**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** *Roles of cellular interactions within neural stem cell lineages during niche morphogenesis and lineage progression***.**

**Keywords:** Neural stem cells; lineage; niche; glia; communication; morphogenesis; *Drosophila*

**Department:** Developmental and Stem Cell Biology

**Name of the lab:** Brain plasticity in response to the environment

**Head of the lab:** Pauline Spéder-Murphy

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***Doctoral school affiliation and University*:** Complexité du vivant, ED515 **-** Université Pierre et Marie Curie

Presentation of the laboratory and its research topics:

Our team is a young and highly motivated group that is interested in understanding how the brain senses and reacts to a changing environment. We are particularly investigating the dynamics and roles of interactions between neural stem cells and their niche under various challenges. We are using the power and versatility of *Drosophila* genetics, combined with advanced microscopy techniques and state-of-the-art transcriptional profiling to tackle these questions. More details on the team members can be find on our website : [http://www.speder-lab.com](http://www.speder-lab.com/).

The Institut Pasteur in Paris is a world-wide renowned research centre, where you will find excellence both in science and techniques. The Department of Developmental and Stem Cell Biology is a dynamic and stimulating environment, that will provide you with a diversity of topics. You will also be part of the Revive LabEx consortium and the *Stem cells in vivo* network, which aim to bring stem cell researchers together.

Description of the project:

Neural stem cells (NSCs) are multipotent progenitors in charge of neurogenesis throughout life. They must regulate their proliferative capacities to parallel neurogenic requirements, ultimately deciding on the number and identity of the new cells, and thus on brain functions12.

Crucially, NSCs inhabit a tailored and complex cellular microenvironment, the niche3. This information-exchanging hub3–5 is known to influence neurogenesis5. To do so, the niche must be able to adapt its architecture to NSC needs and allow successful integration of newborn neurons. However the mechanisms supporting the interactions between niche architecture and NSC lineages are poorly characterised.

We propose to use *Drosophila* as a complete *in vivo* model, with unparalleled genetics, to investigate this question. The *Drosophila* post-embryonic, larval brain contains well-characterised NSCs sharing core features with mammalian NSCs6, and which are found in a genuine, multilayered NSC niche 78,9.

Notably a specific type of glia, the cortex glia, is able to integrate local, systemic and NSC-derived signals to extend membranes which will ultimately enclose each individual NSC lineage. Such architecture is in turn essential for sustaining neurogenesis by promoting survival of newborn neurons (*Spéder and Brand, in revision*).

The PhD project aims to understand i) how individual lineages stay contained within one cortex glia chamber over time and ii) why keeping a lineage together is required. We propose that adhesion and communication mechanisms within the lineages are essential. The project will be divided as follows.

1. Analysis of NSC lineage organisation and communication within a cortex glia chamber

The student will use advanced genetic tools10,11 to determine the extent and dynamics of cellular contacts between NSC, neuronal progeny and cortex glia during chamber life. These patterns will be matched with localisation and expression profiles of selected adhesion and communication proteins (cadherins, integrins, intercellular bridges, gap junctions). This will identify players involved in cellular contacts.

In addition, the student will describe the architecture of lineage progression within a chamber, staining for differentiation and neuronal markers (transcription factors, neurotransmitter, axonal pattern).

1. Impact of cellular contacts on NSC lineages

The student will then choose one or two players and determine how their knockdowns affect NSC lineage organisation and function. Interestingly, a candidate-based approach performed in the lab has already identified a gap junction protein, Inx2, as required within NSC lineages for proper brain development.

The PhD student will have to identify the resulting cellular phenotype (pattern of cellular contacts and adhesions, lineage organisation, fate determination, axonal pairing).

1. Impact of cortex glia architecture on NSC lineages

The student will also assess the extrinsic influence of cortex glia architecture on NSC lineage organisation. She/he will assess the consequence of altering chamber formation on these cellular contacts and adhesion patterns, as well as on lineage organisation and fate determination. In particular, the student will use a multicolour clonal approach12 to track the localisation of individual lineages while staining for adhesion and communication players (1).

1. Interplay between lineage organisation and cortex glia chamber

Strikingly, disrupting *inx2* function either in NSC or neurons also lead to incomplete chamber formation. To better understand this non-autonomous phenotype, the student will determine the temporal window, the cellular and molecular interactors and the cellular features of cortex glia (morphology, division, calcium flux through live-imaging).

Depending on these findings, the transcriptional profiles of cortex glia, NSC or neurons under *inx2* knockdown will be determined using a technique well-mastered in the lab13, as a way to identify underlying mechanisms.

All together these approaches cover a range of practical and theoretical skills, and will provide a comprehensive view of the reciprocal interplay between niche architecture, and NSC lineage architecture and functions.

References:

1. Swartling, F. J., Čančer, M., Frantz, A., Weishaupt, H. & Persson, A. I. Deregulated proliferation and differentiation in brain tumors. *Cell Tissue Res.* **359,** 225–54 (2015).

2. Ernst, C. Proliferation and Differentiation Deficits are a Major Convergence Point for Neurodevelopmental Disorders. *Trends Neurosci.* **39,** 290–9 (2016).

3. Bjornsson, C. S., Apostolopoulou, M., Tian, Y. & Temple, S. It Takes a Village: Constructing the Neurogenic Niche. *Dev. Cell* **32,** 435–446 (2015).

4. Bond, A. M., Ming, G. L. & Song, H. Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. *Cell Stem Cell* **17,** 385–395 (2015).

5. Silva-Vargas, V., Crouch, E. E. & Doetsch, F. Adult neural stem cells and their niche: A dynamic duo during homeostasis, regeneration, and aging. *Curr. Opin. Neurobiol.* **23,** 935–942 (2013).

6. Homem, C. C. F. & Knoblich, J. a. Drosophila neuroblasts: a model for stem cell biology. *Development* **139,** 4297–310 (2012).

7. Freeman, M. R. Drosophila Central Nervous System Glia. *Cold Spring Harb. Perspect. Biol.* **7,** (2015).

8. Hindle, S. J. & Bainton, R. J. Barrier mechanisms in the Drosophila blood-brain barrier. *Front. Neurosci.* **8,** 414 (2014).

9. Spéder, P. & Brand, A. H. Gap Junction Proteins in the Blood-Brain Barrier Control Nutrient-Dependent Reactivation of Drosophila Neural Stem Cells. *Dev. Cell* 309–321 (2014). doi:10.1016/j.devcel.2014.05.021

10. Feinberg, E. H. *et al.* GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. *Neuron* **57,** 353–63 (2008).

11. He, L., Huang, J. & Perrimon, N. Development of an optimized synthetic Notch receptor as an in vivo cell–cell contact sensor. *Proc. Natl. Acad. Sci.* **114,** 5467–5472 (2017).

12. Kanca, O., Caussinus, E., Denes, A. S., Percival-Smith, A. & Affolter, M. Raeppli: a whole-tissue labeling tool for live imaging of Drosophila development. *Development* **141,** 472–480 (2014).

13. Southall, T. D. *et al.* Cell-type-specific profiling of gene expression and chromatin binding without cell isolation: assaying RNA Pol II occupancy in neural stem cells. *Dev. Cell* **26,** 101–12 (2013).

Expected profile of the candidate (optional):

• Strong interest in basic, fundamental science using model organisms

• Strong academic records

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