First block (14 – 22 September)

Project 1: Is lysosome dysfunction a mechanism of synapse aging?
Instructor: Claudia Guimas Almeida (CEDOC, UNL, Lisbon, Portugal)

Aging-cognitive decline increases the risk of Alzheimer’s disease. The cognitive decline that accompanies old age was thought to be due to neurons’ progressive death with aging. However, recently it became apparent that cognitive decline results instead of synaptic decline, likely undermining memory. Morphologically, the aging-cognitive decline has been characterized by loss of synapses and disrupted remodeling of spines. Until now, the initial processes that lead to synaptic dysfunction during aging are not clearly understood. Accumulation of undegradable proteins and lipids in lysosomes is a marker of cellular aging. However, whether lysosomal dysfunction causes synaptic decline with neuronal aging remains unknown. In previous work, we have found that neurons aged in culture show a synaptic decline and lysosomal dysfunction. In this project, we will use quantitative single-cell fluorescence microscopy and synaptic biology assays to investigate if lysosome aging underlies synaptic dysfunction.

Project 2: Physiological signatures of cognitive aging
Instructor: Miguel Remondes (IMM, Lisbon, Portugal)

Impaired perception and retention of sensory information in episodic memory is a hallmark of cognitive decline in aged-impaired mammals, by contrast with young adult animals, which manage to compensate for such defects. A circuit connecting entorhinal cortex (EC), and HIPP stores contextual information as a coherently retrievable construct responsive to the animal’s location, reflected in spatial selectivity of individual EC-HIPP neurons. In this short project we will try to have access to aged and young adult animals, equipped with brain electrode chronic recording devices and, if possible, perform some simple recording sessions for subsequent analysis. If no possible, we will use already acquired data to delve into possible differences between neural activity, namely the presence of SWR, electrophysiological signatures of memory replay.

Project 3: Recording neuronal activity on hippocampal slices from aged mice
Instructor: Paula Pousinha (IPMC, Nice, France)

While neuronal loss has long been considered as the main contributor to age-related cognitive decline, these alterations are currently attributed to gradual synaptic dysfunction driven by calcium dyshomeostasis and alterations in ionotropic/metabotropic receptors. Given the key role of the hippocampus in encoding, storage, and retrieval of memory, the electrophysiological alterations that occur in the major synapse of this network - the glutamatergic synapse - deserve special attention.

In this research project, we will perform and analyses patch-clamp electrophysiological recordings on hippocampal brain slices from adult (2-3 months old) and old mice (~18 months old). We will assess simple parameters of neuronal activity, as intrinsic and synaptic properties, namely neuron firing, spontaneous activity and the ratio of AMPA and NMDA receptors. To do this we will learn the basis of patch-clamp technique and data analysis by using the PClamp software. Students will have the opportunity to learn strategies which allow increasing the viability of neurons from old animals to better perform electrophysiological recordings.
Project 4: Inter-individual variability of declarative memory decline in healthy aging: a brain network analysis in humans based on a virtual radial-maze task.

Instructors: Gwenaëlle Catheline (INcia, Bordeaux), Nicole Etchamendy (Neurocentre Magendie, Bordeaux)

Older people complain mainly about forgetting and confusing “things of everyday life”, i.e., relatively insignificant events, which are both repetitive and changing, such as “where I parked”.

Our group has developed a radial-arm task assessing in mice such a “varying” declarative memory impaired with aging and translated this task to human subjects using a virtual equivalent of the radial maze.

The project will provide practical experience in assessing performance of young and aged humans using our virtual psychometric tool. Using resting state functional magnetic resonance imaging (in collaboration with Gwenaëlle Catheline), we will examine changes in the functional organization of several large-scale brain networks with aging and its potential association with mnemonic performance assessed in our virtual maze.

Project 5: details to come...

Instructor: Jean-Vincent Goncelin (Sorbonne, Paris)

Second block (24 September- 02 October)

Project 6: Immunohistochemistry on brain tissue obtained from aged humans and rodents

Instructor: Miguel de la Flor García (Universidad Autónoma de Madrid, Spain)

This project will overview several techniques to minimize autofluorescence and improve specific signal in immunohistochemistry performed on aged brain tissue obtained from both rodents and humans. We will use STED super-resolution microscopy to analyze murine and human tissues, and we use confocal microscopy to compare the rate of adult hippocampal neurogenesis in young versus aged rats that present good or bad spatial learning abilities.

Background: Immunohistochemistry on aged brain tissues present remarkable methodological difficulties mainly related to the accumulation of autofluorescent elements such as lipofuscin. The accumulation of these particles is specifically abundant during the course of neurodegenerative diseases such as Alzheimer’s disease (AD). On the other hand, one of the most remarkable features of the aged brain is the decrease in the rate of adult hippocampal neurogenesis, namely the incorporation of new neurons to the hippocampal circuitry.

Methods: We will use state-of-the-art procedures to reduce background and improve signal in immunohistochemistry performed on aged brain tissues obtained from transgenic mice, rats, and humans. After completion of free-floating triple immunohistochemistry, we will compare the fine structure of Amyloid-Beta plaques in patients with AD and a transgenic mouse model of the disease, and will perform morphometric analyses on the obtained images. In parallel, we will examine the rate of adult hippocampal neurogenesis in aged rats with good and bad learning abilities. Attendees will be taught to use a confocal microscope to perform stereological cell counts on brain rat tissue.
Project 7: Unveiling the transcriptomic signatures of human brain ageing
Instructor: Nuno Barbosa-Morais (IMM, Lisbon, Portugal)
The advent of next-generation sequencing technologies has enabled the genome-wide profiling of transcriptional activity in biological samples. They have been instrumental in studying the molecular mechanisms of neural ageing and cognitive decline in animal models but the obvious impossibility of sampling brain tissues from living individuals makes similar efforts quite challenging in humans. However, profiling the transcriptomes of post-mortem human brain samples has been shown to still provide an informative snapshot of the patterns of gene expression underlying tissue specificity. Public repositories of sequencing data include an impressive wealth of clinically annotated human brain transcriptomes from donors of all ages (some having neurodegenerative disorders) that allows scientists to start unveiling what happens at the transcriptional level as human neural tissues age. Moreover, single-cell RNA sequencing has enabled the definition of neural cell type-specific gene expression signatures that can be used to estimate the cellular composition of brain tissues from their bulk transcriptomes.
During this project, we will cover the basic computational biology approaches for the analysis of gene expression from RNA sequencing data and apply them to post-mortem transcriptomes of different brain areas of adult human individuals spanning several decades of age, some of them annotated as having a neurodegenerative disorder. We will also explore machine learning methodologies that employ cell type-specific gene expression signatures to estimate the cellular composition of those brain samples from their bulk transcriptomes. Finally, we will perform in silico functional enrichment analyses, not only to facilitate the biological interpretation of the changes in brain gene expression associated with physiological and pathological ageing, but also to infer their molecular causes and identify compounds with the potential to drive or revert them.

Project 8: In vivo optogenetic to manipulate adult neurogenesis
Instructors: Nora Abrous (Neurocentre Magendie, Bordeaux, France) Nicolas Blin (Neurocentre Magendie, Bordeaux, France),
In this project students will be taking advantage of the use of optogenetic in vivo in behaving rats. Neurons born in the adult Dentate Gyrus will be tagged with ChR2/Arch to allow modifications of their activity during the performance of a hippocampal-dependent learning task: the Morris Water Maze. During the project, students will be taught how to prepare of optic fiber implants and to perform stereotaxic surgeries to implant the fibers. Students will perform optogenetic in vivo while the animals are engaged in the behavioral task. We will then visualize adult-born neurons using immunohistochemistry.

Project 9: Microglia phenotyping in aged animals
Instructor: Joana Coelho (IMM, Lisbon, Portugal)
Changes in microglia’s ability to perform their basic surveillance functions have been implicated in the triggering, maintenance or exacerbation of cellular dysfunction upon aging. While these changes have consequences to the overall performance of cognitive function, assessing microglia specific changes is carried out by histological and morphological techniques or using measures of cell mixed extracts to quantify gene and protein expression. More recently flow cytometry and fluorescence activated cell sorting (FACS) have been used with success to analyze microglia populations isolated from CNS.

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The question guiding this project is whether microglia response to an inflammatory stimulus is different in young and aged animals.

The project will provide practical experience in obtaining microglia cells from rodent adult and aged brain, using flow cytometry to identify and isolate microglia populations but also possible phenotypic changes using antibody labeling. To challenge microglia cells \textit{in vivo} we will also perform intra-cerebro-ventricular (i.c.v.) injection of Lipopolysaccharides (LPS) in aged and young rodents prior to isolation and analysis.

We hope to gain insight into the phenotypic changes that occur in microglia cells within the aging brain.

\textbf{Project 10: Inter-individual variability of declarative memory decline in healthy aging: a brain network analysis in mice on a radial-maze task.}

\textbf{Instructors: Aline Marighetto (Neurocentre Magendie, Bordeaux, France), Azza Sellami (Neurocentre Magendie, Bordeaux, France)}

Aline Marighettos’ group has developed specific radial-arm maze tasks to assess aging-related decline of “relational/declarative memory” in mice and translated the tasks to human subjects using a virtual equivalent of the radial maze. Gwenaelle Catheline has developed MRI facilities in human aging brain. They joined their expertise to set up this translational mini-project including two workshops: one dedicated to mice and the other dedicated to humans with a common paradigm to assess the age-related memory decline.

\textbf{Workshops}

- Workshop 1: Aging in a dish. Instructor: Claudia Guimas Almeida (CEDOC, UNL, Lisbon, Portugal)
- Workshop 2: Approaches to functional and structural imaging analysis. Instructor: Jenny Rieck (Baycrest & Univ. Toronto, Canada)
- Workshop 3: Big data in aging. Instructor: Nuno Barbosa-Morais (iMM, Lisbon, Portugal)