



EUROPEAN  
CURRICULUM VITAE  
FORMAT



PERSONAL INFORMATION

Name **NINFA LICCARDI**

Address

Telephone Mobile

E-mail

LinkedIn

Nationality

Date of Birth

Gender **F**

WORK EXPERIENCE

- Dates (from - to) **04.11.2022 – 05.06.2023**
- Name and address of the employer **University of Madrid (UAM), School of Medicine, Department of Pharmacology, Spain.**
- Type of business or sector **Research Fellowship within an Italian Master at the University of Pavia (*see next point*).**

I carried out this stage in the laboratory of Prof. Jesus Hernandez Gujjo, a long-time collaborator of Prof. Pietro Baldelli, Dept. of Exp Medicine, University of Genova.

The research group of Prof Hernandez has a strong scientific background in the study of catecholamine release from chromaffin cells that they study with various electrophysiological approaches. Many of the molecular players in the stimulus-secretion chain are similarly active in neuro-secretion and catecholamine release. Therefore, studying chromaffin cells uncovered many details of the synaptic processes of docking, priming, and calcium-dependent exocytosis of vesicles.
- Occupation or position held **Researcher**
- Principal subjects/occupational skills covered **In this period in the laboratory of Prof. Jesus Hernandez Gujjo, I learned the following technique:**
  - Preparation and maintenance of culture of primary culture of bovine chromaffin cells. The primary culture of individual chromaffin cell is widely used and well characterized useful model system for neuroendocrine secretory cells neurons. Indeed, the structure and function of the components of the neurotransmitters secretory apparatus appear to be quite analogous in chromaffin cells and neurons. Hence, the ability to obtain reasonably homogeneous chromaffin cells in culture and their characteristic calcium-dependent secretory responses have led to their widespread use in neurobiology and in particular in the study of neurotransmitter biosynthesis, storage and release. In fact, these cells once isolated and cultured can be the subject of a variety of experimental procedures such as, electrophysiological, biochemical, pharmacological, radiochemical and morphological experiments.
  - Recordings and analysis of voltage-gated ion channels (in particular  $Ca^{2+}$ ,  $K^+$  and  $Na^+$ ) by voltage-clamp configuration of patch-clamp method. This technique, using different recording configuration, allows the investigation of a small set or even single ion channels useful to investigate the electrical properties of excitable cells and network.

- Recordings and analysis of single-cell firing properties by current-clamp configuration of patch-clamp method.
- Pharmacological modulation of electrophysiological parameters studies by patch-clamp method.
- Recordings and analysis of neurotransmitter release under physiological and pathological conditions by experimental electrochemical method. This method allows to measure neurotransmitters exocytosis induced by different substance studying the oxidation/reduction, tested by an electrochemical detector. Hence, in our experiments, the release of catecholamines were obtained by stimulation of chromaffin cells with acetylcholine or high concentration of  $K^+$  and the release of catecholamines was measured in real time by an amperometry.
- Calcium imaging experiments using different fluorescent dyes.

The main subject of research activity done in this period concerns as  $Pb^{2+}$  effects the Central Nervous System (CNS) homeostasis. Because  $Pb^{2+}$  inside CNS have severe deleterious effect, such as alteration of neuronal conduction, dose-dependent disorders, headaches and coma, as well as, in children, alteration of brain development and memory formation, the main objective of the scientific activity done in this stage was to study the  $Pb^{2+}$  effects on both neurotransmitters release of neurotransmitters and on neuronal excitability. In this context, we have studied as  $Pb$  modify both the  $Ca^{2+}$  conductance important for synaptic vesicle release and the voltage-gated ion channels involved in the generation and conduction of action potentials.

My own contribution to this scientific topic was to study the effects of  $Pb^{2+}$  on bovine chromaffin cells (BCCs) electrophysiological properties. These cells representing an easy and excellent model of neurons for their neuron-like electrical properties, and hence, used as model to investigate the molecular machinery that underlying cell excitability and neurotransmitter release. In particular, I recorded the amount of catecholamine release from BCCs induce by different concentration of  $Pb^{2+}$  by patch-clamp electrophysiological recordings. Different work configurations of this technique were used such as, (I) the voltage-clamp to study the macroscopic currents and the amount of neurodermitis release, (II) the current-clamp configuration to study the firing cellular excitability. Moreover, I used also a particular version of patch clamp recording, the perforated-patch configurations. The perforated whole-cell patch clamp configuration also allows the recording of whole cell membrane currents. However, at variance with the whole-cell configuration, the electrical access to the cell interior is not achieved through the rupture of the membrane patch but by the addition of an antibiotic agent into the recording pipette that form small holes in the membrane leaving unaltered the intracellular compartment of cell.

In addition, I collaborated with the laboratory technician to prepare the primary culture of BCCs, optimizing the conditions of maintenance to reduce the cellular lost during the maintenance of culture.

I also participated to the recordings of the intracellular  $Ca^{2+}$  concentration and variation by technique of functional imaging with Fluo-4-AM as probe using a microplate reader (Fluostar Optima).

Finally, I analysed all data obtained and used to finalize my Master experimental thesis started at the university of Pavia.

## EDUCATION AND TRAINING

<ul style="list-style-type: none"> <li>• Dates (from - to)</li> <li>• Name and type of organisation providing education and training</li> <li>• Principal subjects/occupational skills covered</li> </ul>	<p><b>05.02.2022 – 06.06.2023</b></p> <p>University of Pavia, Department of Science of Drug</p> <p>Second level Master with the title of "Design and development of Drugs". <a href="https://psfmaster.unipv.it">https://psfmaster.unipv.it</a></p> <p>The master lasts one year and implies the acquisition of 60 university credits (CFU), for a total of 1500 hours. The first 20 CFU were acquired with a didactic lesson in the classroom, carried out both in a traditional and interactive mode. The last 40 CFU were acquired with a practical training/internship at companies, research institutions or even at Italian universities. At the request the training courses can be made also in international research centers of excellence or universities.</p> <p>The didactic section consisted of course about (a) Drug design and Development, (b) Pharmaceutical Synthesis and Biocatalysts, (c) Advance Pharmaceutical Chemistry and (d) Physiological methods used for drug characterization. The practical training was conducted at the University of Madrid (UAM), School of Medicine, Department of Pharmacology, Spain.</p>
---	---

In this context the title of Experimental Thesis carried out in the laboratory of Prof. Jesus Hernandez Guijo was: "Effects of Pb<sup>2+</sup> on bovine chromaffin cells". This thesis was discussed at University of Pavia at 06.06.2023.

- Title of qualification awarded Master in Design and development of Drugs
  - Dates (from - to) **2015 – 2020**
  - Name and type of organisation providing education and training University of Catania. Italy
  - Principal subjects/occupational skills covered Physiology, Pharmacology, Chemistry, Biology, Biochemistry  
Experimental Thesis. Supervisor: Prof. Angelo Spadaro, at the Department of Pharmacy, University of the study of Catania.
- Title: Progettazione, sintesi e valutazione biologica di nuovi derivati 2-benzothiazolylimino-5-arylidene-4-thiazolidinones ad attività antimicrobica e antinfiammatoria.
- In this period in the laboratory of Prof. Angelo Spadaro, I learned the following technique:
- Synthesis of heterocyclic thiazolic compounds "Multi-Target-Directed Ligands" with antimicrobial and anti-inflammatory activity. Characterization by Infrared (IR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy.
  - Determination of anti-inflammatory activity in mouse model. The drug effect was evaluated using carrageenan to produce inflammation in the animals under study.
  - Determination of antibacterial and antifungal activity in mice
- This Thesis concerns the synthesis and the characterization of new chemical compounds with anti-inflammatory and antimicrobial activity. In particular, after the synthesis and the purification of five new molecules, the anti-inflammatory activity of the drugs was determined using intradermal injections of carrageenan to induce an acute inflammatory response in mice's paw and, subsequently, it was administrated by intraperitoneal injection. The modulatory effect of the compounds was evaluated measuring the wight of the mice's paw and was compared to the positive control group treated with carrageenan alone. Instead, the antimicrobial and antifungal activity were determined measuring the minimum inhibitory concentration and the minimum bactericidal concentration, using six bacterial species. The microdilution method was employed.
- This thesis was conducted in collaboration whit Prof. Geronikani Athina at Department of Pharmacy, Aristotle University of Thessaloniki, Greece. In particular the first part of study, syntheses and design of drugs were carried out in the laboratory of prof. Angelo Spadaro and the pharmacological/physiological characterization of compounds were done in the laboratory of Prof. Geronikani Athina.
- Title of qualification awarded Master's degree in Pharmacy
  - Level in national or international classification (if relevant) Final mark: 110/110 with honors.  
**07.2021**: National Qualification to exercise the profession of Pharmacist  
**01/08/2019 – 31/01/2020**: Internship in a Pharmacy (Farmacia Vaccaro)
  - Dates (from - to) **12.02.2019 – 27.06.2019**
  - Name and type of organisation providing education and training Aristotle University of Thessaloniki, Greece, laboratory of Prof. Geronikani Athina at Department of Pharmacy.
  - Principal subjects/occupational skills covered During this stage, I worked on a different topics of my Pharmacy course experimental thesis. In particular, pharmacological/physiological characterization of drugs were carried out in accordance with my supervisor Prof. Angelo Spadaro in the laboratory of Prof. Geronikani Athina.
  - Title of qualification awarded Erasmus Traineeship
  - Dates (from - to) **2010 – 2015**
  - Name and type of organisation providing education and training High School at Liceo Scientifico "E. Medi", Leonforte (EN), Italy

• Title of qualification awarded Baccalaureate Degree, 98/100

## PERSONAL SKILLS AND COMPETENCES

MOTHER TONGUE

ITALIAN

OTHER LANGUAGES

ENGLISH

- Reading skills
- Writing skills
- Verbal skills

B2

B2

B2

(First B2-Cambridge on December 2020)

SPANISH

- Reading skills
- Writing skills
- Verbal skills

B1

B1

B1

(Levels: A1-A2: Basic user; B1-B2: Independent user; C1-C2: Proficient user)

SOCIAL SKILLS AND COMPETENCES

Motivation, spirit of adaptation to new environment, great ability to work in an international context, and team spirit.

ORGANISATIONAL SKILLS AND COMPETENCES

Great attitude to plan the work for objective, coordination and administration of people; Active member of AGIFAR Catania (Associazione Giovani Farmacisti di Catania); Voluntary work in ANPAS (Associazione Nazionale Pubblica Assistenza)

TECHNICAL SKILLS AND COMPETENCES

- Electrophysiology with patch-clamp method and its different configuration of analysis. Perforated whole-cell patch-clamp that is a variant of the patch-clamp technique used to measure the activity of ion channels in the plasma membrane of a single cell. The electrical access to the cell is obtained through inclusion of a pore-forming antibiotic in the patch pipette which perforates the sealed patch of membrane in contact with the patch pipette.
- Catecholamines release recordings with amperometry method.
- Biochemical study of heavy metals cellular toxicity.
- Cell culture of bovine chromaffin cells.
- Different techniques of molecular biology.
- Organic chemistry.
- Drugs synthesis and determination by differ approach (nuclear magnetic resonance, Infrared spectroscopy, Thin-layer chromatography)

Different skills in informatic: Microsoft Office, use of programs for analysis of electrophysiological data and statistic (OriginPro 8.5, HEKA programs; GraphPad Prism, Past3).

DRIVING LICENCE(S)

A, B

COURSE

"Sicurezza e Salute nei Luoghi di Lavoro ai sensi del D.Lgs. 81/08 Formazione Specifica per Attivita' a Basso Rischio".

1.Organization of security in the University

2.The use of VDT and the office working environment

## ADDITIONAL INFORMATION

Flexibility, motivation, creativity and spirit of adaptation are some of the elements that characterize me. My goals are improving myself, my working career and my life. Consequently, my ambition leads me to think "I can do It". Once I have recognized my objective, I focus on that one with interest and perseverance. I have had working experiences in different European countries as Italy, Spain and Greece, and that's the reason why I feel comfortable in an international context, believing that diversity could stimulate a working group.

1. Andrés M. Baraibar, Ricardo de Pascual, Victoria Jiménez Carretero, **Ninfa Liccardi**, Natalia Hernández Juárez, Jesús M. Hernández-Guijo. (2023) Aluminum alters excitability by inhibiting calcium, sodium, and potassium currents in bovine chromaffin cells. *Journal of Neurochemistry*. DOI: 10.1111/jnc.15784

Aluminum ( $Al^{3+}$ ) has long been related to neurotoxicity and neurological diseases. This study aims to describe the specific actions of this metal on cellular excitability and neurotransmitter release in primary culture of bovine chromaffin cells. Using voltage-clamp and current-clamp with the whole-cell configuration of the patch clamp technique, online measurement of catecholamine release, and measurements of cytosolic calcium concentration with Fluo-4-AM, we have observed that  $Al^{3+}$  reduced intracellular calcium concentrations around 25% and decreased irreversibly catecholamine secretion in a time- and dose-dependent manner. Moreover,  $Al^{3+}$  produced a bigger blockade on N-, P-, and Q-type calcium channels subtypes than on L-type channels subtypes. Sodium currents were also inhibited reversibly by  $Al^{3+}$  in a time- and concentration-dependent manner. Voltage-dependent potassium currents were low affected by  $Al^{3+}$ . Nonetheless, calcium/voltage-dependent potassium currents were inhibited in a concentration-dependent manner. This inhibition was related to the depression of calcium influx through voltage-dependent calcium channels subtypes coupled to BK channels. Hence, the blockade of these ionic conductance altered cellular excitability that reduced the action potentials firing and so, the neurotransmitter release and the synaptic transmission.

My own contribution to this scientific activity was the participation on the electrophysiological and live imaging experiments.

In particular, I performed the electrophysiological recordings by use of whole cell and current clamp configuration of patch clamp technique as well as by perforated patch recordings using amphotericin to make possible the registrations of whole cell currents without a loss of intracellular pathways. Hence, I study the modulation of  $Al^{3+}$  on both the firing properties of cells studying the  $Na^+$  and  $K^+$  currents and the release of neurotransmitters release through the study of alteration of  $Ca^{2+}$  homeostasis by  $Ca^{2+}$  imaging experiments. Finally, I contributed to the off-line analyse all the data acquired.

#### WORKSHOP AND CONGRESS:

1. FARMADRID 29 - REUNIÓN DE LOS FARMACÓLOGOS DE LA COMUNIDAD DE MADRID, 1 JUNE 2023. **Ninfa Liccardi**, Ricardo de Pascual, Victoria Jiménez Carretero, and Jesús M. Hernández-Guijo "Lead exerts a depression of neurotransmitter release through a blockage of voltage dependent calcium channels in chromaffin cells" **ORAL PRESENTATION**

The most important findings of the work presented as an oral presentation in this congress point out the neurotoxicity of Lead ( $Pb^{2+}$ ) studied in primary culture of bovine chromaffin cells. In this work we have observed that the neurotoxic effect of  $Pb^{2+}$  was due to a dramatic change of neurotransmitter release by alteration of  $Ca^{2+}$  homeostasis. In particular, we have observed a dose- and voltage-dependent inhibition of  $Ca^{2+}$  currents operated by  $Pb^{2+}$ . This effects on  $Ca^{2+}$  currents dramatically affect the neurotransmitter release. This toxic effect was not accompanied by changes on macroscopic currents of  $Na^+$  and  $K^+$  hence the firing properties of cells were unchanged, as confirmed by current clamp recordings. All experiments were conducted with patch clamp technique and live imaging experiments.

2. FARMADRID 29 - REUNIÓN DE LOS FARMACÓLOGOS DE LA COMUNIDAD DE MADRID, 1 JUNE 2023. Victoria Jiménez Carretero, **Ninfa Liccardi**, Iris Alvarez-Merz, José M. Solís, Jesús M. Hernández Guijo. "Intracellular accumulation of non-excitatory amino acids induces an irreversible synaptic depression under hypoxia" Oral presentation by Victoria Jiménez Carretero.

I collaborated with Victoria Jiménez Carretero for the electrophysiological measures carried out in this project.

The aim of this project was to study the pathological effects of non-excitatory amino acids alone or in combination with hypoxia on hippocampal neurons. In particular, using rat hippocampal slices, we made extracellular electrophysiological measures in the CA1 region: We tested seven non-excitatory amino acids (L-alanine, L-glutamine, glycine, L-histidine, L-serine, taurine, and L-threonine) to study their effect on synaptic transmission. The results indicate that the intracellular

accumulation of non-excitatory amino acids using yet undescribed transport systems, contributes to cellular damage during cerebral edema under hypoxic conditions.

*According to law 679/2016 of the Regulation of the European Parliament of 27th April 2016, I hereby express my consent to process and use my data provided in this CV*

AGIRA 07.07.2023