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Laboratorio congiunto internazionale - Scienze Biomediche

Denominazione Laboratorio: Inter-neuronal transfer of clostridial neurotoxins and tau: cell specificity and relevance for brain disorders

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Nominativi dei Responsabili: Matteo Caleo (University of Padua, CNR Pisa associated) and Laura Restani (CNR Pisa) - Giampietro Schiavo (UCL, Londra)

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In the third year of the project “Joint Lab”, the two labs were able to exploit at maximum the relative expertise, and the results lead to a productive research studies, important for basic science as well as for pre-clinical knowledge, presented at International conferences. The data obtained from this grant pave the way to subsequent new research projects for the following years.

In the following section, we reported the summary of the last results achieved.

A) Activity-dependent transcytosis of Tau in central neurons and in vivo

In tauopathies such as Alzheimer’s disease (AD), tau aggregates are known to propagate across functionally connected neuronal networks, but the mechanisms underlying this process are poorly understood.

During the third year of the project we focused on the trafficking and released of Tau, both the “normal” and the “pathological” variant of Tau were analyzed, taking advantage of the experiments and results obtained on clostridial neurotoxins in the first two years



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of the project. The close collaboration we have with the lab of Prof. Schiavo (UCL, London), allow us to test an interesting hypothesis. Indeed, several lines of evidence support the hypothesis that Tau release is dependent on neuronal activity, since pathological tau can be found inside synaptic vesicles, and other synaptic compartments. These phenomena are strictly close to mechanisms underpinning spreading of clostridial neurotoxins and functional consequences. Thus, during this third year we collaborated with Prof. Schiavo's group to test in vitro and in vivo the effect of selected botulinum neurotoxins (BoNTs) and tetanus neurotoxin (TeNT) on the release of tau from synaptic terminals.

BoNTs and TeNT enter synapses, where they cleave different synaptic SNARE proteins, thus impairing synaptic vesicle fusion and neurotransmitter release. Different clostridial neurotoxins allow us to determine whether the inhibition of synaptic vesicle release is effective in limiting tau spread, both in neuronal cultures in microfluidic chambers (MFCs) and in brain tissue. Schiavo's lab prepared all the viral construct containing split GFP and tau variants (as planned in the project presented) and set up an in vitro system to preliminary test the modulatory effects of botulinum neurotoxins on the spread of pathological tau. Primary neurons were cultured in the central part of three-compartment MFCs and transduced with lentiviruses expressing human tau (hTau) isoforms. Cells were then treated with BoNTs in the lateral chambers and stimulating agents in the central compartment. The content of exogenous Tau (hTau) in the culture media was quantified with an enzyme-linked immunosorbent assay (ELISA). In the in vitro assay, our collaborators found out that botulinum neurotoxin type A (BoNT/A) can decrease the release of the 1N4R mutant hTau (P301S), but not the wild-type form. Moreover, neuronal stimulation significantly increases the release of P301S hTau, while-wild type hTau is not strongly affected. TeNT apparently increases hTau release, particularly of P301S hTau.

Regarding in vivo experiments, all performed in the Pisa lab, we injected Adeno-associated viral vectors (AAVs) expressing hTau isoforms in the eye's vitreous of mice,



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and following one month the superior colliculus (SC) and lateral geniculate nucleus (LGN) areas were analysed through immunohistochemistry and western blotting. The AAV constructs were correctly expressed in retinal ganglion cells (RGCs) and positive terminals were found in brain sections of SC and LG. In a following set of experiments, we delivered BoNT/A in the SC of mice previously injected with the AAV constructs in the eye, in order to validate the results obtained in neuronal cultures and determine the mechanisms of Tau release by neurons using BoNTs as tools. Analyses of brain tissues are currently on-going.

The real, close and long-lasting collaboration between our Lab and Schiavo's Lab allow us to highlight how clostridial neurotoxins represent a powerful tool for the study of neurons and synapses. Using BoNT/A, we showed that hTau release is modulated by specific SNARE complex components and differs depending on the isoform. The use of other BoNT types in both in vitro and in vivo models will help us to identify the SNARE proteins involved in tau release. This approach will provide novel insights on the mechanisms controlling tau release from synaptic terminals and identify novel molecular targets for the development of therapeutic interventions to treat tauopathies.

Moreover, the results obtained during these years, pave the way to new future projects. Indeed, our findings may have implications not only for AD, but also for other neurodegenerative diseases, such as amyotrophic lateral sclerosis, prion disease and Parkinson's disease, whose pathomechanism involves the neuron-to-neuron spread of toxic proteins.

B) Cortical rewiring following peripheral injection of clostridial neurotoxins

In the third year of the project we focused on structural and functional consequences of BoNT type A and TeNT central action. Both toxins are able to block neurotransmission, but BoNT/A, given its main local action, it is wide use in human therapy for treating



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neurological conditions characterized by neuronal hyperactivity. Recently, fMRI studies on human patients with dystonia have indeed demonstrated wide and long-lasting changes in the cortical activity after BoNT/A injection, at timescales that are not compatible with the peripheral blockade at the level of the neuromuscular junction (NMJ). Thus, we performed a set of follow-up experiments aimed to investigate if the toxins were able to spread across two subsequent synapses via retrograde transport following peripheral injection and to affect cortical areas. We injected the toxins in the WP of mice and then we decided to look at the structural plasticity of the cortical areas, e.g. motor areas. To visualize dendritic spines, we injected BoNT or TeNT in Thy1-GFP mice, expressing GFP in layer V pyramidal neurons. Ex vivo dendritic spine analysis revealed a striking decrease in spine density in cortical motor areas 30 days after BoNT/A injection, while whisker paralysis lasted only around 10 days. Moreover, we observed an increase in stubby spines, known to be an immature spine type that could either be new or in the process of being eliminated. To understand the mechanism underlying spine loss, we then measured spine dynamics longitudinally in awake mice using two-photon microscopy. Imaging of apical dendrites in the motor cortex before and after BoNT/A injection revealed a decrease in spine density already 15 days after the peripheral insult, confirming our ex vivo data. Moreover, we observed an increase in spine elimination at day 15 after BoNT/A injection. TeNT peripheral injection does not produce similar changes, thus spine plasticity is not due simply to the peripheral whisker paralysis.

Overall, our data reveal profound morphological changes in cortical neurons after intramuscular BoNT/A injection, which persist longer than the peripheral effect at the NMJ. Our hypothesis is that cortical spine remodeling plays a key role in the therapeutic action of BoNT/A in neuropathologies and strongly contributes to the long-lasting benefits observed in patients. In addition, these set up of experiments were preparatory for the morphological 2 photons imaging experiments and analysis in the Tau injected mice.



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To better characterize functional consequences of these spine changes, we decided to perform a behavioural task, the t-NORT. BoNT/A injected animals perform poorly in this whisker-dependent task, both in the short and in the long-term analyses, suggesting functional consequences of the cortical rewiring. These results could represent the mechanism underlies the beneficial and long lasting effects of BoNT/A in neuropathologies.

Laura Restani, PhD

Responsabile Progetto,

Tecnologo TI, Neuroscience Institute - CNR, Pisa