Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral :

Role of acetylcholine/glutamate co-transmission in the regulation of the striatal neural network. Anatomical and functional heterogeneity of synaptic vesicles in cholinergic interneurons.

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

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Unité de Recherche :
Intitulé : Neuroscience Paris Seine
Code *(ex. UMR xxxx)* : UMR8246

ED158-Cerveau, cognition, comportem

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant :

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 0

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Unité de Recherche :
Intitulé : Laboratoire des Biomolécules
Code *(ex. UMR xxxx)* : UMR7203

ED406-Chimie Moléculaire Paris Centre
Ecole Doctorale de rattachement : Ou si ED non Alliance SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1 (première inscription en 2019, 100%)

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences

Doctoral Program i-Bio 2020 : Life Sciences and Interfaces

Role of acetylcholine/glutamate co-transmission in the regulation of the striatal neural network. Anatomical and functional heterogeneity of synaptic vesicles in cholinergic interneurons.

Co-supervision: Véronique Bernard (UMR8246, ED158) and Nicolas Pietrancosta (UMR7203, ED406)

Summary

The striatal network is regulated by cholinergic interneurons (CINs) that express the vesicular transporters for acetylcholine (VAChT) and glutamate (VGLUT3). Therefore, CINs signal with both acetylcholine (ACh) and glutamate (Glu). We recently showed that VAChT and VGLUT3 may be targeted to distinct synaptic vesicles. This finding suggests that CINs may differentially release ACh and Glu. In the present proposal, we will combine the use of super-resolution microscopy, new fluorescent ligands and optogenetics coupled to electrophysiology to : 1. Characterize the heterogeneity of synaptic vesicles in cholinergic varicosities, 2. Develop new pharmacological tools to label VGLUTs, 3. Analyze the electrophysiological consequences of ACh/Glu co-transmission. Our project will provide a deeper understanding of the striatal functions and will open the door to improve treatment of striatal disorders.

Background

The striatum plays a central role in the regulation of locomotor activity and reward behavior[1]. Dysfunctions of the striatum lead to a wide range of neurological or psychiatric disorders such as Parkinson's disease and addiction[1]. Striatal cholinergic interneurons (CINs) play a key role in the regulation of striatal activity and in particular in reward circuits. They use glutamate (Glu), in addition to acetylcholine (ACh), to regulate striatal homeostasis[2,3]. The ACh/Glu balance depends on the activity of their respective vesicular transporters (VAChT and VGLUT3) in axonal varicosities[2,4]. We have recently shown that ACh and Glu play opposite roles in the regulation of reward behaviors[5]. The co-transmission of ACh/Glu by CINs thus represents a sophisticated level of regulation of striatal circuitry and provides a novel basis for understanding striatal pathology. In rewarding conditions, CINs burst in prediction of reward and pause during dopaminergic bursting [6]. However, whether these different modes of firing differentially trigger acetylcholine or glutamate secretion is not elucidated yet.

Our main working hypothesis is that there would be a differential release of ACh or Glu that would depend on the discharge profiles of CINs. This hypothesis is supported by recent work showing that cholinergic neurons of the interpeduncular nucleus, co-expressing VGLUT1 and VAChT, differentially release Glu and ACh according to their discharge frequency[7]. Moreover, our preliminary results in STimulated Emission Depletion (STED) and STochastic Optical Reconstruction Microscopy (STORM) super-resolution microscopy suggest that VGLUT3 and VAChT would be partially present on distinct SVs (Fig. 1), which would be consistent with differential release of the two neurotransmitters by CINs. These surprising results change our view of the organization of SVs, suggesting an unsuspected anatomical and functional heterogeneity of SVs in cholinergic axonal varicosities.

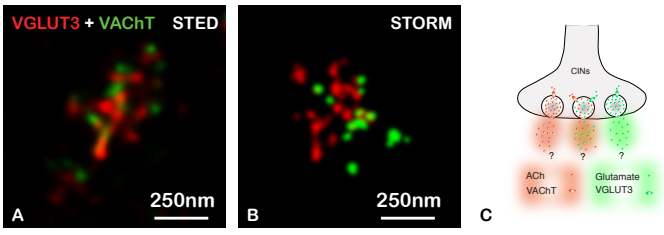

Figure 1: Detection of VAChT and VGLUT3 in striatal cholinergic varicosities. STED (A) and STORM (B) microscopies allow the visualization of highly resolved spots that could correspond to SVs. The VGLUT3- and VAChT-positive spots do not overlap, suggesting that cholinergic and glutamatergic SVs are distinct. (c) Different hypotheses suggested. V. Bernard (unpublished).

Objectives

1. **Characterization of SV heterogeneity in cholinergic striatal axonal varicosities and its functional consequences.**
2. **Development of new neuro-pharmacological tools to detect and localize VGLUT in living cells with increased spatial resolution.**
3. **Analysis of the impact of different CINs discharge profiles on the release of ACh or Glu.**

We will develop for this purpose a multidisciplinary approach combining 1) a **morpho-functional** approach: super-resolution microscopy, optogenetics coupled with electrophysiology in mice and 2) a **neurochemical** approach: Molecular modelling (interaction between fluorophores and 3D model of VGLUTs / Analysis of the spectroscopic properties of the identified compounds and study of their metabolic stability.

This work will be co-supervised: for the morpho-functional part, by Véronique Bernard (IBPS, NPS, UMR8246), a specialist in the study of the distribution and trafficking of neuroactive molecules using high-resolution approaches (electron and super-resolution microscopy) and for the neurochemical aspect, by Nicolas Pietrancosta (LBM, UMR7203), an expert in neuropharmacology and the development of specific molecules of VGLUTs.

1. **Characterization of the heterogeneity of SVs in "cholinergic" striatal axonal varicosities and its functional consequences. (Work carried out at IBPS under the direction of Dr V. Bernard)**

The first part of the project aims at analyzing the anatomical heterogeneity of the SVs of CINs. To that end, different super-resolution microscopy approaches (STED, STORM) will be used, allowing the detection of subcellular elements with a resolution compatible with the visualization of single SVs. These experiments will be conducted on mouse striatum sections, isolated SVs or cultures of neurons[8,9].

This part of the project is divided into two sub-parts :

1.1 We will confirm 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. **1.2** We will investigate whether SVs expressing VAChT or VGLUT3 are associated with a specific panel of proteins involved in neurotransmitter release, such as SNARE proteins. This should provide us with key information about the mode of release of ACh or Glu.

These experiments should allow a first complete description of the heterogeneity of the SVs of CINs.

2. **Characterization of new neuro-pharmacological tools to locate and detect VGLUTs with increased spatial resolution. (Work carried out at the LBM under the direction of Dr. N. Pietrancosta)**

Our preliminary experiments as well as the first part of the project involve the use of anti-VAChT or anti-VGLUT3 antibodies. However, the size of the complex of primary and secondary antibodies coupled to the fluorochrome may increase the distance between fluorochromes and vesicular transporters, making difficult the interpretation of our images. To circumvent this limitation, we will develop and characterize new tools with limited steric bulk. These pharmacological markers will allow us to more accurately analyze the distribution of VAChT and VGLUT3 in our various preparations.

In a long-standing collaboration between the two teams, Nicolas Pietrancosta has developed about a hundred of "LEAD" ligands targeting the VGLUT's family[10-13], some of which are naturally fluorescent and extremely small compared to the primary-secondary antibody-fluorochrome complex. These compounds therefore allow a real gain of resolution in the detection of VGLUTs. We have shown that some of these compounds recognize VGLUT3 in BON cell cultures (Fig. 2).

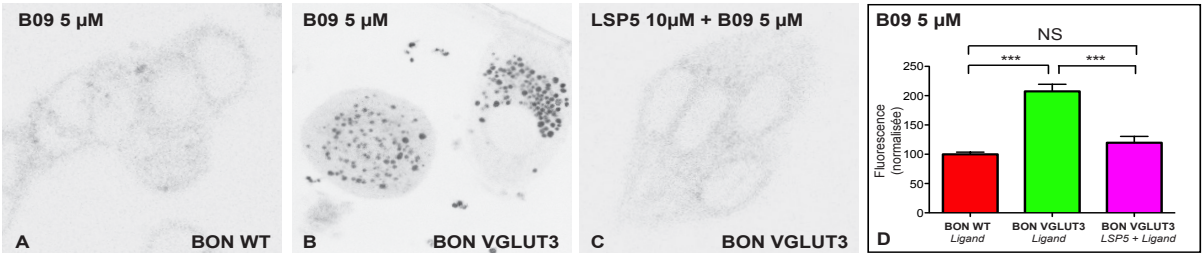

Figure 2: Labeling of VGLUT3 with a fluorescent ligand. Living BON control cells (A) and stably expressing VGLUT3 (B) were incubated with the ligand (B09 5µM; 1h at 37°C). B: Confocal microscopy revealed fluorescent labeling specifically in VGLUT3-expressing BON cells. C ,D: This labelling is abolished when cells are previously incubated with the VGLUT inhibitor LSP5 (V. Bernard and N. Pietrancosta, unpublished).

This part of the project is subdivided into 5 sub-parts :

2.1. Characterization of the physico-chemical properties of the molecules (absorption and emission wavelengths, quantum yield) to select the best compounds for further studies on in vitro models.

2.2. In order to enrich the pharmacology of fluorophores targeting VGLUTs, we will perform a virtual screening on a 3D model of VGLUTs already generated in the laboratory but still requiring adjustments.

2.3. We will validate the specificity and stability of these molecules in BON cell cultures with dense granules[2] expressing the different VGLUT subtypes, primary neuron cultures or striatum slices. We will determine whether these compounds are ligands or substrates (false fluorescent neurotransmitters) and evaluate their stability by mass spectrometry.

2.4. Development of ligands compatible with super-resolution microscopy: A proof of concept has recently been established for the use of such compounds as fluorescent markers of vesicular transporters in neurons under STED microscopy[14]. 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 in an iterative manner.

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.

The development and characterization of these ligands requires to test their use and compatibility with different techniques, under normal and/or pathological conditions. This part of the project will therefore involve a collaboration with Manon Guille-Collignon's team (ENS, chemistry department) which will consist in determining the electroactive properties of the compounds in order to measure the release of fluorescent ligands by amperometry. This team has already carried out this kind of work on monoaminergic false fluorescent neurotransmitters.[15]

3. Analysis of the impact of different CINs discharge profiles on the release of ACh or Glu. (Work carried out at the NPS under the direction of Dr. V. Bernard)

The existence of 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. Cholinergic neurons exhibit a complex discharge pattern. Recent work has shown that the release of ACh or Glu in the interpeduncular nucleus results from different discharge frequencies of these neurons[7]. What about striatal CINs?

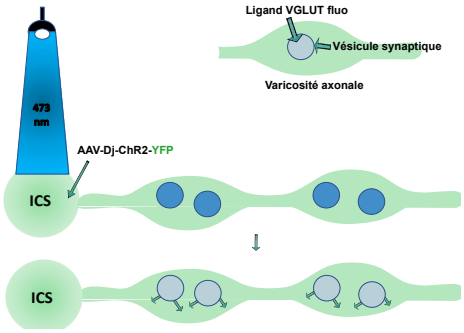

Figure 3: Measurement of the intensity of the fluorescent signal after optogenetic stimulation of CINs. The decrease in intensity would reflect the release of the false fluorescent substrate from the SVs.

To answer this question, we will use an optogenetic approach. We will inject an AAV expressing the rhodopsin channel receptor (ChR2) coupled to YFP into the striatum of CHAT-IRES-CRE mice (Fig. 3). We will specifically stimulate CINs using variable stimulation durations and frequencies. We will measure changes in the intensity of the fluorescent signal, which will reflect the release of glutamate using the fluorescent ligands of VGLUT[11]. On the other hand, in collaboration with Dr. El Mestikawy's team in Montreal, we will measure the release of ACh by measuring cholinergic currents in CINs target neurons, as well as using fluorescent ACh sensors[16].

This part of the project will allow us to track ex vivo the fate of SVs and their content[16,17] 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 profound changes in our understanding of neurotransmitter release. It could have important consequences for a better understanding and for the treatment of pathologies involving dysfunctions of striatal cholinergic transmission such as Parkinson's disease or addiction.

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