Experimental projects (First block)

Project 1: Visualization and quantification of cellular complexity of the CA1 region of the mouse brain

Instructor: Milda Valiukonyte (*Karolinska Institutet, Stockholm, Sweden*)

Background

Understanding the function of a tissue requires knowledge of the spatial organization of its constituent cell types. The use of single-cell RNA sequencing in brain tissue has revealed the genome-wide expression patterns that define many, closely related neuronal types. But this method cannot reveal their spatial arrangement nor provide an accurate count of cells. To reveal both location and number of the different cell types researchers have to perform transcriptomic directly in tissue.

Aim

For this project the students will learn a method for cell typing by in situ sequencing. Hippocampal area CA1 is a part of the trisynaptic circuit that has been central to neuroscience. In this area, in addition to two pyramidal populations at least 23 classes of GABAergic neurons have been proposed to date based on electrophysiology and connectivity in rodents. It was recently shown that distinct scRNAseq profiles of CA1 cells can be attributed to these 23 functional classes in mice. The students will use spatial transcriptomics to identify the location of and quantify the relative numbers of each of these classes in sections of mouse CA1 brain tissue.

Main techniques

Multiplexed in situ based cell type identification
Automated wide-field microscopy

Project 2: In Vivo Perturbation of developmental gene expression trajectories

Instructor: Christian Mayer (*Max Planck Institute of Biological Intelligence, Germany*)

Background

The central nervous system consists of diverse types of inhibitory neurons that vary widely in morphology, physiology, connectivity and molecular markers. During development, molecular diversity is initially reflected in the regional expression of a narrow set of transcription factors in mitotic progenitors. Transcriptional signatures that distinguish mature neuronal subtypes emerge around cell-cycle exit and become more sharply defined during postnatal development. However, a major challenge remains in identifying how these early sources of heterogeneity generate the vast diversity of adult interneurons remains. One way to address this question is to perturb gene expression during development and study the effect of the perturbation on development trajectories.

Aim

In this project, students will learn how to induce Cas9-mediated deletions of a developmental transcription factor by in utero electroporation and how to perform single-cell RNA sequencing of perturbed neurons (Perturb-Seq). In the second part, students will use different computer-based methods to map the developmental trajectories of GABAergic neurons and compare the gene expression of control and perturbed neurons.

Main techniques:

Embryonic brain dissection and dissociation
In utero electroporation

Whole cell single-cell RNA sequencing using 10x system

Project 3: Transcriptional response in activated GABAergic neurons of the visual cortex
Instructor: Hannah Sophie Hochgerner (*Technion – Israel Institute of Technology*)
Background
GABAergic inhibitory neurons, although low in number (around 20% of neurons), are crucial for shaping the output of a circuit. Neurons are plastic entities that adapt their function, and molecular machinery, to changes in network activity.
Aim
This project aims to describe how the diverse GABAergic interneurons of the visual cortex react to sudden input in network activity. Mice will be housed in darkness and then be exposed to light shortly before sacrifice to induce activity. To get a robust coverage and detect the different subtypes of this population it is often necessary to enrich for GABAergic cells prior to single-cell sequencing. The students will learn how to apply single-cell RNA-seq on a functional question in the adult, and use FACS in combination with a transgenic mouse line to enrich for GABAergic cells prior to single-cell RNA-seq.
Main techniques
Adult brain dissection and dissociation
FACS isolation of fluorescently labeled cells
Whole cell single-cell RNA sequencing using 10x system

Project 4: Single whole cells analysis of an Alzheimer's disease mouse model
Instructor: Ana Muñoz-Manchado (*University of Cadiz, Spain / Karolinska Institutet, Sweden*)
Background
The ability to investigate transcriptional changes in individual cells holds great promise in the study of disease. Already now, a few papers, including one on Alzheimer's disease have appeared where this has been used to study disorders in humans (Mathys et al., Nature, 2019). This approach opens up not only the study of diseases but can also be used as a way to understand which aspects of a disorder are recapitulated by mouse models.
Aim
For this project the students will perform whole-cell single-cell RNA-sequencing of the prefrontal cortex of the 3xTg-AD mouse model for Alzheimer's disease (Oddo et al., Neuron, 2003) to investigate similarities and differences to the findings from human subjects.
Main techniques
Adult brain dissection and dissociation
Whole cell single-cell RNA sequencing using 10x system

Project 5: Single nuclei analysis of an Alzheimer's disease mouse model
Instructor: Lisbeth Harder (*Karolinska Institutet, Stockholm, Sweden*)
Background
The ability to investigate transcriptional changes in individual cells holds great promise in the study of disease. Recently, several papers on neurological disorders including Alzheimer's disease (eg Mathys et al., Nature, 2019) have appeared, in which the single cell sequencing technique was develop further to enable the isolation of RNA from nuclei of frozen human samples. This approach opens up not only the study of disease but can also be used as a way to understand which aspects of a disorder are actually recapitulated by mouse models.

However, the current limitation to single-nuclei sequencing when studying human subjects might bias the findings in terms of which mRNAs are captured (Skene et al., Nature Genetics, 2018).

Aim

In this project the students will learn how to isolate nuclei from frozen tissue of the prefrontal cortex using the 3xTg-AD mouse model for Alzheimer's disease (Oddo et al., Neuron, 2003). Samples will be enrich for neuronal populations using antibody-assisted FACS isolation and results will be compare to data from human as well as from whole-cell single-cell RNA-sequencing (project 4).

Main techniques

Isolation of nuclei from frozen tissue
FACS isolation of neuronal populations
Single-nuclei RNA sequencing using 10x system

Project 6: Single nuclei analysis of GABAergic cells in the dorsal horn in a Chronic pain model

Instructor: Danny Kitsberg (*ELSC, Jerusalem University, Israel*)

Background

Chronic pathological pain is a major burden for most societies, with high human, social and economic costs. The development of chronic pain is largely thought to result from a malfunction of the spinal neuron network, and more particularly from a dysfunction of spinal GABAergic neurons located in superficial laminae of the spinal cord. Although the development of RNAseq approaches has enabled a thorough classification of both excitatory and inhibitory neurons in the spinal cord, little is known about the physiologic, morphologic and transcriptomic changes accompanying the development of chronic pain symptoms. For studies on human subjects, we are currently limited to single-nuclei sequencing and a recent study have suggested that using this technique might bias the findings in terms of which mRNAs are captured (Skene et al., Nature Genetics, 2018).

Aim

In this project the students will use neuropathic and control animals to investigate transcriptomic changes in FACS sorted neuronal nuclei from spinal superficial dorsal horn. This will give insights to transcriptomic changes in both excitatory and inhibitory cells.

Main techniques

Isolation of nuclei from frozen spinal cord tissue
FACS isolation
Single-nuclei RNA sequencing using 10x system

Project 7: Single whole cells analysis of GABAergic cells in the dorsal horn in a Chronic pain model

Instructor: Martin Häring (*University Clinic Münster, Germany*)

Background

Chronic pathological pain is a major burden for most societies, with high human, social and economic costs. The development of chronic pain is largely thought to result from a malfunction of the spinal neuron network, and more particularly from a dysfunction of spinal GABAergic neurons located in superficial laminae of the spinal cord. Although the development of RNAseq approaches has enabled a thorough classification of both excitatory

and inhibitory neurons in the spinal cord, little is known about the physiologic, morphologic and transcriptomic changes accompanying the development of chronic pain symptoms.

Aim

The students will use neuropathic and control animals to investigate transcriptomic changes in FACS sorted GABAergic neurons from spinal superficial dorsal horn. Relevance of these changes with respect to previously characterized alterations of morphological and electrophysiological properties will be investigated.

Main techniques

Spinal cord / dorsal horn dissection
FACS isolation
Whole cell single-cell RNA sequencing using 10x system

Project 8: Large scale single-cell RNA-sequencing of brain tissue using SPLiT-Seq
Instructor: Song Cheng (*UK*)

The students will learn how to apply SPLiT-Seq (Split Pool Ligation-based Transcriptome sequencing) which is a technique by which one can perform large-scale single cell (nuclei) sequencing without the need for advanced equipment. We will be open for student's suggestion with regards to which brain area to investigate.

See this webpage for methodological explanation and more information: https://sites.google.com/uw.edu/splitseq.

Main techniques

SPLiT-seq

Project 9: Single-cell profiling of histone modifications in the mouse cortex using scCUT&Tag
Instructor: Marek Bartosovic (*Karolinska Institutet, Stockholm, Sweden*)

Background

It is evident that the gene expression is directly regulated by a combination of various regulatory epigenetic elements such as promoters, enhancers and repressive chromatin. Single-cell ATAC-seq has been routinely used to identify and measure the chromatin accessibility/footprints of transcription factors and thus characterize the active chromatin state at promoters and enhancers.

Aim

In order to investigate the repressive chromatin state, we will use single-cell CUT&Tag of H3K27me3 mark characteristic for heterochromatin or inactive promoters/genomic regions. Single-cell CUT&Tag has been used to generate the profile of H3K27me3 in major cell types in the brain (Bartosovic et al., 2021). In this project we will go one step further and focus on analyzing the repressive chromatin states in the mouse cortex and aim to uncover the patterns of H3K27me3 in the different cortical layers. The students will perform the dissection of mouse cortex, nuclei isolation and custom single-cell CUT&Tag protocol based on the 10x Genomics platform.

Main techniques

Mouse cortex dissection
Single-cell CUT&Tag

Project 10: InCITE-Seq
Instructor: Hattie Chung (*Broad Institute/MIT, US*)
Background
Neurons transform external signals into molecular changes, often encoded by the expression and subcellular localization of regulatory proteins that alter gene expression patterns and subsequent neuronal connectivity. This project aims to measure the gene expression changes associated with the activity-regulated transcription factor c-Fos in neurons of the visual cortex after light exposure. We will use inCITE-Seq to jointly measure quantitative c-Fos protein levels with the transcriptome across thousands of single nuclei, then computationally relate nuclear concentrations of c-Fos to gene expression changes within each cell type.
Main techniques
Adult brain dissection and dissociation
Nuclei preparation and intranuclear antibody stain
FACS isolation
Joint protein and RNA sequencing with inCITE-seq on 10x Chromium platform

Computational projects (Second block)

In the second, computational part, all students will learn state-of-the-art approaches for computational analysis and interpretation of single-cell RNA-seq data. The instructors will explain the statistical and computational underpinnings of different methods, and then proceed with practical examples and walkthroughs. In addition to going through the common exercises, each individual group will then apply the relevant approaches to carry out the analysis of the data they have generated in the first part of the course.
The students will learn how to carry out the initial alignment and demultiplexing steps, calculate and interpret various quality control metrics. Installation and setup of different analysis frameworks, including Scanpy, Seurat, scVI and Conos will be covered. The students will examine different normalization and dimensionality reduction techniques, monitoring for common technical side effects, explore different options for clustering subpopulations and testing for differential expression. The course will cover integration and comparison of multiple datasets, techniques for transferring and automating cell annotations. For the analysis of dynamical processes, students will use trajectory fitting techniques as well as RNA velocity estimations. Many of these techniques will be directly applicable to the experimental data that will be generated in the first part of the course, which will be done under the supervision of the instructors.

Instructors:
Viktor Petukhov (*University of Copenhagen, Denmark*)
Gioele La Manno (*School of Life Sciences, EPFL, Lausanne, Switzerland*)
Christoffer Mattsson Langseth (*Stockholm University, Sweden*)
Lisa Bast (*Karolinska Institutet, Sweden*)