in preparation).

Here, we investigated whether sortilin influences the function and balance between excitatory and inhibitory neuronal activity. We generated mice with conditional deletion of sortilin in glutamatergic and GABAergic neurons, respectively, and found that receptor inactivation in these two neuronal subpopulations affects neuronal activity, network dynamics, proteomic synaptic profiling, and animal behavior in distinct ways. Collectively, our results show a contributing role of sortilin in balancing excitatory and inhibitory neurotransmission.

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Characterizing lateral septum neurocircuits

 # 47

The lateral septum (LS) is an affective hub that receives input from various limbic structures, including the hippocampus (HPC), hypothalamus, and amygdala. The LS is thought to modulate affective state and reward processing through its connections to the limbic system and mesolimbic regions. This study identified brain regions projecting to the LS by retrogradely transported adeno-associated virus expressing a green fluorescent protein in mice. Retrograde tracing to the LS confirmed that the primary afferent inputs are predominantly affective in nature.

Furthermore, we found novel inputs to the LS, such as the interpeduncular nucleus. To quantify the retrograde tracing results, a bioinformatic pipeline incorporating ABBA, CellPose and QuPath was developed for automatic cell detection and efficient data flow. Furthermore, retrograde tracing of an enkephalinergic LS population, using a pseudotyped rabies virus, revealed inputs from the Hippocampus and hypothalamus, as well as a local population of LS interneurons. In conclusion, this study supports the hypothesis that the LS is an affective brain region involved in modulating mood and affective state.

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Molecular genetics toolset for brain microcirculation imaging (and beyond)

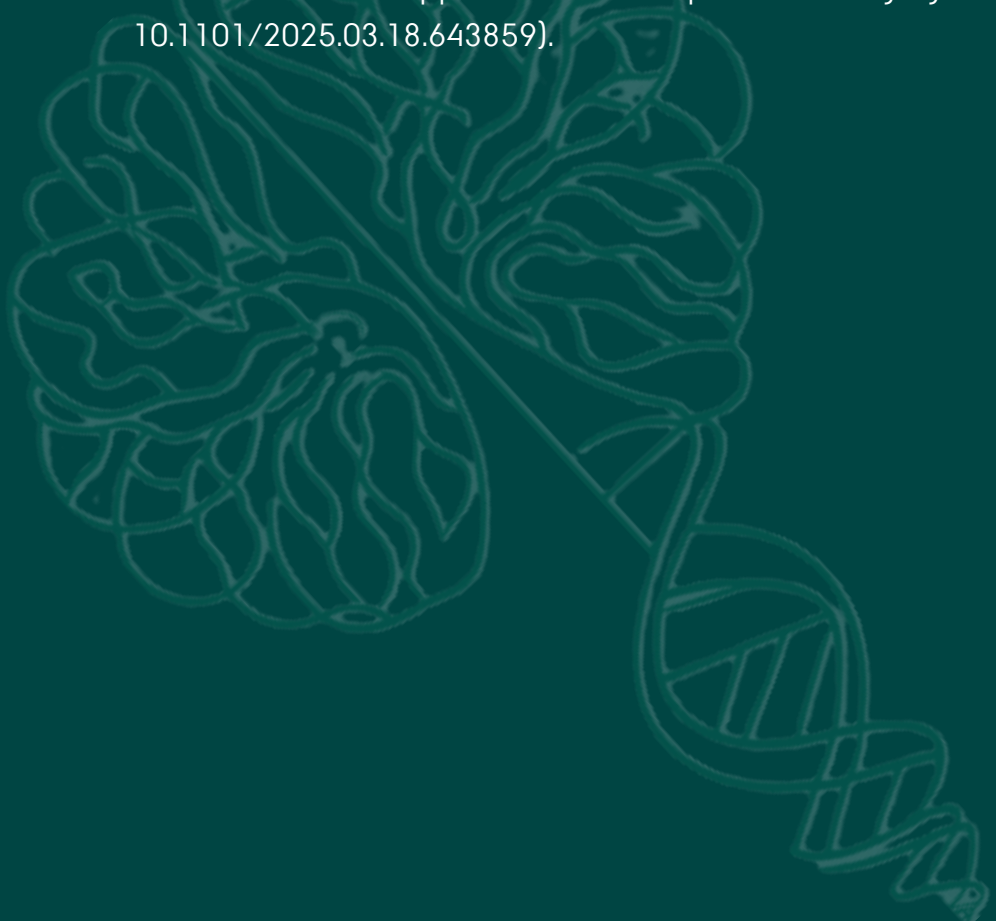
 # 48

Studying blood microcirculation is crucial for understanding vascular diseases. Blood flow is currently imaged following invasive administration of fluorescent dyes that attenuate within one hour. We developed three new molecular genetic approaches for longitudinal vasculature study.

Liver-secreted albumin is the most abundant protein in plasma and cerebrospinal fluid. We created liver-targeting AAVs expressing fluorescent protein-tagged albumin to visualize blood plasma in mice after a single systemic injection (DOI: 0.1016/j.crmeth.2022.100302). While effective in adults, AAV genome dilution in the growing liver limits use in neonates.

To overcome this, we established a virally induced CRISPR/Cas9 knock-in of fluorescent albumin (DOI: 10.1007/978-1-0716-4011-1_6). An AAV carrying ~1 kb homologous arms around Alb exon 14 enabled expression of Alb-mNeonGreen after postnatal day 3 injection, with stable expression for at least three months.

As an alternative, we generated a mouse with mScarlet knocked into Alb exon 14, including a SpyTag, which is utilized for biosensor attachment or blood–brain barrier assessment. These approaches enable powerful imaging of murine vasculature (DOI: 10.1101/2025.03.18.643859).



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Uncovering Early α-Synuclein Oligomerization at Nerve Terminals in Parkinson's Disease

 # 49

Authors: Melisa Faydaver, Mia Rosenkjaer Antorini , Silvia Turchetto, Poul Henning Jensen, Chao Sun

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the accumulation of toxic α-synuclein aggregates, which lead to synaptic dysfunction and neuronal loss. While much is known about late-stage pathology, the early molecular events driving disease onset remain poorly understood. Increasing evidence suggests that α-synuclein oligomers—small, non-fibrillar assemblies—may be the primary toxic species initiating synaptic failure before clinical symptoms appear. However, their precise subcellular origin and toxicity in neurons are still unclear. To address this gap, I am investigating the hypothesis that α-synuclein oligomerisation begins at the nerve terminal and plays a central role in disrupting synaptic function. To test this hypothesis, I will induce endogenous α-synuclein aggregation in rodent primary neurons and organotypic brain slices using engineered pre-formed fibrils and localise emerging α-synuclein oligomers using super-resolution microscopy. My study will provide insight into the earliest stages of α-synuclein pathology.

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Uncovering genetic risk factors and cell-type specific contributions in neurodevelopmental disorders

 # 50

Neurodevelopmental disorders (NDD), such as autism spectrum disorder, are often influenced by multiple genetic factors with small, cumulative effects. Hence, traditional single-marker approaches are not sufficient to investigate the origin, effect, and mechanism of such complex disorders. Genome-wide association studies (GWAS) have reported thousands of statistically-significant associations between single-nucleotide variants (SNVs) and phenotypes linked to complex diseases. However, the molecular foundation, the associated cell types or states through which genes confer disease risk as well as the underlying regulatory mechanisms, are often unknown.

Aiming to translate associations into functional mechanisms and to uncover genetic risk factors and cell-type specific contributions in NDDs, we integrate population-level genetic associations revealed by GWAS with the molecular precision offered by single-cell multi-omics data. We obtained human snRNA sequencing data from 203 unique biological samples and generated a cortex developmental atlas spanning the first trimester to adolescence, covering key events such as neurogenesis, neuronal migration, gliogenesis, synaptogenesis, and myelination. Furthermore, we created an NDD atlas containing brain samples of healthy donors and patients with diverse NDDs as well as scATAC data atlas of the developing brain to determine NDD-specific transcriptional profiles and regulatory mechanisms.

By integration of genetic risk factors, which can be both genes or regulatory elements which we identified by gene-set analyses of GWAS data, with our developmental atlas, we aim to pinpoint the specific trait-associated cell types, unravel disease mechanism, regulatory networks, and finally the relevant genes. This approach will help us to obtain a holistic understanding of NDD development and aid in creating novel precision medicine approaches.