Belgian Society for Cell and Developmental Biology
Spring meeting, June 8, 2018

« Neural stem cells and cortex development »

Information: www.bscdb.be
Venue: Château de Colonster, Liège
Deadline for abstract submission: May 18, 2018

Keynote speakers
Sonia Garel (Paris)
Troy Ghashgai (North Carolina)
Magdalena Götz (Munich)
Simon Hippenmeyer (Klosterneuburg)
Franck Polleux (New York)
David Price (Edinburgh)
Setsuko Sahara (London)
We would like to thank the following University/Aencies/Societies and companies for offering a generous support to the conference.

http://www.uliege.be

http://www.fnrs.be/

http://www.fwo.be/

http://www.isdn-conference.elsevier.com/

http://www.biologists.com/
PLATINUM SPONSOR:

We make it visible.

GOLD SPONSOR:

SILVER SPONSORS:
Adding efficiency to your fluorescence imaging.

ZEISS Celldiscoverer 7

Your automated platform for live cell imaging

Imagine the ease of use and automation of a boxed microscope – combined with the image quality and flexibility of a classical research microscope. Imagine this system calibrates itself, detects and focusses your samples and adaptive optics adjust themselves automatically. ZEISS Celldiscoverer 7 is your reliable automated research platform. No matter if you work with 2D or 3D cell culture, tissue sections or small model organisms. With Celldiscoverer 7 you increase the efficiency of your research. You acquire better data in a shorter time.

www.zeiss.com/celldiscoverer
Where **Resolution** meets **Speed**

3D live-cell
Optogenetics
Multi-scale / Quantitative
High content
Deep imaging

For more information:
microscience.be@nikon.com

A1ways Evolving!
# Cell Culture

## Cells
- ECACC Cell lines
- Primary Cells
- Stem cell lines
- Engineered cell lines
- Induced Pluripotent Stem Cells (EBiSC)

## Prepare
- Serum
- Media
- Buffers
- Supplements
- Antibiotics
- Sterile Filtration
- Growth Factors
- Cytokines
- Extracellular Matrix Proteins (ECM)

## Grow
- General Labware
- Millicell® hanging/standing inserts
- Millicell® 24- and 96-well plates
- HY-Multilayer Flasks
- 3D Cell Culture
- Scepter™ automated Cell Counter
- Millicell® EZ-slide

## Analyze
- Antibodies
- Dyes, Stains
- CellASIC® Onix2 Microfluidic Platform
- Guava® Flow Cytometers
- Muse® Cell Analyzer
- Amnis® Imaging Flow cytometry
- ELISpot plates
- Migration, Invasion, Chemotaxis

---

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.
**PROGRAM**

8:30 – 9:00  Welcome

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
</table>
| 9:00 – 9:30 | Magdalena Götz  
B. Malgrange – C. Alfano   | "Mechanisms governing neural stem cell diversity"                                                               |
| 9:30 – 9:50 | Jerome Bonnefont  
Zeiss                                           | "Bcl6 promotes cortical neurogenesis through repression of multiple self-renewal-promoting pathways"             |
| 9:50 – 10:20 | David Price  
Zeiss                                           | "Actions and interactions of Pax6 and Foxg1 in cerebral cortical cell fate determination"                      |

10:20 – 11:20  Coffee break an Poster session

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
</table>
| 11:20 – 11:50 | Simon Hippenmeyer  
L. Nguyen – C. Silva   | "Mechanisms of Neural Stem Cell Lineage Progression"                                                          |
| 11:50 – 12:05 | Solène Clavreul  
L. Nguyen – C. Silva                                           | "Clonal organization and development of the astroglial network in the mouse cerebral cortex"                      |
| 12:05 – 12:30 | Poster teaser  
L. Nguyen – C. Silva                                           |                                                                                                                 |

12:30 – 14h30 Lunch and poster session

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
</table>
| 14:30 – 15:00 | Franck Polleux  
E. Seuntjens – R. Ivan Gladwyn-NG   | "Human-specific modifiers of synaptic development and cortical circuit function"                               |
| 15:00 – 15h15 | Carla Silva  
E. Seuntjens – R. Ivan Gladwyn-NG                                           | "Cell-intrinsic regulation of interneuron migration drives corticogenesis"                                     |
| 15:15 – 15:45 | Sonia Garel  
E. Seuntjens – R. Ivan Gladwyn-NG                                           | "Microglia at the crossroads of early cortical wiring and environmental signals"                               |
| 15:45 – 16:00 | Elodie Desmaris  
E. Seuntjens – R. Ivan Gladwyn-NG                                           | "DMRT5, DRMT3 and EMX2 cooperatively repress Gsx2 at the pallium subpallium boundary to maintain cortical identity in dorsal telencephalic progenitors" |

16:00 – 16:50  Coffee break and poster session

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Title</th>
</tr>
</thead>
</table>
| 16:50 – 17:20 | Setsuko Sahara  
B. Lakaye – L. Broix   | "Molecular mechanisms generating diversity in cortical progenitors and their progenies"                           |
| 17:20 – 17:50 | Troy Ghashghaei  
B. Lakaye – L. Broix                                           | "Secrets of cortical gliogenesis revealed by comparison of bulk and sparse perturbations"                       |
CONFERENCES
Microglia and prenatal inflammation in the development of cortical circuits

M Thion¹, CA Mosser², D Low³, P Grisel¹, P Squarzoni¹, I Ferezou⁴, F Ginhoux³, E Audinat², and S Garel¹

¹Ecole Normale Supérieure, IBENS, INSERM U1024, CNRS UMR 8197, Brain Development and Plasticity Team, 46 rue d’Ulm, 75005 Paris, France
²Neurophysiologie et Nouvelles Microscopies, INSERM U1128, Université Paris Descartes, 45, rue des Saints Péres, 75006 Paris, France
³Singapore Immunology Network (SiGN), Agency for Science, Technology and Research (A*STAR), Singapore
⁴Unité de Neurosciences Information et Complexité (UNIC), CNRS, 1 Avenue de la Terrasse, Bât. 32/33 91198 Gif-sur-Yvette, France.

Prenatal inflammation and dysfunction of microglia, the brain resident macrophages, have both been associated with the etiology of several neuropsychiatric disorders, including schizophrenia and autism spectrum disorders. Consistently, microglia were shown to regulate neurogenesis, synaptic remodeling and maturation at postnatal stages. However, microglia invade the brain during mid-embryogenesis and could thus exert earlier prenatal and perinatal roles during normal and pathological brain wiring. Here we show that embryonic microglia, which display a transient uneven distribution, regulate the wiring of forebrain circuits. By taking advantage of multiple mouse models, including cell-depletion approaches, we found that perturbing microglia activity affects the development of neocortical inhibitory interneurons, which constitute main actors in neuropsychiatric diseases. In particular, absence, prenatal inflammation or functional perturbation of microglia affects the timely positioning of fast-spiking Parvalbumin-positive interneurons at early postnatal stages and, importantly, their subsequent functional integration in the neocortex. We furthermore found that responses of microglia to environmental signals, including the ones from the microbiome, are sexually dimorphic in males and females. This remarkable finding has major implications for our comprehension of sexual biases in the occurrence of microglia-related diseases, such as the prevalence in males of neurodevelopmental disorders. Our work reveals key roles for immune cells during the normal assembly of cortical circuits and provides novel insights onto how microglia dysfunction or immune risks lead to pathological brain wiring.
Secrets of cortical gliogenesis revealed by comparison of bulk and sparse perturbations

Troy Ghashghaei

Department of Molecular Biomedical Sciences, North Carolina State University

The progenitor cell populations that generate the cerebral cortices give rise to both neurons and glia. Neurogenesis occurs during embryonic development while gliogenesis begins at late stages of embryogenesis and continues during early postnatal life. The degree to which the two distinct lineages are related, and the fundamental principles underlying transition of neural to glial production are critical to understanding cortical development. Bulk conditional deletion of the epidermal growth factor receptor (EGFR) disrupts gliogenesis in mice with robust inflammatory responses that subside after the third postnatal week and are dispensable to normal health and longevity. Thorough analysis of cell types in the bulk model point to oligodendrocyte production as severely affected, while astrocyte production appears largely intact. Surprisingly, sparse and clonal deletions of EGFR using Mosaic Analysis with Double Markers (MADM) revealed three important principles that were not revealed in the bulk model. First, EGFR is unequivocally required for gliogenesis in a cell autonomous manner including both oligodendrocytes and astrocytes. Second, clonal production of glia is stochastic unlike the deterministic program that controls the number of neurons produced in the cortex. Third, wild type siblings of EGFR-null cells in the MADM model respond to the loss of EGFR in their sister cells. This ‘sibling’ response was replicated in postnatal gliogenesis in the olfactory bulbs, but not in neurogenesis that continues in this tissue during postnatal life in mice. Our study highlights a critical cell-autonomous requirement for EGFR in gliogenesis, and reveal technical limitations in bulk- versus sparse-autonomous genetic methods in extrapolating the role of factors involved with cell fate specification in neural tissues. Mechanisms underlying the apparent stochastic nature of glial expansion in the cortex is of great interest and single cell analyses are in process to discover key players.
Mechanisms Generating Cell-Type Diversity in Cerebral Cortex

Simon Hippenmeyer

Institute of Science and Technology Austria

The concerted production of the correct number and diversity of neurons and glia is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling the precise pre-programmed RGP lineage progression through proliferation, neurogenesis and gliogenesis remain unknown. To this end we use quantitative MADM-based experimental paradigms at single RGP resolution to define the sequential non-cell-autonomous and intrinsic cell-autonomous functions of candidate genes and signaling pathways controlling RGP-mediated cortical neuron and glia genesis and postnatal stem cell behavior.
Exploring the structural and functional consequences of humanization of SRGAP2C expression in mouse cortical and hippocampal circuits

Franck Polleux

Department of Neuroscience Mortimer B. Zuckerman Mind Brain Behavior Institute Columbia University New York, NY, 10027, USA

A long-standing question and key focus in the field of neuroscience is to understand how genes shape the development of neural circuits and ultimately behavior. This question is particularly relevant in the context of the human brain. While much of our genetic makeup is shared with our closest relatives, such as chimpanzees and other great apes, specific genetic changes have occurred over the course of human evolution that have been hypothesized to underlie the emergence of human-specific features of brain development and function. However, which genetic changes had a significant impact on human brain evolution and more importantly, what are the critical features of brain development that have been targeted by these human-specific genetic changes remain largely unresolved.

Over the past 8 years, we focused our attention on gene duplications that are unique to the human genome. One such gene is SRGAP2: the ancestral copy, SRGAP2A, promotes excitatory (E) and inhibitory (I) synapse maturation and limits the density of both E and I synapses made onto cortical pyramidal neurons (Charrier et al., 2012; Fossati et al., 2016). Partial duplication of SRGAP2A resulted in a human-specific paralog, SRGAP2C, which binds to and inhibits SRGAP2A function. Deletion of SRGAP2A or humanization of SRGAP2C expression in mouse cortical pyramidal neurons leads to the emergence of phenotypic traits characterizing human cortical neurons, including increased density of E and I synapses (Charrier et al., Cell 2012; Fossati et al., Neuron 2016). The main question emerging from these results is to determine the consequences of these changes in synaptic development on hippocampal and cortical circuit function in vivo. I will report two projects aimed at exploring this important question.

First, using monosynaptic rabies tracing from very sparse populations of layer 2/3 cortical neurons and a novel method for 3D reconstruction and mapping of traced brains, we interrogate how cell-autonomous changes in synaptic density induced by humanization of SRGAP2C expression affect the structural organization of cortical circuits. We combine this approach with our newly developed Cre-dependent SRGAP2C ‘humanized’ mouse model. Our results show that SRGAP2C expression causes neuronal circuit changes both at the level of local connectivity, including changes in the relative contribution of layer-specific inputs, as well as long-range feedback connections from other cortical regions. We are currently probing if the increased circuit connectivity induced by humanization of SRGAP2C expression are accompanied by changes in circuit performance both in the cortex and hippocampus.

This work is supported by NIH RO1 NS067557-05A1 (FP), an award from the Roger De Spoelberch Foundation (FP).
Actions and interactions of Pax6 and Foxg1 in cerebral cortical cell fate determination

David Price

Biomedical Sciences, University of Edinburgh, Edinburgh, UK

Mammalian cerebral cortical circuits comprise two types of neuron: excitatory projection neurons and inhibitory interneurons. Excitatory neurons are glutamatergic and transmit over relatively long distances. Inhibitory interneurons are GABAergic and act locally to enhance function; they stabilize circuits, filter their inputs and outputs and increase the contrast between the activities of linked circuits by mediating negative feedback, feed-forward and lateral inhibition. An imbalance between excitation and inhibition is a likely cause of many neurodevelopmental cognitive disorders. I shall present recent unpublished work showing that Foxg1, whose expression extends through the entire telencephalon, permits and might actively promote pro-GABAergic programs in at least many and possibly all cortical cells whereas Pax6, whose expression is restricted mainly to cortical progenitors, promotes a pro-glutamatergic program and opposes pro-GABAergic programs by blocking Foxg1-dependent pro-GABAergic gene expression.
Molecular mechanisms generating diversity in cortical progenitors

Setsuko Sahara

King’s College London

Self-renewing progenitors acquire their neurogenic competency at the expense of progressive loss of their expansive capacity. We are investigating fate-switching mechanisms that change self-renewing progenitors into neurogenic progenitors. Our previous study identified that one member of the fibroblast growth factor family of proteins, Fgf10, plays a role in inducing the transition of expansive neuroepithelial cells (NEs) to neurogenic radial glial progenitors (RGs). Subsequent RNA-seq analysis comparing gain-of and loss-of function of Fgf10 cortices followed by functional screening in an ES cell based cortical progenitor differentiation assay, led to the identification of several genes functioning downstream of Fgf10, controlling multiple steps of cortical progenitor differentiation. These include: two transcription factors (TFs) regulating NE-RG transition, two TFs for neurogenesis, and one microtubule protein that induces one cortical progenitor subtype (apical intermediate progenitor, aIP/short neural progenitor (SNP)). I will discuss our recent data about the mechanism by which this microtubule protein induces aIP/SNP fate, committing terminal symmetric division by retracting radial fibers from the basal membrane.
ORAL PRESENTATIONS
Bcl6 promotes cortical neurogenesis through repression of multiple self-renewal-promoting pathways

Jerome Bonnefont, Luca Tiberi, Jelle van den Ameele, Delphine Potier, Zachary B Gaber, Xionghui Lin, Angéline Bilheu, Adèle Herpoel, Fausto D. Velez Bravo, François Guillemot, Stein Aerts, Pierre Vanderhaeghen

Université Saint-Louis Bruxelles

Presenting author: Jerome Bonnefont

During neurogenesis, progenitors switch from self-renewal to differentiation through the interplay of intrinsic and extrinsic cues, but how these are integrated remains poorly understood. Here we show that Bcl6, a transcriptional repressor previously reported to repress the Notch target Hes5, acts as a driver of the neurogenic transition through direct silencing of a repertoire of genes belonging to pathways promoting self-renewal, most strikingly belonging to the Wnt pathway. We identify Cyclin d1/d2 as key most downstream targets, in parallel to Hes genes, to promote cell cycle exit and neurogenesis. At the molecular level, Bcl6 represses all its targets through Sirt1 recruitment followed by Histone deacetylation. Our data identify the molecular logic through which a single intrinsic factor ensures robustness of cell fate transition through repression of multiple extrinsic pathways that favor self-renewal.
Clonal organization and development of the astroglial network in the mouse cerebral cortex

Solène Clavreul, Lamiae Abdeladim, Edwin Hernandez, Sio-Hoï Ieng, Jason Durand, Raphaëlle Barry, Farshad Nourbakhsh, Ryad Benosman, Gilles Bonvento, Emmanuel Beaurepaire, Jean Livet*, Karine Loulier*

Institut de la Vision / Université Pierre et Marie Curie

Presenting author: Solène Clavreul

Astrocytes constitute a large and diverse cell population with functional, morphological and molecular differences within or across brain regions. Cortical astrocytes are issued from radial glia progenitors prior birth and from extensive local proliferation during the first postnatal week. However the individual contribution of radial glia progenitors to the astroglial network is still poorly characterized. Here we applied the MAGIC Markers strategy to label cohorts of neighboring cortical progenitors and track the astrocyte descent of multiple cortical progenitors labeled with stable complex color combinations from early postnatal to adult stages. We performed high-resolution large-volume imaging of labeled brains using recently developed chromatic serial multiphoton (Chrom-SMP) microscopy to characterize the spatial distribution of clonally-related astrocytes. Cortical astrocyte clones are highly diverse in terms of cell number, subtypes generated and spatial dispersion, revealing the variable contribution of cortical progenitors to the astroglial network and the plasticity of their progeny. Astrocyte network is established through an initial dynamic phase of proliferation and spatial dispersion during the first postnatal week and matures with an increase in astrocyte complexity and volume during the following two weeks. Taken together our results provide new information on astrocytic network construction and maturation in the mammalian cerebral cortex.
DMRT5, DMRT3 and EMX2 cooperatively repress Gsx2 at the palliumsubpallium boundary to maintain cortical identity in dorsal telencephalic progenitors.

Eric Bellefroid¹, Elodie Desmaris¹, Marc Keruzore¹, Amandine Saulnier¹, Leslie Ratié¹, Stavroula Assimacopoulos², Sarah De Clercq¹, Xinsheng Nan³, Kaushik Roychoudhury⁴, Sadia Kricha¹, Thomas Lingner⁵, David Zarkower⁶, Antonello Mallamaci⁷, Thomas Theil⁸, Tomas Pieler⁹, Meng Li¹⁰

¹ Université Libre de Bruxelles
² University of Chicago
³ Neurosciences and Mental Health Research Institute
⁴ University of Cincinnati College of Medicine
⁵ University Medical Center Kristine Henningfeld, University of Göttingen
⁶ University of Minnesota
⁷ SISSA
⁸ University of Edinburgh Kenneth Campbell, Cincinnati Children’s Hospital Medical Center
⁹ Center of Molecular Physiology of the brain, University of Göttingen
¹⁰ Neurosciences and Mental Health Research Institute Elizabeth Grove, University of Chicago

Presenting author: Elodie Desmaris

Specification of dorsal/ventral regional identity in progenitors of the developing telencephalon is a first pivotal step in the development of the cerebral cortex and basal ganglia. Previously, we demonstrated that the two zinc finger doublesex and mab-3 related (Dmrt) genes, Dmrt5 (Dmrt2) and Dmrt3, which are coexpressed in high caudomedial to low rostrolateral gradients in the cerebral cortical primordium, are separately needed for normal formation of the cortical hem, hippocampus and caudomedial neocortex. We have now addressed the role of Dmrt3 and Dmrt5 in controlling dorsal/ventral division of the telencephalon by comparing the phenotypes of single knock-out (KO) with double KO embryos and by misexpressing Dmrt5 in the ventral telencephalon. We find that DMT3 and DMRT5 act as critical regulators of progenitor cell dorsoventral identity by repressing ventralizing regulators. Early ventral fate transcriptional regulators expressed in the dorsal lateral ganglionic eminence such as Gsx2 are upregulated in the dorsal telencephalon of Dmrt3;Dmrt5 double KO embryos and downregulated when ventral telencephalic progenitors express ectopic Dmrt5. Conditional overexpression of Dmrt5 throughout the telencephalon produces gene expression and structural defects that are highly consistent with reduced GSX2 activity. Further, Emx2;Dmrt5 double KO show a phenotype similar to Dmrt3;Dmrt5 double KO embryos, and both Dmrt3, DMRT5 and the homeobox transcription factor EMX2 bind to a ventral telencephalon-specific enhancer in the Gsx2 locus. Our findings uncover cooperative functions of DMRT3 and DMRT5 in the specification of cortical fate in dorsal telencephalic progenitors, and together with EMX2, in dividing dorsal from ventral in the telencephalon.
Cell-Intrinsic Regulation of Interneuron Migration Drives Corticogenesis


Université de Liège

Presenting author: Carla Silva

Interneurons navigate along multiple tangential paths to settle into appropriate cortical layers. They undergo a saltatory migration paced by intermittent nuclear jumps whose regulation relies on interplay between extracellular cues and genetic-encoded information. It remains unclear how cycles of pause and movement are coordinated at the molecular level. Post-translational modification of proteins contributes to cell migration regulation. Our study uncovers that carboxypeptidase 1, which promotes post-translational protein deglutamylation, controls the pausing of migrating cortical interneurons. Moreover, we demonstrate that pausing during migration attenuates movement simultaneity at the population level, thereby controlling the flow of interneurons invading the cortex. Interfering with the regulation of pausing not only affects the size of the cortical interneuron cohort but also impairs the generation of age-matched projection neurons of the upper layers.
Osteomimetism in MCF-7 breast cancer cells

Absil Lara, Journé Fabrice, Nonclercq Denis

Université de Mons

Presenting author: Lara Absil

Bone is the most common site for metastasis of breast cancer. FXR is present in breast carcinoma where its function is still little known. We have investigated the involvement of FXR in breast cancer cells to determine a relationship between its expression and (1) proliferation (2) in osteomimetism. We evaluated FXR expression in MCF-7. This expression increased after a treatment with CDCA. After activation of FXR/ER by CDCA/estrogens, we observed an activation of cell proliferation in MCF-7 but not in MDA-MB-231. A treatment with anti-estrogens decreased the cell proliferation induced by the CDCA/estrogens. We assessed the expression of RUNX2 and bone proteins. Estrogens/CDCA induced RUNX2 and bone proteins. Tamoxifen in combination with estrogens/CDCA decreased the expression of these proteins. These data support a relationship between FXR and ER expression in the control of cell proliferation in the osteomimetism.
Differentiation towards cortical interneurons starting from a three-dimensional ganglionic eminence stem cell model.

Aerts T, Gomers T, Pancho Yanza A, Seuntjens E

Katholieke Universiteit Leuven

Presenting author: Tania Aerts

In the current study, we aimed to differentiate cells towards MGE-derived interneuron fates starting from a stem cell culture derived from the ganglionic eminences (GEs) of embryonic mice. The GEs are a transient progenitor region present in the subpallium of the developing mammalian brain. They are the main source of cortical interneurons and comprise the medial ganglionic eminence (MGE), the lateral ganglionic eminence (LGE) and the caudal ganglionic eminence (CGE). Within the GE, primary progenitor cells (PPCs) reside in the ventricular zone (VZ), while the subventricular zone (SVZ) and mantle zone (MZ) contain intermediate progenitor cells (IPCs) and immature interneurons, respectively. Isolation of MGE cells of E13.5 mice and subsequent cultivation in the presence of EGF and FGF induces the formation of spheroids that can be kept over several passages. Previous data has shown that these culturing conditions select for PPCs after 4DIV. This PPC identity can be maintained over ten passages, making this stem cell culture an accessible and inexpensive resource of interneuron progenitors amenable to gene-function studies. Whether the capacity to generate cortical interneurons is maintained in this spheroid cell culture system, is unclear. We investigated the effect of XAV939 (a Wnt antagonist) and Purmorphamine (a Sonic Hedgehog agonist) on the differentiation capacity of these stem cells. qPCR and immunocytochemistry for a panel of progenitor and mature cortical interneuron markers revealed that after 7 days, inhibition of Wnt resulted in a similar expression of Dlx5/6 and Lhx6 compared to E13.5 in vivo cells. However, all conditions generated a mixture of various cell types, of which only a small percentage were MGE-derived mature interneurons expressing Somatostatin or Parvalbumin. Additionally, spheroids obtained from higher passage numbers had reduced differentiation success towards these interneuron fates, indicating that a longer cultivation in the presence of EGF and FGF might result in loss of interneuron lineage restriction in the stem cells.
Zika tropism for long range migrating embryonic cells

Alfano C, Gladwyn-Ng I, Darmuzey M, Couderc T, Lecuit M, Nguyen L

Université de Liège

Presenting author: Christian Alfano

Clinical studies have highlighted a strong correlation between Zika infection and neonatal abnormalities, and laboratory research on cell and animal models confirmed a causal link. Recent studies have ascertained the tropism of Zika for many different types of cells: from microvascular endothelial cells to different types of placental cells, from Leydig or Sertoli cells (testis) to brain neuronal progenitors or different types of eye cells (e.g. ganglionic and bipolar cells). However, it is still unclear how the virus reaches and crosses the placental barrier and then spreads into different foetal organs. Our study is aimed at identifying cellular vectors vehiculating Zika virus from the placental tissue to the embryonic brain and/or spreading the infection into the developing cerebral cortex. We are testing Zika tropism for subgroups of cell populations born in extra-embryonic or extra-cephalic territories, which begin to invade the brain at early stages of development, as well as for neuronal or non-neuronal cells migrating to the cerebral cortex from different regions of the telencephalon. To test their possible role in Zika virus spreading we are infecting transgenic mouse lines labelling different cell types and impairing migration and/or maturation of these cells by using different genetic and chemical tools.
The absence of SV2A in interneurons leads to Epilepsy

O. Bartholome, P. Van den Ackerveken, O. de la Brassinne, A. Florio, and B. Rogister

*Université de Liège*

**Presenting author:** Odile Bartholome

The SV2A protein is a glycoprotein present in the membranes of most synaptic vesicles whose physiological role remains unknown. However, it has been demonstrated that levetiracetam, an effective anti-epileptic drug, binds to SV2A. Moreover, SV2A expression is down-regulated in epileptic foci resected in humans with temporal lobe epilepsy, and SV2A full knock-out mice exhibited seizures around post-natal day 7 (P7) and die in status epilepticus around P15. This project aims to understand how SV2A protein may be involved in epilepsy. First, we wanted to test if the absence of SV2A could lead to seizures appearance after complete brain development. We induced recombination in Ubiquitin-creERT2 : SV2A-cKO mice at P70 and observed seizures in cKO mice while WT mice behaved normally. Next, we wished to unravel the sub-population of neurons responsible for seizures onset. We compared Dlx5,6 : SV2A-cKO mice (targeting GABAergic interneurons) with Nex : SV2A-cKO (targeting glutamatergic neuron) and observed that Dlx5,6 : SV2A-cKO mice exhibited spontaneous seizure around P15 and die around P21 while Nex : SV2A-cKO had no phenotype. Furthermore, comparison of PV : SV2A-cKO, SST : SV2A-cKO and VIP : SV2A-cKO mice revealed that only one interneuron subtype is linked to spontaneous seizure around P18 and with a death between P20 and P45.
Characterising the role of p27 on microglial motility

Jolien Beeken, Bert Brône, Laurent Nguyen

*Universiteit Hasselt*

**Presenting author:** Jolien Beeken

Microglia, the resident immune cells of the CNS, play key roles in brain development and homeostasis. During synaptogenesis, they are responsible for the pruning of excessive connections in the brain through a process termed phagocytosis. Microglial cells perform their tasks in a spatio-temporal regulated manner, which requires a fine control of the cellular movement or "motility". However, the molecular mechanisms regulating this microglial motility remain largely unknown. In developing neurons on the other hand p27 (cyclin dependent kinase inhibitor p27kip1) is identified as a protein that regulates migration through modulation of the cellular skeleton. We detected the presence of p27 in microglia and since microglial motility involves rearrangements of the cellular cytoskeleton, our aim is to characterize and gain insight in the influence of p27 on microglial motility. To achieve this, we will investigate the influence of a p27 knock-out and a p27 mutant version that no longer binds to cyclins and CDKs on the morphology, the migration, phagocytosis and intracellular transport processes in microglia. With this project we aim to improve the understanding of brain development regarding dysfunction of microglia in neurological disorders.
Regional oligodendrocytopathy and astrocytopathy precede myelinolysis and blood-brain disruption in osmotic demyelination syndrome

Joanna Bouchat, Bruno Couturier, Fabrice Gankam-Kengne, Catherine Marneffe, Benoît Balau, Kathleen De Swert, Jean-Pierre Brion, Jacques Gilloteaux and Charles Nicaise

Université de Namur

Presenting author: Joanna Bouchat

Osmotic demyelination syndrome (ODS) is a non-inflammatory disorder of the central nervous system (CNS) myelin that occurs following too rapid correction of chronic hyponatremia. The physiopathology remains unclear although hypothetical mechanisms include blood-borne myelinotoxic factors or glial cells inability to adapt to osmotic shift. To unravel ODS physiopathology, we generated a novel murine model of ODS in our laboratory. Demyelination in the thalamus, mesencephalon, pons and subcortical regions was observed at 48 hours post-correction in ODS mice brains. Concomitant with demyelination, we demonstrated a disruption of blood-brain barrier (BBB) in thalamus. Histological and ultrastructural analysis revealed thalamic oligodendrocytopathy and astrocytopathy starting at 12 hours after the correction of chronic hyponatremia. Following osmotic insult, thalamic Iba1+ microglial cells infiltrated the brain tissue within 12 hours post-correction, while acquiring an activated morphology, from quiescent type A to types B, C and D at latter time points. This new mouse model indicates an early implication of glial cells in ODS physiopathology, while it discards BBB disruption as a primary cause of demyelination. It also raises new queries about glial cells heterogeneity in susceptible brain regions (Bouchat et al., 2018).
Understanding the role of Elongator in synaptogenesis during cortical development

Loic Broix, Laurent Nguyen

Université de Liège

Presenting author: Loic Broix

Genetic variants of Elongator subunits (Ep1-Elp6) have been associated with neurological disorders characterized by synaptic deficits. Therefore, we postulate that Elongator controls the development and function of synapses bridging cerebral cortical neurons and that Elp2 variants may cause cognitive deficits in patients by interfering with these synaptic processes. The project aims to decipher how Elongator controls synaptogenesis and test whether and how human Elp2 mutations impair these processes. For this purpose, we first show the localization of Elp subunits at the synapses using purified synaptosomes and its specific localization at both pre- and postsynaptic terminals of excitatory and inhibitory synapses in cultured neurons. We also demonstrate that deletion of Elongator leads to a decrease density of synaptic markers in the dendrites. We will next study synapse morphology and activity in vivo in the Elp3 conditional KO mouse model and Elp2 KI mouse model harboring variants associated with neurological disorders. Moreover, we will try to shed new light on the role of local translation regulation whose alteration may underlie a broad array of neurological disorders characterized by synaptogenesis defects. Our results will help us understanding the pathophysiological mechanisms that underlie the neurodevelopment defects and cognitive deficits observed in Elp2 patients.
Non-pulsed sinusoidal electromagnetic field rescues animals from severe stroke through induction of angiogenesis

Annelies Bronckaers, Lena P. Font, Miriam M. Cardonne; Hannelore Kemps; Raf Meesen; Oneida F. Salmon; Fidel G. González; Ivo Lambrichts; Jean-Michel Rigo; Bert Brône

Presenting author: Annelies Bronckaers

Despite the high prevalence and devastating outcome, only a few treatment options for cerebral ischemic stroke exist. We evaluated whether Non-Pulsed Sinusoidal Electromagnetic Field (NP-SEMF) is able to increase survival and neurological outcome in a rat model of cerebral ischemia. After ischemic injury, induced by occlusion of both common carotid arteries, animals received daily 20 minutes NP-SEMF treatment of either 10 or 60 Hz for 4 days. NP-SEMF dramatically increased survival, reduced the size of the infarcted brain area and significantly improved the neurological score of the surviving rats. In the hippocampus, a significant increase of blood vessels was found after treatment. In addition, NP-SEMF enhanced angiogenesis in the chicken chorioallantoic membrane assay, while it also stimulated proliferation, migration and NO production of endothelial cells in vitro. The importance of NO as a key signaling molecule in the beneficial effect of NP-SEMF was highlighted by inhibition of the NO synthase. Our results indicate for the first time that NP-SEMF exposure (13.5mT at 60 Hz and 10Hz) induces NO-mediated angiogenesis in vitro and improves the survival and neurological outcome of cerebral ischemia in rats underlining its therapeutic potential in stroke.
Unravelling the pathophysiological mechanisms of LIS1-associated human cortical malformations

Cordón-Barris L., Reiner O., Ladewig J., and Nguyen L

Université de Liège

Presenting author: Lluís Cordón Barris

LIS1 is a keystone protein in neuronal development that controls various biological activities, such as cellular transport, proliferation of neuronal progenitors and neuronal migration. Functional disruption of this protein is responsible for Miller Dieker syndrome (MDS), characterized by cortical malformations such as different lissencephalic grade of severity and subcortical band heterotopia. In order to decipher the pathological mechanisms resulting from LIS1 mutation, we engineered dorsal cortical progenitors (DCP) from CRISPR/Cas9 edited human embryonic stem cells (hESC) that express reduced levels of LIS1 protein. This cellular model will be used to uncover the molecular pathways and the correspondent cellular functions dysregulated when LIS1 is not properly expressed in early cortical progenitors. The candidate genes connection to LIS1 will be confirmed in this model by gain of function (GOF) or lose of function (LOF) experiments and screened in the exome of different lissencephalic patients. This study will bring into light the pathophysiology of lissencephaly with new molecular pathways associated to LIS1 disruption that can be target by drugs to prevent, rescue or ameliorate the prognosis of this disease.
Smad-interacting-protein 1 (Sip1, Zeb2) orchestrates migration and differentiation of olfactory bulb interneurons


Katholieke Universiteit Leuven

Presenting author: Astrid Deryckere

Neurogenesis in the mouse brain is continued after birth in two distinct niches being the ventricular-subventricular zone (V-SVZ) lining the lateral ventricle and the dentate gyrus of the hippocampus. Neuroblasts produced in the V-SVZ migrate tangentially through the rostral migratory stream towards the olfactory bulb (OB) where they integrate and give rise to a variety of OB interneurons. Smad interacting protein 1 (Sip1/Zeb2) is a transcription factor that binds E2-box-type sequences and mutations in one allele cause Mowat-Wilson syndrome, a severe multi-spectrum neurodevelopmental disorder. Presence of Zeb2 has been found embryonically in the LGE and postnatally in the V-SVZ. We previously showed that conditional deletion of Zeb2 in either the LGE or V-SVZ results in a postnatal decrease in proliferation capacity as well as migration and differentiation defects. Moreover, we found that postnatal overexpression of Sox6 results in a similar phenotype. Here, we evaluate the outcome of Zeb2 deletion on the proliferative capacity of the V-SVZ using Cre-drivers that target different populations of LGE progenitors. Furthermore, we show that most OB interneuron types (Calbindin, Calretinin, ST4, Tyrosine Hydroxylase, Somatostatin and Parvalbumin) are affected after Zeb2 deletion using a Gsh2 cre-driver and that in this process, Zeb2 also acts non-autonomously.
The neuroregenerative effect of human dental pulp stem cells in vitro: revealing the potential of IGF-II

Yörg Dillen, Hannelore Kemps, Sara Lambrichts, Pascal Gervois, Ivo Lambrichts, Esther Wolfs, Annelies Bronckaers

Universiteit Hasselt

Presenting author: Yörg Dillen

Ischaemic stroke is a severe condition which is defined by loss of brain function due to impaired blood flow to the brain. Cell-based therapy is considered as a promising approach to minimize neurological damage and enhance functional recovery. The goal of this study is to evaluate the neuroregenerative effect of human dental pulp stem cells (DPSC) in vitro and identify the key paracrine factors mediating this effect. The effect of DPSC on the migration of NSC was investigated. Therefore, a transwell migration assay with mouse NSC was performed. The conditioned medium (CM) of DPSC was able to significantly increase the migration of NSC (n = 8). Various growth factors, including BDNF, NGF, GDNF, NT-3 and IGF-II were shown to be secreted by DPSC and their particular effect on NSC migration was investigated. IGF-II significantly attracted NSC in the transwell system (n = 7), revealing a potential role for IGF-II to stimulate the neuroregenerative process after stroke. To investigate the contribution of IGF-II to the stimulatory effect on NSC migration by CM of DPSC, the transwell migration experiments was performed while the function of IGF-II is inhibited. Preliminary experiments show a reduction in migration of NSC when the IGF-II function is blocked in the CM of DPSC. Taken together, our data reveal a promising role for IGF-II as a neuroregenerative strategy.
The role of intestinal cell kinase in interneurons, a protein mutated in Juvenile Myoclonic Epilepsy

Maxime Gilsoul, Bernard Coumans, Antonio-V Delgado-Escueta, Laurence de Nijs, Laurent Nguyen, Bernard Lakaye

*Université de Liège*

**Presenting author:** Maxime Gilsoul

Heterozygous mutations in intestinal cell kinase (ICK) have been associated with juvenile myoclonic epilepsy (JME). Considering epilepsy as an imbalance between excitation and inhibition, it appeared interesting to study the effect of JME mutations on inhibitory side and so to determine the role of ICK in interneurons. The presence of ICK in the ganglionic eminence (GE) was revealed by ISH on mouse brain section at E14.5. After dissection of the medial ganglionic eminence (MGE), the level of expression has been determined by RT-qPCR on FACS sorted MGE-derived cells obtained from Dlx5,6-Cre-GFP mouse brain at E13.5. The presence of ICK in the GE could suggest a role plays by ICK in the interneurons. Invalidation of ICK in the GE was obtained by crossing ICKfloxed mouse with Dlx5,6-Cre –GFP mouse. The genetic mouse model showed that absence of ICK affects the size and growth of mouse. Indeed, after birth evidence growth delay, as body size and skin color, was observed for 2 pups (Dlx5,6 Cre/+; ICK L+/+) in two littermates. At P25, mice were still smaller (body weight = 9.1± 0.8g compared to others = 14.5 ± 1.7g) but brains looked similar.
Potential Therapeutics for ZIKV-Induced Microcephaly

Ivan GLADWYN-NG, Christian ALFANO, Lluis CORDON-BARRIS, Giovanni MORELLI, Catherine CREPPE, Thérèse COUDERC, Marc LECUIT and Laurent NGUYEN.

Université de Liège

Presenting author: Ivan GLADWYN-NG

While the causal link between Zika virus (ZIKV) infection during gestation and congenital microcephaly is increasingly supported by recent publications, the underlying mechanisms remain poorly elucidated. Our recent publication showed that ZIKV triggers endoplasmic reticulum (ER) stress in the cerebral cortex through combinatorial analyses of ZIKV-infected post-mortem human foetuses, mouse embryos and cultured human neural stem cells. The virus perturbs a physiological unfolded protein response (UPR) within cortical progenitors that controls neurogenesis, which leads to diminished generation of projection neurons. Here, we demonstrate, via cellular and genetic assays, that intracerebral administration of different pharmacological inhibitors of distinct arms of the UPR counteracts the pathophysiological up-regulation of the ER stress within ZIKV-infected mouse embryonic brains. Concomitantly, the pharmacological inhibition of ZIKV-induced ER stress / UPR by different compounds was associated with the prevention of microcephaly in infected embryonic mice. In conclusion, we developed an in vivo model for ZIKV infection, as well as validated it for the testing of potential pharmacological compounds. Our results suggest the pharmacological inhibition of ER stress-induced UPR may contribute towards a treatment strategy in ZIKV congenital infections.
HDAC6 inhibition rescue axonal transport defects in motor neurons derived from ALS patients

Wenting Guo, Catherine Verfaillie, Ludo Van Den Bosch

Katholieke Universiteit Leuven

Presenting author: Wenting Guo

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder due to selective loss of motor neurons (MNs). Previous research to understand pathogenic ALS mechanisms is mainly based on rodent models overexpressing a mutant gene and many potential therapies for ALS have been unsuccessful in human clinical trials. The cell reprogramming technology which enables the generation of human induced pluripotent stem cells (hiPSCs) from human somatic cells, gave researchers a new opportunity to obtain human MNs by adding small molecules. As a patient derived in vitro model, the iPSC model is convenient for investigating mechanism, selecting potential biomarkers as well drug screening. Mutations in the fused in sarcoma (FUS) gene can cause both juvenile and late onset ALS. We generated and characterized induced pluripotent stem cells (iPSCs) from ALS patients with different FUS mutations, as well as from healthy controls. Patient-derived MNs show progressive axonal transport defects which is in line with clinic symptoms of ALS. Axonal transport defects are rescued by CRISPR/Cas9-mediated genetic correction of the FUS mutation in patient-derived iPSCs. Moreover, these defects are reproduced by expressing mutant FUS in human embryonic stem cells (hESCs), whereas knockdown of endogenous FUS has no effect, confirming that these pathological changes are mutant FUS dependent. Pharmacological inhibition of histone deacetylase 6 (HDAC6) increase α-tubulin acetylation and restore the axonal transport defects in patient-derived MNs. Here we propose HDAC6 inhibitors which has been used for cancer as a promising drug candidates for treating ALS disease.
The CXCL12/CXCR4/integrinβ1 signaling axis in microglial migration during brain development

Sofie Kessels, Sophie Smolders, Silke Smolders, Kaline Arnauts, Jean-Michel Rigo, Pascal Legendre and Bert Brône

Universiteit Hasselt

Presenting author: Sofie Kessels

BACKGROUND During embryonic brain development microglia, myeloid-derived cells originating from the yolk sac, already invade the brain at embryonic day (E)10.5 in mice. CXCL12 signaling through its receptor CXCR4 was found to recruit microglial cells to the cortex. The β1 integrin is a downstream target of CXCR4 in cancer and immune cells and increases adhesion and invasion after CXCL12 signaling. HYPOTHESIS As we previously demonstrated the involvement of α5β1 integrin during microglial migration in the embryonic mouse cortex, we hypothesized that the CXCL12/CXCR4/β1 integrin signaling axis drives microglial migration during embryonic brain development. RESULTS We found that BV-2 cells, a microglia cell line, showed increased migration towards CXCL12 in the presence of fibronectin, which was inhibited by blocking CXCR4. However, this was not observed in primary cultures of microglia. Blockage of integrin β1, PI3K and MEK1/2 reduced BV-2 cell migration towards control levels, while β2 integrin blockage did not affect migration. Blockage of integrin β1, PI3K and MEK1/2 in acute brain slices at E13.5 and E17.5 differentially affected microglial migration speed, while CXCR4 blockage did not have any effect. CONCLUSION Our results support the presence of a CXCL12/CXCR4/β1 integrin signaling axis in BV-2 cells in vitro only. They argue against an involvement of CXCR4 signaling in regulating microglial migration speed in vivo after these cells have invaded the brain parenchyma in the developing embryo.
Loss of Elp3 induces postnatal hydrocephalus by inducing endoplasmic reticulum stress and dysregulation of Notch signaling


Université de Liège

Presenting author: Sophie Laguesse

Ependymal cells line the ventricle of the adult brain and bear multiple motile cilia at their apical surface. Planar polarization of ependymal cells determines the orientation of motile cilia whose coordinated beating drives directional cerebrospinal fluid (CSF) flow. Defects in planar polarity establishment and impaired ciliogenesis have been associated with hydrocephalus and developmental abnormalities. Although several proteins have been identified as planar cell polarity core components, the precise regulation of the establishment of ependymal planar cell polarity and cilium assembly during cortex development remain poorly understood. Here, using a conditional knock-out mouse model, we showed that a deletion of Elp3, the catalytic subunit of the Elongator complex, in the embryonic telencephalon disrupts the coordinated motile cilia beating and the cerebrospinal fluid flow, leading to enlarged ventricles and postnatal hydrocephaly. Indeed, Elp3 depletion impairs the rotational and translational polarities of ependymal motile cilia, as well as the organization of ciliary tufts at the surface of the lateral wall. Ependymal cells are derived from embryonic radial glial cells, where Elp3 is also required for the coordinated planar polarity and the growth of the primary cilium. The primary cilium has been suggested to participate in the organization of ependymal cells planar polarization and we showed that loss of Elongator activity leads to the accumulation of misfolded proteins that trigger endoplasmic reticulum stress and activate the unfolded protein response. This in turn inhibits Notch signaling and impairs primary ciliogenesis. These concomitant defects in ciliogenesis and planar polarity of ependymal cells perturb the coordinated beating of motile cilia, leading to impaired CSF flow and severe hydrocephalus.
p27Kip1 modulates axonal transport by regulating α tubulin acetyltransferase 1 stability

Giovanni Morelli, Aviel Even, Ivan Gladwyn-Ng, Romain Le Bail, Michal Shilian, Juliette D. Godin, Elise Peyre, Bassem A. Hassan, Arnaud Besson, Jean-Michel Rigo, Miguel Weil, Bert Brône, Laurent Nguyen

Université de Liège

Presenting author: Romain Le Bail

The protein p27Kip1 plays roles that extend beyond cell-cycle regulation during cerebral cortex development, such as the regulation of neuronal migration and neurite branching via signaling pathways that converge on the actin and microtubule cytoskeletons. Microtubule-dependent transport is essential for the maturation of neurons and the establishment of neuronal connectivity through synapse formation and maintenance. Here we show that p27Kip1 controls the transport of vesicles and organelles along the axon of mice cortical projection neurons in vitro. Moreover, suppression of the p27Kip1 ortholog, dacapo, in Drosophila melanogaster disrupts axonal transport in vivo, leading to reduction of locomotor activity in 3rd instar larvae and adult flies. At the molecular level, p27Kip1 stabilizes the α tubulin acetyltransferase ATAT1, thereby promoting the acetylation of microtubules, a post-translational modification required for proper axonal transport.
The impact of prenatal exposure to androgens on gametogenesis in rats

Elise Marescaux, Annica Frau, Denis Nonclercq

Université de Mons

Presenting author: Elise Marescaux

Hyperandrogenization is a major characteristic of the polycystic ovary syndrome (PCOS). Testosterone exposure during prenatal life seems to be the most reproducible animal model for this syndrome. Pregnant rats (Sprague-Dawley) received subcutaneous injections of testosterone (1mg/kg and 3 mg/kg) between day 16 and 19 of gestation. Male and female offspring exposed in utero were sacrificed on day 4 of postnatal life to study early alterations of gametogenesis. In females, hyperplasia of the primordial follicles and an acceleration of follicular maturation have been highlighted. In males, a decrease of Sertoli cells and any alteration of Leydig cells has been detected. These alterations found in females and males show that the in utero exposure to androgens induces early disturbances of gonad development.
Unveiling the roles of Down Syndrome Cell Adhesion Molecules in mammalian forebrain development

Manuela Dimitra Mitsogiannis, Eve Seuntjens

Katholieke Universiteit Leuven

Presenting author: Manuela Dimitra Mitsogiannis

Down Syndrome (DS) Cell Adhesion Molecules (DSCAMs) are transmembrane proteins of the immunoglobulin superfamily. Human DSCAM is located on chromosome 21’s DS critical region, and mutations or copy-number variations of this gene have also been associated to Fragile X syndrome, intellectual disability, autism, and bipolar disorder. The DSCAM paralogue DSCAM-like 1 (DSCAML1) maps to chromosome 11q23, implicated in the development of Jacobsen and Tourette syndromes. Additionally, a spontaneous mouse DSCAM deletion leads to motor coordination defects and seizures. Previous studies have revealed the involvement of DSCAMs in several neurodevelopmental processes, including synaptogenesis, neural proliferation, dendritic self-avoidance, cell sorting, and axon growth/guidance. However, their functions in mammalian forebrain development have yet to be completely elucidated. DSCAMs are known to be expressed in the developing neocortex, and to regulate radial cortical migration and callosal projections development. However, these proteins are also present in embryonic ventral forebrain regions, and DSCAMs-related neurodevelopmental disorders present both cortical and basal ganglia dysfunction features. Our research therefore aims at unraveling DSCAMs roles in the developing mouse brain, with a focus on ventral telencephalic formation and function, through the phenotypical analysis of DSCAM/DSCAML1 knockout mice, and via targeted genetic manipulation during embryonic and early postnatal mouse development.
Deciphering the role of NEDD4-2 in physiological and pathological interneurons migration

Martin MOÏSE, Laurent NGUYEN

Université de Liège

Presenting author: Martin Moïse

The aim of this project is to identify the pathomechanisms triggered by mutations in NEDD4-2, a gene coding for a HECT-domain E3-ubiquitin ligase recently associated to malformations of cortex development (MCD), mental retardation and epilepsy as well as less constant malformation disorders. Both spatial and temporal patterns of expression are consistent with a role of Nedd4-2 in post-mitotic interneurons during their tangential migration to the cortex. We created a new conditional KO mouse model crossing Nedd4-2flox/flox mice with Dlx5,6:CRE-GFP mice to generate cKO embryos. In these settings, we didn't find any defect in IN progenitors proliferation nor in survival of pre- and post-mitotic INs but were able to highlight a precocious defect of migration marked at early stages by a decreased number of INs exiting the subpallium. We further performed ex vivo functional analysis (time lapse recordings) to assess single-cell migration features. cKO migrating INs exhibit a decreased frequency of nukleokinesis resulting in a decreased speed of migration. Further biochemical and electrophysiological explorations are required to identify relevant E3 substrates such as calcium ion channels, calcium influx being a pivotal event of INs migration.
Role of the centrosomal protein CDK5RAP2 in human cochlear development

Anaïs Mounier, Amandine Czajkowski, Kevin Hanon, Bilal Mughal, Sandrine Passemard, Laurent Nguyen, Brigitte Malgrange

Université de Liège

Presenting author: Anaïs Mounier

Hearing impairment (HI) affects almost 250 million people worldwide and is mainly caused by damage to cochlear hair cells (HCs) that convert mechanical stimuli of sound into electrical signals. At least 60% of the people with early-onset HI have hereditary hearing loss (HHL) due to genetic mutations. In this context, we are studying the Cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (CDK5RAP2). Mutations in CDK5RAP2 gene are associated to autosomal recessive primary microcephaly 3 (MCPH3) and recent observations showed that they can also be associated to congenital hearing loss. In order to study the mechanisms leading to HHL in MCPH3 patients, we are using the technology of induced pluripotent stem cells (iPSCs). Patients with mutations in CDK5RAP2 gene and showing a neuro-sensory deafness are recruited at the Neuropaediatry Service of the Robert Debré Hospital. Fibroblasts from these patients and healthy individuals are then reprogrammed into iPSCs. These iPSCs are differentiated into otic progenitors (OSCs) which can be differentiated into HCs. We are also generating inner ear organoids from iPSCs using a 3D differentiation protocol mimicking human cochlear development. We observed that CDK5RAP2 protein is not detected at the centrosome as it should be in the mutated OSCs. They also show a lower proliferation rate and lower expression of otic progenitor markers when compared with WT OSCs. A lower expression of otic progenitors was observed in the mutated organoids as well. These results suggest that the mutation of CDK5RAP2 could affect the cell division in the otic progenitors, which can explain the deafness observed in the patients.
Deciphering the action mechanism of Pcdh19 during cortical development


Katholieke Universiteit Leuven

Presenting author: Anna Gabriela Pancho Yanza

PCDH19 is a gene that codes for the transmembrane protein Pcdh19. More importantly, this gene is the cause of an X-linked neurodevelopmental disorder, epilepsy with mental retardation limited to females (EFMR). Strikingly EFMR only affects females while males are spared from epilepsy. It has been suggested that the mosaic absence of Pcdh19 triggered by X-random inactivation leads to the onset of the disease. During brain development connections between excitatory projection neurons and inhibitory neurons are established via proper neuronal migration. As epilepsy is one consequence of disturbed interneuron migration we suggest that Pcdh19 is important within this process. We therefore analyzed Pcdh19 expression during brain development and found it within important regions for origin of interneurons and in the neocortex. Furthermore, we mimicked the mosaic absence in knockdown mouse models and detected perturbed cortical interneuron migration. As we think that downstream signaling pathways and protein interaction partners of Pcdh19 are key for the disorder we employed CRISPR-CAS9 to endogenously label Pcdh19 in order to perform future proteomic studies. Additionally, we are assessing potential nuclear translocation of the intracellular domain of Pcdh19 which might be crucial for nuclear signaling. We expect to reveal the functional role of Pcdh19 during brain development.
The neural environment is a key determinant for directing iPSC-derived myeloid progenitors into the CX3CR1+CCR2-microglia-like phenotype

Alessandra Quarta¹, Debbie Le Blon¹, Tine D’Aes¹, Vincent Pasque², Somayyeh Hamzei Taj³, Mathias Hoehn³, Zwi Berneman¹, Peter Ponsaerts¹

¹Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (Vaxinfectio), University of Antwerp, Antwerp, Belgium
²Stem Cell Biology and Embryology, Department of Development and Regeneration, KU Leuven, Leuven, Belgium.
³In-vivo-NMR Laboratory, Max Planck Institute for Metabolism Research, Cologne, Germany.
⁴Department of Radiology, Leiden University Medical Center, Leiden, Netherlands.

Presenting author: Alessandra Quarta

Differentiation of microglia from iPSC holds great potential for in vitro neurodevelopment and immunology research. Here, we present a novel protocol to obtain yolk sac-like CX3CR1+CCR2-macrophage progenitors from CX3CR1eGFP/+CCR2RFP/+ murine iPSC, which following co-culture with astrocyte-committed neural stem cells (aNSC) mature into a highly uniform population of CX3CR1+CCR2-iPSC-derived microglia that display typical ramified or ameboid morphology and are able to colonize microglia-depleted organotypic brain slice cultures (mdOBSC). Further characterisation of these iPSC-microglia revealed their inability to upregulate the MHCII activation marker and a lower degree of TNFα secretion following LPS+IFNγ stimulation as compared to CX3CR1-CCR2-iPSC-derived macrophages. This distinct MHCII expression pattern was strikingly similar to the expression pattern of MHCII on endogenous CX3CR1+CCR2- microglia and infiltrating CX3CR1+CCR2+ monocytes following experimental stroke in CX3CR1eGFP/+CCR2RFP/+ mice. Despite distinct phenotype, also CX3CR1-CCR2- iPSC-macrophages, when cultured in the presence of aNSC or on mdOBSC, were subject to rapid conversion into CX3CR1+CCR2-microglia-like cells with ameboid or ramified morphology and reduced expression of MHCII. These results suggest that a neural environment is the main determinant for myeloid progenitors to adopt the CX3CR1+CCR2-microglia phenotype, although it remains to be investigated whether both populations described here are identical or different microglia-like cells.
Embryonic DNA damage results in premature neuronal differentiation via an EMT-like mechanism

André C.M. Mfossa, Mieke Verslegers, Tine Verreet, Elke Gabriel, Winnok H. De Vos, Jay Gopalakrishnan, Wilfred van Ijcken, Rafi Benotmane, Danny Huybrechts, Roel Quintens

Belgian Nuclear Research Centre

Presenting author: Roel Quintens

The developing brain is very sensitive to DNA damage as exemplified by the frequent occurrence of microcephaly in DNA repair deficiency syndromes and after in utero exposure to ionizing radiation. Unlike primary microcephaly, DNA damage-induced microcephaly has not yet been associated with premature neuronal differentiation. Here, we show that a transient induction of DNA damage by irradiation of mice at embryonic day 11 was accompanied by a G2/M cell cycle block followed by massive apoptosis of cortical cells. The induction of premature neuronal differentiation was demonstrated by a decrease of radial glia cells and the occurrence of ectopic postmitotic neurons while no change was observed in the number of intermediate progenitors. Moreover, the number of horizontal mitoses was increased. This was associated with a disruption of the apical adherens junction belt, a reduction of epithelial cell markers and reduced expression of Qki5, a regulator of epithelial-to-mesenchymal transition. The importance of p53 in the observed phenotype was shown by a strong p53-dependent induction of gene expression and a partial rescue of the brain size reduction in irradiated Emx1-cre+/-; Trp53fl/fl mice. Finally, preliminary experiments showed delayed growth of irradiated human brain organoids, suggesting their suitability as a model for radiation-induced microcephaly.
Characterizing the dynamics of the gene regulatory network (GRN) driving hepatocyte development is essential for understanding how cell fate decisions are made, and for optimizing in vitro production of hepatocytes. The GRN driving hepatocyte differentiation comprises eight liver-enriched transcription factors (LETFs). Current functional studies cannot predict how each factor quantitatively controls the expression of all other members of the GRN. Our goal is to develop a computational tool to capture the temporal dynamics of the GRN and to predict how quantitative variations of individual GRN members impact on the global function of the network. We measured LETF expression at several stages in developing hepatocytes, using total RNA from whole liver and FACS-purified hepatocytes, from wild-type and mutant mouse embryos. Our measurements were used to calibrate a mathematical model describing the temporal expression of the GRN members throughout development. The model was validated by comparing the in silico-predicted and experimentally-measured effect of a miRNA inhibiting LETFs. We currently generate mouse hepatoblasts in vitro by forced expression of LETFs and verify if the GRN obtained in vitro matches with that from developing liver. We will adapt the mathematical model to provide a tool for improving LETF-driven differentiation of human hepatocytes in vitro.
Unveiling the neurogenesis defects induced by prenatal alcohol exposure.

Laura Van Hees, Sophie Laguesse, Laurent Nguyen

Université de Liège

Presenting author: Laura Van Hees

Prenatal alcohol exposure (PAE) is known to damage the fetal brain and leads to life-long cognitive and behavioral dysfunctions. Fetal Alcohol Spectrum Disorders (FASD), which collectively describes the constellation of effects resulting from alcohol consumption during pregnancy, is a complex syndrome that affects up to 5% of children and is the leading cause of preventable intellectual disability. Despite prevention campaigns discouraging alcohol drinking during pregnancy, the number of children suffering from FASD has not decreased over the past years. The consequences of PAE has become a global public health problem and understanding the alcohol-related mechanisms is crucially needed to develop new pharmacological strategies and treatments. Studies have shown that alcohol interferes with the cerebral cortex development in a variety of ways, including defects in neurogenesis, impaired cell proliferation and cell migration, reduced survival and disrupted neurotransmission. However, the precise pathophysiological mechanisms underlying alcohol's actions on cortical development are yet poorly understood. In this study, we set up a mouse model of FASD, using an alcohol consumption paradigm in which mice voluntarily drink high amounts of alcohol throughout pregnancy. Importantly, this model avoids any bias resulting from maternal stress that could be introduced by stressful alcohol consumption procedures such as gavage or injection. We first showed that this model accurately reflects alcohol consumption in human, as mice reach blood alcohol concentration levels comparable to those reported in binge-drinking humans. In order to investigate alcohol-dependent corticogenesis defects, we are analyzing the number, proliferation and specification of glutamatergic projection neurons during embryonic development and at postnatal stages. By using in utero electroporation, we are investigating the migration pattern of projection neurons during neurogenesis. Our preliminary results reveal an abnormal accumulation of neurons in deep layers of the cortex of alcohol-exposed embryos, suggesting impaired neuronal migration or dysregulated layer specification. In addition, we are conducting behavioral testes on newborn mice, evaluating the acquirement of developmental milestones between alcohol-exposed newborn mice and the water controls. We will also evaluate adolescent mice behavior and alcohol consumption in order to determine whether PAE has a middle-term impact on adolescent behavior and drinking pattern.
Prdm12 is essential for nociceptor development

Vermeiren Simon, Desiderio Simon, Van Campenhout Claude, Malki Elisa, Tsibos Panagiotis, Kricha Sadia, Pieler Tomas, Henningfeld A. Kris, Nagy Vanja and Bellefroid Eric

Université Libre de Bruxelles

Presenting author: Simon Vermeiren

The ability to detect harmful stimuli is crucial for survival and constitute the main function of nociceptors, a diversified group of sensory neurons located in the trigeminal (TG) and dorsal root ganglia (DRG), innervating the head and body. Any event that interferes with the development and function of nociceptors can lead to incurable diseases. Recently, mutations in the epigenetic regulator coding gene Prdm12 were found in patients with congenital insensitivity to pain. Prdm12 is expressed during vertebrate development in the brain and spinal cord, but also in TG and DRG, where its function is not well known. In this study, we characterized Prdm12 expression pattern in TG and DRG during mouse development, and its function using Knock Out (KO) and conditional KO approaches. We observed that Prdm12 expression starts in sensory neuron progenitors, then restricted to nociceptors expressing TrkA, and remains in several subtypes of mature nociceptors. Analysis made on Prdm12 KO and cKO embryos revealed that Prdm12 is important to initiate and maintain the expression of TrkA in TG and DRG, a receptor crucial for nociceptor survival and maturation. We also highlighted differences in the mechanisms leading to the loss of nociceptors in Prdm12 KO TG and DRG.
Unravelling the role of the CAK complex in the morphological development of cortical neurons

Sébastien Verteneuil, Quentin Marlier, Mariano Barbacid, David Santamaría, Laurent Nguyen, Renaud Vandenbosch and Brigitte Malgrange

Université de Liège

Presenting author: Sébastien Verteneuil

Aim: Cdk-activating kinase (CAK) complex is a trimeric (Cdk7, cyclin H and Mat1) protein complex mostly known for its positive role on proliferation through cell cycle Cdns phosphorylation. Besides its role on Cdns, CAK complex also regulates transcription both directly by phosphorylating RNA polymerase II, promoting thereby transcription initiation, and indirectly by phosphorylating several nuclear receptors, leading to specific genes transcription. Despite this plural transcriptional role, CAK complex disruption does not impact global transcription but affects different subsets of genes in specific tissues. As morphological development of cortical neurons relies on a tight regulation by a complex transcriptional program, we investigate the potential role of the CAK complex in this process. Methods: To decipher CAK complex function(s), we use a conditional knock-out mouse model (NexCre) in which Cdk7, the CAK complex catalytic member, is deleted from postmitotic cortical and hippocampal neurons. We also perform in vitro loss-of-function assays using primary cortical neurons cultures. Results: Our results indicate that Cdk7 conditional invalidation in postmitotic cortical neurons induces cortical layer-unspecific neuronal packing without affecting total number of neurons in vivo. This phenotype is associated to dendritic size and complexity impairments in those neurons, observed in both in vivo and in vitro experiments. Conclusion: Together, our data highlight a role for the CAK complex in the morphological development of cortical neurons. Further investigations will be conducted to determine 1) if the phenotype is restricted to dendrites and 2) the mechanisms underlying this phenotype.
Unraveling NeuroD1 mechanism of action

Reuter AS, Stern DG, Peers B, Manfroid I, Voz ML

*Université de Liège*

**Presenting author:** Anne-Sophie Reuter

ARP/ASCL factors are key determinants of cell fate specification and differentiation in a wide variety of tissues, notably in the nervous system where they act as proneural factors, coordinating the acquisition of generic cell fates and of specific subtype identities. In the digestive system, ARP/ASCL factors act also as cell fate determinants and cross-species comparison amongst vertebrates highlighted that the identity of these determinants depends not only on the organ but also on the species. As zebrafish is a good and easy model to perform phenotypic rescue experiments, we tested in this model whether expression of other members of the ARP/ASCL family could rescue the intestinal secretory defects of the ascl1a-/- mutant. We showed that any ARP/ascl factor is able to initiate the first step of the secretory cascade but the subsequent step(s) of the endocrine differentiation program requires Neurod1. In fact, it is the only one able to rescue enteroendocrine cells while other ARP/Ascl tested only rescue goblet intestinal cells. By constructing several hybrid proteins between Ascl1a and Neurod1, we could highlight a domain, highly conserved in all NeuroD subfamily members but not present in the atonal, neurog and ascl members, which is necessary and sufficient to rescue the enteroendocrine cells.