ESN-ISN Neurochemistry School

Faro, Portugal | 2023

Hands-on Labs

1.

Title: **Comet Assay: a robust tool to evaluate DNA damage in neurons**

Organizer(s) name(s):
Rodrigo Paulino
Anderson Araújo
Clévio Nóbrega

Brief description:
The comet assay, also known as single-cell gel electrophoresis, is used to evaluate DNA damage in neurons. Neurons are isolated and embedded in agarose gel on a slide. Electrophoresis causes damaged DNA to migrate more rapidly, forming a comet-like shape with a tail, while undamaged DNA remains closer to the nucleus. Staining and microscopy allow visualization of comets, and software analyses parameters like tail length and intensity, providing a quantitative measure of DNA damage. This assay helps researchers understand the extent of DNA damage in neurons and its implications for neuronal health.

Syllabus (e.g., procedures, techniques):
Cell collection and suspension in low melting agarose
Slides lysis and balancing
Electrophoresis
Comet microscopy
Comet (DNA damage) analysis

Time (hours):
3h

Maximum participants:
6-8

2.

Title: **An introduction to human brain organoids' preparation and maintenance**

Organizer(s) name(s):
Mónica Fernandes
Sofia Calado

Brief description:
Organoids are human iPSC-derived 3D cell culture models that are revolutionizing life sciences due to their great potential to model complex systems in an *in vitro* setup. Brain organoids recapitulate different human brain regions and are used to study brain development and related diseases. Moreover, although being more challenging, these organoids are being applied in modelling neurological diseases of the adult, including neurodegenerative diseases and cancer.

Syllabus (e.g., procedures, techniques):
Introduction to iPSCs and organoids in research
How to initially set up a "brain" in a cell culture dish: iPSCs and the reagents and materials needed
How to maintain cultures of brain organoids
How to recover and prepare the organoids for histological analysis
How to analyze

Time (hours):
3h

Maximum participants:
4-6

3.

Title: **R-Mind: Unlocking the Potential of R Data Analysis for Biomolecular Research**

Organizer(s) name(s):
Isabel Duarte
Ramiro Magno

Important note:
This workshop is not for R absolute beginners.
Basic R programming knowledge is required: R basic syntax, import data into R, install packages, load packages, set and change working directory, saving code as R scripts.

Brief description:
R is a powerful programming language and software environment for statistical computing and graphics. It provides a wide range of statistical and graphical techniques, making it an essential tool for data analysis and visualization. With a vast collection of packages and libraries, R enables researchers to implement advanced statistical models and algorithms. Its flexibility allows for seamless integration with other programming languages, facilitating data manipulation and preprocessing. R's popularity among statisticians and data scientists makes it an indispensable tool for conducting research and analyzing complex datasets.

Syllabus (e.g., procedures, techniques):
Tidy Data, Data Wrangling, and Reproducible Analysis
What is tidy data, and why should I care?
Basic data wrangling with dplyr, tidyr and other tidyverse packages
Data analysis reporting using R Markdown for reproducible research
Data Analysis and Visualization
Exploratory data analysis (descriptive statistics) and data visualization
Basic statistical inference in R (t-tests, ANOVA, PCA, linear regression, correlation...)
Creating publication-ready visualizations with ggplot2 and patchwork
Differential gene expression analysis
Functional enrichment analysis (pathways)

Bring your own data day:
Brief data analysis consulting: Identification of challenges in data analysis using R, and help with overcoming them.
Beforehand, you must provide a detailed description of the data you plan to bring, to allow the tutors time to prepare for productive consulting.

Time (hours):
9h

Maximum participants:
8

4.

Title: **Behavioral Paradigms: Exploring the Analysis of Motor Function in Rodents**

Organizer(s) name(s):
Tiago Gomes
Marta Vitorino

Brief description:
Motor function analysis in rodents is a pivotal component of understanding the progression and underlying mechanisms of neurodegenerative disorders. This analysis allows researchers to assess and quantify motor impairments in rodent models (Mus musculus and Acomys cahirinus), providing valuable insights into disease pathology and potential therapeutic interventions. Through various behavioral paradigms, such as gait analysis, rotarod performance, and open field testing, motor function analysis enables the precise evaluation of motor coordination, balance, and locomotion. By studying motor deficits in rodent models, researchers can gain a deeper understanding of the impact of neurodegeneration on motor behavior and develop novel strategies for intervention and treatment.

Syllabus (e.g., procedures, techniques):
Brief explanation about neurodegenerative disease model
Explanation about each behavioral test:

- Beam Walking test
- Swimming test
- Rotarod
- Grip strength test (cannot be done with Acomys)
- Footprint analysis

Explanation why some of the tests cannot be done with Acomys, or are not challenging enough for Acomys. Explain the necessity of behavioral test adaptation with the characteristics of each rodent species.
Outcomes of behavioral tests
Challenges and Troubleshooting
Analysis of behavioral paradigms

Time (hours):
6h (3h+3h)

Maximum participants:
15

5.

Title: **Brain-derived extracellular vesicles in health and disease**

Organizer(s) name(s):
Rafael G. Costa
Clévio Nóbrega

Brief description:
Extracellular vesicles (EVs) are released by cells, carrying several biomolecules such as proteins, nucleic acids, and lipids. They serve as intercellular messengers, facilitating communication between neurons and influencing disease progression. By analyzing the content and composition of EVs, researchers can identify disease-specific biomarkers, monitor disease progression, and uncover novel therapeutic targets. The study of EVs in neurodegeneration research holds great promise for understanding disease mechanisms and developing innovative strategies for early diagnosis and treatment.

Syllabus (e.g., procedures, techniques):
Introduction to extracellular vesicles' (EVs) biology in health and disease
EVs biogenesis
EVs in cell-cell communication
EVs in neurodegenerative diseases
EVs as biomarkers and therapeutic agents
Methods for EV isolation and characterization:

- Isolation techniques
- Ultracentrifugation
- Polymer co-precipitation
- Immunoaffinity enrichment
- Characterization techniques
- Electron microscopy
- Western blot
- Flow cytometry

Hands-on activity: "Isolation of extracellular vesicles (EVs) derived from rodent brains using ultracentrifugation as a gold standard technique"

Time (hours):
3h

Maximum participants:
6

6.

Title: **Rodent brain processing**

Organizer(s) name(s):
Inês Afonso
Clévio Nóbrega

Brief description:
Brain processing and immunohistochemistry are vital components of neuroscience research, enabling a deeper understanding of brain structure and function. Brain processing involves the careful extraction and preparation of brain tissue for analysis. This process includes tissue fixation, sectioning, and mounting onto slides, ensuring optimal preservation of cellular and molecular components. Immunohistochemistry, on the other hand, is a technique that utilizes specific antibodies to label and visualize target proteins within brain tissue sections. By combining immunohistochemistry with microscopy, researchers can identify and study various cellular markers, neurotransmitters, and pathological changes in the brain. This powerful technique provides valuable insights into the localization and distribution of specific molecules, contributing to our understanding of brain function and neurodegenerative diseases.

Syllabus (e.g., procedures, techniques):
Brain processing and sectioning
Immunohistochemistry
Biochemical stanning's to access neuronal structure.

Time (hours):
3h

Maximum participants:
10

7.

Title: **Hands-on on zebrafish as a model to study the brain**

Organizer(s) name(s):
Tatiana Varela
Débora Varela
Natércia Conceição

Brief description:
The zebrafish have emerged as a model for the study of human neurological diseases, but also as a system for the screening of compounds with the potential to treat or prevent these diseases. Beyond the technical advantages over traditional models (e.g. rodents), the high similarity of targets, physiology, drug metabolism and pharmacology in comparison to humans further highlight the potential of zebrafish as a robust model for drug screenings.

Syllabus (e.g., procedures, techniques):
Visit to the zebrafish facilities
Manipulation of zebrafish embryos and identification of embryonic stages using a bright-field stereomicroscope
Imaging of transgenic zebrafish expressing fluorescent markers of different proteins using a fluorescence stereomicroscope
Monitor the spontaneous swimming behavior (traveled distance and trajectory) of WT zebrafish larvae

Time (hours):
3h

Maximum participants:
5

8.

Title: **Seeing is believing: time-lapse analysis of neuronal activity using fluorescent probes**

Organizer(s) name(s):
Inês Araújo
Sónia Simão
Rafaela Agostinho

Brief description:
Participants will have the opportunity to perform time-lapse confocal imaging in cultured neurons, using fluorescent probes for specific signaling phenomena. This methodology will allow participants to:

1) Assess changes in intracellular calcium concentration.
2) Assess the production of intracellular gasotransmitters.

Syllabus (e.g., procedures, techniques):
Loading cultured neurons with fluorescent probes
Time-lapse imaging of intracellular calcium or H2S signaling
Single-cell analysis of changes in calcium or H2S concentration
(https://doi.org/10.1016/j.dyepig.2023.111304)

Time (hours):
3h

Maximum participants:
4

9.

Title: **Targeting the brain with stereotaxic surgery: From theory to practice**

Organizer(s) name(s):
David Brito
Clévio Nóbrega

Brief description:
Stereotaxic surgery is a precise technique used in neurochemistry research to target specific areas of the brain with accuracy. By employing a three-dimensional coordinate system, this surgical approach allows researchers to manipulate and study processes within targeted brain regions. Using specialized instruments and a stereotaxic atlas, researchers can guide their interventions to precise coordinates, enabling them to implant electrodes, deliver drugs, or collect samples with minimal damage to surrounding structures. This methodology provides invaluable insights into the intricate mechanisms underlying brain function and behavior.

Syllabus (e.g., procedures, techniques):
Applications for stereotaxic surgery in the rodent brain:
Systemic vs brain targeting advantages and disadvantages.
Common methods that require brain delivery (i.e. pharmacology, cannulas and viral particles).
Targeting specific mouse brain regions: determining coordinates, volume, and injection speeds.
Troubleshooting.
Hands-on brain delivery using stereotaxic frames.
Practical introduction to material, settings, and variables in stereotaxic surgery.

Time (hours):
3h

Maximum participants:
4

10.

Title: **Viral plasmid design for neurochemistry applications: what comes before the wet lab**

Organizer(s) name(s):
Ricardo Reis
Clévio Nóbrega

Brief description:
Viral plasmid design allows for customization of plasmid elements, such as promoters, enhancers, transgene, and Inverted terminal repeats (ITRs), that will ultimately impact the expression of your transgene. Furthermore, designing your own viral plasmids enable you to fine tune plasmid size, and thus circumvolute viral vector packaging size problems. Custom plasmids enable researchers to have their exact needs met instead of relying on third party plasmids. In this workshop, participant will learn to design custom Adeno-Associated Virus (AAV) plasmids employing restriction cloning in the free online platform Benchling.

Syllabus (e.g., procedures, techniques):
In this workshop, participants will learn to employ restriction cloning with to introduce multiple plasmid elements such as a promoter, regulatory elements, and a transgene into an AAV backbone plasmid. Three Plasmids containing the elements of interest, including the backbone will be used to generate a functional AAV expression plasmid. All procedures will be performed in silico with the free online platform, Benchling.
Contemplated topics summary:

- Determining plasmid elements of interest.
- Scanning the backbone plasmid and inserts for compatible restrictions sites.
- Determining which restriction enzymes to use.
- Creation of compatible restrictions sites in PCR amplified inserts
- Digesting plasmids/PCR products with chosen enzymes and ligating fragments of interest thought the Benchling Assembly Wizard feature.

Time (hours):
3h

Maximum participants:
20

11.

Title: **Light microscopy imaging for biosciences**

Organizer(s) name(s):
Rui Silva

Brief description:
The aim is to offer participants a comprehensive basic light microscopy course, that includes fundamentals of light microscopy, introduces some the state-of-art of advanced fluorescence microscopy techniques, and an overview on image analysis.

Syllabus (e.g., procedures, techniques):
The course comprises two parts:

1. A theoretical lecture that provides a concise historical overview of microscopy, introduces fundamental microscopy concepts, explores different types of microscopes, and covers the basics of image analysis.
2. A hands-on activity on image analysis using the freely available FIJI software. This session will focus on basic image analysis operations, including segmentation and fluorescence quantification.

Participants are expected to bring their own laptops with FIJI software already installed. No prior experience or microscope images are necessary for the course.

Time (hours):
6h

Maximum participants:
15 participants

12.

Title: **Live imaging of zebrafish neurovascular development**

Organizer(s) name(s):
Marco Campinho
Clévio Nóbrega

Brief description:
The proposed activity aims at carrying out live image of zebrafish neurovascular development. Brain function is dependent on great amounts of energy and gas exchange at the tissue is essential to enable it. Therefore, understanding blood-brain-barrier (BBB) development is essential to fully understand brain development and function. Given this being a highly dynamic process live imaging is essential to study these processes.
This activity will use zebrafish double transgenic (Tg(GFAP:GFP; kdrl:mCherry)) that simultaneous illuminate glial cells (GFP) and endothelial cells (mCherry) to register by confocal microscopy live imaging this dynamic process.

Syllabus (e.g., procedures, techniques):
Zebrafish transgenics, live imaging, confocal microscopy, image analysis

Time (hours):
6h

Maximum participants:
4

13.

Title: ***In vivo* electroporation of the chicken embryo neural tube**

Organizer(s) name(s):
Gil Carraco
Raquel Andrade
Nísia Martins

Brief description:
Electroporation is an experimental technique that relies on the application of electric pulses for the incorporation of foreign genetic material (eg. expression vectors, RNAi) into cells/tissues. The chicken (Gallus gallus) embryo is a reference animal model system for the study of early neurogenesis and brain development due to its extraordinary similarities to the Human embryo and because it is easily amenable to *in vivo* procedures, both *in ovo* and in *ex ovo* culture conditions.
During this hands-on practice, participants will manipulate chicken embryos incubated for 48h (HH10-12) and perform *in ovo* and *ex ovo* electroporation of expression plasmids containing fluorescent reporters. Participants will become acquainted with the HH chick embryo staging system (Hamilton and Hamburger, 1951), will learn to inject genetic material into the developing neural tube and brain cavities and to apply an electric current to modify gene expression in one half of the neural tube (while the contralateral tissue serves as control). The participants will also employ the EC Culture system (Chapman et al., 2021) for manipulation of early embryos outside the eggshell. Successful tissue electroporation will be monitored by examining fluorescent signal (GFP or RFP) after 24h.

Syllabus (e.g., procedures, techniques):
Chicken embryo manipulation and staging
Ex ovo EC Chapman culture
Plasmid DNA microinjection in the developing neural tube and brain cavities
Electroporation of the developing neural tube and brain
GFP/RFP observation using a SteREO LUMAR V12 Stereoscope.

Time (hours):
3 hours plus 1 hour on the following day for result visualization and image acquisition.

Maximum participants:
6

14.

Title: **Whole-mount in situ hybridization for gene expression analysis**

Organizer(s) name(s):
Gil Carraco
Raquel Andrade
Nísia Martins

Brief description:
Gene expression is very dynamic during embryo development. Whole-mount in situ hybridization allows the detection of RNA transcripts within an intact tissue/embryo, evidencing where a specific gene is expressed. This technique relies on the hybridization of a labelled antisense RNA probe to an RNA molecule of interest (mRNA, lncRNA, miRNA, etc). Then, an immunohistochemistry approach is used to detect the labelled probe in situ.
During this hands-on practice, participants will learn how to establish and work in an RNase-Free environment. They will synthesize antisense digoxigenin-labelled RNA probes *in vitro* and perform whole-mount in situ hybridization in embryos in different neurulation stages. Image acquisition and analysis will be performed using a SteREO Discovery V12 Stereoscope.

Syllabus (e.g., procedures, techniques):
Synthesis of digoxigenin labelled RNA antisense probes
Whole mount in situ hybridization in chicken embryos
Image acquisition using a SteREO Discovery V12 Stereoscope

Time (hours):
6

Maximum participants:
6