NENS Exchange Grant Report

An Ultrastructural investigation of neuromodulatory cholinergic axons.

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Duration of stay
01.01.2019 - 30.06.2019

1 Aim

Acetylcholine has been shown to affect VIP+ interneurons with a specificity and temporal resolution presumably too high for pure volume transmission, although synapses of ChAT+ axons onto cortical GABAergic interneurons have not been shown yet. We hypothesize that the cholinergic axons projecting from the BF are making synapse-like connections onto the GABAergic interneurons of the barrel cortex. We will focus on L2/3 VIP+ and PV+ cells. To assess our hypothesis, we will use two transgenic mouse lines where EYFP and td-Tomato mark the ChAT+ axons and VIP+ or PV+ cells respectively. These structures are imaged simultaneously, allowing for interaction studies. To understand these connections, we will use correlated light and electron microscopy (CLEM) where regions of interest imaged in fluorescence microscope are assessed at a ultrastructural level via electron microscopy and later reconstructed to 3D models. Besides characterizing these reconstructed neuronal structures, this technique will allow us to investigate the connectivity of cholinergic axons onto GABAergic interneurons at nanoscale resolution.

2 Overview

The neurotransmitter acetylcholine has wide-ranging effects on brain functions, by modulating and shaping neuronal activity, synaptic transmission and plasticity. The main source of cholinergic axons in the cortex is the basal forebrain, whose neurons project widely across the brain. However, despite physiological evidence that suggests a direct effect of acetylcholine on the interneurons that inhibit pyramidal neurons, there is no structural evidence of these specific synaptic contacts. To address this question and characterize the nature of cholinergic contacts onto interneurons, we used a correlative light and electron microscopy technique to target two types of inhibitory cell types: vasoactive intestinal peptide (VIP) and parvalbumin (PV) expressing interneurons, and investigated their interactions with cholinergic axons. We reconstructed and characterized the VIP- and PV-expressing cells, analysing their synaptic inputs and possible connections with cholinergic axons. Results show that dendritic spines are absent from PV+ neurons with only few spines on the VIP+ cell, although both had similar numbers of excitatory inputs on the dendritic shaft. Regarding the cholinergic connectivity with these cell types, axons appear to be closely apposed to the VIP+ and PV+ interneurons, particularly at their somata. However, there is limited evidence of typical synaptic connections with clear features that would allow us to classify these sites as synapses. Nevertheless, the abundance of vesicles and contacts with the membranes could represent regions of possible communication. This finding suggests that the connectivity between the cholinergic neurons and these interneurons in the cerebral cortex may not appear as typical synaptic connections, raising questions about how the connectivity of neuromodulatory axons should be characterized.

3 Figures

Here are some of the data I obtained during my staying at EPFL.

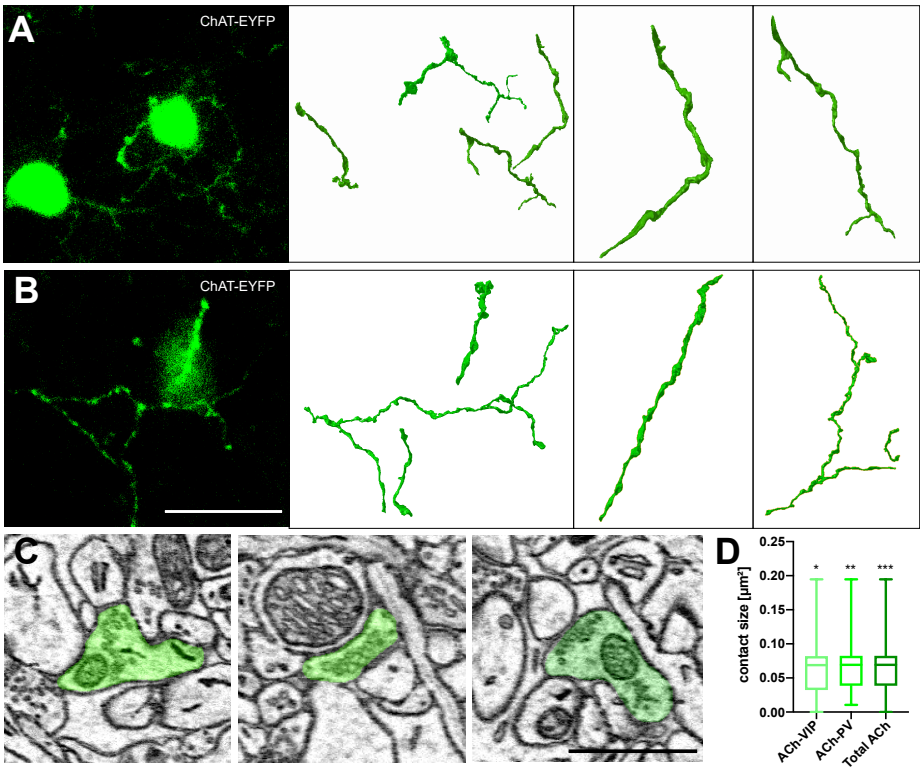

Figure 1: ChAT-expressing axons. A and B. Representative images of ChAT-positive axons showing their fluorescent images and the EM reconstructions. C. Representative EM images of cholinergic synaptic-like appositions. D. A quantification of the surface of the synaptic-like structures found along the cholinergic axons. They tend to follow a normal distribution.

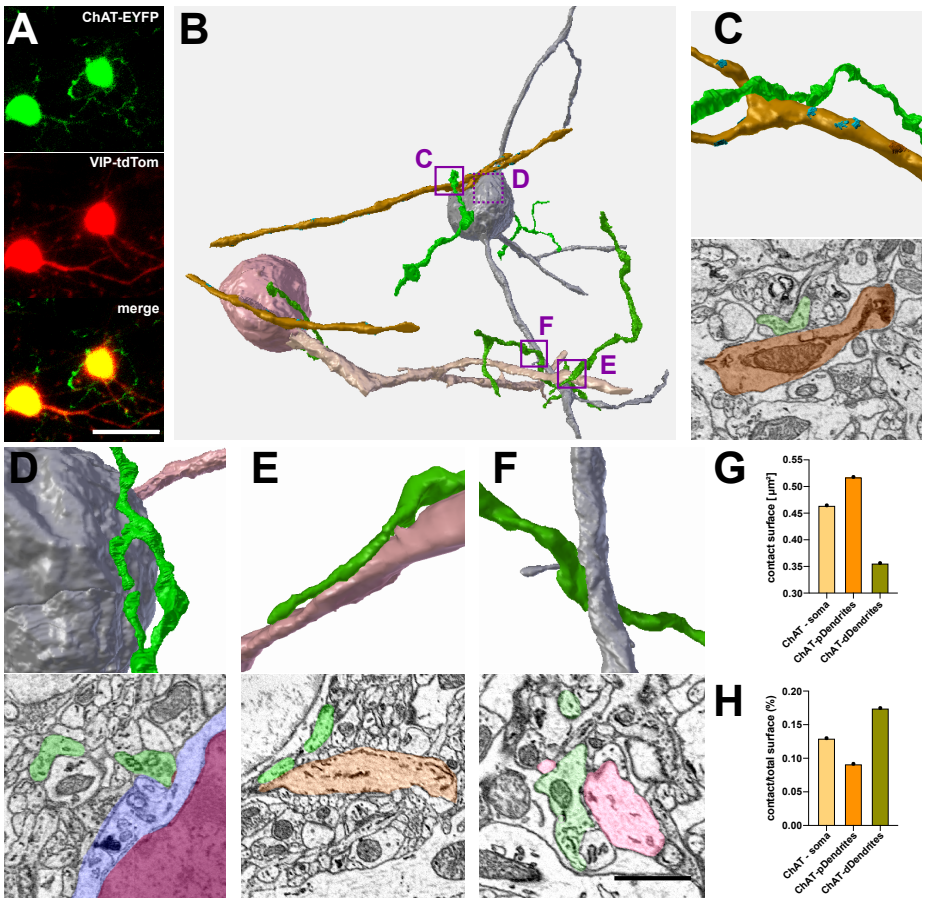

Figure 2: VIP-positive interneurons and acetyl cholinergic axons interaction. (A) ChAT+ axons (top), VIP+ neurons (mid) and merged confocal 40x images (bottom). Scale bar = 20 µm. (B) 3D EM reconstructions of VIP+ neurons and surrounding ChAT+ axons seen in A. Boxes and letters indicate contact locations shown in C-F. C-F 3D reconstructions and EM micrographs of contacts between ChAT+ axons and VIP+ proximal dendrites (E and F), distal dendrites (C) and soma (D). (G and H). Quantification of ChAT-VIP interaction in different regions of the neuron, as measured in total contact surface (G) and relative contact surface (H) normalized against total axonal surface.

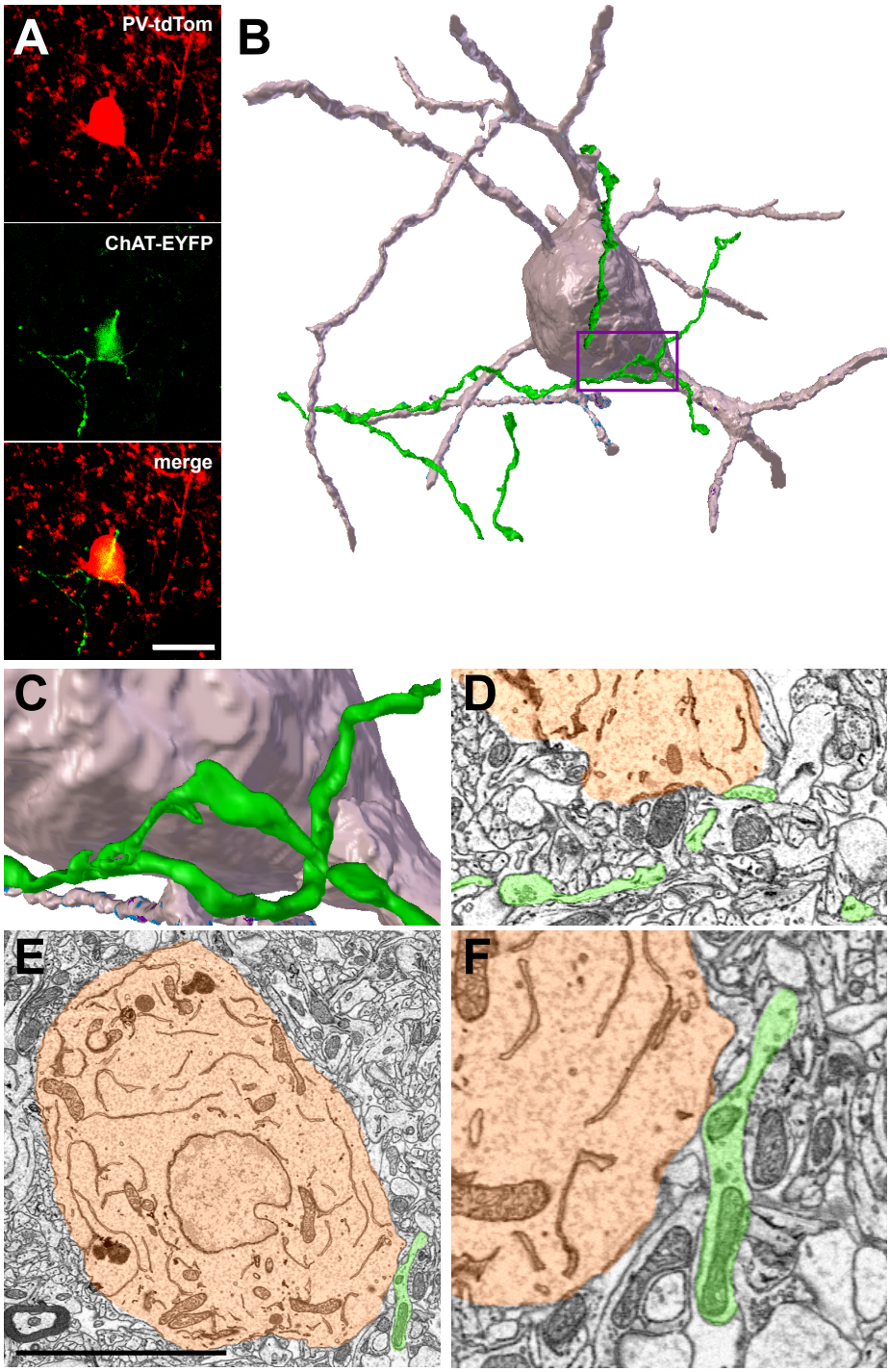

Figure 3: PV-positive interneuron and cholinergic axon interactions. (A) Representative fluorescent images of a PV+ cell (top), an adjacent ChAT+ axon (mid) and merged channels (bottom).4 Scale bar = 20 μm. (B) 3D EM reconstruction of the imaged PV+ cell and the ChAT+ axon in close proximity, indicated by the box. (C-F) 3D reconstruction and EM micro of ChAT+/PV+ contacts. Scale bar = 5 μm.

4 Methodology and experience

During my stay at EPFL, I worked on correlative light and electron microscopy (CLEM), a very challenging method. The project was mainly conducted by myself, making me more independent and organized. I learned how to organize the day-to-day experiments and how to analyze and present the data. Moreover, the used CLEM technique requires high accuracy as we are not using any laser marks (NIRB) to identify the region of interest but only natural landmarks such as blood vessels and cell bodies. The skills I obtained during this project will be very useful to me in my future career. It was very important to me to work so closely with the people that developed this technique and learn directly from them. I enjoyed a lot discussing with Graham, his office door was always opened, and I would just knock and ask him questions directly. Everyone in the lab was very helpful and I really liked the relaxed group environment especially the 10 am coffee breaks. Thanks to NENS, I not only had the chance to work with one of world-leading electron microscopist but to join the EPFL scientific life and live in Lausanne. EPFL is one of the most exciting places in Europe for science, and everyday there were conferences or seminars going on in the whole campus. On the other side, Lausanne presents breath-taking views from each corner making the whole experience even more valuable.

Figure 4: A photo of me and the lab people in the ultra-microtome room. From left to right: Maria Croisier, Daniela Doda, Graham Knott, Jerome Blanc, Stine Hasselholt, Stephanie Rosset.