



Copenhagen, March 2021

Dear participant,

A warm welcome to The Brain Prize Meeting– Neurodevelopmental disorders- mechanisms and pathways to treatment.

The Lundbeck Foundation Brain Prize meetings aim to bring senior and early career investigators together to discuss progress and challenges in the field of the current year's Brain Prize winners.

The Brain Prize in 2020 was awarded to Professors Huda Y. Zoghbi and Adrian Bird for their fundamental and pioneering work on Rett syndrome, a rare neurodevelopmental disorder that primarily affects girls during their early childhood. The work of the two prize winners established the importance of MeCP2 and epigenetic regulation in both brain development and the maintenance of normal adult brain function. Their work has also challenged the idea that neurological developmental disorders are irreversible and has pointed to novel opportunities for treatment of Rett syndrome and other neurodevelopmental disorders.

With international keynote speakers, including the 2020 Brain Prize winners, this year's Brain Prize meeting focusses on key topics within the field of neurodevelopmental disorder research. The meeting will include sessions on epigenetics, regulation of gene expression, genomics, neuronal and circuit dysfunction, diagnosis, and pathways to treatment of neurodevelopmental disorders.

At the time of writing, we are still in the midst of a Covid-19 pandemic. This year, The Brain Prize meeting is therefore a virtual one. While virtual meetings will never replace or replicate the atmosphere of an in-person meeting, we felt that a significant upside of virtual meetings - their accessibility, is something we wanted to capitalize on. We therefore made the meeting free to attend and are looking forward to welcoming nearly 800 registrants from 55 countries around the globe.

We sincerely hope you enjoy the meeting and congratulations to The Brain Prize winners 2020, Huda and Adrian.

With our best wishes,

Lundbeck Foundation and the scientific organizers.

**Anne-Marie Bisgaard Pedersen
Rigshospitalet**

**Anders Børghlum
Aarhus University**

**Gitte Moos Knudsen
University of Copenhagen**

**Konstantin Khodosevich
BRIC, University of Copenhagen**

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Practical details

To enter the meeting please use the following links:

Monday, 1st of March: https://stream.dvc.dk/Brain-Prize_01/

Tuesday, 2nd of March: https://stream.dvc.dk/Brain-Prize_02/

Wednesday, 3rd of March: https://stream.dvc.dk/Brain-Prize_03/

Thursday, 4th of March: https://stream.dvc.dk/Brain-Prize_04/

***Please note that the meeting starts every day at 15:00 CET – except for Thursday, where we start the meeting at 14:00 CET.**

How to ask questions during the sessions.

In the chat window on the right side of the screen, you can post questions for the speakers.

The first time you want to ask a question, you will be asked to fill in your name (or an alias).

Your question is sent to the moderator, who will accept or decline the question.

Once a question has been accepted, it is visible in the chat window to all other attendees at the meeting.

Participants can choose to give a question a thumb up  to indicate that they like the question.

The moderator will choose the questions for the Q&A session.

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Session 1/Monday - March 1, 2021:

Epigenetics and regulation of gene expression in neurodevelopmental disorders.

15:00 – 15:05	Welcome – Martin Meyer, the Lundbeck Foundation
15:05 – 15:10	Intro by session chair, Carmen Sandi, EPFL, Switzerland
15:10 – 15:45	Adrian Bird, Wellcome Centre for Cell Biology, University of Edinburgh, UK - The genetics and epigenetics of Rett Syndrome.
15:45 – 16:20	Elisabeth Binder, Max Planck Institute of Psychiatry, Germany - Prenatal stress - implications for risk trajectories in psychiatry.
16:20 – 16:35	<p>FLASH TALKS</p> <p>Anna Starnawska, Aarhus University, Denmark: Integrative genomic and epigenomic analysis of autism spectrum disorder.</p> <p>Leandros Boukas, Johns Hopkins University, USA: Leveraging the Mendelian Disorders of the Epigenetic Machinery to Systematically Map Functional Epigenetic Variation.</p> <p>Mandy Meijer, Radboud University Medical Center, The Netherlands: Cell type-specific DNA methylation is associated with childhood Attention-Deficit/Hyperactivity Disorder symptoms.</p>
16:35 – 16:40	Q&A for all three flash talks
16:40 – 17:15	Jonathan Mill, University of Exeter, UK - (Epi)genomic trajectories to neuropsychiatric disease.
17:15 – 17:45	Moderated panel discussion with all speakers from the session Epigenetics and regulation of gene expression in neurodevelopmental disorders: Where do we want to be in 10 years' time and how do we get there?

All times are in CET.



Adrian Bird

Wellcome Centre for Cell Biology, University of Edinburgh, UK

The Genetics and Epigenetics of Rett syndrome.

Cytosine residues in DNA can be modified post-synthetically and this affects local protein-DNA interactions. For example, the MeCP2 protein specifically binds to methylated sites in the genome, potentially allowing it to interpret this “epigenetic” mark. Several clinical disorders are caused by mutations in the *MECP2* gene, including the profound neurological disorder Rett syndrome. The equivalent phenotype in animal models can be reversed, suggesting that the protein fine-tunes neuronal function and that the human disorder may also be curable. Evidence will be presented that the root cause of Rett syndrome is failure of the primary function of MeCP2, which is to restrain gene expression in a DNA methylation-dependent manner. In addition to symmetrically methylated CG sites, MeCP2 targets non-CG methylation, which is unusually abundant in neurons. Genetic experiments that evaluate the relative importance of these two modes of binding will be presented.



Elisabeth Binder

Max Planck Institute of Psychiatry, Germany

Prenatal stress - implications for risk trajectories in psychiatry.

Early adverse exposures, including maternal stress during pregnancy, have been shown to result in long-lasting consequences on neural circuit function and stress hormone regulation and ultimately in an increased risk for psychiatric but also medical disorders later in life. This presentation will focus on increased exposure to stress hormones, i.e glucocorticoids (GCs) in utero as one mechanism mediating such increases in risk.

The presentation will first highlight data from a human hippocampal cell line that identify long lasting changes in DNA methylation in response to GCs that increased transcriptional sensitivity to future stress exposure, suggesting that prenatal GC exposure could prime the transcriptional response to subsequent stress exposure. Data from human brain organoids and single cell sequencing will then delineate that specific nervous system cell subtypes show differential sensitivity to early GC exposure during brain development. Specifically, GC-responsive transcripts in neurons are significantly enriched among genes with associations with behavioral traits and psychiatric diseases in large GWAS or carrying rare variants found in neurodevelopmental disorders. The GC-responsive transcription factor ZBTB16 will be presented as a downstream candidate potentially mediating GC-induced changes in neural differentiation. Overall, the presentation will outline how in utero stress-exposure can have lasting effects on cell and tissue function and how this relates to risk or resilience to stress-related disorders in the context of common genetic variation.

Anna Starnawska

Aarhus University, Denmark

Integrative genomic and epigenomic analysis of autism spectrum disorder.

Starnawska A.^{1,2,3}, Hansen C. S.^{1,4}, Demontis D.^{1,2,3}, Grove J.^{1,2,3}, Als T.^{1,2,3}, Mors O.^{2,5}, Børglum A. D.^{1,2,3}, Staunstrup N. H.^{1,2,5}

¹Department of Biomedicine, Aarhus University, Denmark

²The Lundbeck Foundation Initiative for Integrative Psychiatric Research, iPSYCH, Denmark

³Center for Genomics and Personalized Medicine, CGPM, and Center for Integrative Sequencing, iSEQ, Aarhus, Denmark

⁴Department of Psychiatry, Icahn School of Medicine at Mount Sinai, United States

⁵Psychosis Research Unit, Aarhus University Hospital, Aarhus, Denmark

Background: Autism Spectrum Disorder (ASD) is a group of complex neurodevelopmental disorders characterized by a pervasive impairment of social and communication skills along with repetitive and restrictive behaviors. ASD risk is influenced by a combination of genetic and environmental factors. Both genetic polymorphism and environmental exposures can alter DNA methylation (DNAm), which plays a pivotal role in neuronal development, proper brain functioning, and thereby mental health.

Aim: Identify DNAm signatures of ASD at the time of birth and specifically investigate the impact of common genetic ASD risk variants on the newborn epigenome.

Methods: DNAm levels were quantified with Infinium EPIC array >850,000 loci in the human genome in 2,065 neonatal dried blood spots from the Danish iPSYCH 2012 cohort.

Genotyping data were available from Infinium PsychChip v1.0 array. Three different statistical methodologies were applied: 1) epigenome-wide association study (EWAS) of ASD to identify loci with differential DNAm between 843 cases and 688 controls; 2) EWAS of ASD polygenic risk score (PRS) to identify DNAm changes associated with polygenic burden of the disorder; and 3) methylation Quantitative Trait Loci (mQTL) analysis for common variants located within ASD risk loci to determine if and where in the human genome they impact epigenetic regulation.

Results: ASD EWAS identified 9 differentially methylated positions and 34 differentially methylated regions mapping to genes crucial for neuron development, neuron differentiation, and synapse formation e.g. *MYT1L*, *MAD1L1*, *SHANK2* genes. EWAS of ASD PRS replicated previous findings where differential DNAm at birth was associated with elevated polygenic burden for the disorder and identified additional differentially methylated regions associated with this phenotype. mQTL analyses confirmed that ASD risk variants impact DNAm levels and identified new loci associated with the disorder, not indicated by the ASD GWAS itself.

Conclusions: We identified differential DNAm at birth to be associated with ASD diagnosis later in life. Moreover, by combining epigenomic and genomic data we identified differential DNAm at birth to be associated with ASD polygenic burden and provided evidence that common ASD risk variants impact neonatal epigenetic landscape, which may mediate part of the genetic risk of this neurodevelopmental disorder.

Leandros Boukas

Johns Hopkins University, USA

Leveraging the Mendelian Disorders of the Epigenetic Machinery to Systematically Map - Functional Epigenetic Variation.

L.Boukas^{1,2,*}, T.R. Luperchio^{1,*}, K.D.Hansen^{1,2} and H.T. Bjornsson^{1,3,4}

1 Department of Genetic Medicine, Johns Hopkins School of Medicine

2 Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health

3 Department of Pediatrics, Johns Hopkins School of Medicine

4 Faculty of Medicine, University of Iceland

***** Equal first-author contribution

A long-standing problem in epigenetics is the identification of specific changes that causally mediate disease phenotypes through the alteration of transcriptional states. While it is now clear that epigenetic changes can perturb normal central nervous system development and cause neurodevelopmental disease, it is challenging to pinpoint the exact disease-driving changes among a potentially large number of passenger changes and noise.

We propose an approach to address this issue, by leveraging the Mendelian Disorders of the Epigenetic Machinery (MDEMs). In MDEMs, a genetic variant disrupts an epigenetic regulator, leading to epigenetic abnormalities, which give rise to the phenotype, likely through a perturbation of the transcriptome. We use mouse models of Kabuki syndromes type 1 and 2 (KS1 and 2; caused by haploinsufficiency of histone methyltransferases KMT2D and KDM6A, respectively), and Rubinstein Taybi type I (RT1; caused by haploinsufficiency of histone acetyltransferase CREBBP). Despite distinct causative genes, all three syndromes share common phenotypes, including intellectual disability and immune dysfunction. We hypothesize that this phenotypic convergence results from shared epigenetic/transcriptomic alterations. Therefore, we seek to identify abnormalities shared across the three MDEMs, in order to pinpoint locations where epigenetic variation is truly causally related to these phenotypes.

We develop a new statistical approach for this kind of analysis, building on recent work in covariate-powered multiple testing. As a first proof-of-principle, and because of the tractability of immune cells, we focus on the immune dysfunction. We perform ATAC- and RNA-Seq in B cells from mouse models of KS1, KS2 and RT1. We show that disruption of chromatin accessibility at promoters often leads to disruption of downstream gene expression and identify 463 loci and 249 genes with shared disruption across the three MDEMs. As an example of how widespread dysregulation leads to specific phenotypes, we show that subtle expression alterations of multiple genes lead to IgA deficiency in KS1 and RT1. In contrast, we predict that KS2 does not have IgA deficiency, and confirm this pattern in mice.

We propose that the joint study of MDEMs offers a principled approach for systematically mapping epigenetic variation causal for a variety of disease phenotypes, including neurodevelopmental ones.

Mandy Meijer

Radboud University Medical Center, The Netherlands

Cell type-specific DNA methylation is associated with childhood Attention-Deficit/Hyperactivity Disorder symptoms.

Mandy Meijer^{1,2}, Marieke Klein³, Barbara Franke^{1,4}, William Copeland⁵, Karolina Aberg⁶, Edwin van den Oord⁶

¹Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, The Netherlands

²Laboratory of Behavioral Genetics, Brain Mind Institute, School of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

³Department of Psychiatry, University of California, San Diego, La Jolla, CA, 92093, USA

⁴Department of Psychiatry, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, The Netherlands

⁵Vermont Center for Children, Youth, and Families, Department of Psychiatry, University of Vermont, Burlington

⁶Center for Biomarker Research and Precision Medicine, Virginia Commonwealth University, Richmond, Virginia

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder with a prevalence of 5% in children. Both genetic and environmental adversities, such as early life stress, play an important role in the onset of ADHD. The interplay between genome and environment can be studied via DNA methylation of CpG sites, which is thought to influence gene expression levels and, ultimately, behavior.

Here, we performed a methylome-wide association study (MWAS) of ADHD symptoms (N=583 children, age<16 years with mean age 13.5, 50% boys) in the longitudinal Great Smoky Mountain Study (GSMS). Associations of sequencing-based DNA methylation levels in whole blood of the majority of 28 million CpG sites in the human genome were calculated for parent-rated ADHD symptoms via linear regression using the RaMWAS R package. The model included covariates for age and squared age, sex, ethnicity, blood cell type, smoking, socioeconomic status, trauma, technical batch effects, and four principal components to account for remaining major sources of unmeasured variation.

Results on bulk cells did not show any methylome-wide significant hits (q-value<0.05). With the use of epigenomic deconvolution, MWAS was performed on specific blood cell types. For robustness, the cohort was randomly divided in three sub-cohorts and results were meta-analyzed. This study identified the first 2, 24, and 16 methylome-wide significant hits for ADHD (q-value<0.05) in T cells, monocytes, and granulocytes, respectively. Enriched Gene Ontology (GO) terms in top hits per cell type (p-value<1*10⁻⁵) identified metalloproteinase, ribonuclease, and endonuclease activity for T cells. Differentially methylated genes in monocytes were enriched for calcium ion binding and channel activity, and granulocytes showed enrichment of DNA methylation in genes related to neuropeptide receptor activity.

Replication and further analysis of these preliminary results should reveal the possible biological role of blood cell type-specific DNA methylation markers in childhood ADHD symptoms. Currently, we are working on a meta-analysis with harmonized quality control of multiple cohorts with a total sample size ~5000 individuals.



Jonathan Mill

University of Exeter, United Kingdom

(Epi)genomic trajectories to neuropsychiatric disease.

The research in my group is focused on understanding both the ‘causes’ and ‘consequences’ of genomic variation in the brain, and the role this plays in neuropsychiatric and neurodegenerative disease. Despite major advances in understanding the risk factors (both genetic and environmental) for these diseases, the mechanisms involved in the onset and progression of pathology are not fully understood and long-term treatments to reverse cellular disease processes in the brain remain elusive. Although genetic studies have been highly successful in identifying variants associated with brain disorders, there remains uncertainty about the specific causal genes involved and how their function is dysregulated during the progression of neuropathology. Increased understanding about the functional complexity of the genome has led to recognition about the role of non-coding regulatory variation in health and disease. Our work aims to characterise the regulatory regions, epigenetic modifications and transcriptional patterns defining the different brain regions and cell-types in the human central nervous system, and assess their role in neurodevelopment, ageing and disease. In this talk I will present on-going work aimed at identifying regulatory genomic variation and transcriptional diversity associated with a diverse range of brain phenotypes. I will describe the dynamic nature of DNA modifications across human brain development and ageing and describe the impact of genetic variation on the epigenome during the life-course. Novel tools mean that it is now feasible to examine epigenetic variation across the genome in large numbers of samples, and I will give an overview of our recent analyses of brain disorders including schizophrenia and autism. Finally, I will outline some of the issues related to regulatory genomic studies of neuropsychiatric disease and explore the feasibility of identifying peripheral biomarkers of disease phenotypes manifest in inaccessible tissues such as the brain.

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Session 2 / Tuesday - March 2, 2021:
Genomics, genetics, identification of mutations.

15:00 – 15:05	Intro by session chair Isabelle Mansuy, Laboratory of Neuroepigenetics, University and ETH Zürich, Switzerland
15:05 – 15:40	Matthew E. Hurles, Wellcome Sanger Institute, UK - Genetic architecture of neurodevelopmental disorders.
15:40 – 16:15	Mark Daly, Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland - Progress in the genetics of schizophrenia.
16:15 – 16:30	FLASH TALKS Daniel G. Calame, Baylor College of Medicine, USA: Biallelic loss-of-function variants in the splicing regulator NSRP1 cause a severe neurodevelopmental disorder with spastic cerebral palsy and epilepsy. Stamatina Tzanoulinou, University of Lausanne, Switzerland: Inhibition of TRPV4 rescues circuit and social deficits unmasked by acute inflammatory response in a SHANK3 mouse model of autism. Andrea Asenjo-Martinez, BRIC, University of Copenhagen, Denmark: Identification of diseased neuronal cell types in 15q13.3 schizophrenia mouse model.
16:30– 16:35	Q&A for all three flash talks
16:35 – 17:10	Joseph Buxbaum, Icahn School of Medicine Mount Sinai, USA - From gene discovery to novel therapeutics in autism spectrum disorder.
17:10 – 17:40	Moderated panel discussion with all speakers from the session Genomics, genetics, identification of mutations: Where do we want to be in 10 years' time and how do we get there?

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Matthew Hurles

Wellcome Sanger Institute, UK

Genetic architecture of neurodevelopmental disorders.

I will describe our UK-wide study of thousands of parent-offspring trios with undiagnosed developmental disorders, the Deciphering Developmental Disorders (DDD) study (www.ddduk.org). ninety percent of these children have neurodevelopmental disorders. Using genome-wide genetic assays (array-CGH and exome sequencing) we are currently able to provide genetic diagnoses for 35% of these children, most of which are new mutations causing dominant diseases. We have also identified more than 50 novel developmental disorders, and described the relative contributions of different classes of genetic disease to the overall genetic architecture of these disorders. Most recently, we have analysed exome sequences in over 31,000 trios as part of an international collaboration and I will describe the results of that study, and how it informs our perspective on the likely genetic causes in the patients that remain undiagnosed.



Mark Daly

Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland

Progress in the genetics of schizophrenia.

Schizophrenia is well-established to have a substantial genetic component with contributions from across the allele frequency spectrum. Initially theorized more than 50 years ago, the high heritability, consistency of prevalence across populations and increasing risk observed for individuals in more densely affected families suggested that polygenic inherited predisposition should play a dominant role in defining schizophrenia risk in the population.

Over the past decade, after years of underpowered linkage and association studies, the high heritability has been converted into hard evidence of associated variants. In this talk I will review progress in genome-wide association studies (GWAS) which have now, identified 270 common (minor allele frequency [MAF] > 1%) risk loci of individually small effect and which explain a significant fraction of disease liability. Rare (MAF < 0.1%) recurrent copy number variants (CNVs) were the first robustly associated genetic loci with schizophrenia, as exemplified by the dramatically higher rates of schizophrenia in 22q11.2 deletion carriers. This suggests a role for rare gene-disrupting mutations with much larger effects on individual risk (OR 2 - 60). Because these are often de novo mutations under strong selection, they contribute to a much lesser extent to genetic heritability than common variation, though they represent the strongest individual risk factors identified to date.

Both of these variant types have proven challenging to convert to specific actionable biological clues as both most often implicate regions without hard evidence of one specific responsible gene. We have therefore embarked on a global effort, now beyond 25,000 cases, to perform exome sequencing in schizophrenia using the collaborative model of the PGC. I will describe the newest results from this effort which have revealed already 10 clearcut genes in which rare, disruptive mutations confer strong risk to schizophrenia, providing some of the strongest biological and mechanistic clues to disease pathogenesis to date.

Daniel G. Calame

Baylor College of Medicine, USA

Biallelic loss-of-function variants in the splicing regulator *NSRP1* cause a severe neurodevelopmental disorder with spastic cerebral palsy and epilepsy.

Daniel G. Calame¹; Somayeh Bakhtiari²; Rachel Logan³; Zeynep Coban-Akdemir⁴; Haowei Du⁵; Tadahiro Mitani⁶; Jawid M. Fatih⁵; Jill V. Hunter⁶; Isabella Herman¹; Davut Pehlivan¹; Shalini N. Jhangiani⁷; Richard Person⁸; Rhonda E. Schnur⁸; Sheng Chih Jin, PhD⁹; Kaya Bilguvar¹⁰; Jennifer E. Posey⁵; Sookyong Koh¹¹; Saghar G. Firouzabadi¹²; Elham Alehabib¹³; Abbas Tafakhori¹⁴; Sahra Esmkhani¹⁵; Richard A. Gibbs^{5,7}; Mahmoud M. Noureideen¹⁶; Maha S. Zaki¹⁷; Dana Marafi¹⁸; Michael C. Krueer²; Hossein Darvish¹⁹; James R. Lupski^{5,7}

1. Division of Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA
2. Pediatric Movement Disorders Program, Division of Pediatric Neurology, Barrow Neurological Institute, Phoenix Children's Hospital, Phoenix, AZ, USA
3. Division of Neurosciences, Children's Healthcare of Atlanta, Atlanta, GA, USA
4. Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX, USA
5. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
6. Department of Radiology, Baylor College of Medicine, Houston, TX, USA
7. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA
8. GeneDX, Gaithersburg, MD, USA
9. Department of Genetics, Washington University School of Medicine, St. Louis, MO, USA
10. Department of Genetics, Yale University, New Haven, CT, USA
11. Department of Pediatrics, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, GA, USA
12. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
13. Student Research Committee, Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
14. Iranian Center of Neurological Research, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
15. Department of Basic Oncology, Division of Cancer Genetics, Oncology Institute, Istanbul University, Istanbul, Turkey
16. Department of Pediatrics, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt
17. Department of Clinical Genetics, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt
18. Department of Pediatrics, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait
19. Department of Medical Genetics, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Purpose: Alternative splicing plays a critical role in mouse neurodevelopment, regulating neurogenesis, cortical lamination, and synaptogenesis, yet few known human neurodevelopmental disorders result from pathogenic variation in splicing regulator genes. Nuclear Speckle Splicing Regulator Protein 1 (NSRP1) is a ubiquitously expressed splicing regulator not known to underlie a Mendelian disorder.

Methods: Exome sequencing (ES) and rare variant family-based genomics was performed as a part of the Baylor-Hopkins Center for Mendelian Genomics Initiative. Additional families were identified via the online matchmaking database GeneMatcher.

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Results: We identified six patients from three unrelated consanguineous families with homozygous loss-of-function variants in *NSRP1*. Clinical features include developmental delay (6/6), epilepsy (6/6), axial hypotonia (6/6), appendicular spasticity (6/6), microcephaly (5/6, Z-score -0.95 to -5.60), dysphagia (4/6), and dysmorphic features (4/6). Brain abnormalities include simplified gyral pattern, under-opercularization, and/or cerebellar vermal hypoplasia. Molecular analysis identified three pathogenic *NSRP1* predicted loss-of-function variant alleles: c.1359_1362delAAAG (p.Glu455AlafsTer20), c.1272dupG (p.Lys425GlufsTer5), and c.52C>T (p.Gln18Ter). c.1359_1362delAAAG and c.52C>T are absent in gnomAD, and c.1272dupG is found only once in the heterozygous state. The two frameshift variants result in a premature termination codon in the last exon, and the mutant transcripts are predicted to escape nonsense mediated decay and cause loss of a C-terminal nuclear localization signal required for NSRP1 function. c.52C>T results in a premature termination codon within the second exon (total seven) and has a CADD score of 37.

Conclusions: We establish *NSRP1* as a disease gene for a severe autosomal recessive neurodevelopmental disorder characterized by developmental delay, epilepsy, microcephaly, and spastic cerebral palsy.

Stamatina Tzanoulinou

University of Lausanne, Switzerland

Inhibition of TRPV4 rescues circuit and social deficits unmasked by acute inflammatory response in a Shank3 mouse model of autism.

S. Tzanoulinou^{1,2,4}, S. Musardo^{1,2}, A. Contestabile^{1,2}, S. Bariselli¹, G. Casarotto¹, E. Magrinelli¹, Y.H. Jiang³, D. Jabaudon¹ & C. Bellone¹

¹ Department of Fundamental Neuroscience, CMU, University of Geneva, Geneva, Switzerland.

² Equal contribution.

³ Department of Pediatrics, Duke University, Durham, North Carolina 27710, USA

⁴ Current address: Department of Biomedical Sciences (DSB), FBM, University of Lausanne, Lausanne, Switzerland.

Autism spectrum disorder (ASD) is a neurodevelopmental disease characterized by social deficits and repetitive behaviors. The high heterogeneity of the disease may be explained by gene and environmental interactions and potential risk factors include immune dysfunctions and immune-mediated co-morbidities. Mutations in the *SHANK3* gene have been recognized as a genetic risk factor for ASD. While heterozygous *SHANK3* mutations are usually the types of mutations associated with idiopathic autism in patients, heterozygous deletion of *Shank3* gene in mice does not commonly induce ASD-related behavioural deficit. In this study, we used *in-vivo* and *ex-vivo* approaches to demonstrate that region-specific neonatal downregulation of *Shank3* in the nucleus accumbens (NAc) promotes hyperexcitability of dopamine receptor D1-expressing Medium Spiny Neurons and upregulates *Trpv4* to impair social behavior. Interestingly, genetically vulnerable *Shank3*^{+/-} mice, when challenged with Lipopolysaccharide to induce an inflammatory response, showed similar circuit and behavioural alterations that were rescued by acute *Trpv4* inhibition. Altogether our data demonstrate shared molecular and circuit mechanisms between ASD-relevant genetic alterations and environmental insults, which can ultimately lead to sociability dysfunctions.

Andrea Asenjo-Martinez

Biotech Research and Innovation Center (BRIC), University of Copenhagen, Denmark

Identification of diseased neuronal cell types in 15q13.3 schizophrenia mouse model

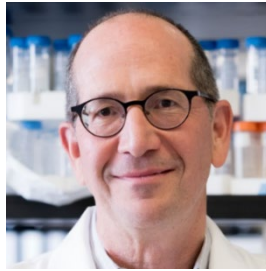
A. Asenjo-Martinez, R. Rydbirk, N. Vasistha, K. Dragicevic and K. Khodosevich

Many neurodevelopmental disorders such as schizophrenia arise due to impaired brain development and neuronal maturation. While the exact molecular mechanisms underlying this pathology are still largely unknown, there is now strong evidence that an imbalance in excitatory and inhibitory signals, caused by dysfunctional inhibitory signalling, leads to cognitive impairment.

Inhibitory GABAergic interneurons (INs) are extremely diverse and distinct subtypes of GABAergic interneurons have large differences in morphology, physiology and gene expression. The highest diversity of GABAergic interneurons is in the cortex where > 60 transcriptomic subtypes of GABAergic interneurons have been reported for mouse brain. Over recent years accumulating evidence from mouse models and patient material shows that only some neuronal subtypes are affected in a certain psychiatric disorder, whereas other subtypes might function normally.

Using single-nuclei RNAseq, we can identify the different cortical transcriptomic cell types from mouse cortex and by comparing the single-nuclei transcriptomes of neuronal cells between wild-type and one of the most robust genetic models of schizophrenia-like phenotype (15q13 copy number variation (CNV), Df(h15q13)/+, that robustly reproduces symptoms in humans) we were able to identify which specific subtype(s) of INs are affected first in schizophrenia and determine the gene regulatory networks involved in the diseased phenotype, leading to the identification of specific pathways that might be used as potential targeted therapies.

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Joseph Buxbaum

Icahn School of Medicine Mount Sinai, USA

From gene discovery to novel therapeutics in autism spectrum disorder.

There have been very significant strides in identifying both rare and common genetic variants that are associated with ASD. More than 100 genes have been identified that, when mutated with rare deleterious variants, are associated with high risk for ASD and associated disorders. These genes, and the associated mutations, can be studied in model systems to provide windows into the biology processes that lead to ASD. Such insights, in turn, can lead to novel therapeutic approaches for these rare genetic disorders. Genetics, therefore, provides one mechanism to stratify ASD subjects and to develop precision medicine approaches. Other biomarkers are being sought that can also be used for stratification and precision medicine. This presentation will summarize the status of genetic discovery in ASD and give examples of how gene discovery can contribute to novel therapeutics.

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Session 3 / Wednesday - March 3, 2021:
Neuronal dysfunction in neurodevelopmental disorders

15:00 – 15:05	Intro by session chair Konstantin Khodosevich, BRIC, University of Copenhagen, Denmark
15:05 – 15:40	Claudia Bagni, University of Lausanne, Switzerland - Molecular Complexes Underlying Motor and Social Skill Development.
15:40 – 16:15	Jessica Cardin, Dept. of Neuroscience, Yale University, USA - GABAergic contributions to neurodevelopmental disorders.
16:15 – 16:30	FLASH TALKS Ali H. Rafati, Aarhus University, Denmark: Modelling of the Neuronal Excitatory/Inhibitory Imbalance. Mykhailo Batiuk, University of Copenhagen, Denmark: Selective vulnerability of supragranular layer neurons in schizophrenia. Katrin Linda, Donders Institute for Brain, The Netherlands: Imbalanced autophagy causes synaptic deficits in a human model for neurodevelopmental disorders.
16:30 – 16:35	Q&A for all three flash talks
16:35 – 17:10	Flora M. Vaccarino, Child Study Center and Dept. of Neuroscience, Yale University, USA - Cell fate trajectories in cortical organoids from autism spectrum disorders.
17:10 – 17:40	Moderated panel discussion with all speakers from the session Neuronal dysfunction in neurodevelopmental disorders: Where do we want to be in 10 years' time and how do we get there?

All times are in CET.

**The Brain Prize Meeting
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Mechanisms and pathways to treatment
1-4 March 2021**



Claudia Bagni

University of Lausanne, Switzerland

Molecular Complexes Underlying Motor and Social Skill Development

Individuals with the 15q11.2 microdeletions, which include the CYFIP1 gene, can present with a diverse array of symptoms such as cognitive disturbances, epilepsy and motor problems. The core behavioral features and the underlying molecular mechanisms of this genetic condition, however, remain unclear. In brain, CYFIP1 regulates synapse structure and plasticity by orchestrating at least two processes: actin remodeling and protein synthesis. I will discuss how Cyfip1 haploinsufficiency causes deficits in functional brain connectivity, social and motor behaviours and the molecular and cellular mechanisms underlying those deficits. Finally, I will frame the importance of our findings in the context of different neurodevelopmental disorders.

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Jessica Cardin

Department of Neuroscience, Yale University, USA

GABAergic contributions to neurodevelopmental disorders.

Developmental disruption of inhibitory function in brain circuits is a common element of several neurodevelopmental disorders, including schizophrenia and autism. However, GABAergic interneurons exhibit diverse developmental trajectories and mature roles, suggesting potentially variable vulnerability to developmental perturbation. Recent work has suggested that vasoactive intestinal peptide-expressing (VIP) interneurons may play a critical role in the proper development and function of cortical circuits, making them a potentially key point of vulnerability in neurodevelopmental disorders. We find that loss of MeCP2 from VIP interneurons replicates key neural and behavioral phenotypes observed following global *Mecp2* loss of function mutations in mouse models of Rett Syndrome. Indeed, similar perturbations of cortical function are observed following VIP-specific mutation of the gene *ERBB4*, a risk gene for schizophrenia and a key element of a signaling pathway critical for GABAergic interneuron development. Together, our data suggest convergent perturbations of state-dependent cortical function following developmental dysregulation of key inhibitory populations from multiple sources.

Ali H. Rafati

Department of Clinical Medicine - Translational Neuropsychiatry Unit, Aarhus University, Aarhus, Denmark

Modelling of the Neuronal Excitatory/Inhibitory Imbalance

A. H. Rafati ¹, M. Ardalan ^{1,2}, C. Mallard ² and G. Wegener ¹

1. Department of Clinical Medicine - Translational Neuropsychiatry Unit, Aarhus University, Aarhus, Denmark

2. Centre for Perinatal Medicine and Health, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

The dynamic of neurites complexity is an essential concept in theoretical neuroscience by modelling the mechanisms underlying the initiation and propagation of functional synaptic plasticity, which can be viewed from different perspectives such as shape characterization of the particle using the eigenvectors of the particle (neuron), however, is not rather helpful to show the mathematical framework behind the complexity of neurites. We now define the term complexity and hyper-complexity, which denote the number of neurite branches in the natural and pathological settings. Accordingly, we hypothesized that the general distribution of neurites per se is either straight-line, curved-oriented, or complex in the standard-setting, which can be modelled by Lagrange Interpolation Polynomial,

$$f(x) = p_n(x) = \sum_{k=0}^n \frac{l_k(x)}{l_k(x_k)} f_{(k)}.$$

This model predicts where in 3D model, the interconnecting branches oriented either linear or curved, which may further help clarify dynamic competition between excitatory and inhibitory neuronal populations as a critical hypothesis behind neurodevelopmental disorders such as autism. Here we introduce the well-known mathematical model used for generating neural networks (NN) and in diverse biologically adapted models such as neuronal dynamics. It seems that 'linear interpolation' is neurobiologically adapted for connectivity, which can model neurite connections. We can plot in 3D the whole map. To a limited extent, we will analyse the neurites on Golgi-stained sections and rendered in Imaris, besides mathematical simulation using Matlab to investigate how neurite connection is oriented and fits our proposed model of Lagrange interpolation function.

Mykhailo Y. Batiuk

Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, University of Copenhagen Denmark

Selective vulnerability of supragranular layer neurons in schizophrenia

Mykhailo Y. Batiuk^{1*}, Teadora Tyler^{2*}, Shenglin Mei³, Rasmus Rydbirk¹, Viktor Petukhov¹, Dora Sedmak⁴, Erzsebet Frank², Virginia Feher², Nikola Habek⁴, Qiwen Hu³, Anna Igolkina^{3,5}, Lilla Roszik², Ulrich Pfisterer¹, Zdravko Petanjek⁴, Istvan Adorjan^{2†}, Peter V. Kharchenko^{3†}, Konstantin Khodosevich^{1†}

¹Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

²Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary

³Department of Biomedical Informatics, Harvard Medical School, Boston, Massachusetts, 02115, USA

⁴Croatian Institute for Brain Research & Center of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb, Zagreb, Croatia

⁵St. Petersburg Polytechnical University, St. Petersburg, Russia

* authors have contributed equally

† corresponding authors

Schizophrenia is one of the most wide-spread mental brain disorders with complex and largely unknown etiology. To characterize the impact of schizophrenia at a cellular level, we performed single nucleus RNA sequencing of >170,000 neurons from the dorsolateral prefrontal cortex of patients with schizophrenia and matched controls (7 vs 11, respectively). In addition, to correlate data with cortical anatomy, >100,000 neurons were analyzed topographically by immunohistochemistry in an extended cohort of cases with schizophrenia and controls (10 vs 10). Compositional analysis of RNA sequencing data revealed reduction in relative abundance across all families of GABAergic neurons and a concomitant increase in principal neurons, which was most pronounced for supragranular subtypes (layers 2-3). Moreover, supragranular subtypes of GABAergic interneurons showed most dramatic transcriptomic changes. These results were substantiated by histological analysis, which revealed a reduction in the density of calretinin, calbindin and parvalbumin GABAergic interneurons particularly in layer 2. Common effect of schizophrenia on supragranular neuronal networks was underlined by downregulation of protein processing genes and upregulation of neuronal development/plasticity genes across supragranular subtypes of principal neurons and GABAergic interneurons. *In situ* hybridization and spatial transcriptomics further confirmed supragranular layer neuron vulnerability, revealing complexity of schizophrenia-affected cortical circuits. These point towards general network impairment within supragranular layers being a core substrate associated with schizophrenia symptomatology.

Katrin Linda

Department of Human Genetics, Radboudumc, Donders Institute for Brain, Cognition, and Behavior, The Netherlands

Imbalanced autophagy causes synaptic deficits in a human model for neurodevelopmental disorders.

Katrin Linda¹, Elly I. Lewerissa¹, Anouk H. A. Verboven¹, Michele Gabriele^{2,3,4}, Monica Frega^{1,5}, Teun M. Klein Gunnewiek^{1,6}, Lynn Devilee¹, Edda Ulferts¹, Astrid Oudakker¹, Chantal Schoenmaker¹, Hans van Bokhoven^{1,6}, Dirk Schubert⁷, Giuseppe Testa^{2,3}, David A. Koolen¹, Bert B.A. de Vries¹, Nael Nadif Kasri^{1,6*}

¹Department of Human Genetics, Radboudumc, Donders Institute for Brain, Cognition, and Behavior, The Netherlands.

²Department of Oncology and Haemato-Oncology, University of Milan, Milan, Italy.

³Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy.

⁴Massachusetts Institute of Technology, Department of Biological Engineering, Cambridge, MA, USA.

⁵Department of Clinical Neurophysiology, University of Twente, The Netherlands.

⁶Department of Anatomy, Radboudumc, Donders Institute for Brain, Cognition, and Behaviour, The Netherlands

⁷Department of Cognitive Neuroscience, Radboudumc, Donders Institute for Brain, Cognition and Behavior, The Netherlands.

Autophagy is a finely tuned process of programmed degradation and recycling of proteins and cellular components, which is crucial in neuronal function and synaptic integrity. Mounting evidence implicates chromatin remodelling in fine-tuning autophagy pathways. However, this epigenetic regulation is poorly understood in neurons. Here, we investigate the role in autophagy of KANSL1, a member of the nonspecific lethal complex, which acetylates histone H4 on lysine 16 (H4K16ac) to facilitate transcriptional activation. Loss-of-function of KANSL1 is strongly associated with the neurodevelopmental disorder Koolen-de Vries Syndrome (KdVS). Starting from KANSL1-deficient human induced-pluripotent stem cells, both from KdVS patients and genome-edited lines, we identified superoxide dismutase 1, an antioxidant enzyme, to be significantly decreased, leading to a subsequent increase in oxidative stress and autophagosome accumulation. In KANSL1-deficient neurons, autophagosome accumulation at excitatory synapses resulted in reduced synaptic density, reduced AMPA receptor-mediated transmission and impaired neuronal network activity. Furthermore, we found that increased oxidative stress-mediated autophagosome accumulation leads to increased mTOR activation and decreased lysosome function, further preventing the clearing of autophagosomes. Finally, by pharmacologically reducing oxidative stress, we could rescue the aberrant autophagosome formation as well as synaptic and neuronal network activity in KANSL1-deficient neurons. Our findings thus point towards an important relation between oxidative stress-induced autophagy and synapse function and demonstrate the importance of H4K16ac-mediated changes in chromatin structure to balance reactive oxygen species- and mTOR-dependent autophagy.



Flora M. Vaccarino

Child Study Center, Yale University, New Haven, CT, USA

Department of Neuroscience, Yale University, New Haven, CT, USA

Cell fate trajectories in cortical organoids from autism spectrum disorders.

Human induced pluripotent stem cell (iPSC)-derived organoids are a promising, tractable tool to investigate how gene regulatory events drive cellular diversity in the brain, and how developmental disorders impact these regulatory processes. I will discuss the characterization of epigenomes and transcriptomes of human cortical organoids and, in parallel, isogenic human fetal brains. We defined networks of enhancer elements and their target genes exhibiting dynamic changes during the process of differentiation from stem cells to telencephalic neurons. Modules involved in neuronal and synaptic differentiation were downregulated in autism spectrum disorders (ASD). To understand gene regulatory activity in different cell types, we analyzed epigenomes and transcriptomes of human cortical organoids at the single cell level. In single cell transcriptome analyses involving over 650,000 cells from 26 individuals, organoids progressively differentiated into radial glial precursors and then excitatory and inhibitory neuronal lineages and were patterned into regional identities similar to human fetal brains. Neuronal differentiation trajectories were differentially altered in ASD organoids derived from macrocephalic or normocephalic individuals. Thus, organoids reveal gene regulatory networks driving cell fate and brain patterning that may shed light onto normal and abnormal neurodevelopment.

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Session 4 / Thursday - March 4, 2021:

Diagnosis and treatment of neurodevelopmental disorders.

14:00 – 14:05	Intro by session chair Laura Andreae, King's College London, UK
14:05 – 14:40	Jeffrey Neul, Vanderbilt University Medical Center, USA – title tbd
14:40 – 14:55	<p>FLASH TALKS</p> <p>Allan Bayat, University of Southern Denmark, Denmark: Genetic testing and its impact on therapeutic decision making in childhood-onset epilepsies – a study in a tertiary referral centre.</p> <p>Sydney Leaman, King's College London, UK: GABAB receptor agonist - R-baclofen - lowers resting-state functional connectivity in dorsal attention and salience subnetworks in adults with Autism Spectrum Disorder but not neurotypical controls.</p> <p>Rikke Hahn Kofoed, Sunnybrook Health Sciences Centre, Canada: Non-invasive gene delivery to the brain mediated by transcranial focused ultrasound allowing intravenous adeno-associated virus to cross the blood-brain barrier.</p>
14:55 – 15:00	Q&A for all three flash talks
15:00 – 15:35	Huda Y. Zoghbi, Baylor College of Medicine, Duncan Neurological Research Institute, HHMI, USA - Epigenetics and Brain Plasticity: Lessons from Rett Syndrome and other MECP2 disorders.
15:35 – 16:05	Moderated panel discussion with The Brain Prize winners Huda Y. Zoghbi and Adrian Bird - their science, and their outlook on the future of research into neurodevelopmental disorders.
16:05 – 16:10	Round off and closing remarks by Martin Meyer, the Lundbeck Foundation.
16:15 – 17:00	ANNOUNCEMENT OF THE WINNERS OF THE BRAIN PRIZE 2021

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Jeffrey Neul

Vanderbilt University Medical Center, USA

Development of clinical trial readiness in Rett syndrome using natural history studies and animal models.

**Jeffrey Lorenz Neul MD, PhD, Director, Vanderbilt Kennedy Center
Annette Schaffer Eskind Chair, Professor of Pediatrics, Pharmacology, and Special Education, Vanderbilt University Medical Center, Vanderbilt University**

Rett syndrome (RTT) is a severe neurodevelopmental disorder that primarily affects girls and has a characteristic set of disease features and progression. In general, people with Rett syndrome appear unaffected at birth and during infancy, but after a period of some degree of developmental delay they undergo a period of regression in which they experience a loss of acquired hand skills and spoken language, develop characteristic repetitive hand movements (stereotypies), and have gait problems or do not acquire the ability to walk. The regressive period is limited, and eventually affected individuals enter a stationary phase in which skills do not continue to be lost and they display a variety of additional clinical features such as seizures, autonomic abnormalities, and growth failure. Unfortunately, the skills lost are usually not regained and affected individuals are dependent on others for activities of daily living.

The discovery that mutations in the X-linked gene, MECP2, is the genetic basis of RTT by Huda Zoghbi provided an opportunity to develop cellular and animal models to further the understanding of the pathophysiology of the disease and develop new therapeutic approaches. With the demonstration by Adrian Bird that the phenotypic abnormalities observed in a mouse model of RTT could be reversed, even in symptomatic mice, by reexpression of MECP2 provided great hope that truly disease-modifying or even reversing therapies might be able to be developed for RTT. Significant effort is underway to develop such therapies and a number of clinical trials of potentially disease-modifying therapies have been conducted, are ongoing, or are being considered.

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Although this work has provided great hope, there remains significant challenges with regards to the execution of effective clinical trials of novel therapies. Notably, there exists few validated clinical outcome measures for people with RTT and there are no validated biomarkers of disease progression or, importantly for clinical trials, biomarkers of early treatment response. Shortly after the discovery of the genetic basis of RTT, a longitudinal natural history study (NHS) of RTT was initiated in the United States. Over the course of this study, over 1500 individuals have participated in longitudinal assessments at multiple sites, leading to insights into the variety of clinical features of the disorder, disease progression, and genotype-phenotype relationships. Recent work has focused on developing clinical trial readiness, including clinical research sites capable of conducting interventional trials, creating and validating outcome measures, and identifying putative biomarkers. The existence of the excellent animal models allows a ready flow of information from clinical research and laboratory translational research which is facilitating the advancement of biomarkers, especially biochemical and neurophysiological biomarkers. With focused, multidisciplinary, and collaborative effort we hope that these efforts will allow the identification of truly disease-modifying therapy in RTT, fulfilling the great hope provided by animal work.

Allan Bayat

Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, Denmark

Genetic testing and its impact on therapeutic decision making in childhood-onset epilepsies - a study in a tertiary referral centre.

Bayat A^{1,2}, Fenger CD^{1,3}, Rubboli G^{1,4}, Møller RS^{1,2}

¹Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre, Denmark.

²Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, ³ Amplexa Genetics A/S, Denmark, ⁴ University of Copenhagen, Copenhagen, Denmark

Purpose: The Danish Epilepsy Centre, Filadelfia is the only tertiary hospital in Denmark, specialized in the treatment of epilepsy. The aim was to assess the distribution of genetic epilepsies and accordingly adjusted medical treatment at a tertiary epilepsy referral centre.

Method: The records of all children born between 2006 and 2011 and followed at the Danish Epilepsy Centre in 2015 were systematically analysed regarding: seizure classification, epilepsy syndrome, age at seizure onset, brain magnetic resonance imaging findings, co-morbidities, genetic diagnosis, and medical treatment adjustments following the genetic diagnosis.

Results: A total of 357 children were included, however 7 % turned out not to have epilepsy, and 11 % had epilepsy due to an acquired brain lesion. These two groups of patients were excluded from further analysis. The remaining 290 children were most frequently diagnosed with developmental and epileptic encephalopathies (35 %), focal or multifocal epilepsy (41 %), and generalized epilepsy (8 %). 211/290 patients consented for genetic testing aiming to find a genetic explanation for their illness. Genetic approaches used were karyotyping, array and next-generation sequencing (NGS) approaches such as gene panels and whole-exome sequencing. A genetic cause was found in half of the consented patients across 36 different genes; 14 cases were explained by a copy-number variant while 86 cases had a single-nucleotide variant. Commonly affected genes affected were *SCN1A* and *TSC1/2* followed by the *PCDH19*, *CDKL5*, *SCN2A*, and *SCN8A*. Tailored treatment approaches were possible in half of patients with a molecular diagnosis. This applied to the children with a *SCN1A*, *TSC1/2*, *DEPDC5*, *KCNQ2*, *PNPO*, *SCN2A*, *SCN8A*, or *SLC2A1* related disorder. Exome sequencing led to molecular diagnosis in several genetically unexplained children and identified three new candidate genes (*TRA2B*, *CELSR1* and *ADGRL1*).

Conclusion: Almost 50% of our cohort have reached a genetic diagnoses. NGS approached have much higher yield than an array (detection rate of 40% vs 7%). Our data shows that 1 out of 4 patients referred to a tertiary epilepsy centre can benefit from early genetic testing in order to receive tailored treatment and prevent unnecessary and potentially harmful diagnostic procedures and management.

Sydney Leaman

Department of Forensic and Neurodevelopmental Science, Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom

GABA_B receptor agonist - R-baclofen - lowers resting-state functional connectivity in dorsal attention and salience subnetworks in adults with Autism Spectrum Disorder but not neurotypical controls.

Sydney Leaman*, Nichol Wong, Andreia Carvalho Pereira, Hester Velthuis, Mihail Dimitrov, Maria Francesca Ponteduro, Charlotte Pretzsch, David Lythgoe, Dafnis Batalle, Declan Murphy, Eileen Daly, Grainne McAlonan

Department of Forensic and Neurodevelopmental Science, Institute of Psychiatry, Psychology and Neuroscience, King's College London

*sydney.leaman@kcl.ac.uk

Although highly heritable, it is clear from genetic studies that the etiology of autism spectrum disorders (ASD) shows great heterogeneity. Convergence of the broad molecular underpinnings of this neurodevelopmental disorder have been posited to occur at a higher, functional level in the dynamic balance between excitation and inhibition (E:I balance), affecting multiple scales of neural circuit and brain network activity. Drugs acting on receptors that mediate excitatory or inhibitory neurotransmission can be used as an indirect and non-invasive method to challenge E:I balance in humans with neurodevelopmental disorders, such as ASD.

In a randomized cross-over design, we compared whole-brain network functional connectivity from resting-state fMRI data in 17 typically developing adult males and 14 with ASD, during placebo conditions and following oral administration of 30mg R-baclofen - an agonist for the ubiquitously expressed, G-protein coupled, GABA_B receptor.

Acute administration of GABA_B receptor agonist - R-baclofen - lowered intrinsic functional connectivity in a subnetwork comprising regions within dorsal attention and salience networks, including the medial prefrontal cortex, in individuals with ASD but not in TD controls.

We propose that neurotypical and autistic brains show differences in the maintenance of network-level functional homeostasis when challenged with increased metabotropic inhibitory drive. Our results further promote the idea that pharmacological challenges to neurotransmitter or synaptic functions may reveal important phenotypic differences in neurodevelopmental disorders, which may otherwise be obscured or very subtle. Future work will incorporate MR spectroscopy to more directly probe E:I balance through quantification of excitatory and inhibitory neurotransmitter concentrations in affected functional brain regions.

Rikke H. Kofoed

Sunnybrook Health Sciences Centre, Canada

Non-invasive gene delivery to the brain mediated by transcranial focused ultrasound allowing intravenous adeno-associated virus to cross the blood-brain barrier.

R.H. Kofoed^{1,2}, K. Noseworthy^{1,2}, C. Dibia¹, N. Vacaresse¹, K. Hynynen^{3,4}, and I. Aubert^{1,2}

¹ Sunnybrook Research Institute, Biological Sciences, Toronto, ON, Canada

² University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, ON, Canada

³ Sunnybrook Research Institute, Physical Sciences, Toronto, ON, Canada

⁴ University of Toronto, Department of Medical Biophysics, Toronto, ON, Canada

INTRODUCTION A single gene therapy delivery to the brain can offer a life-long treatment for neurological disorders. Advances in the use of adeno-associated virus (AAV) as a gene carrier recently led to the U.S. Food and Drug Administration approval of the first AAV-based treatment for a neurological disorder. Brain delivery of AAVs in the clinic, however, still depends on invasive intracranial injections or high systemic doses. To achieve efficient and non-invasive gene delivery to the brain, the blood-brain barrier (BBB), which limits the entry of therapeutics to the brain, has to be circumvented.

METHODS We used transcranial focused ultrasound (FUS) combined with intravenously injected microbubbles to increase the permeability of the BBB in a safe and controlled manner; i.e. non-invasively, reversibly and locally. FUS-BBB modulation allows for the passage of intravenously administered AAVs from the blood to the brain, only at FUS-targeted sites, hereby providing a non-invasive AAV delivery to the brain. The increased BBB permeability also decreases the AAV dosage needed for BBB crossing and brain delivery. We conducted a comprehensive study to establish the standards of FUS-mediated delivery of well-characterized AAVs to the brain.

RESULTS We found significant differences in gene delivery efficacy to neurons and astrocytes in the brain depending on both AAV serotype, the properties of the brain tissue and the uptake of the AAVs in peripheral organs. Furthermore, we combined FUS-mediated delivery with novel, engineered AAVs. By utilizing the unique features of these novel AAVs, we showed that local FUS-BBB modulation, i.e. in a small volume of the brain, is necessary and sufficient to achieve gene delivery that can be: (1) widespread in the brain; and (2) simultaneous across multiple brain regions.

CONCLUSION We have demonstrated that gene delivery efficacy using AAV and FUS depends on the choice of AAV serotype and the brain region targeted. Our results provide a standardized base to inform and improve future pre-clinical and clinical studies utilizing AAV and FUS for non-invasive gene delivery to the brain. FUS-mediated gene delivery can be tailored for each treatment and mediate brain delivery to specific regions or widespread to multiple regions, as needed.



Huda Y. Zoghbi

Baylor College of Medicine, Duncan Neurological Research Institute, HHMI, USA

Epigenetics and Brain Plasticity: Lessons from Rett Syndrome and other *MECP2* disorders

Huda Y. Zoghbi, MD, Investigator, Howard Hughes Medical Institute

Ralph D. Feigin Professor, Baylor College of Medicine and Director, Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital

Rett syndrome is a delayed-onset childhood disorder, typically found in girls, that causes a broad range of severe neurological disabilities, including loss of the ability to speak and socialize, and the development of tremors, ataxia, seizures, autonomic dysfunction, and stereotypic hand-wringing movements. We discovered that loss-of-function mutations in the *MECP2* gene cause Rett syndrome, and before long it became clear that milder mutations in *MECP2* can also cause other neuropsychiatric phenotypes ranging from autism to bipolar disorder. Using genetically-engineered mice, we learned that the brain is acutely sensitive to MeCP2 levels; both decreases and increases in the amount of MeCP2 protein can lead to neurological problems that are also observed in humans. Nonetheless, normalizing MeCP2 levels reverses symptoms in a humanized mouse model of *MECP2* duplication syndrome, a disorder that results from excess MeCP2 and typically affects boys. Most recently we have found an approach that could delay onset of Rett symptoms, suggesting that earlier diagnosis through screening may be worthwhile for these disorders.

Annemette Bondo Lind

Institute of psychology, Aarhus University, Denmark

A qualitative study of stress experience and coping strategies among people with somatoform disorders in life history and everyday life before and after an intervention with mindfulness therapy.

Background: People suffering from psychosomatic diseases like e.g. chronic fatigue syndrome, chronic pain syndrome, fibromyalgia, irritable bowel disease and somatoform disorders seems to be stress vulnerable. However, how the exact mechanisms are interacting between the characteristics of these patients' way of experiencing stress, their bodily and mental reactions to stress, and the general way they cope with stressful conditions, is not known. Research support there could be strong iatrogenic components at stake due to the contested nature of these illnesses, uncertain illness perceptions, lack of knowledge and insufficient treatment.

Aim: The study aimed to explore the interrelation between experienced stress, the use of coping strategies, psychosomatic symptoms and the psychosocial context in 1) a life history perspective, 2) a daily life perspective and 3) a treatment perspective - seen from the patients' points of view.

Methods: Epistemologically the study was based on social construction. The main data-source was 24 pre-treatment interviews, conducted 1-3 months before treatment, and 22 post-treatment interviews, conducted 9-14 months after end of treatment. Data were coded in relation to the three sub studies mentioned (using Nvivo 8/10). Central codes, concepts, hypothesis and theories were developed in dialogue in the research group. The analyses of the daily life perspective were done based on the principles of constructivist grounded theory including theoretical sampling. Whereas the analysis of the life history and post-treatment data were done based on thematic analytic procedures.

Results: Exploring the life histories of these patients before they became serious ill we identified how all patients were raised in cultures (in their home and in school) where stressful conditions and events and related feelings and distress generally were not communicated verbally - thus stress-related emotions, concerns and feelings were not processed/shared by words. The patients had learnt to handle the stress they experienced generally by using avoidant strategies. Pre-treatment patients were struggling in an existential crisis of insecurity. The patients' primary daily concern was their longing for existential recognition of their illness, needs, feelings and vulnerability. Patients experienced difficulties recognizing their vulnerability, needs and feelings of distress themselves, and therefore they were depending on the recognition from their surroundings.

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Post-treatment patients felt existentially recognized as "really ill", experienced it more easy to relax, used more flexible coping strategies finding it easier to manage their symptoms more actively, and experienced enhanced body and self-awareness. Some patients also experienced how they communicated more openly in close social interactions, thus transforming the emotional avoidance culture they were rooted in.

Conclusions: Based on the patients' narrations we developed new hypothesis and theories of how psychosomatic illness like somatoform disorders could be developed in the specific patterns of growing up in an emotional avoidance culture, which seems to create stress-vulnerability. Before treatment the patients were suffering in crisis of insecurity, not knowing the exact nature, development and solution for their suffering, longing for existential recognition of their suffering, feelings, vulnerability and needs. After treatment patients had in various degrees gained more secure perceptions of their illness - feeling existentially recognized as "really ill", increased relaxation ability - reducing their symptoms, increased awareness connecting differently to mind and body - creating more insight and meaning in life. Some patients also experienced to improve their communication of needs, vulnerability and distress in close social interactions.

Anthi C. Krontira

Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry

Environmental effects on neurodevelopment via ZBTB16

Authors: Anthi C. Krontira^{1,2}, Cristiana Cruceanu¹, Christina Kyrrousi³, Silvia Cappello³, Elisabeth B. Binder^{1,4}

Affiliations: ¹Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Germany; ²International Max Planck Research School for Translational Psychiatry, Max Planck Institute of Psychiatry, Germany; ³Research group of Developmental Neurobiology, Max Planck Institute of Psychiatry, Germany; ⁴Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, USA.

Abstract:

The brain undergoes rapid growth and maturation during the prenatal period, thus it is highly susceptible to environmental stimuli. During this period increased glucocorticoid exposure of the embryo has been associated with adverse neurobehavioral, e.g. increased attention deficits, and physiological outcomes, e.g. brain structural and functional changes, later in life. Glucocorticoids are one of the main factors mediating stress effects prenatally and one of the main systems found dysregulated in stress-related psychiatric disorders. In addition, glucocorticoids treatment is often used in pregnancies at risk for premature birth. To investigate these processes in a human specific and reactive system, we used induced Pluripotent Stem Cells-derived 3-dimensional cerebral organoids to model prenatally mediated risk and response to excess of glucocorticoids and combined them with *in vivo* mouse models of neurodevelopment. We found a very promising candidate gene for neurodevelopmentally-relevant glucocorticoids response in *ZBTB16*, which had previously been shown to respond to glucocorticoids in human blood samples. *ZBTB16* is expressed dynamically throughout human neurodevelopment with high expression in the progenitor cells (PAX6+ cells) early on and downregulation when the first neuronal markers (MAP2, DCX) appear. Dexamethasone exposure (100nM) of organoids for 7 days results in a robust increase of *ZBTB16* expression in the progenitor cells. We used *in utero* electroporations in mice at different developmental time-points and *ex vivo* electroporations in human cerebral organoids to overexpress *ZBTB16*, mimicking the effect of glucocorticoids on the transcription factor. Overexpression of *ZBTB16* leads to increased proliferation of progenitor cells, which seem to stay at an intermediate state expressing both PAX6 and TBR2. This increased proliferation results in increased production of deep (TBR1) and upper layer (CTIP2) neurons. We are working on the molecular signaling pathway of glucocorticoids through *ZBTB16*. We identified *ZBTB16* as a mediator of altered neuronal differentiation processes after glucocorticoids stimulation. The molecular mechanisms and pathways we shed light on in this work could have profound implications for our understanding risk to stress exposure during early brain development, and consequently psychopathology vulnerability.

Asami Oguro-Ando

The Institute of Biomedical and Clinical Science, University of Exeter, United Kingdom

Dysregulation of cytokine signaling via JAKMIP1 in syndromic autism spectrum disorders.

J. Gandawijaya ¹, RA. Bamford¹ and A. Oguro-Ando¹

Autism Spectrum Disorder (ASD), which affects approximately 1% of the world's population, is a developmental disorder with a strong genetic component characterized by 1) difficulties in interpersonal relationships, 2) communication disorders, and 3) patterning of interests and activities. The pathogenesis of ASD is largely unexplored and the molecular mechanisms of its cause need to be elucidated in order to develop new drugs. Research to date suggests that several genetic abnormalities are associated with ASD, including chromosomal abnormalities, spontaneous mutations and Fragile X syndrome. Classifying ASD patients on the basis of these specific genetic causes may ultimately allow the identification of more similar subgroups and hasten our understanding of the mechanisms involved in the pathogenesis of ASD. We therefore focused on the gene JAKMIP1, which previous studies have shown to be highly expressed in neurons and found that its blood levels were markedly reduced in patients with both Fragile X syndrome and chromosome 15q duplication, both of which are associated with genetic abnormalities associated with ASD. Furthermore, JAKMIP1 has been shown to be involved in both syndromic and idiopathic ASD and elucidating the function of JAKMIP1 will be important in understanding the neural signaling pathways involved in ASD.

Through its C-terminus, JAKMIP1 interacts with members of the Janus Kinase (JAK) family of non-receptor tyrosine kinases, key components of the intracellular signaling cascades initiated by cytokines. We hypothesize that given its high expression in neurons, JAKMIP1 may coordinate neuronal responses to cytokines by interacting with JAKs and cytokine receptors. Our preliminary experiments suggest that JAKMIP1 may regulate signals in the inflammatory system. We are now investigating how JAKMIP1 modulates the neuronal response to cytokines, identifying novel JAKMIP1 regulatory pathways and exploring the potential for pharmacological treatment with STAT3 agonists in ASD. This research will pave the way for the discovery of new drugs for specific ASDs that target the neural functions regulated by the JAK/STAT cascade.

Adebisi Benjamin Temidayo
Osun State University, Nigeria

Cerebrospinal fluid cell count, as an indicator for inflammation and neurological conditions.

Cerebrospinal Fluid, CSF, cell count is the number of blood cells (both red blood cells and white blood cells) in the CSF.

CSF is produced by the choroid plexus into the subarachnoid space. The CSF cushions the brain and spinal cord and provides them with nourishment and metabolic support.

CSF could be gotten from an individual by lumbar puncture or Spinal tap.

The procedure is performed by inserting a needle through the back, in a position lower than the first or second lumbar vertebra. (That is the terminal point of the spinal cord).

Preferable the level of 3rd and 4th lumbar vertebra is safe for the procedure.

The needle gets into the spinal sub-arachnoid space to aspirate the CSF.

The subarachnoid space is the space between the Arachnoid and pia maters (these are brain and spinal cord coverings, the outermost matter is the Dura matter.).

Between 5 to 20 mls of CSF might just be enough for examination. (The average total CSF in adult is between 170 -240 mls) and interestingly, CSF is produced daily.

Approximately 500mls of CSF are produced daily at the rate of 25mls per hour but they are constantly reabsorbed; therefore, at a given time, between 125-150mls, of CSF, are present in the Subarachnoid space.

Measurements of CSF components could be used in diagnosis of the health of the Central nervous system and its diseases.

Normally, Red blood cell should not be in the CSF except there's a rupture resulting from bleeding while a higher than 5 white blood cell per ml in Adult is indication of inflammation or infections in the brain. Increase in number of white blood cells in the CSF is called Pleocytosis.

The blood–cerebrospinal fluid barrier (BCSFB) is a fluid–brain barrier that is composed of a pair of membranes that separate blood from CSF, at the capillary level, and CSF from brain tissue.

The Blood-CSF barrier could also be inflamed, in Meningitis and other inflammatory conditions of the nervous system and compromised leading into higher White blood cells (Lymphocytes) count in the CSF.

Burak Ozgur

University of Copenhagen, Denmark

Brain capillary endothelial cell monolayers cultured under hypoxic conditions maintain barrier integrity and display increased expression of Glut1, Lat1 and transferrin receptor.

Burak Ozgur, Hans Christian Cederberg Helms, Erica Tornabene & Birger Brodin

Department of Pharmacy, The Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100, Copenhagen, Denmark.

Introduction and aim: Brain capillary endothelial cells (BCECs) are exposed to hypoxic conditions during the early phases of brain development. However, little is known of the effect of hypoxia on barrier properties of the endothelium and blood-brain barrier (BBB) function. A number of studies have suggested breakdown of the barrier function, whereas others have claimed beneficial effects of the hypoxic conditions. The study aimed at investigating if BCECs cultured under hypoxic conditions-maintained BBB function.

Method: Primary monocultures of bovine BCECs were cultured on permeable supports under normoxic (90% atmospheric air-10% CO₂) and hypoxic (1% O₂, 10% CO₂ and 89% N₂) conditions for 6 days. The resulting endothelial cell monolayers were investigated regarding functional tightness and expression of BBB phenotype markers.

Results: In cell monolayers exposed to hypoxia, we observed translocation of HIF-1 alpha from cytosol to nucleus, indicating a cellular hypoxic response via the HIF-signaling pathways. Cell monolayers exposed to hypoxia throughout a 6-day culture period did not show decreased monolayer integrity. Trans-endothelial electrical resistance and localization of claudin-5 and ZO-1 were not affected by hypoxia. GLUT-1, LAT-1, TfR were significantly upregulated on transcriptomic level in cells cultured under hypoxic conditions. The upregulation of TfR and GLUT-1 could be confirmed at protein level, which translated into a higher glucose uptake. The effects of hypoxia on the endothelial cells were found to be HIF-dependent as stabilizing HIF-1 α under normoxic condition with 10 μ M deferoxamine (DFO) revealed similar effects on BCECs.

Conclusion: In conclusion, BCECs develop monolayer with well-developed tight junctional complexes under hypoxic conditions, and increase expression of GLUT-1, LAT-1 and TfR. This correlates with the evidences suggesting that the BBB function of the brain capillaries is present at the early stages of brain vasculature development, where the brain environment is hypoxic.

Dongik Park

Aarhus University, Denmark

Mechanisms of sortilin in regulation of emotion and memory

D. Park^{1,2}, U. Bølcho^{1,2}, C.W. Turck³, A. Nykjær^{1,2}

¹ Danish Research Institute of Translational Neuroscience, Department of Biomedicine, Aarhus University, Denmark

² The Danish National Research Foundation Center, PROMEMO, Department of Biomedicine, Aarhus University, Denmark

³ Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Germany

Mental and cognitive disorders are one of the main leading causes of disability with high lifetime estimate prevalence of 20% in Europe. While the major culprit of the illnesses has been shown to be dysregulated neurodevelopmental process during gestation and early postnatal stage, their etiologies are still poorly understood precluding targeted development of efficient therapeutics.

Accumulating evidence has suggested that Vps10p domain receptor family is strongly associated with diverse mental and cognitive disorders. The receptors control synaptic trafficking processes and regulate critical functions such as neuronal development, wiring, and synaptic plasticity. Particularly, sortilin, the archetype receptor governs neuronal cell fate and synaptic strength in interaction with several neurotrophic factors and receptors. To investigate the roles of sortilin in regulation of molecular pathways and behaviors related to emotion and memory, we employed interdisciplinary approaches including electrophysiology, quantitative (phospho)proteomics, metabolomics, and mouse behaviors. We found that sortilin deficiency and K269E mutation significantly altered glutamatergic, mitochondrial, and ubiquitin-proteasome pathways in mouse hippocampus. In addition, hippocampal long-term potentiation was impaired in both groups. In line with this, sortilin deficient and K269E mutation carrying mice exhibited significantly impaired emotion and cognition-related behaviors. Our study suggests that sortilin-associated molecular pathways may have a crucial role in regulation of emotion and memory, thus providing important insight into the mode of action of sortilin in psychiatric and neurodevelopmental disorders.

Elena Perenthaler

Erasmus MC – University Medical Center, Rotterdam, The Netherlands

Characterization of the functional enhancers in human neural stem cells

Elena Perenthaler, Soheil Yousefi, Ruizhi Deng, Anita Nikoncuk, Tahsin Stefan Barakat

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

The development of the cerebral cortex is a complex and dynamic process. Alterations at any stage can result in a wide range of neurodevelopmental disorders (NDDs), that are a common cause of developmental delay, intellectual disability, and epilepsy. Exome sequencing greatly increased the diagnostic yield of genetic forms of NDDs, allowing the identification of variations in hundreds of genes. Nevertheless, many cases remain genetically unexplained, hinting at variations in the non-coding genome. Among these non-coding regions are the understudied enhancers, cis-acting elements that control gene-expression in a temporal and tissue-specific manner during many key-developmental processes.

Here, we combined analysis of transcription factor binding sites, histone modifications (ChIPseq) and open chromatin regions (ATACseq) with the massively parallel reporter assay ChIP-STARR-seq to identify the subset of functional enhancers in human neural stem cells, an in vitro model reflecting early brain development. This led to a genome-wide, quantitative map of enhancer activity that can be of relevance for neurodevelopmental disorders.

Enrico Castroflorio

Harwell Institute, Harwell Campus, Oxfordshire, United Kingdom

The Ncoa7 locus regulates V-ATPase formation and function, neurodevelopment and behaviour.

E.Castroflorio¹, J.den Hoed², D.Svistunova², M.J.Finelli², A.Cebrian-Serrano³,
S.Corrochano^{1,4}, A.R.Bassett⁵, B.Davies² and P.L.Oliver²

¹ MRC Harwell Institute, Harwell Campus, Oxfordshire, OX11 0RD, UK.

² Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford, OX1 3PT, UK.

³ Wellcome Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK.

⁴ Present Address: Hospital Clinico San Carlos, Instituto de Investigación Sanitaria San Carlos, Calle del Prod Martín Lagos 9/n, 28040, Madrid, Spain.

⁵ Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SA, UK.

Members of the Tre2/Bub2/Cdc16 (TBC), lysin motif (LysM), domain catalytic (TLDc) protein family are associated with multiple neurodevelopmental disorders, although their exact roles in disease remain unclear. For example, nuclear receptor coactivator 7 (NCOA7) has been associated with autism, although almost nothing is known regarding the mode-of-action of this TLDc protein in the nervous system. Here we investigated the molecular function of NCOA7 in neurons and generated a novel mouse model to determine the consequences of deleting this locus in vivo. We show that NCOA7 interacts with the cytoplasmic domain of the vacuolar (V)-ATPase in the brain and demonstrate that this protein is required for normal assembly and activity of this critical proton pump. Neurons lacking Ncoa7 exhibit altered development alongside defective lysosomal formation and function; accordingly, Ncoa7 deletion animals exhibited abnormal neuronal patterning defects and a reduced expression of lysosomal markers. Furthermore, behavioural assessment revealed anxiety and social defects in mice lacking Ncoa7. In summary, we demonstrate that NCOA7 is an important V-ATPase regulatory protein in the brain, modulating lysosomal function, neuronal connectivity and behavior; thus our study reveals a molecular mechanism controlling endolysosomal homeostasis that is essential for neurodevelopment.

Eva Medico Salsench

Erasmus MC University Medical Center, Rotterdam, The Netherlands

Expanding the mutational landscape and clinical phenotype of the *YIF1B* related brain disorder

Eva Medico Salsench¹, Reza Maroofian², Ruizhi Deng¹, Kristina Lanko¹, Belén Pérez Gonzalez³, Obdulia Sanchez-Lijarcio³, Salvador Ibáñez-Mico⁴, Antonina Wojcik⁵, Marcello Vargas⁵, Audrey Schroeder⁶, Chin-To Fong⁷, Amber Begtrup⁸, Ibrahim H Kaya⁹, Mohammad AlMuhaizea^{9,10}, Dilek Colak¹¹, Henry Houlden², Robert-Jan Galjaard¹, Namik Kaya¹², Tahsin Stefan Barakat¹,

¹Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, The Netherlands. ²Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, United Kingdom. ³Centro de Diagnóstico de Enfermedades Moleculares, Centro de Biología Molecular. Universidad Autónoma de Madrid. CIBER Enfermedades Raras. IdiPAZ, Madrid, Spain. ⁴Pediatric Neurology Unit, Arrixaca University Hospital, Murcia, Spain. ⁵Gillette Children's Specialty Healthcare, St. Paul, USA. ⁶Division of Medical Genetics, University of Rochester Medical Center, Rochester, USA. ⁷Departments of Pediatrics and of Medicine, University of Rochester Medical Center, Rochester, USA. ⁸GeneDx, Gaithersburg, USA. ⁹College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia. ¹⁰Department of Neurosciences, King Faisal Specialist Hospital and Research Centre (KFSHRC), Riyadh, Kingdom of Saudi Arabia. ¹¹Department of Biostatistics, Epidemiology and Scientific Computing, KFSHRC, Riyadh, Kingdom of Saudi Arabia. ¹²Department of Genetics, King Faisal Specialist Hospital and Research Centre, Riyadh, Kingdom of Saudi Arabia.

Intracellular proteins involved in mediating vesicular trafficking in eukaryotic cells have been implicated in brain disorders, showing the relevance of the process for neuronal development in human. *YIF1B* is an essential protein involved in the anterograde trafficking from the endoplasmic reticulum to the cell membrane, and in Golgi apparatus architecture. We recently described a neurodevelopmental disorder caused by recessive variants in *YIF1B*, which has now been recognized by OMIM as Kaya-Barakat-Masson syndrome (KABAMAS, OMIM# 619125). So far, our study (Almuhaizea *et al.*) and that of Diaz *et al.* reported 16 affected individuals from 11 independent families. These individuals presented with a progressive encephalopathy with various degrees of movement disorders, microcephaly, and epilepsy. In all but one family, bi-allelic protein truncating variants were identified in *YIF1B*, with only a single bi-allelic missense mutation assumed to be causative. Here, we describe 6 additional individuals from 6 families harboring protein altering variants in *YIF1B*, 4 of which are homozygous or compound heterozygous missense variants. Interestingly, all *YIF1B* missense variants encountered localized in, or close to, the transmembrane domains, which were previously shown to be essential for *YIF1B* function. To investigate the function of these missense variants, we performed site-directed mutagenesis followed by expression and interaction studies, providing functional evidence from *in vitro* studies that these missense variants impact on *YIF1B* function. In addition, we compare the clinical phenotype between all currently known *YIF1B* cases to further delineate the mutational landscape and the clinical phenotype associated with this new disease entity.

Helena Ferreira

University of Coimbra, Portugal

Longitudinal study of ultrasonic vocalizations in mouse model of autism spectrum disorder.

H. Ferreira¹, H. Ferreira^{2,3}, S. Santos¹, J. Martins^{2,3}, M. Castelo-Branco^{2,3*}, J. Gonçalves^{2,3*}

¹University of Coimbra, Faculty of Medicine, Master's in biomedical research, Portugal

²University of Coimbra, Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), Portugal

³University of Coimbra, Institute of Nuclear Sciences Applied to Health (ICNAS), Portugal

*These authors share senior authorship.

Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting 1 in 59 children. It is characterized by deficits in social interaction and by stereotyped behaviours. Moreover, impaired communication is a hallmark of ASD. Another feature of ASD is the prevalence of disease in males, being necessary to unravel the potential manifestation dimorphisms that may translate the female protection to the disease. Since the onset of ASD symptoms occurs at a young age in the individual, it becomes necessary to explore developmental, cognitive and social impairments since an earlier stage to disclose effect of biological sex on ASD-like behaviour.

In order to explore a possible influence of sex in the link between deficiencies in communication, development, social impairment and repetitive behaviours, we used an established model for the study of ASD, the mouse model of *Tsc2*^{+/-}. Here, we investigated male and female mice in a longitudinal study by assessing pup developmental milestones as well as social and repetitive, stereotyped behaviours in juveniles, paired with USVs analysis throughout.

We found an overall delay in milestone development in females, as well as a genotype-dependent evolution of sensory and motor skills, and a delay in posture and coordination in *Tsc2*^{+/-} animals. Additionally, we found a less complex vocal repertoire in *Tsc2*^{+/-} females during neonatal development, which is carried through juvenile age. Moreover, *Tsc2*^{+/-} presented a more repetitive, stereotyped behaviour, paired with a less complex vocal repertoire at this stage. Interestingly, we also found that while social tasks elicit communication differences between *Tsc2*^{+/-} and WT females, repetitive tasks elicit communication differences between *Tsc2*^{+/-} and WT males.

These results uncover a link between behaviour, communication and development, and suggest the use of USVs in neonatal periods as biomarkers for social abnormalities and stereotyped behaviours at later ages.

Hussein Ghazale

Sunnbryook Research Institute and University of Toronto, Canada

Neurogenin 2/Mbt1 Coupling Regulates Cortical Neurogenesis: An Early Vital Event in Neocortical Development

Sisu Han^{1,2*}, Hussein Ghazale^{1,2*}, Grey Wilkinson³, Saiqun Li³, Yaroslav Ilnytskyi⁴, Lata Adnani^{1,3}, Rajiv Dixit^{1,2}, Dawn Zinyk^{1,2}, Jeff Biernaskie⁵, Igor Kovalchuk⁴, Carol Schuurmans^{1,2,3}

¹Sunnbryook Research Institute, Toronto, ON, Canada

²Department of Biochemistry, University of Toronto, Toronto ON, Canada

³Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada

⁴Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada.

⁵Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.

*Co-authors

Abstract: During fetal brain development, neural progenitor cells (NPCs) in the dorsal telencephalon generate excitatory projection neurons that populate the six layers of the neocortex in a sequential inside-out fashion. The proneural gene *Neurog2*, which encodes a basic-helix-loop-helix (bHLH) transcription factor, is a key intrinsic determinant of a glutamatergic projection neuron identity. However, *Neurog2* proneural activity is temporally regulated, as this transcription factor is only necessary and sufficient to promote the differentiation of early-born, deep-layer and not, later-born, upper-layer neurons. We performed a TAP-tagging experiment to identify interacting partners that might control *Neurog2* proneural activity, and identified polycomb associated protein, Mbt1 (lethal(3) malignant brain tumor-like protein; L3mbtl3) as a *Neurog2* binding partner. In vivo gain-of-function experiments revealed that Mbt1 suppresses neurogenesis in early cortical NPCs. Conversely, loss-of-function studies performed in *Mbt1*^{-/-} mutant mice revealed an expansion of early-born, deep layer neurons and a reduction of upper layer neurons, along with a significant expansion in the number of cortical NPCs. Our data thus suggests that Mbt1 may suppress *Neurog2* mediated deep layer cortical neurogenesis by recruiting polycomb repressive complexes to a specific subset of *Neurog2* target genes. Understanding how Mbt1 regulates *Neurog2* proneural activity could be important in understanding how development goes awry in neurodevelopmental disorders, such as autism spectrum disorder, which is associated with increased neurogenesis.

Isabella Herman

Baylor College of Medicine, United States

Quantitative dissection of multilocus pathogenic variation in neurodevelopmental disorders.

I. Herman^{1,2,3}, A. Jolly^{2,4}, H. Du², M. Dawood^{2,4,5}, G.M.H. Abdel-Salam⁶, D. Marafi^{2,7}, T. Mitani², D.G. Calame^{1,2,3}, Z. Coban-Akdemir^{2,8}, J.M. Fatih², I. Hegazy⁶, S.N. Jhangiani^{2,5}, R.A. Gibbs^{2,5}, D. Pehlivan^{1,2,3}, J.E. Posey², and J.R. Lupski^{2,3,5,9}

¹Section of Pediatric Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, 77030, USA - ²Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, 77030, USA - ³Texas Children's Hospital, Houston, Texas, 77030, USA - ⁴Medical Scientist Training Program, Baylor College of Medicine, Houston, TX, 77030, USA - ⁵Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, 77030, USA - ⁶Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt - ⁷Department of Pediatrics, Faculty of Medicine, Kuwait University, P.O. Box 24923, 13110 Safat, Kuwait - ⁸Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas, USA - ⁹Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030

Objective: Genomic sequencing and clinical genomics have demonstrated that substantial subsets of atypical and/or severe disease presentations result from multilocus pathogenic variation (MPV) causing blended phenotypes. Using the Human Phenotype Ontology (HPO), we quantitatively dissected the blended phenotype of an infant with severe neurodevelopmental disorder, brain malformation, dysmorphism, and hypotonia.

Methods: Family-based exome sequencing (ES) with rare variant analysis was completed. HPO analysis with semantic similarity was implemented to determine phenotypic contribution of each implicated gene.

Results: ES revealed deleterious variants in *CAPN3* (c.259C>G:p.L87V), *MUSK* (c.1781C>T:p.A594V), *NAV2* (c.1996G>A:p.G666R), and *ZC4H2* (c.595A>C:p.N199H). *CAPN3*, *MUSK*, and *ZC4H2* are established disease genes linked to limb-girdle muscular dystrophy (OMIM# 253600), congenital myasthenia (OMIM# 616325), and Wieacker-Wolff syndrome (OMIM# 314580), respectively. *NAV2* is a retinoic-acid responsive novel gene candidate with biological roles in neurite outgrowth and cerebellar dysgenesis in mouse models. Using semantic similarity, we show quantitatively that no gene individually explains the proband phenotype but rather the totality of the clinically observed disease is most parsimoniously explained by disease-contributing effects of all four genes. These data reveal that the combination of variants results in a blended phenotype with each gene affecting a different part of the nervous system and nervous system-muscle connection.

Conclusions: In patients with MPV and complex blended phenotypes resulting from multiple molecular diagnoses, HPO analysis allows for dissection of phenotypic contribution of both established disease genes and novel gene candidates not yet proven to cause human disease with marked implications for prognosis, treatment, and family counseling for the most complex genetic patients.

Jelena Radulovic

Department of Biomedicine, Aarhus University, Denmark

Stress-related memories disrupt sociability and associated patterning of hippocampal activity: a role of hilar oxytocin receptor-positive interneurons.

Mariah A A Meyer¹, Max Anstötz², Lynn Y Ren¹, Michael P Fiske², Samantha L Schroth², Ana Cicvaric¹, Katsuhiko Nishimori³, Gianmaria Maccaferri², Jelena Radulovic^{1,4,5}

¹Department of Psychiatry and Behavioral Sciences, Northwestern University, Feinberg School of Medicine, Chicago, IL, 60611, USA

²Department of Physiology, Northwestern University, Feinberg School of Medicine, Chicago, IL, 60611, USA.

³Department of Obesity and Internal Inflammation, Fukushima Medical University, Fukushima, 960-1295, Japan.

⁴Department of Biomedicine, Aarhus University, Aarhus, Denmark

⁵Department of Neuroscience and Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, The Bronx, NY, 10461, USA.

In susceptible individuals, memories of stressful experiences can give rise to debilitating socio-affective symptoms. This occurs even when the ability to retrieve such memories is limited, as seen in patients suffering from traumatic amnesia. We therefore hypothesized that the encoding, rather than retrieval, mechanisms of stress-related memories underlie their impact on social and emotional behavior. To test this hypothesis, we used combinations of stress-enhanced and state-dependent fear conditioning, which engage different encoding mechanisms for the formation of stress-related memories. We found that the encoding of stress-enhanced state-dependent memories robustly and sex specifically impairs sociability in male mice and disrupts the asymmetry of dentate gyrus (DG)/CA3 activity accompanying social interactions. These deficits were restored by chemogenetic inactivation of oxytocin receptor-positive interneurons localized in the hilus (Oxtr-HI), and by inactivation of dorsohippocampal efferents to the caudal lateral septum. Together, our data suggest that disrupted patterning of dorsohippocampal DG/CA3 activity underlies stress-induced sociability deficits, and that Oxtr-HI can be a cellular target for improving these deficits.

Joshi V. Shrilaxmi

Jawaharlal Nehru Centre for Advanced Scientific Research, India

Microtubule-based abnormalities due to variants in *EFHC2*, a gene implicated in Juvenile Myoclonic Epilepsy.

Shrilaxmi Joshi V¹, Praveen Raju K¹, Parthasarthy Satishchandra³, Sanjib Sinha³, and Anuranjan Anand^{1,2}

¹Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, India

²Neuroscience Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, India

³Department of Neurology, NIMHANS, India

Juvenile myoclonic epilepsy (JME) is a neurological disorder characterized by frequent myoclonic seizures often accompanied by generalized tonic-clonic seizures during adolescence. We present, our work on contribution of the *EFHC2* gene to JME. *EFHC2*, a gene on the X-chromosome encodes a protein containing three DM10 domains and a putative EF-hand motif. During cell division, it localizes to the spindle poles and midbody in cultured mammalian cells. We have identified seven potentially pathogenic *EFHC2* variants present almost exclusively among 500 JME patients. When expressed in cultured cells, *EFHC2* harboring these variants lead to significant levels of spindle abnormalities, multi-polarity, and chromosome segregation defects. We observe that *EFHC2* co-precipitates with α -tubulin and γ -tubulin suggesting that it is a microtubule-associated protein and may have a role in microtubule organization. We examine the contribution of the DM10 domains to *EFHC2* subcellular localization and function. *EFHC2* is expressed in the mammalian brain regions: cerebral cortex, hippocampus, hypothalamus, and cerebellum.

Julie Donskov

Aarhus University, Denmark

Cholesterol signaling in mental illness—from genetic risk to psychopathology.

J. Donskov^{1,4,5,6}, M. Denham^{1,2}, M. Ernst³, A. Børglum^{1,4,5,6} and P. Qvist^{1,4,5,6}

¹Department of Biomedicine, Aarhus University

²Danish Research Institute of Translational Neuroscience (DANDRITE), Nordic EMBL Partnership for Molecular

Medicine, Aarhus University

³Section for Clinical Mass Spectrometry, Danish Center for Neonatal Screening, Department of Congenital Disorders,

Statens Serum Institut, Copenhagen

⁴iPSYCH, The Lundbeck Foundation Initiative for Integrative Psychiatric Research, Aarhus

⁵Centre for Integrative Sequencing, iSEQ, Aarhus University

⁶Center for Genomics and Personalized Medicine, Aarhus University

Psychiatric disorders comprise a heterogeneous group of disabling conditions collectively characterized by abnormal patterns of feelings, thoughts, and behaviors. Their phenotypes are shaped by a concerted interplay between hereditary risk and adverse exposures, and overlap in their clinical and therapeutic profiles suggests that their etiologies are interconnected. However, limited insight into the biological mechanisms underlying mental illness hampers the development of improved diagnostic tools and treatment. Identification of core molecular pathways is thus paramount for progress in the management of mental illness.

Several findings implicate cholesterol signaling in the pathoetiology of mental illness: cholesterol and steroidogenic imbalances are common; pharmacological targeting of cholesterol signaling has clinical efficacy in treatment; epidemiological risk factors (e.g. vitamin D deficiency, stress, and sex) involve a steroid response; and steroid-activated nuclear receptors are dysregulated in postmortem brain-tissue from patients. In addition, there is unacknowledged genetic support for cholesterol signaling through nuclear receptors as a central pathway in mental illness.

Particularly, a significant proportion (~1/10) of risk loci identified across diagnostic entities harbors genes encoding nuclear receptor co-regulators that direct the cellular specificity and genetic risk-enriched genomic action of steroids.

Collectively, nuclear receptor-mediated signaling appears uniquely suited to bridge environmental and genetic risk in psychopathology, and the genetic burden on key modulators along with associated molecular pathways may determine the vulnerability and clinical outcome in response to epidemiological risk in mental illness.

Through interlinked studies of unique clinical, pre-clinical, and non-clinical samples, I aim to uncover the biology and clinical implication of brain cholesterol signaling in mental health and provide a framework for assessing and targeting nuclear receptor biology profiling in precision medicine.

Kristina Lanko

Erasmus MC University Medical Center, Rotterdam, The Netherlands

Delineating the molecular and phenotypic spectrum of the SETD1B-related syndrome.

M. J.A. Weerts¹, K. Lanko¹, F. J. Guzmán-Vega², A. Jackson³,... (94 others)...., S.T.Arold^{2,4}, T.S.Barakat^{1,#}

¹ Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, The Netherlands

² King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Division of Biological and Environmental Sciences and Engineering (BESE), Thuwal, 23955-6900, Saudi Arabia

³ Manchester Centre for Genomic Medicine, St. Mary's Hospital, Oxford Road, Manchester , M13 9WL. U.K.

⁴ Centre de Biochimie Structurale, CNRS, INSERM, Université de Montpellier, 34090 Montpellier, France

***SETD1B* encodes a lysine-specific histone methyltransferase that methylates histone H3 at position lysine-4 (H3K4me1, H3K4me2, H3K4me3) and thereby is involved in the regulation of gene expression. Pathogenic variants in *SETD1B* have been associated with a syndromic neurodevelopmental disorder including intellectual disability, language delay and seizures. To date, clinical features have been described for eleven patients with (likely) pathogenic *SETD1B* sequence variants. We perform an in-depth clinical characterization of a cohort of 36 unpublished individuals with *SETD1B* sequence variants, describing their molecular and phenotypic spectrum. By means of computational protein modelling we predict potential functional effect of *SETD1B* variants. Selected variants located in different functional domains of SETD1B were functionally tested using *in vitro* and genome-wide methylation assays, confirming *in silico* predictions.**

Our data present evidence for a loss-of-function mechanism of *SETD1B* variants, resulting in a core clinical phenotype of global developmental delay, language delay including regression, intellectual disability, autism and other behavioral issues, and variable epilepsy phenotypes. Developmental delay appeared to precede seizure onset, suggesting SETD1B dysfunction impacts physiological neurodevelopment even in the absence of epileptic activity. Interestingly, males are significantly overrepresented, and thus we speculate that sex-linked traits could affect susceptibility to clinical penetrance and the clinical spectrum of *SETD1B* variants.

Together, this work expands the phenotypic and molecular spectrum associated with *SETD1B* variants, provides insights into their functional effects and will ultimately facilitate the counseling regarding the clinical spectrum of newly diagnosed patients with the *SETD1B*-related syndrome.

Mallika Chatterjee

Amity University, India

Heparan sulfate modifications determine navigation properties of thalamocortical axons in the developing mouse forebrain.

Mallika Chatterjee^{1*}, Rana², James Y.Li^{2*}.

Affiliation: ¹Amity Institute of Neuropsychology and Neurosciences, Amity University, Noida, U.P, India, ²Department of Genetics and Genome Sciences, University of Connecticut Health Center, CT, USA. * Co-corresponding authors.

Abstract: Development of precise topographical connections between the thalamus and cortex is imperative for accurate sensory and motor functioning of the vertebrate body. Thalamocortical axons (TCAs) navigate complex territories before reaching their final cortical destinations. This complex route is designed by the intricate, context dependent function of various guidance molecule-receptor complexes like Slit-Robo, Erbb-neuregulin, Nrp2-semaphorin etc.

Of late, heparan sulfate proteoglycans (HSPGs) has been shown to be key functional interactors of signaling and axon- guidance molecules. Various post-translationally modified HSPGs have been shown to play important roles in determining corpus callosum and optic chiasm development. However, their function in determining the trajectory of forebrain projection fibers has not been yet looked into. Gbx2, a homeodomain containing transcription factor is expressed in the developing mouse thalamus. Our microarray data shows that Gbx2 regulates thalamic expression of all 3 isoforms of Hs6st -a key enzyme of the heparan sulfate synthesis pathway known to be involved in the 6 Ortho (6O) sulfation of heparan sulfate. Gbx2 loss causes significant down-regulation of expression of all 3 isoforms resulting in aberrant sulfation pattern within the mutant TCAs. Analyses of Hs6st1/2 mutants reveal significant trajectory defects with some of these mutant axons being directed ventrally towards the hypothalamus – a partial phenocopy of Gbx2 mutants. This behavior also recapitulates Slit/Robo mutant TCA defects. Using explant cultures we show that Slit/Robo interaction is indeed compromised in Hs6st1/2 mutants. The binding kinetics of Slit/Robo/HSPG in presence and absence of 6O sulfation are presently being characterized through in silico molecular dynamics simulation.

Marie Sønderstrup-Jensen

Bispebjerg-Frederiksberg Hospital, Denmark

Enrichment of gene pathways related to critical time-windows of neurodevelopment in a genetic rat model of schizophrenia-relevant features.

Marie Sønderstrup-Jensen¹, Mykhilo Batiuk², Panagiotis Mantas³, Carles Tapias-Espinosa⁴, Rasmus Rydbirk², Konstantin Khodosevich², Alberto Fernandez-Teruel⁴, and Susana Aznar¹

¹Research Laboratory for Stereology and Neuroscience, Department of Neurology, Bispebjerg-Frederiksberg Hospital, Denmark - ²Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, University of Copenhagen, Denmark - ³Department of Health Technology, Technical University of Denmark (DTU), Denmark - ⁴Department of Psychiatry and Forensic Medicine, School of Medicine, Universidad Autónoma de Barcelona, Spain

Schizophrenia is considered a neurodevelopmental disorder. The Roman High-Avoidance (RHA-I) inbred rat strain is a suitable model for investigating the neurobiology behind schizophrenia-relevant traits. These animals display behaviours and brain alterations associated with schizophrenia vulnerability when compared to their counterpart, the Roman Low-Avoidance (RLA-I) rat strain. Recent evidence suggests a more immature state of the frontal cortex (FC) in the RHA-I. We want to further explore this by performing a transcriptomic analysis and look for enrichment of gene pathways related to neurodevelopment in the RHA-I. FC samples from 8 RHA-I and 8 RLA-I were sequenced on an Illumina NextSeq 500 and differential expression analysis performed, resulting in 223 significantly differentially expressed genes. There was a significant overrepresentation of genes involved in synaptic function. Next, to identify gene sets related to critical time-windows (TWs) of neurodevelopment, we used the opensource BrainCloud® transcriptomic dataset. It consists of 269 samples from prefrontal cortex of healthy humans throughout ageing. 227 of these samples were included in a weighted gene co-expression analysis, from which 32 functional gene modules were identified. To determine which of these were differentially regulated during critical TWs of neurodevelopment, the samples were grouped according to four TWs, the critical TWs being infancy (TW1) and childhood and early adolescence (TW2). We identified 21 functional gene modules differentially regulated during TW1 and TW2, extracted genes with a module membership >0.6, identified their RGD homolog, and created gene lists that were converted to gene sets for gene set enrichment analysis. Five gene sets were significantly enriched in the RHA-I, all corresponding to functional gene modules differentially regulated during TW1 and significantly enriched for myelination, cellular stress response, mitochondria, synaptic function, and immune response. Genes of interest representative for each significant gene set will be validated by qPCR in the RHA-I and RLA-I. Our results point to gene pathways differentially regulated during neurodevelopment whose expression is shifted in the RHA-I compared to the RLA-I strain. This observation adds support to our belief that the RHA-I strain presents a halted FC maturation which may be associated with their schizophrenia-like behaviour.

Marta Garcia-Forn

Icahn School of Medicine at Mount Sinai, United States

Developmental and behavioral phenotypes in a new mouse model of DDX3X syndrome

Marta Garcia-Forn^{1,2,3,4}, Andrea Boitnott^{1,2,3,4}, Dévina C Ung^{1,2,3,4}, Kristi Niblo^{1,2,3,4}, Yeaji Park^{1,2,3,4}, Danielle Mendonca^{1,2,3,4}, Michael Flores^{1,2,3,4,5}, Sylvia Maxwell^{1,2,3,4,6}, Jacob Ellegood⁷, Lily R Qiu⁸, Dorothy E Grice^{1,2}, Jason P Lerch^{7,8,9}, Mladen-Roko Rasin¹⁰, Joseph D Buxbaum^{1,2,3,4,11}, Elodie Drapeau^{1,2}, Silvia De Rubeis^{1,2,3,4}

¹ Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ² Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ³ The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ⁴ Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ⁵ Department of Biology, New York University, College of Arts and Science, New York, NY 10003, USA. ⁶ The Bronx High School of Science, NY 10468, USA. ⁷ Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, ON M5T 3H7, Canada. ⁸ Wellcome Centre for Integrative Neuroimaging, FMRIB, Nuffield Department of Clinical Neuroscience, University of Oxford, Oxford, OX3 9DU, UK. ⁹ Department of Medical Biophysics, University of Toronto, Toronto, Ontario, ON M5T 3H7, Canada. ¹⁰ Department of Neuroscience and Cell Biology, Rutgers University, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA. ¹¹ Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

Mutations in the X-linked gene DDX3X account for ~2% of intellectual disability in females, often in co-morbidity with behavioral problems, motor deficits, and brain malformations. DDX3X encodes an RNA helicase with emerging functions in corticogenesis and synaptogenesis. Here, we present the first characterization of a Ddx3x haploinsufficient mouse (Ddx3x+/-) with construct validity for DDX3X loss-of-function mutations. Ddx3x+/- mice show physical, sensory, and motor developmental delays that evolve into behavioral anomalies in adulthood, including hyperactivity, anxiety-like behaviors, cognitive impairments, and motor deficits. Motor function further declines with age. These behavioral changes are associated with a reduction in brain volume postnatally, with some regions (e.g., cortex and amygdala) disproportionately affected. Cortical thinning is accompanied by defective cortical lamination, indicating that Ddx3x regulates the balance of glutamatergic neurons in the developing cortex. These data shed new light on the developmental mechanisms driving DDX3X syndrome and support face validity for a novel pre-clinical mouse model.

Marziyeh Jabbari

Aarhus University, Denmark

Role of hippocampal neuroinflammation in the resistance to brain infarction in a genetic animal model of depression.

M. Jabbari¹, M. Ardalani^{1,2}, V. Bay¹, A. Safi², R. Afsharpour², T. Chumak², R.A. Tasker¹, C. Malard², G. Wegener¹

¹Department of Clinical Medicine, Translational Neuropsychiatry Unit, Aarhus University, Aarhus, Denmark

²Centre for Perinatal Medicine and Health, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Background: The incidence of depression and stroke are frequently co-morbid, which may suggest an overlap of the underlying etiologies of each condition. A previous study from our group showed that a genetic animal model of depression, the Flinders Sensitive Line rats (FSL), were resistant to transient occlusion of the middle cerebral artery (MCAO) through an unknown mechanism. Thus, the main aim of this study was to examine the role of hippocampal neuroinflammation in the resistance of FSL rats to focal brain ischemia.

Method: Adult male FSL and Sprague-Dawley (SD) rats underwent MCAO surgery/sham and utilized for investigation of neuroinflammation in the hippocampus. Using Nissl, ionized calcium-binding adaptor molecule 1 (Iba1) and glial fibrillary acid protein (GFAP) staining, hippocampi volume, and volume of the inflammatory cells (microglia and astrocytes) in connection with their activity were measured applying stereological methods.

Results: We found no significant difference between ipsi and contralateral brain hemispheres volume within each group ($p > 0.05$). The volume of hippocampal astrocytes also did not significantly differ between ipsi and contralateral hemispheres within each group of rats ($p > 0.05$). Interestingly, we found that the microglia volume was significantly bigger (as an indicator of activation) in the ipsilateral hippocampus than the contralateral one in SD.MCAO group ($p = 0.001$), while the size of microglia was not different between ipsi and contralateral hippocampi within FSL.MCAO rats ($p > 0.05$).

Conclusion: Our results suggest microglia activation as one of the possible cellular mechanisms underlying FSL rats' resistance to an infarction and may shed light on pharmacological targeting of microglial activation to better understand the relationship between depression and stroke.

Meike van Der Heijden

Baylor College of Medicine, Houston, Texas, USA

Maturation of Purkinje Cell Firing Properties Relies on Neurogenesis of Excitatory Neurons

M.E. van der Heijden^{1,5}, E.P. Lackey^{1,2,5}, R. Perez⁵, F.S. İşleyen^{1,3}, A.M. Brown^{1,2,5}, T Lin^{1,5}, H.Y. Zoghbi^{2,3,5,6} and R.V. Sillitoe^{1,2,3,4,5*}

¹Department of Pathology & Immunology, Baylor College of Medicine, Houston, Texas, USA

²Department of Neuroscience, Baylor College of Medicine, Houston, Texas, USA

³Program in Developmental Biology, Baylor College of Medicine, Houston, Texas, USA

⁴Development, Disease Models & Therapeutics Graduate Program, Baylor College of Medicine, Houston, Texas, USA

⁵Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, Texas, 77030, USA

⁶Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA

Preterm infants have a higher likelihood for suffering insults to the cerebellum during a period of highly dynamic cerebellar development and often develop motor disorders and cognitive difficulty as a result of the insult. This dynamic period is hallmarked by exponential neurogenesis of excitatory granule cells that drives cerebellar size expansion, cellular lamination, and foliation. At the cellular circuit level, the dynamic period is characterized by reorganization of excitatory inputs onto Purkinje cells, which integrates all incoming excitatory signals and form the sole output cell of the cerebellar cortex. Despite evidence suggesting that the cerebellar is exceptionally vulnerable to insults during the dynamic period, it is unclear how the anatomical changes influence cerebellar function and whether they are dependent on the integration of the late born granule cells. Here, we tracked the structural and functional changes in Purkinje cells, as a read-out of cerebellar function, in control mice and mice lacking granule cell neurogenesis. We focused our studies on P7-P14 mice as this is the developmental equivalent in mice of dynamic cerebellar changes that occur in third trimester infants. We reveal that structural reorganization of excitatory inputs is paired with a switch in *in vivo* Purkinje cell firing properties from slow and irregular firing patterns in P7-P10 cells to fast and rhythmic, yet intermittent, firing rates in P11-P14 cells. Interestingly, when we used intersectional genetics to prevent integration of granule cells into the Purkinje cell microcircuit, we found that Purkinje cells that do not undergo the anatomical reorganization of excitatory inputs and also fail to make the developmental switch in firing patterns. Specifically, Purkinje cells recording in P14 mice lacking late born granule cells have firing patterns that are indistinguishable from P7-P10 control Purkinje cells. Finally, we show that mice without excitatory cerebellar neurons have impaired motor behaviors and vocal skills that mice usually acquire during the period of dynamic development. These data argue that late born excitatory cerebellar neurons set the maturation time window for Purkinje cell function that is necessary to refine cerebellar-dependent behaviors.

Mette Q Ludwig

Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark

A genetic map of the dorsal vagal complex and its role in obesity in rodents.

Mette Q Ludwig¹, Wenwen Cheng², Desiree Gordian², Julie Lee³, Sarah J Paulsen⁴, Stine N Hansen⁴, Kristoffer L Egerod¹, Pernille Barkholt⁵, Christopher J Rhodes⁶, Anna Secher⁴, Lotte Bjerre Knudsen⁴, Charles Pyke⁴, Martin G Myers, Jr² and Tune H Pers¹

¹ Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

² Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48105, USA

³ Novo Nordisk Foundation Center for Stem Cell Biology, University of Copenhagen, Copenhagen, Denmark

⁴ Global Drug Discovery, Novo Nordisk A/S, Måløv, Denmark.

⁵ Gubra, Hørsholm, Denmark

⁶ Research and Early Development, Cardiovascular, Renal and Metabolic Diseases, BioPharmaceuticals R&D, AstraZeneca Ltd, Gaithersburg, Maryland, USA

The brainstem dorsal vagal complex (DVC) is known to regulate energy balance and is the target of appetite suppressing hormones, such as glucagon-like peptide-1 (GLP-1). Here we provide a comprehensive genetic map of the DVC and identify neuronal populations that control feeding. Combining bulk and single-nucleus gene expression and chromatin profiling of DVC cells, we reveal 25 neuronal populations with unique transcriptional and chromatin accessibility landscapes and peptide receptor expression profiles. GLP-1 receptor (GLP-1R) agonist administration induces gene expression alterations specific to two distinct sets of *Glp1r* neurons – one population in the area postrema (AP) and one in the nucleus of the solitary tract (NTS) that also expresses calcitonin receptor (*Calcr*). Transcripts and regions of accessible chromatin linked to obesity-associated genetic variants are enriched in AP and NTS neurons that express *Glp1r* and/or *Calcr*, and activation of several of these neuronal populations decreases feeding in rodents. Thus, DVC neuronal populations associated with obesity predisposition suppress feeding and may represent targets for therapy of obesity.

Michael J. Courtney

University of Turku and Åbo Academy University, Turku, Finland

A high-throughput pipeline for dynamic single-cell biology applied to model the impact of SynGAP1 deficiency on neurons in synaptically-connected networks.

L.-L. Li^{1,2} and M.J. Courtney^{1,2}

¹Neuronal Signalling Lab, Turku Bioscience Centre, University of Turku and Åbo Academy University, Turku, Finland.

²Turku Screening Unit, University of Turku and Åbo Academy University, Turku, Finland.

SynGAP1 is associated with a range of neurodevelopmental and other neurological disorders, most notably SynGAP syndrome, a non-syndromic intellectual disability disorder. SynGAP1 constrains Ras-family activation of the ERK pathway that regulates neurotransmitter receptor traffic and synaptic plasticity. Normal levels of connectivity ensure functionality of synaptic networks while ensuring inputs are sufficiently limited to prevent epileptic activity. Epilepsies are among the symptoms of SynGAP syndrome, presumably as a result of excess Ras-ERK signaling. Therefore, strategies to limit Ras-ERK signaling may have potential for treating SynGAP syndrome and approved drugs such as statins have been used off-label for treating conditions associated with epilepsy including SynGAP syndrome.

The identification of compounds and druggable targets with potential for neurodevelopmental disorders such as these would benefit from more physiologically relevant high-throughput readouts. To this end, we developed an automated cell physiology pipeline. This records single-cell dynamics, of at least 3 to 4 pathways simultaneously, in developing neural circuits cultured in 384 well plates delivered from an automated incubator while cells are stimulated by chemical or optogenetic means. Pathways are monitored by spectrally separable panels of reporters. These are delivered by AAV vectors and expressed under control of cell-specific promoters. Image data are processed to extract pathway dynamics together with spatial features. Genuine single cell trajectories are further analysed and visualised to reveal neuronal subpopulations with distinct signaling circuit behaviours, for which the relationships between measured parameters is determined.

We first validated this pipeline with neural cultures exposed to standard neuron-activating stimuli. We confirmed the ability of the pipeline to measure impact of SynGAP1 knockdown on neuronal ERK signaling. Subsequently, we determined the performance of the pathway in quantifying the impact of selected statins in conjunction with the absence and presence of elevated synaptic activity. Finally, we used the pipeline to evaluate the hypothesis that statins might normalise neuronal function perturbed by SynGAP knockdown. We conclude that this automated cell physiology pipeline has the potential to efficiently identify distinct subpopulation behaviours, evaluate the effects of small molecules and genetic knockdowns and may have potential for developing advanced phenotypic screens for neurodevelopmental disorders.

Mie Kristensen

University of Copenhagen, Denmark

Cell-penetrating peptide-mediated modulation of the blood-brain barrier integrity for brain drug delivery.

Patrick Frøslev¹, Emma Lisa Al Humaidan^{*1}, Sidse Lund Pedersen^{*1}, Burak Ozgür¹, Birger Brodin¹, Mie Kristensen¹

¹*Faculty of Health and Medical Sciences, Department of Pharmacy, University of Copenhagen, Denmark*

^{*}*Authors contributed equally*

RATIONALE: Cell-penetrating peptides efficiently deliver various cargos into cells. They may also serve as shuttles for delivery of brain therapeutics across the blood-brain barrier (BBB). One example is NR2B9c; which relieves neuronal damage after ischemic stroke¹. NR2B9c is conjugated to the cell-penetrating peptide Tat² to facilitate BBB permeation and internalization into neurons. Recently, we demonstrated that Tat and Tat-NR2B9c permeates the BBB in live mice, and that Tat and Tat-NR2B9c affect the BBB integrity *in vitro*³. Thus, Tat-mediated drug delivery across BBB may take place through the paracellular space in addition to transcytosis as earlier suggested⁴.

With this study we investigate the mechanism of cell uptake and effects on BBB integrity when exploiting cell-penetrating peptides, namely Tat (YGRKKRRQRRR) and penetratin⁵ (RQIKIWFQNRRMKWKK), as shuttles for brain drug delivery.

METHODS: Tat and penetratin were labelled with the fluorophore TAMRA for visualization and quantification purposes. A human induced pluripotent stem cell-based BBB model (BIONi-010C⁶) was used to evaluate the barrier interacting and permeating potential of Tat and penetratin. Effects on the barrier integrity was monitored in real time using a cellZscope device, and the underlying mechanism of cell uptake was studied using live cell microscopy.

RESULTS: Tat and penetratin efficiently adhered to the plasma membrane and internalized into monolayers of BIONi-010C cells. Tat, but not penetratin, permeated the barrier. Live cell microscopy revealed that endocytosis drove the cell uptake of Tat, whereas penetratin appeared to directly translocate across the plasma membrane. Both Tat and penetratin affected the barrier integrity in a reversible manner, which lead to a marked increase in barrier permeation of the paracellular marker mannitol (182 Da) upon co-incubation. In contrast, no effect on barrier permeation was observed for the transcellular marker propranolol (259 Da) upon co-incubation with Tat or penetratin.

CONCLUSION: We have demonstrated that the cell-penetrating peptides Tat and penetratin modulate the BBB integrity, which may be exploited for brain delivery of small hydrophilic drugs as well as for delivery into brain endothelial cells.

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Mikhail Paveliev

DANDRITE, Aarhus University, Aarhus, Denmark

GRASP imaging of auditory inputs to the basolateral amygdala.

Mikhail Paveliev¹, Jinhyun Kim², Sadegh Nabavi¹, Anders Nykjær¹

¹DANDRITE, Aarhus University, Aarhus, Denmark

²Korea Institute of Science and Technology, Seoul, Korea

Wiring of the brain synaptic networks determines adaptive behavior of animals and humans and undergoes pathologic changes in a range of psychiatric and neurologic diseases. Studies of the brain functional connectivity at the level of single synapses is complicated by the fact that synaptic inputs from multiple sources are often intermingled at target neurons. Basolateral amygdala represents an important example of such kind as it integrates sensory inputs of several modalities as well as multiple inputs from regulatory networks to govern the emotional associative memory. In the present study we aimed at identifying the synaptic contacts comprising the auditory input to the basolateral amygdala. We took advantage of the mammalian GFP Reconstruction Across Synaptic Partners (mGRASP) technique to detect synaptic inputs from the auditory cortex and medial geniculate nucleus (MGN) to the basolateral amygdala of adult mice. We report clearly visible expression of the preGRASP construct containing neurexin-1beta fragment in the auditory cortex and MGN as detected by Cerulean fluorescence following stereotaxic injection of the preGRASP-bearing adenoassociated (AAV) vector. The post-GRASP construct contained the esterase-truncated neuroligin-1 and its expression in the basolateral amygdala was visualized by the fluorescence of dimeric Tomato included within the same AAV vector via the self-cleavable 2A peptide. We further report GRASP signal with characteristic shape of synaptic puncta visualized with confocal imaging at Tomato-positive dendritic branches within the lateral amygdala. The GRASP imaging of auditory synaptic inputs reported here can be used for further studies of the auditory signal processing within the basolateral amygdala.

Mohsina Mukti

Translational Neuropsychiatry Unit, Aarhus University, Denmark

Does obesity increase susceptibility to stress? Neuropeptide Y as a possible biomarker.

Mohsina Mukti^{1,2}, Karen Johanne Pallesen³, Lise Juul³, Lone Overby Fjorback³, Michael Winterdahl¹

¹Translational Neuropsychiatry Unit, Aarhus University, Denmark

²Department of Pharmacy, University of Padova, Padova, Italy

³Danish Center for Mindfulness, Aarhus University, Denmark

Although it is known that stress can cause obesity, the actual mechanism of how central pathways mediate this effect is unclear. However, the interaction between neural circuits involved in regulating energy homeostasis and body composition with the physiological responses to stressors are both closely linked to neuropeptide Y (NPY). In an attempt to shed light on this matter, the current project investigates plasma neuropeptide Y levels in stressed individuals as a possible biomarker of resilience.

A parallel random clinical trial was conducted in 50 adults who contacted a Danish municipal health care center due to stress-related problems. The trial consisted of two intervention groups and a waitlist group. The serological, physical, and psychological parameters such as plasma NPY level, BMI and self-reported perceived stressed level (PSS), respectively, were collected.

A negative correlation between initial plasma NPY level and initial BMI (Pearson's $r = -0.363$, $p = .01$) and initial plasma NPY level and the change in plasma NPY (Pearson's $r = -0.521$, $p < .01$) during intervention/waitlist was observed.

Our findings support that plasma NPY is closely related to BMI. However, we were not able to confirm that plasma NPY was correlated to PSS or change in PSS. However, this could be due to a low number of subjects divided into several groups as well as our population consisting of both females and males. Furthermore, the self-reported PSS may not be sensitive towards all aspects of stress. An extensive study must be carried out in a larger sample to strengthen and validate our findings.

Molly Bond

Barts and the London School of Medicine and Dentistry, United Kingdom

Vitamin D levels in children and adolescents with chronic tic disorders: a multicentre study.

Molly Bond^{1*}, Natalie Moll^{2*}, Alicia Rosello^{3,4}, Rod Bond⁵, Jaana Schnell⁸, Bianka Burger⁸, Pieter J. Hoekstra⁶, Andrea Dietrich⁶, Anette Schrag⁷, Eva Kocovska¹, Davide Martino^{11,12}, Norbert Mueller⁸, Markus Schwarz², Ute-Christiane Meier^{1,10} and the EMTICS collaborative group

¹Blizard Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry - ²Institute of Laboratory Medicine, University Hospital, LMU Munich, Germany - ³Statistics, modelling and economic department, National Infection Service, PHE, London, UK - ⁴Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, UK - ⁵University of Sussex, Brighton, UK - ⁶University of Groningen, University Medical Center Groningen, Department of Child and Adolescent Psychiatry, Groningen, The Netherlands - ⁷University College London, Institute of Neurology, London, UK - ⁸Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Germany - ¹⁰Institute of Psychiatry, Psychology and Neuroscience, Kings College London - ¹¹Department of Clinical Neuroscience, Cumming School of Medicine, University of Calgary, Calgary, Canada - ¹²Hotchkiss Brain Institute, Calgary, Canada

*Joint first authors

Aim: This study investigated whether vitamin D is associated with the presence or severity of chronic tic disorders and their psychiatric comorbidities.

Methods: This cross-sectional study compared serum 25-hydroxyvitamin D [25(OH)D] (ng/ml) among three groups: children and adolescents (3-16 years) with CTD (n = 327); first-degree relatives (3-10 years) of individuals with CTD who were assessed for up to a period of 7 years for possible onset of tics (n = 31); and first-degree relatives who remained unaffected and were ≥10 years old at their last assessment (n = 93). The relationship between 25(OH)D and the presence and severity of tics, as well as comorbid obsessive-compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD), were analysed controlling for age, sex, season, centre, latitude, family relatedness and comorbidities.

Results: When comparing the CTD cohort to the unaffected cohort, the observed result was contrary to the one expected: a 10ng/ml increase in 25(OH)D was associated with higher odds of having CTD (OR 2.08, 95% CI 1.27-3.42, p<0.01). There was no association between 25(OH)D and tic severity. However, a 10ng/ml increase in 25(OH)D was associated with lower odds of having comorbid ADHD within the CTD cohort (OR 0.55, 95% CI 0.36-0.84, p=0.01) and was inversely associated with ADHD symptom severity ($\beta = -2.52$, 95% CI -4.16--0.88, p<0.01).

Conclusion: Lower vitamin D levels were not associated with a higher presence or severity of tics. However, low serum 25(OH)D may represent an underlying vulnerability for comorbid ADHD in children and adolescents with CTD.

Manireh Mansouri

Shahid Beheshti University, Tehran, Iran

Correlation between the infra-limbic neuronal plasticity and autism like behaviours in the maternal separated rats.

M. Mansouri¹, H. Pouretmad¹, M. Roghani², G. Wegener³, M. Ardalan^{3,4}

¹ Department of Cognitive Psychology, Institute for Cognitive and Brain Sciences, Shahid Beheshti University, Tehran, Iran

² Department of Psychology, Shahid Beheshti University, Tehran, Iran

³ Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

⁴ Centre for Perinatal Medicine and Health, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Introduction: Negative environmental experiences in early life play an essential role in the development of various neurodevelopmental disorders, including autism, through influencing the proper postnatal brain development. Oxytocinergic system defects have been reported in people with autism and low mother-child interactions. The positive effects of oxytocin on improving social behaviors in clinical and pre-clinical studies have also been observed. This study aimed to investigate the impact of maternal separation on autistic-like behaviors and structural changes in the brain and to assess the possible therapeutic effects of oxytocin on the above parameters.

Research method: Pups were exposed to the maternal separation from postnatal day (PND) 1 to PND14. After weaning, Intraperitoneal injections of oxytocin at a dose of 1 mg/kg (5 times from PND22 to PND30) were administered to the animals, followed by an examination of autism-related behaviors at adolescence (PND 42-50), brain structural plasticity using stereological methods.

Findings: We found that maternal separation induced autistic-like behaviors, which was associated with an increase in the infralimbic brain region volume and the number of the pyramidal neurons in the same brain region. Treatment with oxytocin improved autistic-like behaviours normalized the number of neurons and the volume of the infralimbic region ($p < 0.05$).

Conclusion: This study demonstrated the effect of maternal separation on the development of autistic-like behaviors and plasticity changes in the infralimbic area of the prefrontal cortex. In general, the present project results indicated the significant role of infralimbic in development and response to the treatment of autism and the high potential of oxytocin in modulating the related abnormalities.

Mykhailo Y. Batiuk

BRIC, University of Copenhagen, Denmark

Selective vulnerability of supragranular layer neurons in schizophrenia.

Mykhailo Y. Batiuk^{1*}, Teadora Tyler^{2*}, Shenglin Mei³, Rasmus Rydbirk¹, Viktor Petukhov¹, Dora Sedmak⁴, Erzsebet Frank², Virginia Feher², Nikola Habek⁴, Qiwen Hu³, Anna Igolkina^{3,5}, Lilla Roszik², Ulrich Pfisterer¹, Zdravko Petanjek⁴, Istvan Adorjan^{2†}, Peter V. Kharchenko^{3†}, Konstantin Khodosevich^{1†}

¹Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

²Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary

³Department of Biomedical Informatics, Harvard Medical School, Boston, Massachusetts, 02115, USA

⁴Croatian Institute for Brain Research & Center of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb, Zagreb, Croatia

⁵St. Petersburg Polytechnical University, St. Petersburg, Russia

* authors have contributed equally

† corresponding authors

Schizophrenia is one of the most wide-spread mental brain disorders with complex and largely unknown etiology. To characterize the impact of schizophrenia at a cellular level, we performed single nucleus RNA sequencing of >170,000 neurons from the dorsolateral prefrontal cortex of patients with schizophrenia and matched controls (7 vs 11, respectively). In addition, to correlate data with cortical anatomy, >100,000 neurons were analyzed topographically by immunohistochemistry in an extended cohort of cases with schizophrenia and controls (10 vs 10). Compositional analysis of RNA sequencing data revealed reduction in relative abundance across all families of GABAergic neurons and a concomitant increase in principal neurons, which was most pronounced for supragranular subtypes (layers 2-3). Moreover, supragranular subtypes of GABAergic interneurons showed most dramatic transcriptomic changes. These results were substantiated by histological analysis, which revealed a reduction in the density of calretinin, calbindin and parvalbumin GABAergic interneurons particularly in layer 2. Common effect of schizophrenia on supragranular neuronal networks was underlined by downregulation of protein processing genes and upregulation of neuronal development/plasticity genes across supragranular subtypes of principal neurons and GABAergic interneurons. *In situ* hybridization and spatial transcriptomics further confirmed supragranular layer neuron vulnerability, revealing complexity of schizophrenia-affected cortical circuits. These point towards general network impairment within supragranular layers being a core substrate associated with schizophrenia symptomatology.

Nana Svane

University of Copenhagen, Denmark

Cryopreservation of Brain Microvascular Endothelial Cells (BMEC) derived from the human Induced Pluripotent Stem Cell line BIONi010, for *in vitro* modelling of the Blood-Brain Barrier.

N. Svane¹, B. Ozgür¹, C. Goldeman¹, L. Saaby^{1,2}, B. Brodin¹.

¹ Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

² Bioneer A/S, Hørsholm, Denmark

Introduction and aim: Brain microvascular endothelial cells (BMECs) derived from human induced pluripotent stem cells (hiPSCs) are frequently used for *in vitro* modelling of the blood-brain barrier (BBB). Differentiation of hiPSC's to BMEC's is a time demanding process as it requires complete differentiation prior to each experiment (13 days). An approach to decrease culture-time prior to an experiment, is to cryopreserve partly differentiated BMEC progenitors [1], and thaw them shortly before use. The aim of this work was to investigate the possibility of using cryopreservation/thawing of partly differentiated BIONi010-hiPSC cells as a method to generate monolayers of hiPSC-BMEC's for *in vitro* BBB modelling.

Methods: The BIONi010-hiPSC line was differentiated according to an established protocol for a similar iPSC line [2]. Transendothelial electrical resistance of BMEC monolayers and immunocytochemistry was performed two days post-thaw after culture on permeable supports and compared with non-frozen controls.

Results: Cryopreservation was initiated by resuspending partly differentiated cells in endothelial cell media supplemented with 30% FBS and 10% DMSO and transferred to cryogenic vials. The cells were placed in -80°C for 24 hours followed by prolonged storage in liquid nitrogen tank. Two days prior to experiments, cells were thawed, suspended in endothelial cell media with 10 µM Y27632 and retinoic acid and seeded on permeable supports coated with 40% collagen and 10% fibronectin in water. The cell monolayer exhibited a high barrier tightness of $5208 \pm 440 \Omega \cdot \text{cm}^2$ post-thaw regardless the length of cryopreservation (7 days to 1.5 years), as compared to $5829 \pm 354 \Omega \cdot \text{cm}^2$ (control). Cell morphology and localization of F-actin, claudin 5 and GLUT1 was assessed with immunocytochemistry. The cells exhibited monolayer formation and both claudin 5 and GLUT1 were expressed at cell-cell borders, in a pattern comparable to controls.

Conclusion and perspectives: In conclusion, partly differentiated BIONi010-derived BMEC could be cryopreserved and thawed without loss of barrier properties and with cell morphology, Claudin-5 and Glut-1 expression retained. Future studies will address functional expression of key transporters. The study indicate that the cryopreservation procedure could be an interesting approach to accelerate throughput of hiPSC-BMEC cultures for blood-brain barrier permeation experiments.

Natasha J. Anstey

University of Edinburgh, United Kingdom

Imbalance of flight-freeze responses and their cellular correlates in the *Nlgn3*^{-/-} rat model of autism.

Natasha J Anstey^{*1, 2, 3, 5}, Vijayakumar Kapgal^{*2, 5, 7}, Shashank Tiwari^{2, 5}, Thomas C Watson^{1, 2, 3}, Anna KH Toft^{1, 2, 3, 5}, Owen R Dando^{1, 2, 3, 4}, Felicity H Inkpen^{1, 2, 3}, Paul S Baxter^{1, 4}, Zrinko Kozić^{1, 2, 3}, Adam D Jackson^{1, 2, 3, 4}, Xin He^{1, 2, 3, 4}, Mohammad Sarfaraz Nawaz^{2, 5}, Aiman Kayenaat^{2, 5, 7}, Aditi Bhattacharya^{2, 5}, David JA Wyllie^{1, 2, 3, 5}, Sumantra Chattarji^{*2, 5}, Emma R Wood^{*1, 2, 3, 5}, Oliver Hardt^{*2, 5, 6}, Peter C Kind^{*1, 2, 3, 5}

*** These authors contributed equally**

1. Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, EH8 9XD, UK
2. Simons Initiative for the Developing Brain, University of Edinburgh, Edinburgh, EH8 9XD, UK
3. Patrick Wild Centre for Autism Research, University of Edinburgh, Edinburgh, EH8 9XD, UK
4. Dementia Research Institute, University of Edinburgh, Edinburgh, EH8 9XD, UK
5. Centre for Brain Development and Repair, inStem, National Centre for Biological Sciences, Bangalore, Karnataka, 560065, India
6. Department of Psychology, McGill University, Montreal, Quebec, H3A 1B1, Canada
7. The University of Transdisciplinary Health Sciences and Technology, Bangalore, Karnataka, 560065, India

Autism spectrum disorders (ASDs) and intellectual disabilities (IDs) are a complex, heterogeneous range of disorders that are poorly understood in terms of their underlying cellular and circuit pathophysiology. Single-gene mutations account for a large proportion of the genetic causes of ASD/ID, and of these, mutations in synaptic proteins have been repeatedly implicated. One such protein is the postsynaptic transmembrane protein, NEUROLIGIN-3 (NLGN3), mutations in which are highly correlative with ASDs and IDs. Fear learning is well studied in models of ASD and ID, however differences in fear response behaviours are often overlooked. Whilst examining fear in a rat model of ASD/ID lacking *Nlgn3*, we observed that have intact fear and spatial learning abilities, however they display a greater propensity to exhibit flight responses in contrast to classic freezing seen in wildtypes during fearful situations. Consequently, we examined the physiological underpinnings of this in neurons of the periaqueductal grey (PAG), a midbrain area involved in flight-or-freeze responses. In *ex vivo* slices from *Nlgn3*^{-/-} rats, dorsal PAG (dPAG) neurons showed intrinsic hyperexcitability. Further analysis of this revealed lower magnitude *in vivo* dPAG stimulation evoked flight behaviour in *Nlgn3*^{-/-} rats, indicating the functional impact of the increased cellular excitability. These physiological phenotypes may give rise to unusual expression of fear responses seen in *Nlgn3*^{-/-} rats. This study provides new insight into potential pathophysiologies leading to emotional disorders in individuals with ASD.

Navneet A. Vasistha

BRIC, University of Copenhagen, Denmark

Cell type dependent impact of maternal inflammation on cortical interneuron development.

Navneet A Vasistha¹, Matej Andelic¹, Susmita Malwade¹ and Konstantin Khodosevich¹

¹Biotech Research and Innovation Center, University of Copenhagen

Maternal immune activation (MIA) due to viral infection is described to impair sensorimotor development in the offspring and linked to neurodevelopmental disorders such as schizophrenia and autism. Such abnormalities are thought to result from the impact of maternally derived cytokines on different stages of neuronal development though the mechanistic insights have not been studied hitherto. Further, while it is known that cortical GABAergic interneurons (cINs) play an important role in neuronal network function, the impact of MIA on development of cINs is also not described.

We inject time-mated pregnant dams with Poly I:C (a viral dsRNA mimic) to induce activation of the maternal immune system at different gestational stages. Despite robust activation of maternal immune system at E9.5, we observed a perturbation of only medial ganglionic eminence (MGE)-derived Nkx2.1+ progenitors. Ultimately, this led to a reduction of PV+ and SST+ but not VIP+ interneurons at P15. Conversely, injecting Poly I:C at E12.5 impacted caudal ganglionic eminence (CGE)-derived Nr2f2+ progenitors alone and a reduction in VIP+ interneurons at P15. Finally, immune activation at later stages of embryonic development i.e., E16.5 had an effect on both MGE and CGE-progenitors but with the opposite effect of increasing BrdU labelling. This indicates maternal inflammation to impact fetal neurodevelopment in a stage and cell-type dependent manner. Clustering of these cells using Pagoda and Conos provided us with

We sought to investigate the cell-type impact of MIA on early fetal stages by employing single-nucleus transcriptomics. We injected pregnant dams with Poly I:C on E9.5 and collected the developing ganglionic eminence and dorsal cortex 24 hours later. Nuclei were extracted and processed according to the 10X Genomics protocol. This resulted in a total of 3298 control and 4061 treated cells spread across 16 distinct cell clusters. Poly I:C frequently resulted in dysregulation of cell differentiation pathways across neural progenitor subtypes. Further, in both MGE and CGE progenitors, MIA affected pathways related to regulation of mRNA splicing. However, we found a higher gene fraction affected in gene ontology terms related to cell division in MGE progenitors as compared to CGE progenitor.

Our results hence indicate a stage and cell type dependent impact of MIA on progenitor niches. These differences are likely to influence the identity, cellular behaviour and function of neurons and thus play a role in sculpting cortical circuits. Efforts are underway to analyze how such differences in gene expression result in cell type specific impact of MIA. This will be crucial to unravel the developmental basis of psychiatric disorders.

Sara Bandiera

Department of Physiology Anatomy and Genetics, University of Oxford, United Kingdom

Characterization of the earliest thalamocortical interactions in the human fetal brain.

S. Bandiera¹, Z. Molnár¹

¹ Department of Physiology Anatomy and Genetics, University of Oxford

The degree to which intrinsic versus extrinsic factors control the development of the cerebral cortex is the subject of sustained research. While there is evidence for an early control of patterning intrinsic to the neocortex, a major extrinsic source of patterning is provided by the early thalamo-cortical afferents (TCA). In the human brain, the thalamo-cortical interactions start from very early stages and they take place over a prolonged period, therefore potentially influencing the intrinsic neurogenic programme and early circuit formation.

The first developmental compartment reached by the TCA is the mature and largely expanded subplate zone (SP), that serves as a transient “waiting station” for the incoming axons, which establish their earliest synaptic contacts before innervating the cortical plate. Several studies showed that these early transient synaptic circuits are crucial for the correct development of the functional connectivity of the adult brain, and their impairment has been associated with neurodevelopmental disorders, such as schizophrenia and autism.

We traced early thalamocortical projections with carbocyanine dyes in fixed post-mortem human brains at 17 post conceptional week, a stage of development when the TCAs already reached their prospective cortical area, however most of the cortical plate neurons have not been born yet. We observed that the TCAs not only innervated the overlying subplate, but they also developed projections towards the outer subventricular zone (OSVZ), a zone that contains outer radial glial cells (oRGCs) and intermediate progenitors. In addition to the labelled TCAs that entered the germinal zone and ended in growth cones, we also observed a few oRGC bodies stained by the carbocyanine dye possibly due to transneuronal labelling. The early close association of OSVZ progenitors with TCA raise important questions about the possible mechanisms that regulate cortical neuronal production and emergence of cytoarchitectonic differences in human cortex. SP and the OSVZ both represent transient compartments that can mediate and integrate the influence of intrinsic and extrinsic regulatory events. The characterization of the molecular and cellular influence exerted by TCA on cortical progenitors and on early circuits is not only important to understand cortical specialisation but shall also reveal mechanisms of neurodevelopmental disorders.

Shivang Khadelwal

Indian Institute of Technology Jodhpur, India

Vibrational visual sensory substitution,

The visual sensory substitution's underlying objective is to develop a device that can help the people who are destitute of vision to see and visualize the world. The sensory substitution technology has paved the way for new developments in compensating for sensory loss. People who go blind don't lose their ability to see. They lose their sensory system, which acts as a mode to see the retina, but they retain central visual mechanisms. In your brain, you have different parts assigned for various purposes. This is called Brain Phrenology. When one of your sensory receptors die out or stop getting inputs, the neurons responsible for that ones are taken over by sensory information from other senses, which ultimately increases the sensing capabilities of those senses.

So the neurons responsible for carrying messages from the retina tend to remain even after the loss of eyesight. If stimulated again with similar signals, they can re-activate themselves to send and perceive information. Your brain does not receive what you see as an image when photons strike your retina. Instead, the image is sent in the form of electrical signals that the brain interprets. Suppose we generate similar kinds of electrical impulses with the help of another sensory organ such as the skin or tongue, or ear. In that case, we might raise the action potential in the brain's visual area allowing it to perceive the signal.

The idea is to make a device that will include a camera, transducer, Arduino and quite a few vibration generators. First, the camera will identify the objects in the vicinity. The images will go through the Pixelsynth that will convert images into sound. Those sounds, characteristic of the item, will go through real-time compression and mapped to touch using the Arduino which will actuate the vibration generators placed on the jacket that the person will be wearing. The generator will create characteristic vibrations that will provoke the visual neurons sending the information to the brain as electrical impulses. By this, the person will be able to visualize the object in front of it.

Silvia Monari

Ecole Polytechnique Fédérale de Lausanne, Switzerland

Fear extinction impairments and sleep abnormalities in rats selected for blunted glucocorticoid responsiveness.

Silvia Monari* (1), Sophie Walker (1), Jocelyn Grosse (1), Olivia Zanoletti (1), Isabelle Guillot de Suduiraut (1), Diana Cash (2), Simone Astori (1) & Carmen Sandi (1)

1) Laboratory of Behavioral Genetics, Brain Mind Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

2) Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

Humans show inter-individual differences in vulnerability to develop post-traumatic stress disorder (PTSD) following exposure to traumatic events. Although initial observations linked low cortisol levels to PTSD pathophysiology, whether inter-individual differences in glucocorticoid responsiveness are implicated in the development of PTSD is not yet clear. We have addressed this question in lines of Wistar rats genetically-selected for their differential habituation of glucocorticoid responses to repeated stress exposure at puberty. We observed that rats with blunted stress-induced corticosterone responses exhibit strong deficits in the consolidation of fear extinction, when compared to rats with 'normative' glucocorticoid responses. Notably, similar to PTSD patients, rats with blunted corticosterone exhibit smaller hippocampi, regardless of trauma exposure. Given that glucocorticoids are known to modulate distinct hippocampal memory consolidation processes in sleep and wake, we examined sleep/wake rhythms through polysomnographic (EEG/EMG) recordings. Low corticosterone stress-responder rats display an altered sleep architecture, with markedly longer bouts of REM sleep, reminiscent of the excessive REM sleep described in PTSD patients. Finally, we tested the causal involvement of corticosterone in the fear extinction consolidation and found that post-extinction corticosterone treatment largely prevents deficits in extinction memory and attenuates fear relapse. Our findings strongly support an involvement of blunted glucocorticoid responsiveness in biobehavioral traits indicative of higher vulnerability to PTSD, supporting a causative role of low cortisol levels in PTSD pathophysiology.

Sofia Santos

University of Coimbra, Portugal

Novel call found in the perinatal period of two mouse models as a potential biomarker for early development.

S. Santos¹, H. Ferreira¹, H. Ferreira^{2,3}, J. Martins^{2,3}, J. Gonçalves^{2,3*}, M. Castelo-Branco^{2,3*}

¹University of Coimbra, Faculty of Medicine, Master's in biomedical research, Portugal

²University of Coimbra, Coimbra Institute for Biomedical Imaging and Translational Research (CIBIT), Portugal

³University of Coimbra, Institute of Nuclear Sciences Applied to Health (ICNAS), Portugal

***These authors share senior authorship**

Rodent communication represents an essential component in animals' socialisation and for the transmission of various messages, such as presence of stressors or threats. This communication happens through ultrasonic vocalizations (USVs). The vocal repertoire exhibited by mice is varied, and is influenced by factors such as environment, mouse strain, genetic background and state of development of the animal. As such, analysis on rodents' USVs provides an important insight into communication ability, emotional state and developmental stage. A longitudinal analysis of USVs allows for the outlining of a vocal developmental profile of the animal. However, often studies on USVs choose to only focus on a short number of timepoints, or even skip the neonatal stage entirely, thus hindering the ability to conclude on vocal development in detail. In this sense, the understanding of the evolution of the mouse's vocal profile across strains and conditions is still scarce. Here, we longitudinally analysed two mouse genetic backgrounds, C57BL/6 and hybrid 129/JxC57BL/6, from postnatal day (PND) 6 to PND18. In addition to the USVs previously described in literature, we found a novel vocalization that had never been reported. This vocalization is composed of two elements (Chevron + Chevron, Chevron + Upward, Chevron + Downward or Chevron + Complex) with a very narrow temporal gap between them, not greater than 0.04 seconds. This is an unprecedented vocalization characteristic, as no classification system in the literature describes such a pattern. Importantly, this new vocalization is only present in the perinatal period and is extinguished around PND20. We compared the relative frequency of this novel call to other vocalization categories and found a nice correlation between the extinction of the novel call and the increase in frequency of Complex vocalizations. This vocalization was also found in the transgenic littermates of the mouse strains used, namely NF1^{+/-} and TSC2^{+/-}, models for autism spectrum disorder, and show promising genotype-dependent differences. Overall, we proposed that this novel USV is a primitive form of the complex USV, which has potential to be a biomarker of early development during neonatal period.

Thomas Theil

University of Edinburgh, United Kingdom

The Joubert gene *INPP5E* acts as a negative regulator of Sonic Hedgehog signaling in human brain organoids.

L. Schembs^{1,2}, A. Willems³, B. Selvaraj³, J. Cooper³, S. Bostrand^{1,2}, K. Hasenpusch-Theil^{1,2}, K. Burr³, S. Chandran³, T. Theil^{1,2}

¹Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh EH8 9XD, United Kingdom

²Simons Initiative for the Developing Brain, University of Edinburgh, Hugh Robson Building, Edinburgh, EH8 9XD, United Kingdom

³Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB, United Kingdom

Joubert Syndrome (JS) is a genetically and phenotypically heterogeneous syndrome that is marked by multiple organ defects. In addition to the characteristic cerebellar abnormalities, JS patients show malformations of the corpus callosum, polymicrogyria and heterotopia formation in the cerebral cortex which have been associated with intellectual disability and with autism spectrum disorder in up to 40% of JS patients but the underlying pathomechanisms remain unclear. The *INPP5E* gene is mutated in JS patients and has key roles in JS pathogenesis by regulating the signalling function and the stability of the primary cilium, a cellular antenna controlling multiple intracellular signalling pathways. To investigate *INPP5E*'s roles in human cortex development, we generated human iPSC lines carrying an *INPP5E* loss of function mutation using Crispr/Cas9 gene editing and grew cortical organoids from these mutant cells. Intriguingly, mutant organoids did not form progenitor cells and projection neurons of the cerebral cortex but express ventral telencephalic markers and produced striatal and cortical interneurons characteristic of the basal ganglia. *INPP5E* mutant organoids also showed increased ciliary expression of SMOOTHENED and an up-regulation of the SHH target genes *PATCHED1* and *GLI1* suggesting an up-regulation of SHH signalling, a key regulator of dorsal/ventral patterning of the telencephalon. Moreover, treating organoids with the SHH antagonist cyclopamine partially rescued the ventralisation leading to the suppression of ventral telencephalic markers and allowing the expression of the cortical progenitor marker PAX6. Taken together, these findings indicate a novel, tissue specific role of *INPP5E* as a negative regulator of SHH signalling during human corticogenesis.

Vanessa Hall

University of Copenhagen, Denmark

The entorhinal cortex (EC) is the spatial processing center of the brain and structurally is an interface between the three layered allocortex and six layered neocortex, known as the periallocortex. Limited studies indicate peculiarities in the formation of the EC such as early emergence of cells in layers (L) II and late deposition of LIII as well as divergence in the timing of maturation of cell types in the superficial layers. Here, we profile the developing entorhinal cortex in detail using an understudied model in neuroanatomy and development, the pig, to examine developmental events of the EC in depth. We determined the pig serves as an excellent anatomical model for studying human neurogenesis, given its long gestational length, presence of a moderate sized outer subventricular zone and early cessation of neurogenesis during gestation. We demonstrate that in the developing medial EC, the deeper layers form first and prior to the superficial layers, but the deeper layers, LVa/b/LVI emerge in parallel and the LII/III emerges later, but also in parallel. We coin this lamination pattern, parallel lamination. The early-born stellate cells in the superficial layers express the classic LV marker, *Bcl11b* and arise from a common progenitor that forms the late deep layer LV neurons. In summary, we characterize the developing EC in a novel animal model and outline in detail the formation of the MEC which provides insight into how the periallocortex forms in the brain, which differs remarkably to the inside-out lamination of the neocortex.

Xiuming Yuan

Radboud University Medical Center, The Netherlands

Epigenetic mechanisms of homeostatic plasticity in a human neuronal model system

X. Yuan¹, B. Franke^{1,2}, N. Nadif Kasri¹

¹Department of Human Genetics, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, The Netherlands

²Department of Psychiatry, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, The Netherlands

Introduction: Neurodevelopmental disorders (NDDs) are conditions of impaired cognitive, behavioural, and/or motor functions stemming from an atypical development of the central nervous system. Among the most frequent NDDs are attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), and intellectual disability (ID). Impairments in NDDs are thought to be associated with disrupted homeostatic plasticity, a process known to stabilize neuronal network activity by balancing neuronal excitation and inhibition. Many studies have shown that homeostatic plasticity is regulated by epigenetic mechanisms, such as DNA-methylation. However, an epigenetic profile involved in the disruption of homeostatic plasticity in NDDs is currently insufficiently defined. This study aims to explore the role of DNA methylation in disrupted homeostatic plasticity and understand how this regulation underlies neurodevelopmental disorders.

Objective: (a) To develop a model of homeostatic plasticity in human induced pluripotent stem cell (iPSC)-derived neurons and identify genes which are differentially methylated during synaptic scaling; and (b) to validate the function of selected differentially methylated gene(s) also robustly linked to NDDs through epigenetic CRISPR-mediated engineering.

Methods: The homeostatic plasticity model is established in human iPSC-derived cortical excitatory neurons. Firstly, WTC-Ngn2 iPSCs are plated on micro-electrode array (MEA) plates and differentiated to induced neurons until day 28. Neurons are then treated with the sodium channel blocker tetrodotoxin (TTX) for 48 h. Neuron network activity is measured on MEAs before and after TTX treatment. As a next step, TTX-treated and untreated samples will be collected for RNA sequencing and DNA bisulfite sequencing. By comparing the DNA methylation and gene expression profiles before and after TTX treatment, genes which are altered in their methylation and in their transcription during the synaptic scaling process are identified. The ones that are also robustly linked to NDDs will be selected for further validation. DNA methylation status will be edited by an inducible CRISPR-mediated DNA-methylation editing system during neuron differentiation, and deficits in homeostatic plasticity

will be analyzed on the MEA. The expression of the target genes will be analyzed by Western blot, quantitative reverse transcription PCR (RT-qPCR), and immunofluorescence staining. DNA methylation level of target region will be validated by bisulfite sequencing.

Results: In accordance with previous work, we found that chronically blocking sodium channels by TTX eliminated all spiking activity and network burst activity for the entire 48h treatment. After TTX was withdrawn, mean firing rate, mean burst rate and mean network burst rate increased, then recovered to basal levels by the end of 48 h post TTX-withdrawal.

Results are presented in Figure 1.

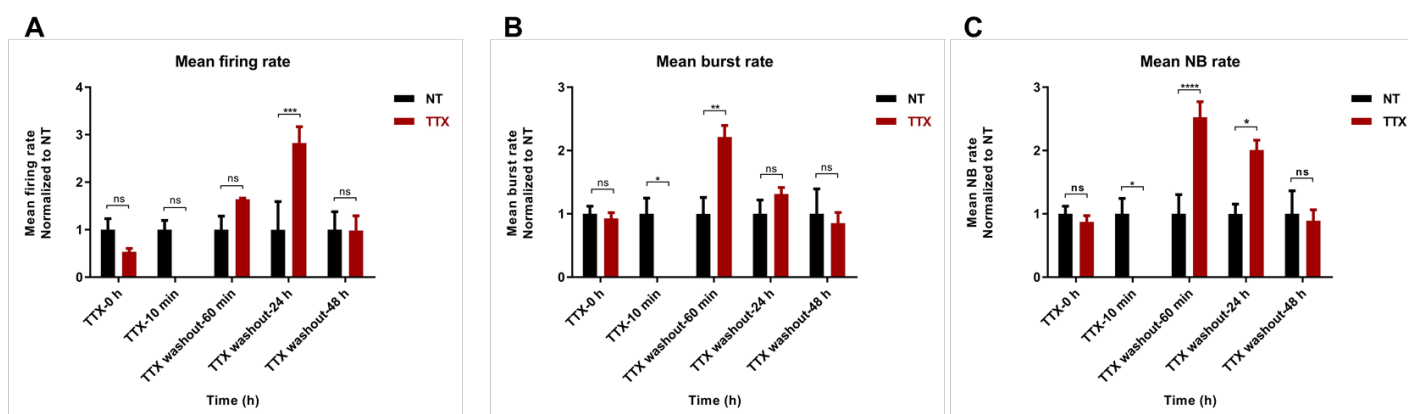


Figure 1. Effect of TTX on Ngn2-induced human neurons network activity. Shown is the effect of 48-hours tetrodotoxin (TTX) treatment and 48-hours TTX withdrawal on mean firing rate (A), mean burst rate (B) and mean network burst (NB) rate (C) for Ngn2-induced human neurons. The values are normalized to the non-treated (NT) data (n=6 for NT, n=5 for TTX-treated). Data represent means \pm SEM. *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.0001; two-way ANOVA test and post-hoc Bonferroni correction was performed between NT and TTX.

Conclusions: This result indicates that Ngn2-induced human neurons can undergo homeostatic plasticity upon TTX treatment. In addition, homeostatic plasticity can be assessed by measuring network activity on MEAs.

Disclosure of potential conflicts of interest

Barbara Franke received educational speaking fees from Medice. All other authors report no conflicts of interest.

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