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 S.E. Abraham, M. Häusser & C. Schmidt-Heiber (University College London)
Parasagittal slice of mouse hippocampus.
Newly generated neurons are red (doublecortin), mature neurons are green (NeuN), and nuclei are blue (DAPI).

PROGRAM 2017-2018
PASTEUR COURSE

DEVELOPMENT & PLASTICITY OF THE NERVOUS SYSTEM

SEPTEMBER 18 - OCTOBER 19, 2017

LOCATION: EDUCATION CENTER AT THE INSTITUT PASTEUR

Pavillon Louis Martin
Building N°9
28 rue du Docteur Roux
75724 PARIS CEDEX 15

LECTURES: Room N° 2
PRACTICAL COURSES: 2nd Floor
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 resolutive 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 olfaction**
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. A morpho-functional and behavioral characterization of drosophila olfaction 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.
**1st Part: Evolution and Development of the Nervous System: Week 1**

**Doctoral School Module 1:**
**From September 18th to September 29th, 2017**

**Monday, September 18**

8:30 - 9:00 Reception of the students
Pierre-Marie Lledo, Alain Trembleau
Isabelle Cloez-Tayarani
*(Institut Pasteur - Paris)* & *(Université Pierre et Marie Curie - Paris)*
Murielle Almouss & Christine Vauthier
*(Centre d'Enseignement, Institut Pasteur - Paris)*

9:15 - 11:00 On the use and protection of animals in research and education
Xavier Montagutelli
*(Institut Pasteur - Paris)*

11:00 - 11:30 Introduction to Practical Courses
Isabelle Cloez-Tayarani
*(Institut Pasteur - Paris)*

13:00 - 13:30 Security Rules
Murielle Almouss
*(Institut Pasteur - Paris)*

13:30 - 19:30 Practical Experiments:
*Module I*: Brain dissection, culture of neurospheres
Richard Belvindrah
*(Institut du fer à Moulin)*
Sandrine Vitry
*(Institut Pasteur - Paris)*

**Tuesday, September 19**

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 III
Paraffin brain sections and staining
Salvador Martinez
*(Instituto Neurociencias Alicante - Spain)*

12:00 - 19:00 Practical Experiments: Module IV - A
Stereotaxic injection of 6-OHDA
Mariana Alonso
*(Institut Pasteur - Paris)*

**Wednesday, September 20**

9:00 - 11:15 General principles of the development and evolution of the central nervous system in Chordates
Philippe Vernier
*(CNRS - Gif sur Yvette)*

14:00 - 19:00 Practical Experiments: Module III
Signal analysis
Salvador Martinez
*(Instituto Neurociencias Alicante - Spain)*

12:00 - 19:00 Practical Experiments: Module IV - A
Stereotaxic injection of 6-OHDA
Mariana Alonso
*(Institut Pasteur - Paris)*
**THURSDAY, SEPTEMBER 21**

9:00 - 11:15  Imaging Infectious Diseases & Light Microscopy for life-Sciences  
Jean-Yves TINEVEZ  
(CITech, Imagopole Institut Pasteur - Paris)

13:00 - 14:00  **Introduction to Fluorescent Microscopy and Image Analysis** (Room 2)  
Sandrine VITRY  
(Institut Pasteur - Paris)

14:00 - 19:00  **Practical Experiments: Module IV-A**  
Stereotaxic injection of 6-OHDA  
Mariana ALONSO  
(Institut Pasteur - Paris)

**FRIDAY, SEPTEMBER 22**

9:00 - 11:15  Genes and Environment in cerebral development  
Yehezkel BEN-ARI  
(INMED / INSERM U29 Marseille)

13:00 - 19:00  **Practical Experiments: Module I & II**  
Adhesion of neurospheres / Neuron fixation (45')  
R. BELVINDRAH & S. VITRY  
(Institut du fer à Moulin - Paris) (Institut Pasteur - Paris)

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**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:** Understanding the brain anatomy in 3D

**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 18th to September 29th, 2017**

**Monday, September 25**

**9:00 - 11:15** Compared embryonic and adult neurogenesis:
Two modes in zebrafish
Laure BALLY-CUIF
(Institut Pasteur - Paris)

**13:00 - 19:00** Practical Experiments: Modules I & II
Immunostaining of NSCs and mouse cortical neurons
R. BELVINDRAH & S. VITRY
(Institut du fer à Moulin) (Institut Pasteur - Paris)

**Tuesday, September 26**

**9:00 - 11:15** The developing cortex and associated malformation
Fiona FRANCIS
(INSERM - Paris)

**13:00 - 19:00** Practical Experiments: Module I
Microscope acquisitions and quantification
Richard BELVINDRAH
(Institut du fer à Moulin)

**Wednesday, September 27**

**9:00 - 11:15** Adult neurogenesis in the olfactory system:
A recapitulation of embryogenesis?
Pierre-Marie LLEDO
(Institut Pasteur - Paris)

**11:15 - 12:15** Introduction to Module V
Béatrice DURAND
(Institut Curie - Orsay)

**13:00 - 19:00** Practical Experiments: Module II
Microscope analysis of labeled neurons and quantification
Sandrine VITRY
(Institut Pasteur - Paris)

**13:00 - 19:00** Practical Experiments: Module V
Egg preparation for mRNA injection and preparation of animal caps
Béatrice DURAND
(Institut Curie - Orsay)

**Thursday, September 28**

**9:00 - 11:15** Molecular-genetic dissection of nicotine addiction
Uwe MASKOS
(Institut Pasteur - Paris)

**13:00 - 19:00** Practical Experiments: Module II
Microscope analysis of labeled neurons and quantification
Sandrine VITRY
(Institut Pasteur - Paris)

**13:00 - 19:00** Practical Experiments: Module V
Egg preparation for mRNA injection and preparation of animal caps
Béatrice DURAND
(Institut Curie - Orsay)
FRIYDAY, SEPTEMBER 29

9:00 - 11:15 Functional architecture of nicotinic receptors and their homologs – consequences in neuronal signaling
   Pierre-Jean CORRINGER
   (Institut Pasteur - Paris)

13:00 - 19:00 PRACTICAL EXPERIMENTS: MODULE V
   Observations and analysis
   Béatrice DURAND
   (Institut Curie - Orsay)

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: Tools to investigate early neural development in Xenopus Leavis
### 2nd Part: Development, Plasticity and Pathologies of the Nervous System: Week 3

**Doctoral School Module 2:**
**From October 2nd to October 13th, 2017**

#### Monday, October 2

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>9:00 - 11:15</td>
<td>The neurons of addiction,</td>
<td>Jean-Antoine GIRAULT (Inserm - Paris)</td>
</tr>
<tr>
<td>13:45 - 19:00</td>
<td><strong>Practical Experiments: Module I</strong>&lt;br&gt;Microscope acquisitions and quantification</td>
<td>Richard BELVINDRAH (Institut du fer à Moulin)</td>
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#### Tuesday, October 3

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<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>9:00 - 11:15</td>
<td>How to built brain maps</td>
<td>Patricia GASPAR (Institut du Fer à Moulin - Paris)</td>
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<tr>
<td>13:00 - 19:00</td>
<td><strong>Practical Experiments: Module IV-B</strong>&lt;br&gt;Behavioral analysis of 60HDA injected mice</td>
<td>Mariana ALONSO (Institut Pasteur - Paris)</td>
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<td>13:00 - 19:00</td>
<td><strong>Practical Experiments: Module VI</strong>&lt;br&gt;Behavioral analysis of mice (+scopolamine treatment)</td>
<td>Maria Eugenia VILARCHAO MARTINEZ Mariana ALONSO (Institut Pasteur - Paris)</td>
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#### Wednesday, October 4

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>9:00 - 11:15</td>
<td>Hearing – or how the cochlea does a hard job using soft parts</td>
<td>Jonathan ASHMORE (University College - London)</td>
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<td>11:45 - 12:30</td>
<td><strong>Round Table “STEM CELLS”</strong>&lt;br&gt;(Lunch included)</td>
<td>Shahragim TAJBAKHSH, Pauline SPEDER Sigolène MEILHAC, Béatrice DURAND, and DPNS Course organizers (Université Pierre et Marie Curie - Paris) (Institut Pasteur - Paris)</td>
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<tr>
<td>13:30 - 19:00</td>
<td><strong>Practical Experiments: Module IV-B</strong>&lt;br&gt;Behavioral analysis of 60HDA injected mice</td>
<td>Mariana ALONSO (Institut Pasteur- Paris)</td>
</tr>
<tr>
<td>13:30 - 19:00</td>
<td><strong>Practical Experiments: Module VI</strong>&lt;br&gt;Behavioral analysis of mice (+scopolamine treatment)</td>
<td>Maria Eugenia VILARCHAO MARTINEZ Mariana ALONSO (Institut Pasteur - Paris)</td>
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THURSDAY, OCTOBER 5

9:00 - 11:15 Evolution and function of Sensory System - I  
Stuart FIRESTEIN  
(Columbia University, New York)

13:15 - 19:00 PRACTICAL EXPERIMENTS: MODULE IV-B  
Data computerizing  
Mariana ALONSO  
(Institut Pasteur- Paris)

13:15 - 19:00 PRACTICAL EXPERIMENTS: MODULE VI  
Data computerizing  
Maria Eugenia VILARCHAO MARTINEZ  
(Institut Pasteur- Paris)

FRIDAY, OCTOBER 6

9:00 - 11:15 Evolution and Function of Sensory Systems - II  
Stuart FIRESTEIN  
(Columbia University - New-York)

11:15 - 12:15 Introduction to Module VII  
Caroline DUBACQ & Thomas CHERTEMPS  
(UMPC, Paris)

13:15 - 19:00 PRACTICAL EXPERIMENTS: Module IV - B  
Statistical analysis  
Mariana ALONSO  
(Institut Pasteur- Paris)

13:15 - 19:00 PRACTICAL EXPERIMENTS: Module VI  
Statistical analysis  
Stéphanie DAUMAS  
(UPMC- Paris)

Maria Eugenia VILARCHAO MARTINEZ  
(Institut Pasteur- Paris)

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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  
(Part B)

Module VI: Effect of cholinergic modulation of short-term memory
Module VII: Fly Olfaction: Morpho-functional and behavioral characterization of drosophila olfaction

2nd Part: Development, Plasticity and Pathologies of the Nervous System: Week 4

Doctoral School Module 2:
From October 9th to October 13th, 2017

Monday, October 9

9:00 - 11:15 Genes, synapses, and autism
Thomas BOURGERON

12:00 - 19:00 Practical Experiments: Module IV-C
Marc DAVENNE & Giuseppe GANGAROSSA
Mice perfusion - Vibratome sections
(UMPC, Paris) & (Collège de France, Paris)

Tuesday, October 10

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
Introduction
CAROLINE DUBACQ & THOMAS CHERTEMPS
(UMPC, Paris)

13:15 - 19:00 Practical Experiments: Module IV-C
Marc DAVENNE & Giuseppe GANGAROSSA
Vibratome sections - Immunohistochemistry
(UMPC, Paris) & (Collège de France, Paris)

14:00 - 19:00 Practical Experiments: Module VII
Caroline DUBACQ & Thomas CHERTEMPS
Dissection, microscopic analysis and exposure to odorants
(UMPC, Paris)

Wednesday, October 11

9:00 - 11:15 Glutamate receptor ion channels: molecular operation
Pierre PAOLETTI
(Ecole Normale Supérieure - Paris)

11:15 - 11:30 Practical Experiments: Module IV-C
Marc DAVENNE
Immunohistochemistry (antibody incubation)
(UMPC, Paris)

14:00 - 19:00 Practical Experiments: Module IV-C
Marc DAVENNE
Immunohistochemistry
(UMPC, Paris)

13:15 - 19:00 Practical Experiments: Module VII
Caroline DUBACQ & Thomas CHERTEMPS
Olfactory behavior in larvae
(UMPC, Paris)
THURSDAY, OCTOBER 12

9:00 - 11:15 Axonal and dendritic local translation and its role in the development and plasticity of neural network

Alain TREMBLEAU
Université Pierre & Marie Curie, Paris)

14:00 - 19:00 PRACTICAL EXPERIMENTS : MODULE IV-C
Microscope observations

Marc DAVENNE
(UMPC, Paris)

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

Caroline DUBACQ & Thomas CHERTEMPS
(UMPC, Paris)

FRIDAY, OCTOBER 13

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
(UMPC, Paris)

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

Module VII: Fly Olfaction : Morpho-functional and behavioral characterization of drosophila olfaction
Week 5

From October 16 to October 19, 2017

Monday, October 16

13:15 - 17:00 Oral Examination (Article Presentation)

Tuesday, October 17

Off (Room 2 available for students)

Wednesday, October 18

13:15 - 17:00 Practical Course Examination

Thursday, October 19

9:00 - 13:00 Written Examination
**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 16, (1:15-5:00 PM)** - 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 18, (1:15-5:00 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. 13).
   (20 points ; 20-min presentation followed by 15-min discussion)

4) **Thursday, October 19, (9:00-1:00 PM)** - 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 16 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 18 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 13 octobre).
   (20 points ; 20 minutes de présentation et 15 minutes discussion)

4) **Jeudi 19 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