Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2018-2019

1. **Host team :**
   Research Unit (e.g. Department or Institute) : Neuroscience Paris Seine
   Research Unit Director : Hervé Chneiweiss
   Research Team Director : Salah El Mestikawy/Stéphanie Daumas
   Team name : Glutamatergic transmission in normal and pathological conditions

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2. Internship project title:

Heterogeneity of synaptic vesicles : Involvement of acetylcholine – glutamate co-transmission in the regulation of reward behavior and addiction.

3. Internship Description :

Context :

Addiction is a devastating disorder linked to alterations in striatal circuits. To tailor treatments against addiction, it is necessary to fully understand striatal circuits. Although dopamine (DA) plays an important role in addiction, striatal activity is exquisitely coordinated by striatal cholinergic interneurons (SCI). SCIs use acetylcholine (ACh) and glutamate (Glu) as neurotransmitters[1]. ACh-Glu co-transmission by SCIs provides a sophisticated level of regulation and opens a new avenue to understand addiction. The balance between secreted ACh and Glu depends on the activity of the vesicular acetylcholine transporter (VAChT) and the vesicular glutamate transporter (VGLUT3)[2,3].

In rewarding conditions SCIs fire tonically (~2HZ), burst (~20HZ) or pause. How these different firing rates regulate secretion of ACh or Glu is critical to understand addiction. Our overarching hypothesis supported by preliminary data suggests that SCIs specific firing patterns differentially release Glu or ACh. This hypothesis is supported by preliminary data obtained in the laboratory using super resolution STED microscopy showing that VAChT and VGLUT3 are expressed into segregated SV subpopulations and thus that ACh and Glu are released by distinct synaptic vesicles (SV) in a same axonal varicosity. These results change our vision of the functional oragnization of SV. They suggest an unexpected anatomical and functional heterogeneous organization of SV in cholinergic axonal varicosities.

Project :

The aim of this internship will be to analyze SV anatomical heterogeneity using complementary new super-resolution microscopy approaches (STED, STORM, PALM) to :

1. Confirm preliminary data showing that VAChT and VGLUT3 are expressed into segregated SV subpopulations.

2. Caracterize these subpopulations with regard to other vesicular and synaptic proteins, including SNARE proteins involved in neurotransmitter release.
3. Determine how modifications of VGLUT3 and VAChT expression in mutant animals modulate subcellular distribution of these transporters [4].

For that, the student will have to develop super-resolution microscopic approaches, especially « *STimulated Emission Depletion* » (STED) and « *Stochastic optical reconstruction microscopy* » (STORM). STED, by visualizing unique SV in 2D, will allow to identify sub-populations of SV expressing different pannels of SV proteins. STORM will allow to visualize these sub-populations and their anatomical relationships with an increased resolution and in 3D. These two approaches, still little used microscopies in Neurozciences, are very powerfull tools to address new questions of cell biology at the level of the unique SV.

Perspectives :

This work shoulp deeply change our vision of the ways of neurotransmitter release. It may also help to better understand the patho-physiology of addiction.

References :

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2. Gras, C. *et al.* The vesicular glutamate transporter VGLUT3 synergizes striatal acetylcholine tone. *Nat Neurosci* **11,** 292–300 (2008).
3. Guzman, M. S. *et al.* Elimination of the vesicular acetylcholine transporter in the striatum reveals regulation of behaviour by cholinergic-glutamatergic co-transmission. *PLoS Biol.* **9,** e1001194 (2011).
4. Janickova H., Prado V.F., Prado M.A.M., El Mestikawy S. and Bernard V. Vesicular Acetylcholine Transporter (VAChT) overexpression induces major modifications of striatal cholinergic interneuron morphology and function. J. Neurochem. 142, 857–875 (2017)