

Virginia Bazzurro

## Report

Supervisors: Prof. Alberto Diaspro, Prof. Mauro Robello

My research activity concerns the study of the action of pharmacological molecules on GABA<sub>A</sub> receptors, activated by the endogenous inhibitory neurotransmitter  $\gamma$  – aminobutyric acid (GABA), in rat cerebellar neurons (cerebellar granule cells) in culture.

The aim of the project is to study the activation and the modulation of these receptors in a well – defined region of a cell for controlling individual neuronal responses with a high spatial resolution thanks to the uncaging of a “caged GABA”.

During the last academic year, I have experimentally characterized the caged molecule RuBi–GABA, a photo-activated molecule, whose activity can be controlled by a pulse of light.

This probe compound is prepared via covalent appendage of a light-sensitive protecting group, called “cage”, to a signalling molecule that is unable to activate its target, until the bond is broken by UV – VIS light; the uncaged molecule becomes active and can interact with its receptor site that induces a neuronal event.

The electrophysiological technique of the patch-clamp in whole-cell configuration was coupled with the confocal and two-photon microscopy, for regulating the release of a controlled quantity of neurotransmitter in a well-defined instant of time and space.

This new approach provides a useful technique to detect a selected biological target in a temporally and spatially confined way by photo activating a caged neurotransmitter.

I have deeply characterized the uncaging method; in particular, I have investigated how the variation of several physical parameters influence the electrophysiological measurements and how they affect the modality and efficacy of GABA release and consequently the GABA<sub>A</sub> response, using photo-activated-GABA.

The physical parameters considered and tested, for characterizing this method, were: excitation sources (one and two-photon), laser power, exposure time, X-, Y-, Z-distance from the target and different holding potentials used during the patch-clamp measurements.

The study of the change of these parameters is crucial to focus the attention on specific biological issues; a further step that can be done is to map the distribution of functional GABA<sub>A</sub> receptors in different regions of neurons: cell body, axons or dendrites.

The future purpose is to study the modulation of GABA<sub>A</sub> receptors by a pharmacological treatment with benzodiazepines, antisecretory factor or other drugs combined with the uncaging of RuBi–GABA.

## Published articles:

**Bazzurro V.**, Gatta E., Cupello A., Lange S., Robello M. (2018). *Antisecretory Factor Modulates GABA<sub>A</sub> Receptor Activity in Neurons*. J Mol Neurosci; 64(2): 312 – 320.

Doi: <https://doi.org/10.1007/s12031-017-1024-8>

Under review:

Gatta E., **Bazzurro V.**, Bisio A., Cupello A., Gambaro M., Pedrelli F., Schiapparelli P., Robello M. *Ursolic Acid and Irradiation induce apoptotic death in neuroblastoma cells, immature and mature rat cerebellar neurons by different mechanisms.*

In preparation:

**Bazzurro V.**, Cozzolino M., Gatta E., Bianchini P., Angeli E., Robello M., Diaspro A.

*3D Modulation of GABA<sub>A</sub> receptor by RuBi-GABA uncaging under precise control of electrophysiology and non-linear photoactivation in rat cerebellar granule cells in vitro.*

- Workshops:

- November 27<sup>th</sup> 2018, "La sicurezza in laboratorio per l'operatore e le colture cellulari", DISTAV, Università degli Studi di Genova.
- December 3<sup>rd</sup> – 6<sup>th</sup> 2018, "5<sup>th</sup> NIC@IIT Nanoscopy 2.0. Practical Workshop on Advanced Microscopy". Steering Committee: A. Diaspro, P. Bianchini, F.Cella Zancchi, G. Vicidomini, C.J.R. Sheppard. Istituto Italiano di Tecnologia, Genova.
- May 23<sup>rd</sup> – 24<sup>th</sup> 2019, Miniworkshop and CIMN Meeting "Protein Misfolding and Amyloidosis XIII", DIFI, Università degli Studi di Genova.

- International Schools:

- June 11<sup>th</sup> – 14<sup>th</sup> 2019, "International School on Nanoscale Optical Microscopy", 3<sup>rd</sup> edition. Directors: A. Diaspro, P. Bianchini, G. Giacometti. Istituto Veneto di Scienze, Lettere ed Arti, Venezia.

Poster presented during the International School on Nanoscale Optical Microscopy:

**Bazzurro V.**, Cozzolino M., Gatta E., Angeli E., Bianchini P., Robello M., Diaspro A. (2019). *Photoactivatable GABA "caged" for mapping GABA<sub>A</sub> receptor in cerebellar granule cells in vitro*

- Conferences:

- July 20<sup>th</sup> – 24<sup>th</sup> 2019, 12<sup>th</sup> EBSA 10<sup>th</sup> ICBP-IUPAP Congress, Madrid.

Poster presented during the EBSA congress:

**Bazzurro V.**, Cozzolino M., Gatta E., Bianchini P., Robello M., Diaspro A. (2019). *Study of the modulation of GABA<sub>A</sub> receptor by RuBi-GABA uncaging using electrophysiology and non-linear photoactivation in rat cerebellar granule cells in vitro.*

- Co – author of the poster:

- Cozzolino M., **Bazzurro V.**, Gatta E., Bianchini P., Diaspro A., Robello M. (2019). *Study of biophysical parameters in RuBi-GABA uncaging using non-linear photoactivation and electrophysiology in cerebellar granule cells.*

## Courses attended during the academic year 2018 – 2019

<b>Courses attended</b>		
Fisica Applicata alla Biomedicina e ai Biomateriali	Dott. Claudio Canale	48 h
Biofisica	Prof. Ornella Cavalleri, Prof. Alessandra Pesce	48 h

## Exams

<b>Courses attended</b>			<b>Exam</b>
Fisica e Statistica Medica	Prof. Sandro Squarcia	48 h	11/06/2018
Metodi Ottici Spettroscopici per lo Studio dei Materiali	Prof. Maurizio Canepa	48 h	23/07/2018
Fisica Applicata alla Biomedicina e ai Biomateriali	Dott. Claudio Canale	48 h	08/03/2019
Biofisica	Prof. Ornella Cavalleri, Prof. Alessandra Pesce	48 h	27/05/2019