Advanced Methods for Preclinical Alzheimer Research Projects

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1. Behavioral study of mouse models of AD

- **When: Block 1**
- **Instructor: Ana Ribeiro**

Cannabinoids cause memory deficits in a wide assortment of behavioral paradigms (Riedel and Davies, 2005). The most commonly reported outcomes of cannabinoid use or exposure are robust short-term memory deficits (Robinson and Riedel, 2004) mediated by CB_1 receptors (**Figure 1**) in the CA1 region of the hippocampus (Wise et al., 2009). Cannabinoids also disrupt long-term spatial memory storage by interfering with memory consolidation processes mediated by CB_1 receptors in the dorsal hippocampus (Yim et al., 2008). It is likely CB_1 receptors in other regions of the hippocampal formation modulate other forms of memory and consolidation processes.

Long-term exposure to exogenous cannabinoids can result in persistent changes in dendritic morphology and spine density (Kolb et al., 2006; Rubino et al., 2009). Changes to dendritic morphology represent potential mechanisms by which cannabinoid exposure may influence behavioral and cognitive processes. Previous studies have demonstrated that chronic treatment with delta-9-tetrahydrocannabinol (THC) after 10–12 days selectively alters dendritic morphology of neurons depending on developmental age and region of interest, but little is known about the effects of acute treatments.

For that, the present study was designed to examine changes in memory and dendritic morphology produced by an acute treatment with a potent cannabinoid agonist WIN 55, 212-2. We will use C57BL/6 mice for this study, and use different techniques, including spine staining and imaging, genotyping and Y-Maze behavior tests.

2. Studying mitochondria-ER contacts in Alzheimer«s disease (AD)

- **When: Block 1**
- **Instructors: Giacomo Dentoni and Nuno Leal**

Several studies have shown that mitochondria-ER contact sites are altered in several neurodegenerative disorders including Parkinson, FTD and AD. Moreover, the Aβ processing machinery as well as Aβ itself are present/produced in this subcellular region. Several biological processes (including calcium handling, apoptosis, autophagosome formation, etc.) have been reported to be mediated in this region; interestingly, all these pathways are dysfunctional in AD. Hence, these contacts appear to be an interesting area worthy of investigation for AD pathology.

Schedule

Day 1 *Introduction on the topic*

Day 1*Transmission electron microscopy (TEM)*: we plan to assess and show using transmission electron microscopy how to recognize several intracellular organelles using EM, specifically we will acquire images of mitochondria-ER contacts from previously processed mouse brain material. Once acquisition is completed, we plan to show students how we normally carry out assessment of mitochondria-ER proximity in cells and different mitochondrial morphological analysis.

(treatment **Day 1**), **Day 2***Proximity Ligation Assay (PLA)* and **Day 3** *confocal acquisition/analysis*: We will treat wild type primary cortical neurons with synthetic oligomeric Aβ preparations for 24 h pre-fixation to induce increased mitochondria-ER proximity. The following day, neurons will be fixed and proximity between mitochondria and ER will be assessed using PLA in treated ad untreated conditions. The final day we will acquire confocal images of the samples and we will then analyze changes in proximity between the two organelles with Duolink®PLA software.

Day 4-6 *Cellular fractionation*: brains from adult mice will be fractionated using a series of centrifugation steps and Percoll gradient in order to separate various intracellular

compartments including: mitochondria, plasma membrane, ER and MAM enriched fraction. We will then assess the purity of these fraction through western blot analysis.
Day 6 Final analysis and discussion.

3. The role of APP and APLP2 in presynaptic mechanisms.

- **When: Block 1**
- **Instructor: Tomas Jorda**

The Amyloid Precursor Protein (APP) is enriched in presynaptic terminals and interacts with protein partners essential to the release of synaptic vesicles (SVs) suggesting a key role in presynaptic mechanisms. By using electrophysiology coupled to Optogenetics we will investigate presynaptic function of APP and APLP2 at the Dentate gyrus (DG) to CA3 region of the mouse hippocampus.
Furthermore we will also study the expression levels of different synaptic markers in AD mouse models (APP/APLP2KO, Presenilin KO and APP.PS1 transgenic mouse) by using immunohistochemistry and posterior imaging with Confocal and STED microscopy.

Schedule:
Day 1, 2 and 3: Immunohistochemistry and Imaging
Day 4 and 5: Slice Electrophysiology

4. Characterizing tauopathy spreading in an AD mice model

- **When: Block 1**
- **Instructor: Miguel Matias**

For many decades now, hyperphosphorylation and aggregation of tau has been known to be a hallmark and key player in Alzheimer's disease (AD). It is clear from Braak staging that it spreads through the brain in a well-defined and predictable pattern. Despite this knowledge, we still lack good animal models to study the spreading of tau pathology and its effect on neuronal and network function.

With this project I propose we explore a novel paradigm to study tau pathology spreading and its consequences on the hippocampal circuit of a tauopathy mice model.
Briefly, to trigger tau pathology, we will perform unilateral stereotaxic injections of "tau seeds" in the entorhinal cortex (EC) of Tau P301L mice. After 2 different time points (2 days and 2 weeks) these mice will be sacrificed, and electrophysiological recordings will be performed in acute hippocampal slices to evaluate synaptic and circuit function. We will record spontaneous and miniature synaptic currents in CA1 pyramidal neurons (one of the main outputs of EC) as well as evoked responses after stimulation of the Perforant Pathway (EC→CA1 projections). Finally, we will recover the recorded slices and do immunohistochemistry (IHC) to stain aggregated tau. After confocal imaging of the slices, we will be able to quantify the amount of tau aggregates in the "seeded" region (EC) as well as if and where does it spread to in the hippocampus.
By triggering the pathology in EC this project could potentially explore a more physiologically-relevant paradigm to study EC-to-Hippocampus spreading of tau pathology and its functional consequence. Students will be able to learn more about AD pathology while having a hands-on approach to several neuroscience-relevant techniques like animal handling and surgery, electrophysiology and IHC and imaging.

5.Stereotaxic injections and direct intraventricular delivery of drugs into the mouse brain using Alzet osmotic pumps

- **When: Block 2**

- **Instructor: Sara Rodrigues**

Animal models, in particular mouse models, have been playing an important role in Alzheimer's disease research, by partly mimicking the pathological changes that occur in the brains of AD patients. One method for expressing proteins of interest in the mouse brain is through stereotaxic injection. This surgical procedure uses the mouse brain coordinates to allow the injection of a protein into the target region.
Another important aspect in AD research regards to the testing of new potential drug candidates for therapeutical interventions. Since several drugs do not cross the blood brain barrier, we must deliver them directly into the brain region. This can be achieved by using the Alzet pump technology combinaed with brain infusion kit.
Therefore, during our project we will perform stereotaxic injections into a specific brain region (eg: dorsal hippocampus). Furthermore, we will implant Alzet osmotic pumps directly into the lateral ventricle, allowing the continuous infusion of a certain drug into the brain at a constant rate. By learning these 2 valuable methods in the AD field, the participants will be able to perform state-of-the art techniques for gene transfer and drug delivery into the mouse brain.

Schedule:
Day 1: introduction to stereotaxic injections + practice + dissection of the mouse brain (place in 4% PFA for fixation)
Day 2: practice stereotaxic injections
Day 3: introduction to osmotic pumps + practice implantation and brain cannulation
Day 4: practice implantation of osmotic pumps + change brains from day 1 to 30% sucrose solution
Day 5: brain sectioning in the vibratome and staining
Day 6: microscopy

6. In vivo imaging of microglia and neuronal activity in a mouse model of tauopathy

- **When: Block 1**
- **Instructors: Thomas Voguels and Greta Vargova**

In this project the students will go through the entire workflow of *in vivo* 2-photon imaging. The first days will consist of implanting the cranial windows that are required for in-vivo imaging in the brain. The tutors will first show how the surgery is performed and then the students will have the opportunity to try for themselves. On day 3 we will also demonstrate how to visualize neurons and induce tau pathology by injecting an AAV that expresses truncated tau and mCherry (this part comes first in the workflow, but cranial windows need to recover for multiple days). Days 4 and 5 will be spent on *in vivo* 2-photon imaging. We will also go through the process of data analysis. Ideally the students will obtain their own data, but the tutors will also bring existing data in case the windows are not sufficiently good for imaging. On the final day the students can finish their presentations and obtain feedback from the tutors.

Schedule:
Day 1: Implantation of cranial windows in CX3CR1-GFP mice.
Day 2: Implantation of cranial windows in GCAMP mice
Day 3: AAV-mediated gene transfer by injecting with glass pipettes in cortex.
Day 4: In vivo imaging of microglia surrounding tau positive neurons, data analysis, and start making presentations.

Day 5: In vivo imaging of neuronal activity in tau positive neurons, data analysis, and start making presentations.
Day 6: Finishing presentations & final feedback

7. Structural and functional connectivity of the default mode network in preclinical stage of AD

- **When: Block 1**
- **Instructor: Catheline Gwenaëlle**

Hippocampal sclerosis is a hallmark of Alzheimer's disease. The specific atrophy of the medial temporal lobe measured on a conventional anatomical T1 weighted sequence has been described several times since the 2 seminal papers of Kesslak et al. (1991) and Jack et al. (1992). The development of neuroimaging technology of the last 2 decades allows a more global description of an AD brain. It has been clearly shown that neurodegenerative process not only impacts temporal regions but also parietal and posteromedial regions of the brain in AD. These connected regions belong to an extended network centered on the Posterior Cingulate Cortex and called the Default Mode Network (DMN).
Functional and structural connectivity of this network has been described in AD patients based on different types of MRI. The recording of BOLD signal at rest allowing to investigate the functional connectivity and the diffusion weighted sequence allowing the description of structural connectivity.
During this mini-project, students will be initiated to the different steps of the preprocessing and of the analysis of the different MRI types.

Schedule
Day 1: Introduction to multimodal imaging
Day 2: Preprocessing of structural data
Day 3: Preprocessing of functional data
Day 4: Tractography of the cingulate fasciculus/Functional connectivity of the PCC
Day 5: Whole brain statistical analysis/ Presentation Prep
Day 6: Presentation Prep

8. Drosophila as a model of neurodegenerative diseases

- **When: Block 1**
- **Instructor: Sandra Soukup**

This mini project is especially suitable for researchers that would like to use *Drosophila* as:

- an additional approach to confirm key findings *in vivo*,
- to perform candidate screens to identify new modifiers of diseases,
- to study novel pathogenic genes or to find novel genetic interactions.

We will learn the basics of *Drosophila* housing, genetics and neurobiology. We will then use this knowledge to explore how to work with *Drosophila* models of neurodegenerative diseases (like AD and PD): we will design and learn how to locally express pathological mutations of the human Tau protein. We will also analyse how our Drosophila disease models show impairment in neuronal circuits by performing behaviour assays like negative geotaxis and phototaxis. Those behaviour assays are easy to set up in the lab and therefore the researchers could rapidly extrapolate what they learn in this mini-project in their current investigation at their host institutions. To complement these experiments, we will learn how to perform fluorescence imaging of synapses and neurons to analyse neuronal dysfunction.

Schedule
Day 1: Fly handling + Neurodegeneration assays
Day 2: Neurodegeneration assays + Behaviour experiments
Day 3: Dissections +Immunostainings
Day 4: Behaviour experiments+ Immunostaining+ imaging
Day 5: imaging + Data analysis
Day 6: Presentation prepar

9. Behavioral study of memory in mouse models of AD

- **When: Block 1**
- **Instructor: Sebastien Therin**

The project focuses on ADAM10, a synaptic enzyme responsible for limiting amyloid generation in Alzheimer Disease and for spine reshaping through N-cadherin cleavage. In neurons ADAM10 activity can be regulated by trafficking mechanisms that are under the control of activity-dependent synaptic plasticity. Indeed, the long-term potentiation (LTP) triggers ADAM10 binding to the clathrin-adaptor AP2 and the endocytosis of the enzyme. On the other hand, the long-term depression (LTD) fosters its association to SAP97, thus promoting ADAM10 forward trafficking towards the postsynaptic compartment and its shedding activity. Remarkably, the LTD-induced ADAM10 delivery to the synapse is required for the LTD-induced spine shrinkage. This is physiological mechanism according to which ADAM10 activity is tightly regulated by synaptic activity because it is critical for activity-induce spine remodelling.
The aim of this project is to analyse the role of the protein partners of ADAM10 in the regulation of its synaptic localization and activity on the spine-shaping substrate N-cadherin. We will take advantage of cell permeable peptides to interfere with protein-protein interaction in primary neuronal cultures and we will analyse their effect by biochemical techniques, such as coimmunoprecipitation experiments and western blotting. To verify the effect of the Cell permeable peptide in disrupting ADAM10/protein partners association, coimmunoprecipitation will be performed. In parallel, we will assess the effect of the treatment on ADAM10 synaptic localization by the biochemical purification of the postsynaptic fraction followed by western blotting. Finally, we will determine the effect of the alteration of ADAM10 synaptic localization on its substrate N-cadherin implicated in spine shaping.

CELL PERMEABLE PEPTIDE (CPP) TREATMENT: ANALYSIS OF THE EFFECT ON THE TARGET

We will analyse the effect of Cell Permeable Peptides on the interaction of ADAM10 with protein partner AP2.

1. Peptide Active is designed to interfere with ADAM10/AP2 interaction.
Can the active peptide reduce ADAM10/AP2 interaction?
- *Coimmunoprecipitation assay.*

2. The effect of the Peptide Pro on AP2 mediated endocytosis of ADAM10.
Can the active peptide increase ADAM10 synaptic levels?
- *Triton-Insoluble Fraction (TIF) purification and analysis of ADAM10 synaptic levels by Western Blot.*

3. Since ADAM10 cleaves several adhesion molecules.

Can the active peptide affect N-cadherin levels?

- *Triton-Insoluble Fraction (TIF) purification and analysis of N-cadherin synaptic levels by Western blot.*

10. CSF biomarkers of Alzheimer's disease – mouse CSF sampling procedure and analysis

- **When: Block 2**
- **Instructors: Una Smailovic and Kina Höglund**

There is an urgent need for identifying reliable and translational biomarkers that have a potential to bridge the gap between basic and clinical research necessitate to early diagnose and effectively test novel drug candidates for Alzheimer's Disease (AD). Cerebrospinal fluid (CSF) has repeatedly proven to be a valuable source of AD biomarkers since it directly reflects biochemical changes in the brain.
Progressive loss of episodic memory encoding ability is among the earliest and more remarkable symptoms in Alzheimer's disease (AD), developing even before hippocampal degeneration, thus pointing to a synaptic dysfunction.
Sampling CSF from mouse models of AD allows for thorough investigation of candidate biomarkers and their correlates with brain neuropathology, behavioral deficits and functional biomarkers. However, sampling CSF from mice poses certain challenges: it requires surgical procedure that yields a very small quantity of the sampled CSF (10 – 20 uL on average). Moreover, a specific and reproducible quantification of biomarkers in complex matrixes such as blood or cerebrospinal fluid is the fundamental basis for the evaluation of candidate biomarkers.
During this mini-project, we will demonstrate the surgical procedure required to sample CSF from mice and acquire knowledge about the process of assay development, covering the theoretical background, hands on work, exemplified by specific steps in the assay development process as well as data interpretation. The particular method will be on the post synaptic protein neurogranin, which has been shown to be increased specifically in CSF from patients with AD, compared to other types of neurodegenerative disease and controls.

Schedule
Day 1: Short theoretical introduction - mouse anatomy and CSF sampling
Day 2: CSF sampling procedure
Day 3: CSF sampling procedure + ELISA preparation
Day 4: ELISA assay development
Day 5: ELISA assay development and presentation preparation
Day 6: ELISA assay development and presentation preparation

11. ADAM10 in Alzheimer's Disease and it's localization in the synapse: how can protein partners regulate its localization and spine-shaping activity?

- **When: Block 2**
- **Instructor: Lina Vandermeulen**

The project will focus on ADAM10, a synaptic enzyme responsible for limiting amyloid generation in Alzheimer Disease and for spine reshaping through N-cadherin cleavage. In neurons ADAM10 activity can be regulated by trafficking mechanisms that are under the control of activity-dependent synaptic plasticity. Indeed, the long-term potentiation (LTP) triggers ADAM10 binding to the clathrin-adaptor AP2 and the endocytosis of the enzyme. On the other hand, the long-term depression (LTD) fosters its association to SAP97, thus promoting ADAM10 forward trafficking towards the postsynaptic compartment and its shedding activity. Remarkably, the LTD-induced ADAM10 delivery

to the synapse is required for the LTD-induced spine shrinkage. This is physiological mechanism according to which ADAM10 activity is tightly regulated by synaptic activity because it is critical for activity-induce spine remodelling.
The aim of this project is divided into two parts. First, we will analyse whether ADAM10 levels are decreased in the synapse in an Alzheimer's Disease mouse model (APP-PS1) and whether the binding to its protein partner SAP97 is altered. For this we will take advantage of brain tissue from wild type mice and comparing them to AD mice and do a biochemical purification of the postsynaptic fraction. With western-blot of the postsynaptic fraction we can detect ADAM10 levels in the synapse and with co-immunoprecipitation we will be able to reveal the binding between the protein partners.
For the second aim, we want to understand whether interfering with SAP97-ADAM10 binding is involved in spine remodelling. Here we will use a cell permeable peptide to interfere with protein-protein interaction in primary neuronal cultures and we will analyse its effect with the imaging technique 'proximity ligation assay'. Finally, we will determine the effect of the alteration of ADAM10 synaptic localization on spines morphology.

Is the ADAM10-SAP97 interaction altered in Alzheimer's Disease, and does it affect ADAM10 functioning?

Protein-protein interaction assays and spine morphology

We know that ADAM10 synaptic levels are decreased in AD patients.

1. Are ADAM10 synaptic levels decreased in APP PS1 mice and is this influenced by SAP97?
    a. Are there differences in ADAM10 synaptic levels in AD mice?
        i. *Subcellular fractionation of the postsynaptic fraction (Triton-insoluble fraction) of AD mice compared with WT mice.*
            1. Is the binding between ADAM10 to SAP97 altered in AD mice model?
        ii. *Co-immunoprecipitation assay homogenate*

We will analyse the effect of Cell Permeable Peptides on the interaction of ADAM10 with protein partner SAP97.

2. Peptide Pro is designed to interfere with ADAM10/SAP97 interaction.
    b. *Can the PRO peptide reduce ADAM10/SAP97 binding?*
        iii. *Proximity ligation assay*

3. Since ADAM10 synaptic levels are decreased after PRO peptide treatment and ADAM10 cleaves several adhesion molecules.
    c. *Can the CPPs treatment affect spine morphology?*
        iv. *Analysis of spines morphology in GFP-transfected neuronal cultures*

12. Mitochondria distribution the organotypic slices in control and in a tau-seeding model

- **When: Block 2**
- **Instructor: Ania Goncalves**

Pathological accumulation of Tau is a common hallmark of Alzheimer's disease (AD) and other tauopathies. Several mouse models expressing different mutated forms of tau have been engineered. Although they constitute precious tools to study pathologies, they come with their lot of constrains. One of them is that tau aggregation only occurs in old animals, which is challenging for slice electrophysiology and imaging. In addition, following the progression of the disease requires the sacrifice of cohorts of animals at multiple time points, which is time consuming and ethically questionable.
We developed an *in vitro* model of AD using hippocampal organotypic slices. Organotypic slices were prepared from mice expressing the human P301S mutant tau protein. K18 tau seeds were added at DIV 3 and induced tau aggregation in 10 days.
Aim: In this project we'll use our *in vitro* model to characterize mitochondrial mislocalization and the activity impairments of the hippocampal network.

Experiment 1: Mitochondria distribution in a tau-seeding model

Aim: Characterize the morphology and distribution of mitochondria in relation to the proximity of pathological forms of tau.

Techniques: Generation of organotypic hippocampal slice cultures, immunostaining, confocal and super resolution microscopy; Image analysis and processing

Experiment 2: Activity of the hippocampal network in a tau-seeding model

Aim: Study of changes in calcium transients in a hippocampal network.

Techniques: Calcium imaging; Image analysis and processing

13. Assessing properties of CA3 neuronal assemblies involved in information encoding in physiological and Alzheimer's disease conditions

- **When: Block2**
- **Instructor: Dario Cupolillo**

Rapid information encoding following a single-time experience is the basis of episodic memory. During the experience, contextual information converges into the hippocampus and gathers onto specific neuronal assemblies, believed to hold memory traces. Computational and behavioral studies point at the hippocampal CA3 region as a key structure for the integration of multimodal information and rapid memory storage, through synaptic plasticity at the level of CA3 recurrent network.
Progressive loss of episodic memory encoding ability is among the earliest and more remarkable symptoms in Alzheimer's disease (AD), developing even before hippocampal degeneration, thus pointing to a synaptic dysfunction.
During this mini-project, we will employ *ex-vivo* slice electrophysiology to address the properties of specific CA3 neurons involved in memory encoding following a one-time learning of an experience, and compare normal and AD conditions.
Neurons involved in information encoding are identified by the increased genetic transcription of Immediate Early Genes (IEGs) such as cFos. For this reason, we will use mice injected with a cFos-based genetic construct, allowing fluorescent labelling of neurons activated during a salient experience. To model a one-time experience, we will subject these mice to contextual fear conditioning. We then prepare hippocampal slices and use patch-clamp recordings to record synaptic activity and intrinsic properties of labeled neurons.

Schedule

Preparation: -Dox food on 14/01/19
-Injection of 4 mice on the 15/01/19. Post-op 2 weeks -> 29/01/18.
-Handling 3 days before CFC (26/01 -> 28/01)

Day 1: Injection (1 for student)
Day 2: CFC + preparing solutions
Day 3: Electrophysiology + CFC
Day 4: Electrophysiology + CFC
Day 5: Electrophysiology + presentation prep.
Day 6: presentation prep

14. Generation of new C. Elegans transgenics as AD model

- **When: Block 2**
- **Instructor: Anais Marsal Cots**

Generation of transgenic constructs for cell culture and C. elegans to model Alzheimer Disease.
AD is a multifactorial disorder. Less than 5% of the cases are associated with the familial form of AD (EOAD), caused by genetic mutations in genes such as APP, PSEN1 and PSEN2. The remaining >95% of the cases comprise the sporadic form of AD (LOAD), where several risk factors like variations in some genes such as APOE4 and MAPT, in combination with lifestyle and environmental factors may increase the susceptibility to develop AD.
Despite years of extensive research, the molecular mechanisms that underlie the pathology of AD remain unclear. Model organisms, such as the nematode, Caenorhabditis elegans, present a complementary approach to addressing these questions. C. elegans has many advantages as a model system to study AD and other neurodegenerative diseases. Like their mammalian counterparts, they have complex biochemical pathways, most of which are conserved.
In this mini project we will employ the Gateway Cloning technology together with SnapGene software to design a strategy to clone human full-length tau protein fused to a GFP (Green Fluorescent Protein) to be expressed under a promoter for the muscle cells of the nematode.
Once the construct is generated, it can be integrated in the C. elegans chromosome. Eventually, with only one more recombination, the same construct can be expressed in a rodent promoter if we want to study it in mammals.

Schedule
Preparation: - LB plates 3-7 days before starting
Day 1: Production and purification of attB-PCR products
Day 2: Creation of Entry Clones
Day 3: Screening of positive colonies + Creation of Expression Clones
Day 4: Creation of Expression Clones (continued)
Day 5: DNA purification and digestions
Day 6: Prepare presentations

15. Hippocampal and cortical atrophy during the preclinical stage of AD

- **When: Block 2**
- **Instructor: Vincent Planche**

Background: The neurodegenerative process causing brain atrophy and cognitive impairment in AD involves both the limbic system and neocortical areas. Hence, MRI plays a key role in the clinical assessment of patients with suspected AD because regional atrophy can provide positive diagnostic information. However, the pattern and dynamics of brain atrophy in AD (and especially in preclinical stages) remain poorly understood and are somewhat different according age at onset, clinical presentation, neuropsychiatric comorbidities, vascular risk factors and rate of decline.

Objectives: In this mini-project, we will study MRI from older adults before they develop AD and we will measure cortical volumes, hippocampal volumes and hippocampal subfields volumes, using manual and automatic segmentation procedures (VolBrain and 3D-Slicer softwares).

Schedule:
Day 1: Introduction to the field of structural MRI & AD in clinical practice and research. Introduction on quantitative structural analyses of brain MRI. Initiation of manual hippocampal segmentation. Presentation of MRI analysis software tools.
Day 2: Analysis of brain MRI from a cohort of aged subjects with the Volbrain software. Handling a viewer to correct automatic segmentation of brain structures. Manual correction of hippocampal segmentation. Volumetric analyses
Day 3: Focus on hippocampal subfields. Hippocampal subfields segmentation. Manual correction.
Day 4: Statistical analyses of a full data-set of imaging data.
Day 5: Statistical analyses. Predictive value of hippocampal volume in AD. Presentation of results.

16. Proteomic alterations between the hippocampal CA1 and CA3 regions

- **When: Block 2**
- **Instructor: Hazal Haytural**

Mass spectrometry-based proteomics is commonly used to identify alterations in the proteome in order to elucidate mechanisms underlying disease pathogenesis. Therefore, there is a growing interest in applying this technique in the field of Alzheimer disease (AD) in order to have a better understanding of AD pathogenesis and to identify potential biomarkers.
The hippocampus plays a crucial role in the formation of episodic memory, and it is well known that episodic memory and executive functions are impaired during the course of AD. In this mini project, we will combine laser microdissection (LMD) and mass spectrometry to detect the differences in the proteome of the hippocampal CA1 and CA3 regions. LMD has a great advantage as it reduces sample complexity and contamination from surrounding material, and thereby, increases the chance of identifying region-specific alterations. Once samples are ready, we will inject to the liquid chromatography-mass spectrometry (LC-MS) and quantify the relative expression levels of identified proteins between the two selected regions.

Schedule:
Preparation: Preparation of frozen rat brain (due to the fact we do not have permission to use frozen human material, in this mini project, we will focus on understanding how to employ these techniques)
Day 1: Cryostat (up to 100 sections) + Staining
Day 2: LMD (CA1 and CA3 - 3 biological replicates)
(If we have the time, we can also practice on dissecting out single neurons)
Day 3: Protein extraction + Protein determination + Sample preparation for mass spectrometry
Day 4: Sample clean-up + Injection to the LC-MS + Theory behind LC-MS
Day 5: Data analysis (PANTHER, Gene set enrichment analysis, Ingenuity pathway analysis)
Day 6: Conclusion + Presentation

17. Microglial function and induced pluripotent stem cells.

- **When: Block 2**
- **Instructor: Jennifer Pocock**

More detailed information soon.

18. Morphological 3D reconstruction of neurons.

- **When: Block 2**
- **Instructor: Fabio Bertan**

Dendritic and synaptic morphology critically determines the intrinsic electrical properties of neurons. The resulting complexity of the dendritic arborization and synapses enable neurons to produce specialized circuits and cognitive functions. To date, dendritic and synaptic morphology is compromised in patient with Alzheimer's disease as well in different models neurodegeneration.
Reconstruction of neurons, from spine morphology to dendritic arborization, is an important task although still challenging. During this mini-project, we will employ primary hippocampal neurons and *ex-vivo*slices to learn how measure in 2D and 3D neuronal structures. We will learn how to culture and transfect hippocampal neurons with low efficiency. Moreover, we will use both confocal and super resolution microscopy (STED) and we will reconstruct neurons using semi-automatic approaches (Fiji, NeuronStudio and Imaris).

Schedule
Preparation hippocampal neurons before the begging of the course
Day 1: Calcium phosphate transfection of neurons (DIV7) + Confocal microscopy
Day 2: Induction of cLTP in primary neurons (DIV14) + Confocal microscopy
Day 3: Immunostaining for STED imaging + Introduction to ImageJ
Day 4: Immunostaining for STED imaging + Introduction to Imaris
Day 5: STED imaging + Introduction to NeuronStudio + presentation prep
Day 6: Presentation preparation