Developmental neurobiology and pathologies (DNP)

in partnership with ERA-NET Neuron

Confirmed Instructors

Filippo del Bene & Shahad Albadri
Institut Curie, Paris, France

Title: Crispr/Cas9 transgenesis in Zebrafish

Zebrafish larval brain has emerged as a powerful model system to study neuronal development. The possibility to precisely modify its genome via CRISPR/Cas9 technology has further expanded the potentiality of this model system. During the curse we will design gRNA to target a precise genomic location and perform microinjections in one cell stage zebrafish embryos of the gRNA/Cas9 protein complex. The results of this injection will be assessed the following days via phenotypic evaluation and sequencing of the targeted locus. Taking advantage of the transparency of the larval brain we will observe in vivo the axonal morphology of single neurons in existing mutants previously generated in our laboratory. Basic axonal parameters such as total length and branching complexity will be evaluated. Finally we will visualize axonal transport in vivo tracking fluorescently tagged organelles such as mitochondria along axon.

Techniques:
- Zebrafish
- Injections of CRIPS/Cas9 in eggs
- time-lapse imaging
- Neuronal arborisation analysis in transgenic fishes
- tracking of fluorescently tagged proteins in axons

Nicolas Dray
Institut Pasteur, Paris, France

Title: Spatio-temporal analysis of neural stem cells activation and division

In this project, we will live image neural stem cells activation and division in adult zebrafish telencephalon during 5/6 consecutive days. We will then analyse the pattern of spatio-temporal activation in normal condition and after a drug treatment that increase the frequency of stem cell divisions.

In practice, everyday day during 5/6 days we will anesthetized some adult zebrafish (about 2 months old) for 1 hour. With a two-photon microscope we will image a Z-stack of 200um of the telencephalon (each day correspond to one time point). Then, with Imaris and Matlab we will align each z-stack and proceed to some analysis of the positioning of stem cell activation in time and space.

In parallel, we will do the same analysis on fish treated with a drug blocking the Notch signalling to test the role of the Notch signalling in regulating neural stem cell activation.

Techniques:
- Zebrafish
- Live-imaging with two-photon microscopy
- Imaris and Matlab analysis
Joaquim Egea  
Lleida, Spain  
Title: *Growth cone collapse assay in dissociated cortical neurons to assess the repulsive effect of axon guidance cues*

In this project we will learn how to assess the repulsive effect of an axon guidance cue in vitro. To do that we will dissect the cortex from embryonic mouse brains at E16.5 and obtain cultures of dissociated cortical neurons. After 2 days in vitro, these cultures will be stimulated with two different repulsive cues, ephrinA1 and ephrinB3, for 30 minutes, and then fixed and stained with the actin cytoskeleton marker phalloidin, bound to fluorophore. The shape of the growth cone and the amount of actin present will be evaluated under a fluorescence microscopy. The repulsive effect will be then quantified scoring the percentage of “collapsed” growth cones (those with a retracted growth cone and less actin) in the culture and the results will be discussed.

Techniques:
- Dissection of embryonic mouse brains
- Dissociated cultures of neurons
- Immunostaining
- Fluorescent microscopy and images analysis of growth cones

Julien Falk  
CNRS, Lyon, France  
Title: *Using the chick embryo to study axon guidance from spinal cord neurons*

Correct wiring between neurons relies on stereotypical navigation of axons. Axon navigation is controlled by environmental cues that attract or repulse growing axons. Commissural neurons of the dorsal spinal cord that connect the other side of the spinal cord have been a model of choice to study axon guidance. Motor neurons are located in the ventral spinal cord and innervate peripheral muscles. During the course, we will combine in vivo and in vitro approaches to visualize and assess axon navigation in the different experimental paradigms. Students will perform in ovo electroporation and analyze motor and commissural axon navigation using various approaches (vibratome sections and open book preparation for commissural neurons). Students will also be taught to how to perform slice cultures and neuron cultures (commissural and motor neurons). Finally, if feasible, we will monitor axon development using live imaging.

Techniques:
- in ovo electroporation
- vibratome sections and open book preparation for commissural neurons
- slice and neuronal cultures
- Live imaging
Sacramento Ferron  
Burjassot, Spain  

**Title: Co-culture of neural stem cells (NSCs) with choroid plexus (CP) to determine the role of CP-born molecules on adult neurogenesis**

The adult subventricular zone (SVZ) is the most active niche in the adult mammalian brain, supporting significant rates of neurogenesis and oligodendrogenesis. The SVZ lines the walls of the lateral ventricles and constitutes a complex microenvironment because of the special cytoarchitecture of this niche that allows NSCs to be in direct contact with the cerebrospinal fluid (CSF) through a non-motile apical primary cilium that extends towards the ventricle lumen. CSF is secreted mainly from the choroid plexus (CP), an intraventricular secretory epithelium continuous with, but functionally distinct from, the ventricle ependyma. CP-derived CSF is enriched with several factors known to support NSC self-renewal, including basic fibroblast factor (bFGF), amphiregulin, insulin growth factor 2 (IGF2), transforming growth factors (TGFα and TGFβ) and several bone morphogenetic proteins (BMPs). Individual cells dissected from the SVZ proliferate in a medium containing basic fibroblast growth factor (FGF2) and/or epidermal growth factor (EGF) to produce multipotent clonal aggregates called “neurospheres” that can be induced to differentiate to generate βIII-tubulin+ neurons, GFAP+ astrocytes and O4+ oligodendrocytes. We will use and in vitro co-culture protocol to investigate the contribution of CP-born molecules on adult neurogenesis putting particular emphasis on how this system can be employed to analyze NSC properties and behavior.

**Techniques:**

- dissection and harvesting of SVZ cells from adult brains
- generation of neurospheres
- treatment with CP-derived molecules
- Analysis of NSC properties and behaviour

Paolo Giacobini  
University of Lille, INSERM UMR-S 1172, Lille, France  

**Title: Manipulation and 3D-imaging of neurons controlling fertility**

Fertility in mammals is controlled by hypothalamic neurons that secrete gonadotropin-releasing hormone (GnRH). These neurons differentiate in the olfactory placodes during embryogenesis and migrate from the nose to the hypothalamus before birth. Alteration in this neuronal migratory process or in the establishment of the correct GnRH connectivity/ hormone secretion result in several human reproductive pathologies.

The goal of this mini-project is to give the basis for understanding how GnRH neurons migrate from the olfactory placode to the target brain areas during embryonic development and how proper neuronal connectivity is established.

**Techniques:**

- siRNA delivery in utero into olfactory pit (embryonic mice)
- whole-brain immunohistochemistry/clearing
- 3D imaging by light-sheet microscopy
- comparison of mouse and human tissues
- whole-brain imaging of non-pathological and pathological mouse models
- in vivo viral tracing (trans-synaptic tracing of GnRH neuronal connectivity)
Yorick Gitton
Institut de la Vision, Paris, France

*Title: Eye morphogenesis: contribution of neural crest cells in chick embryo*

This workshop has been designed as an introductory course for PhD students or postdoctoral researchers who have little or no experience with avian embryogenesis. In this practical course, we will describe the migration path of neural crest cells towards the territory where the eye forms, and how interactions among different cell types control the differentiation of distinct eye structures. Dysregulated interactions account for congenital ocular malformations, which we will experimentally address. Notably, we will learn microdissection and grafting between chicken and quail, as well as beads implantation to disrupt specific developmental mechanisms. We also will observe our samples after clearing with an ultramicroscope.

**Techniques:**
- Dissection of quail embryos, chicken embryos: excisions, grafts
- beads implantation
- 3D imaging by light-sheet microscopy
- Imaris imaging analysis

Eloisa Herrera & Cruz Morenilla
Institute of Neuroscience, Alicante, Spain

*Title: Visualisation and analysis of axonal outgrowth from mouse retinal ganglion cells*

The complex process of axon guidance is largely driven by the growth cone, which is the dynamic motile structure at the tip of the growing axon. During axon outgrowth, the growth cone must integrate multiple sources of guidance cue information to modulate its cytoskeleton in order to propel the growth cone forward and accurately navigate to find its specific targets. How this integration occurs at the cytoskeletal level is still emerging, and examination of cytoskeletal protein and effector dynamics within the growth cone can allow the elucidation of these mechanisms. The growth of retinal ganglion cell axons is a classic and very useful model system for studying developmental neurobiology processes in mammals. In fact, important early insights into growth cone navigation and microtubule dynamics were initially found using this system. In this mini-project, we will prepare retinal explants isolated from E14.5 mouse embryos to culture them during 24 hours and then visualize and analyse their axonal growth and/or their response to repulsive guidance molecules.

**Techniques:**
- Retinal dissection from embryonic mice
- explant cultures and treatments
- immunostaining and microscopy analysis
Julia Ladewig & Olivia Krefft
Institute of Reconstructive Neurobiology, Bonn, Germany
Title: Pluripotent stem cell derived forebrain-type cerebral organoids as a tool to study human cortical development

Cerebral organoids represent a new model to study human brain development outside the human body. The objective of this project is to learn how to generate and analyse pluripotent stem cells (PSC)-derived forebrain specific cerebral organoids cultures. More specifically, you will perform the most crucial steps of the cortical organoid protocol. You will monitor organoid growth and development over time, you will process organoids for cryosectioning and perform immunocytochemical analyses which will include the use of different microscopy techniques. In addition you will apply state of the art quantification methods to analyse the formation and development of the in vitro generated cortical tissue buds. Taken together, this mini-project will equip you with state of the art techniques in the generation and analyses of PSC-derived three dimensional cortical tissue structures.

Techniques:
• Stem Cells cultures
• embryoid body generation
• organoid embedding and cryosectioning
• immunostaining and microscopy analysis

Karine Loulier
Institut de la Vision, Paris, France
Title: Astrocytic coverage of brain vasculature in the mouse embryonic cerebral cortex

Main subtype of glial cells in the central nervous system (CNS), astrocytes play important roles in blood brain barrier (BBB) formation and maintenance. Perivascular astrocytes contribute to the mammalian BBB by secreting angiogenic factors and by covering CNS vasculature through endfeet that wrap all vessels, from small capillaries to large arteries in the postnatal and adult mouse brain. How this extensive coverage of cortical vasculature by early born astrocytes is achieved during development remains unexplored. This mini-project will rely on in utero electroporation of Magic Markersin E15 cortical progenitors to mark clones of astrocytes and isolectin labeling to visualize pre-birth gliovasculature association at E18. Successfully labeled brains will be dissected out, vibratome-cutted or flat-mounted, and stained using Isolectin IB4 to label blood vessels. This method will enable us to determine if i) neighbouring astrocytes that contact identical blood vessel sections share clonal relationships; ii) pre-birth gliovasculature association occurs selectively in one of the distinct cortical compartments (pial surface / cortical plate / ventricular zone) prior to extend to all cortical vessels; iii) prenatal cortical astrocytes contact small or large diameter vessels equally; iv) vasculature coverage by astrocyte endfeet tag along the migration pattern followed by vessels to colonize the embryonic cerebral cortex.

Techniques:
• In utero electroporation
• embryonic brain dissection
• Vibratome sectioning and « En face » / flat mount
• immunostaining & brainbow imaging
• image reconstructions
Javier Morante
Institute of Neuroscience, Alicante, Spain

Title: Larval neurogenesis in Drosophila: analysis of neural progenitors/postmitotic cells

This practical programme has been designed as an introductory course for late PhD students or postdoctoral who have little or no experience with fly neurogenesis and brain maturation. In this practical course we will describe the glial niche/neuroepithelial interaction that takes place during early neurogenesis and are responsible for setting up the adult fly brain size. Also we will analyze how a delayed larval-to-pupal transition (‘delayed puberty’) impact on this glial niche/neuroepithelial interaction, and what are the consequences and the causes of this aberrant neurogenic program.

Techniques:
- Fly husbandry
- RNAi screening
- Larval brain dissection
- Immunostaining & Imaging analysis

Xavier Nicol
Institut de la Vision, Paris, France

Title: Imaging living cells: axon guidance cue-induced growth cone collapse and FRET imaging to monitor intracellular signalling

Living cells are dynamic, both in their morphology, movement and migration, and in their intracellular signaling. This mini projects aims to provide two examples of techniques enabling to track these dynamic changes. The students will perform videomicroscopy experiments to monitor the morphology changes of growing axons in response to guidance molecules influencing their outgrowth. A second approach will be to track second messenger concentration variations in living cells using FRET microscopy, a fluorescent biosensor-based techniques enabling to follow both the kinetics and localization of intracellular signaling events.

Techniques:
- Retinal dissection
- Cell culture
- Videomicroscopy
- FRET acquisition & analysis

Emily Pacary
Bordeaux Neurocampus, Bordeaux, France

Title: In utero electroporation of the cerebral cortex to study radial migration of projection neurons

Brain electroporation is a rapid and powerful approach to study neuronal development. In particular, this technique has become a method of choice for studying the process of radial migration of projection neurons in the embryonic cerebral cortex. During this practical course, students will learn how to perform in utero electroporation of progenitor cells in the ventricular zone of the cerebral cortex. We will then analyse, in fixed sections, migration of electroporated neurons three days after the electroporation.

Techniques:
• In utero electroporation in the ventricular zone of the cerebral cortex
• Tissue dissection and sectioning
• Confocal acquisition & Image analysis

Nicolas Renier  
CRICM, Paris, France  

Title: Brain-wide changes to the distribution of neuronal activity following a long-term sensory deprivation

In the adult brain, axons and dendrite are for the most part stable and insure a consistency over time in neuronal wiring. Recently, it has been shown that large-scale remodeling of a few axonal projections is possible in the adult brain, following for instance peripheral lesions. This phenomenon has been locally studied in the visual and somatosensory area of the cortex. However, it is not yet known whether local changes in activity levels in the primary region affected by a lesion can reverberate to other regions and trigger long-range reorganization of axonal branching. The goal of this preliminary study will be to determine candidate regions undergoing changes in their activity profile after a long-term sensory deprivation of the whiskers inputs. We will be mapping the whole-brain distribution of the reporter Fos using iDISCO+ and ClearMap, an unbiased method of activity mapping in the brain with tissue clearing and light sheet microscopy. These preliminary results will enable us to locate distant regions of the brain potentially undergoing plastic changes following long-term sensory deprivation for further studies.

Techniques:
• Mouse surgery on mouse whiskers
• Behavior assay
• Brain extraction & Tissue clearing c-Fos staining
• c-fos immunostaining & iDISCO
• Light sheet microscopy & ClearMap python analysis of the whole-brain

Rita Sousa-Nunes  
King’s College, London, UK  

Title: Regulation of neural stem cell quiescence

Drosophila neural stem cells (NSC), called neuroblasts, undergo quiescence during a period of about 24 hours that intervenes between embryonic and postembryonic neurogenesis. This offers the advantages of a clear spatiotemporal segregation between cycling and quiescent neuroblasts; and of a conveniently short time of transition between these states. Studies of neuroblast quiescence, including my own, first implicated regulation by the Target of Rapamycin (TOR) pathway and the transcription factor FoxO, homologues of which were subsequently reported to regulate also mammalian NSCs quiescence. Nonetheless, how this pathway then leads to the morphological and functional properties of NSC quiescence remains largely unknown.

In this project, the student will 1/ perturb the timing of neuroblast reactivation from quiescence by overexpressing components of the TOR and Hpo pathways; 2/ compare the effect of two different neuroblast GAL4 drivers towards the outcome.

Techniques:
• Drosophila husbandry
Noelia Urban
Institute of Molecular Biotechnology, Vienna, Austria

**Title: Generation of embryonic and adult neural stem cell cultures for the study of quiescence and differentiation**

The project aims at highlighting the proliferation and self-renewal differences between embryonic and adult neural stem cells, as well as between adult stem cells from the two main neurogenic regions, the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (DG).

Stem cell cultures from primary tissue will be generated from the three “conditions” (embryonic brain, SVZ and DG) and their ability to generate neurospheres quantified. In order to avoid the delays associated with having to generate stable lines for further experiments, an adult hippocampal stem cell (AHSC) line will be available. The ability of AHSCs to enter quiescence and to differentiate will be assessed by immunocytochemistry and quantification.

**Techniques:**

- Brain dissection (embryonic and adult)
- Primary culture of neural stem cells
- Cell culture (induction of quiescence and differentiation)
- Immunostaining and image analysis

Carla Silva
GIGA Neuroscience, Liège, Belgium

**Title: Fine analysis of interneuron migration during the period of corticogenesis in mice**

GABAergic interneurons are born in subpallial structures called ganglionic eminences (GEs) during the embryonic period in mouse. Cortical interneurons are generated in the medial and caudal ganglionic eminences (MGE and CGE). Explants of the GEs can be plated in culture on a feeder layer of cortical cells and the migration properties can be monitored by the aid of genetically encoded or electroporated fluorescent reporters. The movement of the fluorescent cells can be recorded by time-lapse video microscopy. Our laboratory has been recording migrating interneurons invalidated for genes that control cellular movement. The general migration pattern of interneurons, have been characterized. Here I propose that we refine this characterization by electroporating plasmids that code for fluorescent reporters under the promoter for somatostatin and parvalbumin, two markers of different subtypes of cortical interneurons. We would be able to distinguish different subtypes of interneurons born in the same structure and at the same time window. By using this strategy, we would be able to understand if interneuron fate has an influence in their basic pattern of migration.

**Techniques:**

- dissection of embryonic brain
- primary cultures (feeder layer) and explant cultures
- ex vivo electroporation of subpallial structures
- confocal acquisition and analysis