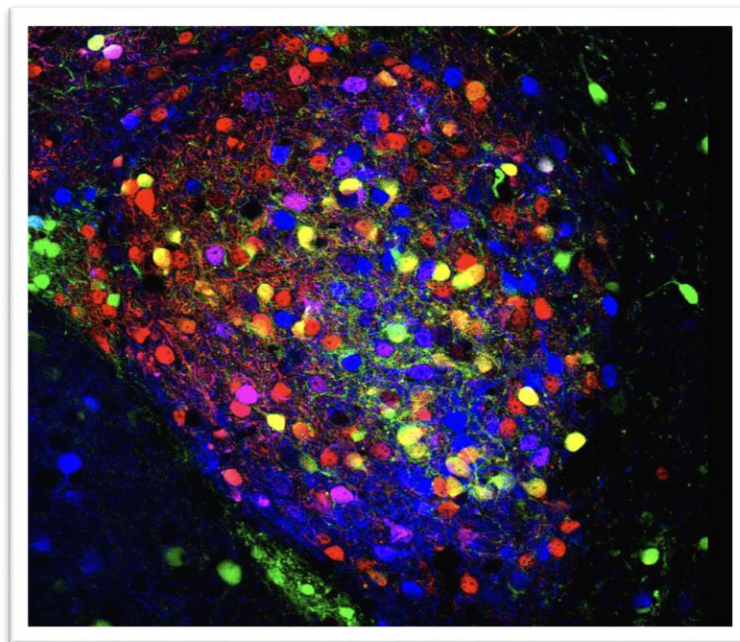


PASTEUR COURSE

DEVELOPMENT & PLASTICITY OF THE NERVOUS SYSTEM

1ST PART: EVOLUTION AND DEVELOPMENT OF THE NERVOUS SYSTEM

2ND PART: DEVELOPMENT, PLASTICITY AND PATHOLOGIES OF THE NERVOUS SYSTEM



From Giuseppe Gangarossa, Paris Diderot University, DPNS Course

PROGRAM 2019-2020

FROM SEPTEMBER 16TH TO OCTOBER 17TH 2019

INTRODUCTION

The course *Development and plasticity of the nervous system (DPSN)* covers the main cutting-edge topics of neuroscience research, at both theoretical and experimental levels. Students following the course will acquire a broad understanding of various topics ranging from molecules to behavior. Various animal models (mouse, frog and drosophila) commonly used in neuroscience research are used, giving students the opportunity to understand the difference between each model, and to further expand their repertoire of practical skills in carrying out experiments. International scientists, expert in their own field of research, provide theoretical and/or experimental courses. During the practical course, students will approach and test stem cell biology, behavioral analyses in mice, neuritogenesis of primary cortical neurons, drug stereotaxic injection, neuroanatomy and gene expression analyses using histochemical techniques, which are further described below.

Preparing neural stem cells and testing their multipotentiality and the influence of exogenous factors on their neural fate approach stem cell biology: Neural stem cells are the most immature progenitor cells in the central nervous system and are defined by their ability to give rise to more stem cells via symmetric division, and to progenitors of all neural lineages by asymmetric division. In this session, students will isolate neural stem cells from various structures of the fetal mouse brain and will subsequently prepare neurospheres. The differentiation pattern of neural stem cells will be studied using the neurospheres they have prepared during the practical course. In addition, students will analyze the influence of exogenous factors on neurosphere differentiation.

Complexity of the brain aims to introduce the students to the basic histological methods that are currently used as the first step of sample analysis in neurobiology. It is necessary to obtain resolute images from morphology of the tissues under study. The simplest way to obtain the picture of cellular structures and complexity is to develop classical staining procedures. Two staining procedures, namely cresyl violet and luxol fast blue, will represent appropriate tools to analyze the distribution of neural cell bodies. During the staining procedure (composed of multiples steps) of rodent brain sections (paraffin embedded), students will be trained to brain anatomy and nuclear ontology. This training will introduce them to the processes of 3D reconstruction and to develop mental representations of anatomical structures and topological references.

Stereotaxic injection and functional consequences: We will study the anatomical and behavioral effects of dopaminergic depletion caused by the injection of a toxin into the striatum. This experimental session is divided into three parts, during which students will be introduced to and will acquire experience in behavioral and statistical analyses and immunohistochemistry techniques. Students will perform stereotaxic injections of a toxin into the striatum of adult mice. Assessing mice abilities during several sensory-motor tasks will test the success of this procedure. Finally, the students will perfuse the mice, slice the brains in serial sections and perform immunohistochemistry to confirm the loss of dopaminergic cells due to the toxin injection.

Behavioral analysis: Effect of cholinergic modulation on short-term memory:

This part is devoted to behavioral analysis after transient inhibition of the cholinergic system in adult mice. Such inhibition will be achieved via intraperitoneal injection of a muscarinic receptor antagonist. Students will then assess the behavioral performances of mice on a short-term memory task, namely the novel object recognition. During this part of the course, students will strengthen

their understanding of behavior testing as well as statistical analysis.

Tools to investigate early neural development in *Xenopus laevis*:

Xenopus are the appropriate tools to study vertebrate embryology and development, and to model human diseases in which the embryonic developments are dysregulated. Research using *Xenopus* embryos takes advantage of a large and abundant production of eggs, and embryos which can be manipulated easily, as well as a conserved cellular, developmental and genomic organization with mammals. For the practical course, experiments will particularly focus on analyzing the development of the neural plate in such models.

Neurite outgrowth using primary rat cortical neurons:

Regional and subregional variations in the size and morphology of neurons occur in the mammalian nervous system. Neuritogenesis corresponds to the sprouting of neurites from neuronal cells and is the first step in the development of the mature neuronal morphologies. Neurite growth and the establishment of the dendrite-axon polarity are major elements to be studied. They involve cytoskeleton and actin polymerization as well as diverse signaling mechanisms allowing the selection of axonal formation among dendrites. Growth factors and calcium play key roles on the speed of neurite outgrowth, neuron morphologies and neurite length and branching. The course will offer the students to study such mechanisms and compare different methods of neurite outgrowth quantification.

***Drosophila* olfactory learning:**

Drosophila flies present an olfactory sensory system allowing the recognition and discrimination of hundreds of discrete odorants. The perception of odorants is crucial for the animal to locate and choose mates, food sources, hosts and oviposition sites that are essential for their survival. Morpho-functional analyses and characterization of *drosophila* olfactory learning will be performed. The study will include an introduction of the nervous system of insects and some genetic concepts. The *drosophila melanogaster* model will be presented as an interesting model for studying olfaction and the role of dopamine in learning behaviors.

LOCATION : *EDUCATION CENTER - INSTITUT PASTEUR*

**28 rue du Docteur Roux
75724 PARIS CEDEX 15**

LECTURES: Room N° 5 Building n°6

PRACTICAL COURSES: 1st Floor Building N°9

1st PART: EVOLUTION AND DEVELOPMENT OF THE NERVOUS SYSTEM: Week 1

**DOCTORAL SCHOOL MODULE 1:
FROM SEPTEMBER 16TH TO SEPTEMBER 27TH, 2019**

MONDAY, SEPTEMBER 16

8:30 - 9:00	Reception of the students	Pierre-Marie LLEDO, Alain TREMBLEAU Isabelle CLOEZ-TAYARANI (Institut Pasteur & Sorbonne Université - Paris) Murielle ALMOUSSA & Catherine MENARD (Centre d'Enseignement, Institut Pasteur - Paris)
9:15 - 11:00	Functional architecture of nicotinic receptors and their Homologs – consequences for neuronal signaling	Pierre-Jean CORRINGER (Institut Pasteur & CNRS, Paris)
11:00 - 11:30	INTRODUCTION TO PRACTICAL COURSES	Isabelle CLOEZ-TAYARANI (Institut Pasteur - Paris)
13:00 - 13:30	SECURITY RULES	Murielle ALMOUSSA (Institut Pasteur - Paris)
13:30 - 19:00	PRACTICAL EXPERIMENTS: Module I : Brain dissection, culture of neurospheres Module II : Brain dissection, culture of neurons	Richard BELVINDRAH (Institut du fer à Moulin) Sandrine VITRY (Institut Pasteur- Paris)

TUESDAY, SEPTEMBER 17

9:15 - 11:15	Imaging infectious Diseases & Light Microscopy for Life Sciences	Jean-Yves TINEVEZ (Institut Pasteur - Paris)
11:15 - 12:00	INTRODUCTION TO MODULE III	Béatrice DURAND (Institut Curie - Orsay)
13:00 - 19:00	PRACTICAL EXPERIMENTS : Module III Egg preparation for mRNA injection and preparation of animal caps	Béatrice DURAND (Institut Curie - Orsay)
12:30 - 19:00	PRACTICAL EXPERIMENTS : MODULE IV -A Stereotaxic injection of 6-OHDA	Mariana ALONSO (Institut Pasteur & CNRS - Paris)

WEDNESDAY, SEPTEMBER 18

9:00 - 11:15	General principles of the development and evolution of the central nervous system in Chordates	Philippe VERNIER (CNRS - Gif sur Yvette)
13:00 -19:00	PRACTICAL EXPERIMENTS : Module III Egg preparation for mRNA injection and preparation of animal caps	Béatrice DURAND (Institut Curie - Orsay)
12:15 - 19:00	PRACTICAL EXPERIMENTS : MODULE IV -A Stereotaxic injection of 6-OHDA	Mariana ALONSO (Institut Pasteur & CNRS - Paris)

THURSDAY, SEPTEMBER 19

9:00 - 11:15 How to build brain maps

Alexandra REBSAM
(Institut du Fer à Moulin – Paris)

12:15 - 13:15 **INTRODUCTION TO FLUORESCENT MICROSCOPY AND
IMAGE ANALYSIS** (Room 5)

Sandrine VITRY
(Institut Pasteur - Paris)

13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE III**
Observations and analysis

Béatrice DURAND
(Institut Curie - Orsay)

13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE IV-A**
Stereotaxic injection of 6-OHDA

Mariana ALONSO
(Institut Pasteur & CNRS - Paris)

FRIDAY, SEPTEMBER 20

9:00 - 11:15 On the use and protection of animals in research
and education

Xavier MONTAGUTELLI
(Institut Pasteur - Paris)

13:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE I & II**
Neuron fixation / Adhesion of neurospheres

R. BELVINDRAH & S. VITRY
(Institut du fer à Moulin - Institut Pasteur- Paris)

Module I: Neural stem cell differentiation: Multipotentiality and influence of endogenous and exogenous factors on the fate of neural progenitors

Module II: How to quantify neurite outgrowth *in vitro*?

Module III: Tools to investigate early neural development in *Xenopus Leavis*

Module IV: Elimination of dopaminergic afferents in mice striatum: Anatomical and functional consequences (**Part A**)

1st PART: EVOLUTION AND DEVELOPMENT OF THE NERVOUS SYSTEM: Week 2

DOCTORAL SCHOOL MODULE 1: FROM SEPTEMBER 16TH TO SEPTEMBER 27TH, 2019

MONDAY, SEPTEMBER 23

- 9:00 - 11:15 Hearing – or how the cochlea does a hard job using soft parts **Pascal MARTIN**
(Institut Curie - Paris)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS : Module I & II** **R. BELVINDRAH & S. VITRY**
Immunostaining of NSCs and mouse cortical neurons (Institut du fer à Moulin - Institut Pasteur- Paris)

TUESDAY, SEPTEMBER 24

- 9:00 - 11:15 Molecular Genetic Dissection of Nicotine Addiction **Uwe MASKOS**
(Institut Pasteur & CNRS – Paris)
- 13:45 - 19:00 **PRACTICAL EXPERIMENTS : Module I** **R. BELVINDRAH**
Microscope acquisitions and quantification (1) (Institut du fer à Moulin - Paris)

WEDNESDAY, SEPTEMBER 25

- 9:00 - 11:15 Genes and Environment in cerebral development **Yehezkel BEN-ARI**
(INMED / INSERM U29 Marseille)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE II** **Sandrine VITRY**
Microscope analysis of labeled neurons and quantification (Institut Pasteur - Paris)

THURSDAY, SEPTEMBER 26

- 9:00 - 11:15 Brain under construction: Molecular managers and cellular boulders **Salvador MARTINEZ**
(Instituto Neurociencias Alicante - Spain)
- 14:00 - 18:00 **PRACTICAL EXPERIMENTS : MODULE V** **Salvador MARTINEZ**
Paraffin brain sections staining and analysis (Instituto Neurociencias Alicante - Spain)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE II** **Sandrine VITRY**
Microscope analysis of labeled neurons and quantification (Institut Pasteur – Paris)

FRIDAY, SEPTEMBER 27

- 9:00 - 11:15 Compared embryonic and adult neurogenesis: Two modes in Zebrafish **Laure BALLY-CUIF**
(Institut Pasteur – Paris)
- 14:00 - 18:00 **PRACTICAL EXPERIMENTS : MODULE V** **Salvador MARTINEZ**
Paraffin brain sections staining and analysis (end) (Instituto Neurociencias Alicante - Spain)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE II** **Sandrine VITRY**
Microscope analysis and quantification (end) (Institut Pasteur - Paris)

Module I : Neural stem cell differentiation Multipotentiality and influence of endogenous and exogenous factors on the fate of neural progenitors

Module II : How to quantify neurite outgrowth *in vitro*?

Module V : Understanding the brain anatomy in 3D

2nd PART: DEVELOPMENT, PLASTICITY AND PATHOLOGIES OF THE NERVOUS SYSTEM: Week 3

DOCTORAL SCHOOL MODULE 2: FROM SEPTEMBER 30th TO OCTOBER 11th, 2019

MONDAY, SEPTEMBER 30

- 9:00 - 11:15 **Genes, synapses, and autism** **Thomas BOURGERON**
(Institut Pasteur-Paris)
- 13:45 - 19:00 **PRACTICAL EXPERIMENTS : MODULE I**
Microscope acquisitions and quantification (2) **Richard BELVINDRAH**
(Institut du Fer à Moulin, Paris)

TUESDAY, OCTOBER 1

- 9:00 - 11:15 **The developing cortex and associated malformation** **Fiona FRANCIS**
(INSERM - Paris)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS: MODULE IV-B**
Behavioral analysis of 60HDA injected mice **Mariana ALONSO**
(Institut Pasteur- Paris)
- 13:00 - 19:00 **PRACTICAL EXPERIMENTS: MODULE VI**
Behavioral analysis of mice (+scopolamine treatment) **Stéphanie DAUMAS**
(Sorbonne Université - Paris)

WEDNESDAY, OCTOBER 2

- 9:00 - 11:15 **Glutamate receptor ion channels: Molecular operation** **Pierre PAOLETTI**
(Institut Pasteur - Paris)
- 11:45 - 13:30 **ROUND TABLE "STEM CELLS"**
Module 3 – Salle 5 – Building 6 (Lunch included) **Ana CUMANO, Sigolène MEILHAC**
B. DURAND, R. BELVINDRAH,
S. VITRY&DPNS Course organizers
(Sorbonne Université & Institut Pasteur - Paris)
- 13:30 - 19:00 **PRACTICAL EXPERIMENTS : Module IV-B**
Behavioral analysis of 60HDA injected mice **Mariana ALONSO**
(Institut Pasteur & CNRS - Paris)
- 13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE VI**
Behavioral analysis of mice (+scopolamine treatment) **Stéphanie DAUMAS/**
Mariana ALONSO
(Sorbonne Université & Institut Pasteur- Paris)

Institut Pasteur - Paris)

THURSDAY, OCTOBER 3

- 9:00 - 11:15 **EVOLUTION AND FUNCTION OF SENSORY SYSTEM - 1** **Stuart FIRENSTEIN**
(Columbia University – New York)
- 13:15 - 19:00 **PRACTICAL EXPERIMENTS: MODULE IV-B**
Data computerizing **Mariana ALONSO**
(Institut Pasteur & CNRS - Paris)
- 13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE VI**
Data computerizing **Stéphanie DAUMAS**
(Sorbonne Université - Paris)

FRIDAY, OCTOBER 4

9:00 - 11:15 **EVOLUTION AND FUNCTION OF SENSORY SYSTEM - II**

STUART FIRENSTEIN
(Columbia University, New York)

11:15 - 12:15 **Introduction to Module VII**

Caroline DUBACQ & Thomas CHERTEMPS
(Sorbonne Université - Paris)

13:15 - 19:00 **PRACTICAL EXPERIMENTS : Module IV - B**
Statistical analysis (Room 5)

Mariana ALONSO
(Institut Pasteur & CNRS - Paris)

13:15 - 19:00 **PRACTICAL EXPERIMENTS: MODULE VI**
Statistical analysis (Room 5)

Stéphanie DAUMAS
(Sorbonne Université - Paris)

Module I: Neural stem cell differentiation: Multipotentiality and influence of endogenous and exogenous factors on the fate of neural progenitors

Module IV: Elimination of dopaminergic afferents in mice striatum: Anatomical and functional consequences Lesions (**Part B**)

Module VI: Effect of cholinergic modulation of short-term memory

Module VII: Learning to fly: Morpho-functional and behavioral characterization of drosophila dopaminergic neurons in associative learning

2nd PART : DEVELOPMENT, PLASTICITY AND PATHOLOGIES OF THE NERVOUS SYSTEM : Week 4

DOCTORAL SCHOOL MODULE 2: FROM SEPTEMBER 30th TO OCTOBER 11th, 2019

MONDAY, OCTOBER 7

- 9:00 - 11:15 **The neurons of addiction** **Jean-Antoine GIRAULT**
(INSERM - Paris)
- 12:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE IV-C** **Marc DAVENNE & Giuseppe GANGAROSSA**
Mice perfusion - Vibratome sections (Sorbonne Université, Paris)

TUESDAY, OCTOBER 8

- 9:00 - 11:15 **The cerebellum: What are the majority of brain neurons good for?** **Clément LENA**
(Ecole Normale Supérieure - Paris)
- 12:30 - 13:00 **PRACTICAL EXPERIMENTS : MODULE VII** **CAROLINE DUBACQ & THOMAS CHERTEMPS**
Introduction (Room 5) (Sorbonne Université, Paris)
- 13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE IV-C** **Marc DAVENNE & Giuseppe GANGAROSSA**
Vibratome sections (Sorbonne Université, Paris)
- 14:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE VII** **Caroline DUBACQ & Thomas CHERTEMPS**
Dissection of Drosophila and microscopic analysis (Sorbonne Université Paris)

WEDNESDAY, OCTOBER 9

- 9:00 - 11:15 **Adult neurogenesis in the olfactory system: A recapitulation of embryogenesis?** **Pierre-Marie LLEDO**
(Institut Pasteur & CNRS - Paris)
- 11:15 - 11:30 **PRACTICAL EXPERIMENTS : MODULE IV-C** **Marc DAVENNE**
Immunohistochemistry (antibody incubation) (Sorbonne Université, Paris)
- 13:15 - 19:00 **PRACTICAL EXPERIMENTS : MODULE VII** **Caroline DUBACQ & Thomas CHERTEMPS**
Analysis of learning behavior (Sorbonne Université, Paris)
- 14:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE IV-C** **Marc DAVENNE**
Immunohistochemistry (Sorbonne Université, Paris)

THURSDAY, OCTOBER 10

- 9:00 - 11:15 **Axonal and dendritic local translation and its role in the development and plasticity of neural network** **Alain TREMBLEAU**
(Sorbonne Université, Paris)

11:15 Course Picture

- 14:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE IV-C** **Marc DAVENNE**
Microscope observations (Sorbonne Université, Paris)

14:00 - 19:00 **PRACTICAL EXPERIMENTS : MODULE VII**
Morpho-functional analysis - Conclusion

Caroline DUBACQ & Thomas CHERTEMPS
(Sorbonne Université, Paris)

FRIDAY, OCTOBER 11

9:00 - 11:15 Initial Events in the Development of
Olfactory Circuits

Charles GREER
(Yale University - New Haven)

13:15 - 14:00 FAREWELL PARTY

14:00 - 17:00 **PRACTICAL EXPERIMENTS : MODULE IV-C**
Conclusion

Marc DAVENNE
(Sorbonne Université, Paris)

Module IV: Elimination of dopaminergic afferents in mice striatum: Anatomical and functional consequences (**Part C**)

Module VII: **Learning to fly:** Morpho-functional and behavioral characterization of drosophila dopaminergic neurons in associative learning

Week 5

FROM OCTOBER 14 TO OCTOBER 17, 2019

MONDAY, OCTOBER 14

13:15 - 17:00 ORAL EXAMINATION (ARTICLE PRESENTATION)

TUESDAY, OCTOBER 15

OFF (Room 5 available for students)

WEDNESDAY, OCTOBER 16

13:15 - 17:00 PRACTICAL COURSE EXAMINATION

THURSDAY, OCTOBER 17

9:00 - 13:00 WRITTEN EXAMINATION

2:00 PM VISIT OF THE MUSEUM OF INSTITUT PASTEUR

DPNS Course Examinations

1) **Practical courses** – Daily evaluation of student participation during the practical courses (Head of Studies and all organizers of practical modules).

(20 points)

2) **Monday, October 14, (13:15-17:00)** - Oral presentation of a scientific article by pairs of students (Students will be provided with published articles at the end of the first week of the DPNS Course).

(20 points; 10-min presentation followed by 10-min discussion)

3) **Wednesday, October 16, (13:15-17:00 PM)** - Oral presentation of a subject related to the practical courses, by groups of 2 pairs of students (Subjects will be given to students on Oct. 11).

(20 points; 20-min presentation followed by 15-min discussion)

4) **Thursday, October 17, (9:00-13:00)** - Written examination.

Some questions may be related to selected topics from the conferences and/or practical courses. It is therefore very important to attend all conferences and assimilate them, as well as the practical courses.

(140 points)

Total : 200 points

Modalités d'examen et barème -

1) **Durant toute la période des TP** - Evaluation journalière individuelle de l'investissement dans les TP, de la qualité et du sérieux du travail (ces évaluations seront effectuées par les responsables des TP et par la Chef de Travaux).

(20 points)

2) **Lundi 14 octobre (13h15-17h00)** - Présentation orale d'un article, en binôme (l'article sera tiré au sort par les binômes à la fin de la première semaine du Cours).

(20 points ; 10 minutes de présentation et 15 minutes de discussion)

3) **Mercredi 16 octobre (13h15-17h00)** - Présentation orale d'une question relative aux TPs en groupe de 2 binômes (la question sera tirée au sort le dernier jour des TP, soit le 11 octobre).

(20 points ; 20 minutes de présentation et 15 minutes discussion)

4) **Jeudi 17 octobre (9h00-13h00)** - Examen écrit individuel, pouvant porter sur des questions de cours (d'où l'obligation de suivre tous les cours, avec le plus grand sérieux) et sur des analyses de résultats.

(140 points)

Total : 200 points