Block 1

Project 1 : *"Live imaging of microtubule dynamics in mouse brain slices"*

Instructor : Alexandre Baffet (Institut Curie, Paris, France)

A large number of genes associated with malformations of cortical development (MCDs) code for regulators of the microtubule cytoskeleton. The organization and regulation of microtubules in the developing neocortex is however still poorly characterized. We have recently established a method for subcellular live imaging within mouse brain slices enabling to monitor microtubule dynamics *in situ*. In the mini-project, the students will ex-utero electroporate E14.5 mouse embryonic cortices with a plasmid coding for EB3-GFP, a factor that dynamically tracks the growing microtubule plus ends. Brains will then be sliced using a vibratome, and 300μm-thick slices put into culture on filters at the air-liquid interface for 24 hours. Slices will then be live imaged using a spinning disk microscope at high spatial and temporal resolution. The students will focus on radial glial progenitor cells, the neural stem cells of the developing neocortex. They will quantify various parameters, including the polarity of microtubules in these cells. They will furthermore test if apically-directed and basally-directed microtubules have different half-lives, in order to test if these two populations are differentially stabilized. Using the same method, the students will image mitochondrial dynamics and, based on their observations, build hypotheses on the mechanisms of mitochondrial transport in radial glia cells.

Techniques:

- Mouse embryonic brain dissection
- *Ex utero* electroporation
- Vibratome sectioning and slice culture
- Subcellular live imaging *in situ*
- Image analysis, including particle tracking

Project 2 : "Role of dopamine-glutamate interplay during synaptogenesis".

Instructor : Nathan Benac (Interdisciplinary Institute for Neuroscience, Bordeaux, France).

Dopamine, one of the major neuromodulators of the brain, has a complex role in the formation of excitatory synapses. In this project, we will investigate how the interplay between glutamate and dopamine modulates the formation of glutamatergic synapses. To do so, we will co-culture hippocampal neurons with genetically-modified heterologous cells in order to examine the synaptogenic properties of selected surface proteins. The formation of synapses will be assessed by super-resolution microscopy and their functionality will be assessed through live calcium imaging.

Techniques :

- Cell culture (dissociated hippocampal neurons, COS-7)
- Cell transfection
- Immunostaining
- Super-resolution microscopy
- Live imaging

Project 3 : "Studying a developmental mosaic brain disorder in human cortical spheroids"

Instructor: Sara Bizzotto (ICM, Paris Brain Institute, France)

The human brain is a mosaic of clones each carrying a unique set of de novo postzygotic somatic mutations that occur during embryonic development. While the great majority of these mutations are neutral, some may cause developmental brain disorders such as focal cortical dysplasia (FCD) by hyperactivating the mTOR pathway. Although disease modeling in the mouse has revealed very useful insights into these pathologies, more specific models are needed to better dissect human-specific disease mechanisms and developmental emergence. Modeling of these disorders requires reproducing the pathogenic mutation at a mosaic state and study its effects on mutant and non-mutant surrounding cells. During this project, we will generate hiPSCs-derived cortical spheroids and transfect them with mutant constructs to mimic the mutation mosaic state. We will then slice the spheroids and perform immunostaining for markers of specific brain cell types, visualize the slides and cleared whole-organoids at the microscope and perform acquisitions for image analysis. We will study the cell type composition differences between control and mutant organoids and explore the differentiation of mutant and non-mutant cells. Furthermore, we will analyze cell morphology and perform staining for phosphorylated ribosomal protein S6 (pS6), a hallmark of mTOR activation.
Single-cell RNA sequencing (scRNA-seq) is particularly useful in order to study a mosaic disorder since it allows to explore transcriptomic perturbations at the single cell level, and thus to identify perturbations specific to mutant cells and discriminate cell autonomous from non-cell autonomous effects. We will thus learn how to prepare cortical spheroid single cell suspensions using fluorescence-activated cell sorting (FACS) for droplet-based (10X Genomics) high- throughput scRNA-seq. We will then visualize scRNA-seq data and go through an example of data analysis and visualization to understand the type of information we can extrapolate from such high-throughput data.

Techniques:
- Human cortical spheroid generation
- Construct transfection
- Immunohistochemistry
- Microscopy
- scRNA-seq

Project 4: "Histological and transcriptional analysis of isochronically labelled cortical neurons".

Instructor: Sergi Roig Puiggros (University of Geneva, Switzerland)

The structure and functions of the cerebral cortex are the result of a developmental process during which neurons are born from apical progenitors (APs), located in a germinal zone lining the ventricles, the ventricular zone (VZ). New-born neurons migrate from the VZ into the cortical plate, where they differentiate and assemble into functional circuits. Interestingly, APs produce cortical neurons following an "inside-out" pattern, with deep layer neurons produced early and superficial ones, late. This highly orchestrated process is key to ensure the proper cortical architecture and neuronal connectivity.
Students will perform in vivo injections of CFSE in order to isochronically label APs and superficial layer neurons. They will analyse the results using cryostat sectioning and light-sheet microscopy. Students will be taught on how to perform CFSE injections and how to process the tissue in order to image it with a light-sheet microscope. Finally, if time allows it, students will have an introduction on how to analyse scRNAseq data on existing datasets using with the same experimental procedure.

Techniques:
- in utero injections

- tissue freezing and cryostat slicing
- tissue clearing and light-sheet imaging
- introduction to bioinformatics and scRNAseq analysis

Project 5: "Studying the morphological properties of developmentally- and adult-born dentate granule neurons"

Instructors: Emilie Pacary & Pierre Mortessagne (Neurocentre Magendie, Bordeaux, France)

In the dentate gyrus (DG) of the hippocampus, the generation of dentate granule neurons (DGNs) starts during the embryonic period, peaks around the time of birth, and continues at low levels during adulthood. The DG is therefore a peculiar brain structure composed of DGNs of different ontogenic origins constituting sub-populations of DGNs that could play different roles in hippocampal physiology and contribute differently to DG functions. Surprisingly, this hypothesis has received little attention and although the morpho-functional properties of adult-born DGNs (Adu-DGNs) have been extensively studied, very little is known about the developmentally-generated ones. In this project, we will analyse the morphological characteristics of these two populations. For this purpose, we will use in vivo electroporation to label DGNs generated in mouse neonates (P0) and stereotaxic injection of retrovirus into the DG of adult mice to tag Adu-DGNs. The morphology (dendrites, spines, axons, AIS) of the two populations of DGNs will be analysed once mature using immunohistochemistry, a semiautomatic neuron tracing system (Neurolucida) and confocal microscopy.

Techniques:

- Postnatal electroporation
- Stereotaxic retrovirus injection
- Vibratome sectioning
- Immunohistochemistry
- Imaging, image analysis

Project 6: "RGC manipulation to modulate CNS regeneration"

Instructor: Homaira NAWABI (Grenoble Institut Neuroscience- GIN- Grenoble FRANCE)

Retina and optic nerve are considered as gold standard model to address the molecular and cellular mechanisms of central nervous system axon regeneration. In this project we will learn how to modulate the expression of different cues within the retina ganglion cells (RGC), the only neurons from the retina that form the optic nerve. To do so, we will inject Adeno associated viruses into the eye. We will also learn to put retina explant in culture, perform immunostaining and imaging. Whole retina and optic nerve clearing, immunostaining and analysis will also be covered.

Techniques:

- Intravitreal injections
- Retina, optic nerve dissection
- Retina explant culture
- Immunostaining
- Imaging

Project 7: "Study of neuronal migration after prenatal alcohol exposure"

Instructor: Julie Stoufflet (Liège University, Belgium)

The cerebral cortex is characterized by a complex cytoarchitectonic, which is progressively established via the tight coordination of key processes such as neurogenesis, cell migration and differentiation. Perturbation of its development often occurs upon exposure to toxic environmental factors, such as alcohol. Fetal alcohol exposure affects some of these developmental steps, including neurons migration. However, how fetal alcohol exposure affects the mechanisms underlying neuronal migration is not fully understood. Here, I propose to perform in utero (to study projection neurons migration) and ex utero (to study interneuron migration) electroporation to label migrating neurons and modulate the expression of candidate genes deregulated after fetal alcohol exposure to try to re-establish a normal migratory rhythm. We will, then, perform organotypic slices or explants culture and time lapse imaging to record their migratory behavior. With these techniques, we will screen selected candidate genes deregulated after fetal alcohol exposure to see if there are involved in the observed neuronal migration defects.

Techniques:

- in utero / ex-utero electroporation
- primary culture for feeder cortical and explant culture
- mouse brain dissections
- organotypic slices/ vibratome sections
- time lapse imaging
- analyses of migration

Project 8 : "Genome-wide profiling of the epigenome and the transcriptome of major cell types from the developing mouse cortex"

Instructor: Boyan Bonev (Helmholtz Zentrum München, Germany)

How epigenome remodeling governs cell fate decisions in the developing neocortex is mostly unknown, largely due to challenges in inferring causal relationships between changes at the epigenome and transcriptional output in a complex and heterogeneous tissue such as the brain. In this project, the students will learn how to perform ATAC-seq to measure chromatin accessibility and isolate high-quality RNA. The students will learn how to dissect and dissociate embryonic mouse brain and how to prepare and evaluate libraries for RNA and ATAC-seq. We will additionally cover the computational aspects of such type of data analysis and work with already sequenced datasets to identify transcription factors associated with changes in the regulatory landscape of the developing cortex.

Techniques:

- Dissection and dissociation of mouse cortex
- RNA isolation and library preparation
- ATAC-seq library preparation
- Mapping and computational analysis of ATAC/RNA-seq data

Project 9: "Emergence of intracortically-projecting neuron diversity"

Instructor: Esther Klinger (University of Geneva, Switzerland)

The cerebral cortex is the most evolved structure of the brain and the site for higher cognitive functions. It consists of 6 layers, each composed of specific types of neurons. Cortical neurons connecting the distinct cortical areas are located in all cortical layers and form a heterogeneous population.
Here, we will study how and when such a heterogeneous population emerge during postnatal development. For this, we will use retrograde labeling to tag intracortically- projecting neurons based on their projections along development. We will then analyze the layer position and marker expression of labeled intracortically-projecting neurons. Finally, we will analyze single-cell RNA sequencing data of cortical neurons to identify the corresponding populations and their transcriptional programs using R.

Techniques:

- Mouse pups
- Stereotaxic injection of retrograde tracers in pups
- Pup perfusion
- Pup brain sectioning using vibratome
- Immunohistochemistry
- Confocal microscope imaging
- Analyses of cell position and protein expression using ImageJ
- Analyses of single-cell RNA sequencing data using R (Seurat package)

Block 2

Project 10 : "Studying the effect of temporal series in the proliferative capacity of Drosophila neural stem cells"

Instructor : Nikos Konstantinides (Institut Jacques Monod, France)

Neuronal diversity relies on the differential capacity of neural stem cells to generate neuronal types at different developmental stages. In the Drosophila visual system, a series of 11 transcription factors (termed temporal transcription factors - tTFs) are expressed sequentially in the neuroblasts and allow them to give rise to different neuronal types. While it has been shown that tTFs affect neuronal output, it is unclear how they influence neuroblast proliferation. In the course, we will use the Drosophila genetic arsenal to generate mutant clones of tTFs and immunohistochemistry and confocal microscopy to address the effect of the mutations on neuroblast lifespan and proliferation capacity. In particular, we will try to answer whether neuroblasts divide faster or slower, how the temporal series is affected, and whether neuroblasts continue to proliferate aberrantly in pupal and adult stages.

Techniques:

- Fly husbandry
- mutant (MARCM) clones + EdU/BrdU staining
- larval brain dissections
- Immunohistochemistry
- Confocal imaging
- Image analysis

Project 11: "MAGIC Markers strategies to investigate neuron-astrocyte anatomical relationships during cortical development"

Instructor: Karine Loulier (Institut for Neuroscience of Montpellier, France)

Because little is known about the early steps of cortical pyramidal neurons (PyrN) and protoplasmic astrocytes (PrA) anatomical interactions establishment, a leading question remains unanswered: are PyrN and PrA interdependent for their respective adequate generation and subsequent functional interactions? In this project, we will focus on PyrN-PrA early anatomical relationships with the hypothesis that a defective cellular layout of PyrN may impact cortical astrogliogenesis. To explore this hypothesis, we will use a color-coded mosaic approach that creates a genetic mosaic in which the status of cells regarding the molecular perturbation is color-coded. This strategy enables to assess simultaneously the cell and non-cell-autonomous effect the chosen molecular perturbation. We will perfom in utero electroporation at two distinct developmental stages (E13 and E15) of a color-coded construct that will induce the clustering of either lower layer or upper layer PyrN and analyze the impact of this induced-PyrN spatial disorganization on the generation, localization and morphology of PrA in the embryonic brain before birth (E18) and at two critical stages of PrA postnatal development (P7 and P21) using a combination of MAGIC Markers imaging and immunostaining on fixed brain sections. This project will help to determine if PrA generation and maturation rely on proper PyrN spatial organization and how early PyrN-PrA anatomical crosstalk is established during cortical development.

Techniques :

- In utero electroporation
- Embryonic brain dissection
- Vibratome sectioning
- Immunostaining
- MAGIC markers imaging
- Image reconstructions and 3D analysis

Project 12: " Differentiation of spinal organoids and motor neuron subtypes from human induced pluripotent stem cells"

Instructors : Stéphane Nedelec & Simona Gribaudo (Institut du Fer à Moulin, Paris, France)

Human pluripotent stem cell differentiation (hPSC) into specific cell types or organoids are complementary powerful models to study human development and disease. The objective of this project is to use hPSC differentiation to study cell fate specification. You will learn how to start 3D differentiation of hPSCs. Then, applying distinct differentiation conditions you will either generate specific cell types (i.e motor neuron subtypes) or spinal organoids. You will dissociate and culture the generated progenitors or process the organoids for cryosectioning, perform immunofluorescence analyses and imaging. You will then learn automated image analysis to quantify the in vitro generated cell types.

Techniques:

- Human induced pluripotent stem cells culture
- Spheroid generation and differentiation
- Neural progenitor culture and differentiation toward postmitotic neurons
- Organoid embedding and cryosectioning
- Immunostaining and microscopy analysis

Project 13: "Inferring ligand-receptor interactions between GABAergic and Glutamatergic cells during Somatosensory Cortex Development"

Instructor: Antoine de Chevigny (Institut de Neurobiologie de la Méditerranée, Marseille, France)

The cerebral cortex contains two main neuronal classes: excitatory glutamatergic neurons (GlutNs) and inhibitory GABAergic interneurons (INs). Each class contains different subtypes that distribute differentially in six layers. During development, GlutNs settle in the cortex before INs, and recent works including our own indicate that the final laminar allocation and connectivity of IN subtypes are extrinsically regulated by already-settled GlutN subtypes. We have hypothesized that a ligand-receptor code between GlutNs and INs might control the laminar allocation and connectivity of IN subtypes. Single cell RNA-seq of both neuron classes during cortical plate invasion of INs (E18-P8), followed by computational analyses allowed us to infer GlutN ligands and IN receptors that might control laminar allocation and connectivity of IN subtypes in S1 of the mouse cortex.
This mini-project will rely on in utero electroporation to test at the functional level whether specific GlutN ligands and IN receptors identified in our analyses might regulate the laminar allocation of INs during perinatal development. We will overexpress selected ligand(s) normally confined to deep GlutNs in superficial GlutNs. Then, immunostainings and semi-automated analyses on postnatal cortex sections will allow us to seek for alterations in the laminar allocation of (receptor-expressing) IN subtypes, potentially with a switch towards electroporated GlutNs. This experiment shall help determine whether the ligand-receptor pair(s) tested is involved in the laminar allocation of IN subtypes.

Techniques:

- In utero electroporation
- Postnatal brain dissection
- Microtome/ Vibratome sectioning
- Immunostaining, (potentially fluorescent in situ hybridization) & confocal imaging
- Image analysis with R and CellPose algorithms

Project 14: "Imaging actin cytoskeleton dynamics during radial migration of projection neurons in the mouse developing cortex".

Instructor : Godin Juliette (IGBMC, Institut de Génétique et de Biologie Moléculaire et Cellulaire, France)

Regulation of actin dynamics is critical for proper migration of projection neurons in the developing cortex. To date, live recordings of actin dynamics rely on actin labelling through fusion of a fluorescent protein to the actin-binding domain of a known actin binding protein. However, overexpression of those probes could induce some bias in the distribution of actin. This project would aim to compare, in migrating cortical neurons, the dynamics of actin using the classic probes or via endogenous tagging of actin thanks to a CRISPR/CAS9 based genome editing approach (ORANGE, MacGillavry lab). The student will get trained with in utero electroporation and perform live recording of brain organotypic slices. They will then analyze actin dynamics in migrating neurons. Students will also discuss the design of guide RNA and other techniques to tag endogenous proteins.

Techniques

- Crispr-CAS9 mediated genome editing to tag endogenous actin

- In utero electroporation applied to mouse embryonic cortices
- Culture of organotypic brain slices
- Videomicroscopy, time lapse recording
- Image analysis

Project 15: "Neurogenesis in the Drosophila optic lobe by temporal patterning"

Instructor: Isabel Holguera (The New York University, United States)

Neural stem cells generate the most diverse cell types in an animal. Temporal patterning is a conserved mechanism of neuronal specification by which stem cells express different transcription factors (termed temporal TFs - tTFs) as they age to generate different neuronal types. In this project, we will study temporal patterning of Drosophila optic lobe neural stem cells (called neuroblasts). Specifically, we will address how different tTFs in neuroblasts generate specific neuronal progeny at each expression window. For this, we will dissect the Drosophila optic lobe at third instar larva (L3) stage and identify the expression pattern of several tTFs in neuroblasts by using immunofluorescence and hybridization chain reaction (HCR) RNA FISH. Additionally, we will assess the effect of loss of function of distinct tTFs in the progression of the temporal cascade using neuroblast mutant clones, and analyze the generation of neuronal diversity in each temporal window by using specific neuronal TFs markers.

Techniques:

- Drosophila brain dissection at L3 stage
- Genetic manipulation of tTFs in neuroblasts using MARCM
- Immunofluorescence
- HCR RNA FISH
- Confocal microscopy and image analysis

Project 16: "Fluid dynamics in the brain of zebrafish and medaka larvae"

Instructor: Nathalie Jurisch Yaksi (KAVLI Center Trondheim, Norway)

Cerebrospinal fluid (CSF) is a clear liquid located within the cavities of the brain and spinal cord. CSF is produced mainly by the choroid plexus and moved across the brain through various physiological processes. Notably, motile cilia of ependymal cells, which are located at the surface of the brain ventricles, move CSF in a directional manner. Ependymal cells are conserved across vertebrates and their dysfunction is associated with brain disorders in human, e.g. hydrocephalus. In this module we will investigate the ependyma and the movement of CSF in vivo in zebrafish and medaka larvae. We will identify the location of the ependymal cells using multiplex in situ hybridization methods (hybridization chain reaction) and immunostaining of whole brains with cilia markers. We will then measure the ventricular size and movement of CSF upon injection of dyes and particles in the brain ventricles. Finally, we will analyze the data using various imaging analysis tools. By comparing zebrafish and medaka larvae, we will identify possible difference in ependymal physiology between these two fish species.

Techniques:

- Zebrafish and medaka
- Live imaging
- Immunostaining and in situ hybridization

- Confocal microscopy
- Data analysis
- Microinjections of dyes and particles in the brain ventricles

Project 17: "Imaging function, connectivity, and development of brain circuits in zebrafish"

Instructor: Emre Yaksi (KAVLI Center Trondheim, Norway)

Due to their small and relatively transparent brains zebrafish are getting increasingly popular for studying brain function and development. Zebrafish has the basic architectural plan of the vertebrate brain with many conserved principles of assembly, cell types, and connectivity. One can also observe evolution at play, especially when studying ancestral paleo-cortex of the zebrafish. In this module we will investigate the basic organizational, development and functional principles underlying the wiring and operation of the zebrafish forebrain. We will use calcium imaging to monitor and analyze the ongoing activity and functional connectivity of distinct forebrain regions. We will use dye injections and tracing to reveal the anatomical connectivity of these structures and reveal neural morphologies. Finally, we will investigate when different components of this network are born and how are they integrated to the rest of the forebrain.

Techniques:

- Functional brain imaging in adult, juvenile and larval zebrafish and associated data analysis
- Dye tracing for determining brain connectivity, in combination with confocal imaging
- Live birth-dating of neurons and functional imaging of their activity

Project 18: "Comparative neurodevelopment of mouse and chick brains"

Instructor: Fernando García-Moreno (Achucarro Basque Center for Neuroscience, Spain)

The brain of vertebrates is generated following stereotyped developmental programs. These programs govern every aspect of development: from the birthdate of neurons in the germinative zone, followed by the migration of these neuroblasts and up to the differentiation of neurons and establishment of mature connections. Many developmental instructions are conserved among the brains of vertebrate species. However, the variations of these programs account for the diversification of current brains. In order to reveal how the different brains evolved, we need to identify which developmental process are distinct to each vertebrate lineage. And so, we need to compare these events between several species, and to discover which ones are conserved or new to each vertebrate taxon.
In this mini-project we will learn to combine two main techniques in neurodevelopment: birthdating of neurogenesis and in vivo electroporation. These techniques will be performed in two species, mouse and chick, to compare neurogenesis, neuronal migration and axonal growth at equivalent stages of development. We will perform birthdating of newborn neurons through species-specific techniques of EdU administration. We will also perform mouse in utero electroporation, and chick in ovo electroporation of a combination of DNA plasmids that will help us analyse neuronal migration and axonal growth.

Techniques:

- Comparative neuroanatomy
- Neuronal birthdating through species-specific EdU administration

- Mouse in utero electroporation
- Chick in ovo electroporation
- Immunostaining and microscopy analysis