Title of the PhD project:

Acetylcholine/Glutamate co-transmission in the striatal network. Anatomical and functional heterogeneity of synaptic vesicles in cholinergic interneurons.

PhD Supervisor

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Title of the team : Neuropharmacology of VGLUTs
Team leader (*if different*) : Nicolas PIETRANCOSTA et Stéphanie DAUMAS
Doctoral School : ED3C – ED 158

Overview of the scientific projects of the team

The Neuropharmacology of VGLUT's team works at the interface between pharmaco-chemistry and neuroscience. The team was a pioneer in the discovery of the vesicular glutamate transporter (VGLUT1-3). In particular we found that cholinergic, serotoninergic and GABAergic neurons express VGLUT3 and therefore communicate with two neurotransmitters. Our research focuses on deciphering the role of acetylcholine or serotonin or GABA and glutamate-cotransmission in normal physiological conditions and in psychiatric disorders. This project will be co-supervised by two leaders in their field. Véronique Bernard has a 20-year experience in the analysis of neurotransmitter receptors, enzymes and vesicular transporters subcellular distribution and trafficking using high resolution microscopies (super resolution and electron microscopies). Nicolas Pietrancosta is an expert in molecules to target VGLUTs. He developed pharmacological tools to target the VGLUTs. NP has a strong expertise in managing Chemical/Biology interface projects. The team developed strong collaborations with laboratories in France and abroad and with imaging platforms and companies.

Main publications since January 1er, 2016

1. Mansouri-Guilani N., **Bernard V.**, Vigneault E., Vialou V., Daumas S., El Mestikawy S. and Gangarossa G. (2019) VGLUT3 gates psychomotor effects induced by amphetamine. J. Neurochem, **148**(6):779-795. doi: 10.1111/jnc.14644.
2. Lambert L., Dubayle D., Fafouri A., Herzog E., Csaba Z., Dournaud P., El Mestikawy S., **Bernard V.** (2018) Endocytosis of activated muscarinic m2 receptor (m2R) in live mouse hippocampal neurons occurs via a clathrin-dependent pathway. Frontiers Cellular Neuroscience (2018), **12**:450, doi: 10.3389/fncel.2018.00450

3. Janickova H., Prado V.F., Prado M.A.M., El Mestikawy S. and **Bernard V.** (2017) Vesicular Acetylcholine Transporter (VAChT) overexpression induces major modifications of striatal cholinergic interneuron morphology and function. J. Neurochem. **142,** 857–875, doi: 10.1111/jnc.14105.
4. Ramet L., Zimmermann J., Bersot T., Poirel O., De Gois S., Silm K., Sakae D.Y., Mansouri-Guilani, N., Bourque M.J., Trudeau L.E., Pietrancosta N., Daumas S., **Bernard V.,** Rosenmund, C. El Mestikawy S. (2017) Characterization of a human point mutation of VGLUT3 (p.A211V) in the rodent brain suggests a non-uniform distribution of the transporter in synaptic vesicles J. Neurosci. **37**(15): 4181-4199. doi: 10.1111/jnc.14105.
5. Chao S., Krejci E., **Bernard V**., Leroy J., Jean L. and Renard P.-Y. (2016) Selective and sensitive near-infrared fluorescent probe for acetylcholinesterase imaging. Chem. Commun., **52**, 11599-11602. doi: 10.1039/c6cc05936h.
6. Autres publications : ORCID : 0000-0003-4748-5167

PhD Co-Supervisor

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Laboratory : umr7203 Laboratoire des Biomolécules
Title of the team : Analyse, Interactions Moléculaires et Cellulaires
Team leader *(if different) : F. Burlina*
Doctoral School : ED406-Chimie Moléculaire

Overview of the scientific projects of the team

Our team is multidisciplinary with expertise in biology, chemistry and physics. We are interested in developing biophysical and chemical biology tools to understand the functions of biomolecules and their interactions in various biological processes. Our projects focus on:- The fundamental understanding of functional biomolecules, such as cell-penetrating peptides,-the design of photolabelling and bioorthogonal ligation-based approaches to study post-translational modifications, - and original biophysical setups to measure biomolecular interactions of charged molecules through membrane;- The invention of new fluorescent biosensors and chemical-genetic tools to observe and study dynamic biochemical events in live cells and tissues with high spatial and temporal resolution.

Main publications since January 1er, 2016

1. Poirel, O.; Mamer, L. E.; Herman, M. A.; Arnulf-Kempcke, M.; Kervern, M.; Potier, B.; Miot, S.; Wang, J.; Favre-Besse, F.-C.; Brabet, I.; Laras, Y.; Bertrand, H.-O.; Acher, F.; Pin, J.-P.; Puel, J.-L.; Giros, B.; Epelbaum, J.; Rosenmund, C.; Dutar, P.; Daumas, S.; El Mestikawy, S.*; **Pietrancosta, N.*** LSP5-2157 a new inhibitor of vesicular glutamate transporters. *Neuropharmacology* 2020, **164**, 107902. ***Co last-authors.**
2. **Pietrancosta, N.*;** Djibo, M.; Daumas, S.; El Mestikawy, S.*; Erickson, J. D.* Molecular, Structural, Functional, and Pharmacological Sites for Vesicular Glutamate Transporter Regulation. *Mol Neurobiol* 2020, **57**, 3118-3142. ***Co last-authors.**
3. Bachollet, S.; Addi, C.; **Pietrancosta, N.;** Mallet, J. M.; Dumat, B. Fluorogenic Protein Probes with Red and Near-Infrared Emission for Genetically Targeted Imaging. *Chemistry* 2020, Volume26, **63**, 14467-14473.

4. Ashraf, U.; Tengo, L.; Le Corre, L.; Fournier, G.; Busca, P.; McCarthy, A. A.; Rameix-Welti, M. A.; Gravier-Pelletier, C.; Ruigrok, R. W. H.; Jacob, Y.; Vidalain, P. O.; **Pietrancosta, N.;*** Crepin, T.; Naffakh, N. Destabilization of the human RED-SMU1 splicing complex as a basis for host-directed antiinfluenza strategy. *Proc Natl Acad Sci U S A* 2019, **116**, 10968-10977. ***Co last-authors.**

5. Smith, N.; **Pietrancosta, N**.*; Davidson, S.; Dutrieux, J.; Chauveau, L.; Cutolo, P.; Dy, M.; Scott-Algara, D.; Manoury, B.; Zirafi, O.; McCort-Tranchepain, I.; Durroux, T.; Bachelerie, F.; Schwartz, O.; Munch, J.; Wack, A.; Nisole, S.; Herbeuval, J. P.* Natural amines inhibit activation of human plasmacytoid dendritic cells through CXCR4 engagement. *Nat Commun* 2017, **8**, 14253. ***Co last-authors.**

Doctoral Project

Title: Acetylcholine/Glutamate co-transmission in the striatal network. Anatomical and functional heterogeneity of synaptic vesicles in cholinergic interneurons.

Abstract:

Striatal activity is regulated by cholinergic interneurons (CINs) that use acetylcholine (ACh) and glutamate (Glu) as neurotransmitters. The ACh/Glu cotransmission depends on the action of the vesicular transporters of ACh (VAChT) and Glu (VGLUT3) in axonal varicosities of CINs. Our hypothesis is that CINs varicosities contain three pools of SVs expressing either VAChT, VGLUT3 or both releasing ACh, Glu or both.

We will explore this vesicular heterogeneity and its functional implications using a multidisciplinary approach combining super resolution microscopy, pharmacological tools targeting VGLUTs and optogenetics stimulation coupled to measures of ACh/Glu release to :

- **Characterize the heterogeneity of SVs in CINs axonal varicosities** using high resolution microscopy to detect organelles with a resolution compatible with visualization of unique SVs (super resolution microscopy and electron microscopy)
- **Develop new chemical tools to locate VGLUT with an increased spatial resolution.** We will continue to develop small size fluorescent sensors to detect VGLUT3.
- **Analyze the impact of various discharge profiles of CINs on ACh or Glu release** coupling optogenetics to stimulate CINs and fluorescent ACh & Glu sensors to evaluate ACh & Glu release.

Context and objective:

Context

GABAergic efferent neurons are the main striatal cell population. Cholinergic interneurons (CINs) (1% of striatal neurons) form an extensively local ramified network. CINs use glutamate (Glu) in addition to acetylcholine (ACh) to regulate striatal homeostasis. The ACh/Glu balance depends on the action of the vesicular transporters of ACh (VAChT) and Glu (VGLUT3) in axonal varicosities of CINs. ACh/Glu cotransmission provides thus CINs with a sophisticated level of regulation of local circuits.

Vesicular synergy, a process where VGLUT3 potentiates ACh uptake into synaptic vesicles (SVs), suggests *the presence of VAChT & VGLUT3 on the same SV*[1]. Alternatively, our super resolution microscopy results and other data, suggest *separate pools of cholinergic and glutamatergic SVs* (Fig.1A,B). **Our hypothesis is that CINs varicosities contain *three pools of SVs expressing either VAChT, VGLUT3 or both and release ACh, Glu or both* (Fig.1C).**

This project will be co-supervised by Véronique Bernard (NPS, UMR8246) for the morpho-fonctional part and Nicolas Pietrancosta (LBM, UMR7203) for the neurochemical aspect.

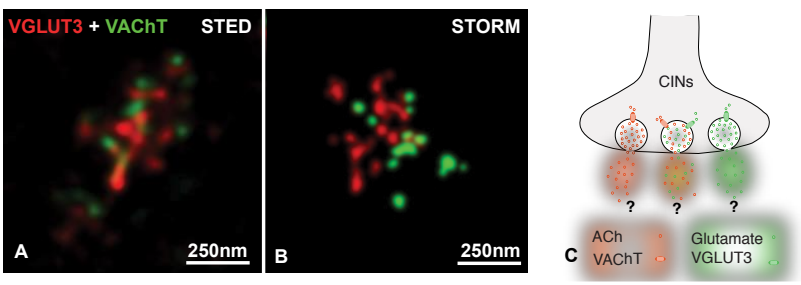

Figure 1. VAChT and VGLUT3 immuno-detection in CINs varicosities using super-resolution microscopy (A,B) and putative model of SV heterogeneity (B).

Objectives

The PhD student will develop a multidisciplinary approach combining a **morpho-functional** approach: super-resolution microscopy, optogenetics coupled with measures of ACh and Glu release and a **neurochemical** approach: molecular modelling (interaction between fluorophores and 3D model of VGLUTs) with analysis of the spectroscopic properties of the identified compounds to study their metabolic stability.

Our proposal will be developed along three axes:

1. Morphological analysis of the heterogeneity of SVs in CINs axonal varicosities (Supervised by V. Bernard, NPS)

The first part of the project aims at analyzing the anatomical heterogeneity of the SVs of CINs. To that end, super-resolution microscopic approaches (STED, STORM) will be used, allowing the detection of subcellular organelles with a resolution compatible with the visualization of single SVs.
We will confirm and enlarge preliminary data suggesting that VAChT and VGLUT3 are largely expressed by separate SVs and that ACh and Glu can therefore be released by separate SVs independently (Fig.1A). We will also determine whether a third subpopulation of SVs may express both VAChT and VGLUT3 and thus release ACh and Glu.
These experiments will allow a first complete description of the heterogeneity of the SVs of CINs.

2. Development of new neuro-pharmacological tools to detect and localize VGLUT in living cells with an increased spatial resolution (Supervised by N. Pietrancosta, LBM)

Our preliminary experiments involved the use of anti-VAChT/VGLUT3 antibodies. However, the size of the complex of primary/secondary/fluorochrome may increase the distance between fluorochromes and vesicular transporters, making difficult the interpretation of images. To circumvent this limitation, we will develop and characterize new pharmacological tools with limited steric bulk and high selectivity. These will enable us to accurately analyze the distribution of VAChT and VGLUT3 in our various preparations.
In a long-standing collaboration between both teams, Nicolas Pietrancosta has developed about a hundred of "LEAD" VGLUT fluorescent ligands (VFLs)[2–5]. Some of them are extremely small and naturally fluorescent. Therefore, these compounds provide a real gain of resolution in the VGLUT's detection. We have shown that some of these compounds recognize VGLUT3 in BON cell cultures (Fig.2).

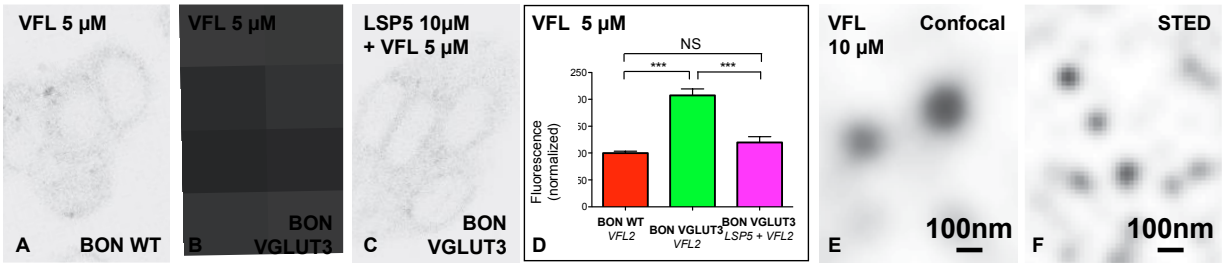

Figure 2. Fluorescent detection of VGLUTs with VFLs by confocal and STED microscopies in live BON cells and isolated SVs. *Untransfected live BON cell (A) or BON cells stably expressing VGLUT3 (B,C) incubated with VFL alone (B) or after preincubation with LSP5, a VGLUT specific inhibitor[5] (C). (D) Quantification of VFL labeling. Confocal microscopy reveals a VFL-dependent specific fluorescence of intracellular vesicles in BON VGLUT3 cells. This labeling was blocked by LSP5. Isolated SVs incubated with VFL observed under a confocal (E) or STED (F) microscope.*

This part of the project is sub-divided into 5 sub-parts :
2.1. Characterization of the physico-chemical properties of the molecules (absorption/emission wavelengths, quantum yield) to select the best compounds for studies on *in vitro* models.
2.2. In order to enrich the pharmacology of VFLs, we will perform a virtual screening on a 3D model of VGLUTs already generated in the laboratory.
2.3. Validation of the specificity and stability of these molecules in BON cells expressing different VGLUT subtypes, primary neuronal cultures or striatum slices.
2.4. Development of ligands compatible with super-resolution microscopy. In collaboration with the organic synthesis chemists associated with the project, the selected molecules will be evaluated and modified to make these compounds super-resolution-compatible.
2.5. We will combine the pharmacological approach to detect VGLUT3 and the immunohistochemical approach to identify VAChT or other molecules in order to analyze the different sub-populations of SVs.
The pharmacological approach coupled with super-resolution microscopy will provide essential information for the anatomical characterization of the mode of release of ACh and Glu at the SV level.

3. Analysis of the impact of different CINs discharge profiles on the release of ACh or Glu (V. Bernard with S. El Mestikawy, NPS)

The existence of at least two sub-populations of SVs containing ACh or Glu represents an anatomical substrate for differential release of these two neurotransmitters depending on the electrical activity of the CINs. A recent work has shown that the release of ACh or Glu in the interpeduncular nucleus results from different discharge frequencies of these neurons[6]. What about striatal CINs?

To answer this question, we will use an optogenetic approach to specifically stimulate CINs with variable stimulation durations and frequencies and measure ACh and/or Glu release using ACh fluorescent sensors (GACh 4.3)[7] and/or VFLs (Fig.3).

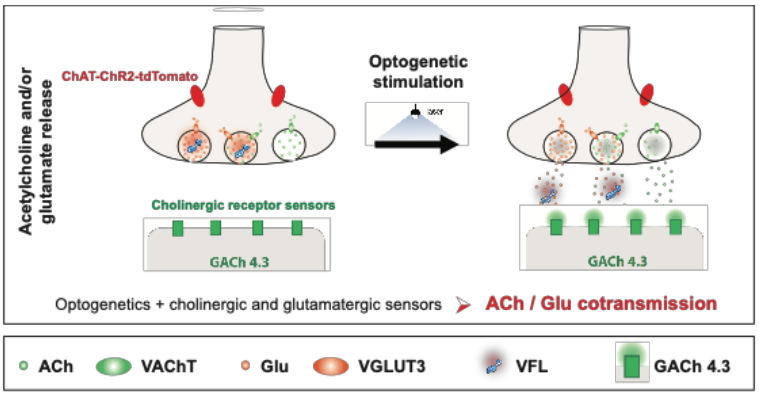

Figure 3. Analysis of the impact of different CINs discharge profiles on the release of ACh and/or Glu.

This will allow us to track *ex vivo* the fate of SVs and their content and determine whether ACh and Glu are released differentially by CINs according to their discharge pattern.

Conclusion

This project will provide novel and key information on the anatomical organization of the protein complex involved in the release of ACh and Glu by CINs. It will ultimately lead to deep changes in our understanding of neurotransmitter release and for the treatment of pathologies involving dysfunctions of striatal cholinergic transmission such as Parkinson's disease or addiction.

References

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12. Lambert, L. *et al.* doi: 10.3389/fncel.2018.00450
13. Dobbertin A. *et al.* doi: 10.1523/JNEUROSCI.3863-08.2009
14. Janickova H. *et al.* doi:10.1111/jnc.14105.
15. Bernard, V. *et al.* Déclaration of invention (2019-33706FR) : Development of Fluorescent Ligand specifically targeting VGLUTs.
16. Bernard, V., Chausset-Boissarie, L., L., Cristofari, P., Djibo, M. & Pietrancosta, N. Declaration of invention (2020 DI 08116) :Development of Fluorescent Ligand specifically targeting VGLUTs for use at super resolution microscopic level.
17. Bernard, V. *et al.* Patent in preparation : Fluorescent Ligand specifically targeting VGLUTs for super resolution imaging.

Justification of suitability for *i-Bio*:

Our PhD proposal falls entirely within the framework of the i-Bio project that aims to promote interdisciplinary research at the interface between Biology and other disciplines; here chemistry. The PhD student will be co-supervised by a neurobiologist and a chemist and will have access to two laboratories and platforms fully equipped for the accomplishment of his/her project.

Role of each supervisor / skills provided:

This project will be co-supervised by two leaders in their field : Véronique Bernard (**VB**, NPS, UMR8246, ORCID : 0000-0003-4748-5167) and Nicolas Pietrancosta (**NP**, LBM, UMR7203, ORCID : 0000-0002-9934-7818).

V. Bernard has long-standing expertise in the study of subcellular localization and trafficking of key molecules of cholinergic and glutamatergic neurotransmission[8–14]. In recent years, she has developed super-resolution microscopy applied to the study of the localization of vesicular proteins, in particular vesicular transporters. VB will supervise the morpho-functional part of this project.

N. Pietrancosta is an expert in medicinal chemistry applied to neuroscience for 10 years[2–5] . has produced a chemical library of fluorescent ligands targeting VGLUTs. Four of these ligands have already been validated in cell cultures, brain slices purified synaptic vesicles and can therefore be used for the planned studies. NP will supervise the neurochemical part of this project.

VB and NP have been developing a fruitful collaboration for four years[15–17].

Profile of the desired student:

The ideal candidate should be highly interested in the interface between neuroscience and chemistry. He/she should have a strong background in neuroscience but also an interest in chemistry in order to interact with chemists who will conceive and synthetize VGLUT ligands. His/her tasks as a PhD student will include high resolution microscopies, detailed analysis and quantification of neuronal tissue images, development and characterization of new VFLs and functional analyses using optogenetics coupled with ACh and Glu release. He/she should have a critical approach to scientific literature. Other selection criteria are to be able to work both in a research team as well as carry out personal research projects.