

# FONDAZIONE CAVALIERI OTTOLENGHI



## *Annual Report 2017*

### **INDEX**

Page 2	OVERVIEW OF THE INSTITUTE
Page 3	Historical notes
Page 4	Organisation at NICO
Page 6	Outreach activities
Page 9	Seminars at NICO
Page 9	On site visit
Page 9	Brain Imaging Center
Page 10	ACTIVITIES OF THE SINGLE GROUPS
Page 10	PI Bertolotto
Page 32	PI Bonfanti- Peretto
Page 41	PI Buffo
Page 56	PI Di Cunto
Page 65	PI Eva
Page 76	PI Geuna
Page 90	PI Panzica
Page 108	PI Tempia
Page 119	PI Vercelli
Page 134	OUTREACH ACTIVITIES
Page 134	Seminars
Page 136	Press report
Page 141	Dissemination (Conferences and events)

# NICO 2017 by the numbers



**9**

Research Groups  
**60** Scientists  
**40** Graduating Students



**76**

Peer-Reviewed  
Publications



**66**

Collaborative Initiatives  
with  
International  
Research Groups



**62**

On-going/Granted  
Research Projects



**12**

Scientific  
Conferences/workshops  
organized  
by NICO members



**14**

Invited speakers



**1**

Spin-off Company



**1**

Biobank



**110**

Outreach Activities  
**72** Invited Talks  
**38** Science Dissemination Initiatives



**14**

trained  
PhD students



**2271**

Facebook Followers

## **BRIEF HISTORICAL NOTES**

The Cavalieri Ottolenghi Foundation is a no-profit organisation recognized by the Piedmont Region committed to supporting research and development of structural and infrastructural Neuroscience. The Foundation comes from the legacy to the University of Turin of Annetta Cavalieri Ottolenghi in the 50s.

After decades in which the Foundation has funded scientific research projects and purchase of scientific equipment, in the 90s an international scientific committee of eminent personalities in Neuroscience proposed to build a centre for Neuroscience and chose Dr. Carlos Dotti, a foreign researcher, as scientific director. For some years, the research group of the Foundation was hosted at the San Luigi Hospital, Orbassano (Torino) while the construction of the building began in 2001 and was completed in 2009. Meanwhile, Dr. Dotti moved abroad with his group.

In 2009, the Board of Administrators identified in prof. Ferdinando Rossi, University of Turin, the figure of the scientific director of the Foundation, decided to issue an announcement of selection, limited to the Piedmont Region, to select the groups to be included in the new building. Eight groups (seven of the University of Turin, one of the San Luigi Hospital) were selected by a national committee (which included Professors Bentivoglio, Bogetto, Cattaneo and Saglio, assisted by Dr. Borio, Administrative Director at the University of Turin) and in May 2010 they moved into the new building. The institute was named Neuroscience Institute Cavalieri Ottolenghi (NICO).

### **Aims of NICO**

1) The complexity of the studies on the brain requires a multidisciplinary approach. For this we combine complementary approaches and experiences, integrating basic research and clinical application. The birth of NICO takes full advantage of both the integration of the wealth of knowledge and the shared use of expensive equipment and laboratories formerly fragmented in university Departments.

2) Our researchers are engaged in many activities of scientific dissemination, dedicated to the public (Open days at the NICO, Stem Cell Day and Night of Researchers, public conferences) and to high school students (Neuroscience Olympics and Scientific Summer Academy). These and other initiatives are designed to bring young people to science, by sharing the commitment and passion that drives scientific research, as well as to communicate with competence and clarity a complex issue such as neuroscience.

The Institute of Neurosciences of the Cavalieri Ottolenghi Foundation (NICO) aims to perform high-level research in neuroscience geared towards the prevention, diagnosis and treatment of neurological disorders. In line with this principle, the research is focused on mechanisms that govern normal neural maturation and defects involved in mental retardation syndromes.

### **THE COLLABORATIVE VISION AT NICO**

Since its foundation in 2011, the NICO adopted a new (relative to the Departments of origin) view of sharing all facilities, supplies and instruments by all groups. Excepted for the clinically-relevant activities, which have to be performed in dedicated and isolated rooms to maintain privacy related to human material, all instruments are located in common facilities which are shared by all NICO members. This has initially created an organizational burden, but it has also obliged people to meet, share decisions, collaborate and interact, also in the formation of new researchers. Internal courses on the use of instruments and facilities have been organised to improve their correct usage. Starting from the practical needs of every day research life, this attitude has boosted collaboration and

exchange of ideas among the individual researchers and ameliorated the scientific production of single researchers. To sum up, it has created a scientific environment which, respecting the peculiarities of single researchers, interacts and operates as a real community to apply for grants, develop multidisciplinary projects, act as a whole institutional body in front of the scientific community and to the public. Finally, it represents a fundamental breakthrough to save money and to exploit the use of expensive instruments.

### **POSITIONING OF NICO IN THE UNIVERSITY OF TURIN**

NICO is part of the University Interdepartmental centre for Neuroscience (called Neuroscience Institute of Turin – NIT), which gathers most researchers active in the field in Turin (even outside the University). NICO researchers are part of doctorate schools (Neuroscience, Molecular Biotechnology and Veterinary Medicine) of the University of Turin and hosts 14 PhD students. Moreover, as lecturers at the schools of Biology, Biotechnology, Pharmacy, Medicine, Psychology and Veterinary Medicine, they are involved in the preparation of many theses for Bachelor and Master' degrees. Currently, NICO laboratories hosts 21 students who are developing their Bachelor or Master thesis projects and 23 stage students. NICO collaborates with several other research centres of the University of Turin, such as the Molecular Biotechnology centre, the IRCCS Candiolo and the Brain Imaging Centre. NICO members belong to the Departments of Neuroscience, Biotechnology, Veterinary Morphophysiology and Systems Biology. NICO members belonging to the Department of Neuroscience of UNITO participate to the project which was recently selected by the MIUR for the Departments of Excellence. The Department of Veterinary Medicine was selected as well. Therefore, NICO will be involved in these five-year projects, with a significant return in terms of personnel, upgrade of instrumentation and translational science collaborations.

Several groups of the NICO have projects and funding in collaboration with the Polytechnic of Turin.

Starting from 2017, microscopy facilities at the NICO are part of the Open Access lab program of the University of Turin.

### **POSITIONING OF THE NICO IN ITALY AND IN THE WORLD**

NICO researchers have several national and international collaborations, as shown by their publication record. They have also a strong rate of exchange of visits and seminars, as it can be argued from their reports in attachment. Also, they participate to exchange programs of bachelor, graduate and doctorate degrees, and NICO is often visited and attended by foreign students. They participated in the international Young Investigators Training Program established in Italy in occasion of the 2011 World International Brain Research Organisation Meeting and of the 2014 European Neuroscience Meeting. Every second year, an international meeting (Steroids and Nervous System) is organized by Prof. Panzica's group (with the cooperation of prof. R.C. Melcangi, University of Milan): the meeting has an average 150 people attendance and more than 40 invited speakers from all over the world. The 2013, 2015 and 2017 editions were organized with the administrative help of the Ottolenghi Foundation. The Clinical Neurobiology group is organising local and national meetings on multiple sclerosis in the San Luigi Hospital.

NICO researchers are/have been members of committees for national and international meetings and societies and acted as referees for international peer review journals and panels of funding agencies.

Researchers of NICO are involved in several collaborative grants at a local (Cassa di Risparmio di Torino, Compagnia di San Paolo), national (PRIN) and international (7-FP and Horizon 2020) level, as detailed in the following reports.

NICO has been recently (July 2015) included by the MIUR (Italian Ministry of University and Research) in the list of Italian Research Institutes which are allowed to hire directly foreign

researchers. Moreover, NICO has successfully applied to the MIUR to receive public funds to support private research institutes.

In 2014 NICO has signed an official agreement of collaboration with Italian Institute of Technology for the exchange of researchers and facilities, and in this frame researchers of the two institutions are already collaborating and preparing joint grant applications. Also, within the frame of NIT, an agreement has been signed with the Istituto Superiore Mario Boella (a research and innovation centre of the Compagnia di San Paolo, operating in the Information and Communication Technologies (ICT) domain) and with the Tohoku University in Japan. As a result of this collaboration, a grant agreement within the Horizon 2020 program has been signed in which the director of NICO is the coordinator.

### **THE NICO SPINOFF**

In 2014 and 2015 some NICO researchers (prof. Eva, Geuna, Panzica, Buffo, Boido and Tamagno) collaborated in preparing the application for an academic spinoff (S&P Brain) of the University of Torino, to provide services to researchers, institution and companies related to behavioral neurosciences. This will allow to provide an income to the NICO, and also to apply for cooperative grants as a company. The spinoff has been approved by the technology transfer committee of the University of Torino and approved by the Academic Senate and Council of Advisors of the University.

**Illustration of the organizational structure and research indicating the current staff, including contractors, and their qualifications, and of the goat educational, scientific and instrumental**

## **Organization of the NICO (Neuroscience Institute Cavalieri Ottolenghi)**

**Scientific Director** is prof. Alessandro Vercelli (appointed March 2014, up to February 2017 then in prorogatio). In addition to the scientific direction he performs also the function of Administrative Director.

Our activities are organized into **nine groups**:

Adult Neurogenesis (PIs Luca Bonfanti and Paolo Peretto)

Brain Development and Disease (PI Alessandro Vercelli)

Clinical Neurobiology (PI Antonio Bertolotto)

Embryonic Neurogenesis (PI Ferdinando Di Cunto).

Nerve Regeneration (PI Stefano Geuna)

Neurobiology of Brain Plasticity (PI Annalisa Buffo)

Neuroendocrinology (PI Giancarlo Panzica)

Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)

Neuropsychopharmacology (PI Carola Eva)

## **Staff**

Employees directly depending from the Foundation consist of **two secretaries** (Maria Lo Grande and Susanna Monteleone) and **two technicians** (Sri Satuti Werdiningsih and Martir Dyrnishi).

We have a contract with a **Press Agent**, dr. Barbara Magnani, who is helping us in all dissemination activities.

According to the Convention with the University of Turin and with San Luigi Hospital of Orbassano, the NICO hosts:

- **University staff**: 4 full professors, 4 associate professors, 8 university research assistants, 1 technician, 17 post-docs and 14 doctoral students;

- **Hospital staff**: 1 Head physician, 1 manager biologist, 4 specialists in Clinical Biochemistry, 3 post-doc fellows, 3 laboratory technicians.

About 40 graduating students of Biology, Biotechnology, Medicine and Psychology perform experiments for their thesis at the NICO.

## **Labs and Equipment**

### ***Molecular and cellular neurobiology, Neuroanatomy***

The laboratory is equipped with several excellent quality research light microscopes, in particular, two confocal microscopes (Leica SP5 and Nikon) and a Nikon ViCo system. Moreover, a two-photon microscope Nikon (A1MP) has recently been acquired in the context of the Open Access laboratories project of the University. An electron microscope is available in the Department of Cell Biology, San Luigi Hospital, adjacent NICO.

There are also various imaging systems with computerized microscopes and photo / digital video cameras that allow morphometric investigations, studies densitometry quantitative autoradiography, image processing and statistical analysis. Two Neurolucida systems are in the microscopy facility.

For neurohistological studies, sliding or rotational microtomes, 3 vibratomes and 4 cryostats are available.

### ***Animal facility***

The structures dedicated to the experimental animals include rooms dedicated to farming and livestock buildings, spaces dedicated to behavioral tests and, finally, rooms equipped for surgery on rodents. The laboratory for behavioral tests is equipped with mazes and infrared camera for the behavioral analysis of locomotor activity, anxiety, depression and memory. There is also a computerized video analysis (Ethovision XT video track system) to analyze scanned images of behavioral tests. Finally, dedicated spaces, equipped for P2 procedures are available to use in animals virus of the corresponding biosafety level.

### ***Cellular and molecular biology***

NICO has excellent facilities for research in the field of molecular and cell biology, and a dedicated and experienced staff for tissue culture experiments and molecular biology.

Tools for cell biology experiments allow cell count, freezing, plating tissue culture and cell transfection. For in vitro and ex vivo cultures (primary cultures, tissue explants, organotypic cultures, neurospheres) inverted microscopes are available and a system that allows the acquisition of images in time-lapse of viable cells.

In addition, NICO provides expertise and services related to molecular biology techniques, such as the preparation and analysis of proteins, DNA, RNA and microRNA. The instrumentation of molecular biology platform includes a semi-automatic system for the purification of nucleic acids, three machines for Real-Time PCR, a electroporator for bacteria or ES, as well as many other instruments as a standard laboratory for extraction and analysis of DNA, RNA and proteins.

### ***Electrophysiology***

The laboratory of neurophysiology provides tools for the preparation of micro-sections of nervous tissue, that can be maintained in vitro for several hours. There are two experimental stations for patch clamp recordings of membrane potential or ionic current of single neurons in sections. These positions are furnished with complete tools for the electrical stimulation of the axons and for application of pharmacological substances. They can also make extracellular recordings to study synaptic plasticity.

### ***Clinical Neurobiology Laboratory (CNL)***

The CNL offers diagnostic services and consulting for the interior (San Luigi Hospital) and external (10 Departments of neurology in the region) diagnosis of multiple sclerosis.

The diagnostic tests offered include cytochemical examination of cerebrospinal fluid, immunoelectrofocusing to search for oligoclonal bands and several essays for the detection of viral nucleic acids. In addition, the laboratory provides a diagnostic service for neuronal paraneoplastic antibodies.

Currently the CNL is one of the few laboratories in Italy capable of providing a diagnostic service for the detection of antibodies NMO-IgG and anti-AQP4.

Finally, the CNL offers various services for monitoring patients with multiple sclerosis treated with different drugs; in this regard the lab performs a service in Italy and Europe for the serological titration of antibodies against interferon-beta (using three different methods) and natalizumab (Tysabri) potentially produced by patients treated with these drugs.

The laboratory is also equipped with a service for the evaluation of the biological activity of interferon-beta through the measurement of gene expression of specific proteins induced by interferon (such as MxA).

### *Common services*

In addition to spaces dedicated to animal facility and laboratories, there are two rooms for the secretariat, a staff kitchen, a room for small meetings (up to 20 people), a seminar room and a room for deep freezers.

## **OUTREACH ACTIVITIES**

From the perspective of educational and scientific dissemination the aims of NICO are:

- to promote scientific culture, and in particular knowledge of neuroscience, in high schools, through multimedia tools that reduce the economic impact of training initiatives;
- to provide basic skills on the normal functioning of the brain and neurodegenerative processes;
- to explain the importance of basic research and the impact on society of tomorrow;
- to create synergies and exchange of expertise / experience in the world of university research, the school and society, represented in this case from the large network of voluntary associations active in the field of disability and dementia.

NICO is engaged in scientific **activities dedicated to high school students** - Scientific Summer Academy, Olympic Neuroscience and Unistem Day, national and international – as well as to general public (Researchers' Night, Open Day and Brain Awareness Week).

These activities were possible thanks to a partnerships network that, starting by the University of Turin has expanded in the years throughout other universities, associations (e.g. Non-profit Associations) and institutions like Centre Agora Science (which brings together the University of Turin and East Piedmont and Polytechnic of Turin). They have allowed to establish direct contacts with teachers and high school students.

NICO is organizing the regional competition of the World Olympics in Neuroscience: every year in the world, high school students participate in a competition to stimulate interest in the study of neuroscience. The competition begins with the sending of educational materials to schools, then a local selection in schools (in Piedmont hundreds of students), regional (at the NICO) and finally a national one in which the Italian "champion" is chosen for the world competition.

The Institute has a strong link with the Piedmontese **Associations of patients** with disabilities (e.g. the Coordination Committee for Tetraplegic and Paraplegic patients of Piedmont) and neurodegenerative diseases and their families (CAAP - Coordination committee of Alzheimer Associations of Piedmont -12 local associations - the Ass. of Parkinson friends of different provinces of the region, the Association Girotondo Onlus for SMA patients in Biella, etc.).

NICO is involved in the organization of a series of **dissemination lectures** for the public, some of which on the occasion of the "Brain Awareness Week " (which is held worldwide in March) at "Circolo dei Lettori" of Turin. The goal is to provide accurate information on scientific topics not easy to understand / disclose - such as the state of research and therapies available on neurodegenerative diseases - and often the subject of simplification and distortion (for example regarding the Stamina affair).

Organization and scientific supervision of **UNISTEM DAY** (yearly, national event; NICO organizes each year the Turin edition), Aula Magna del Rettorato Cavallerizza reale (with 400 students of the secondary school).



### **SCIENTIFIC SEMINARS AT NICO**

Over the last five years, an internal committee (Annalisa Buffo and Silvia De Marchis) has been in charge for the promotion and organisation of the seminar activities at NICO. The committee established a procedure by which speakers to be invited are first proposed by NICO researchers and then selected, based on a poll by the NICO community. A program of seminars exchange is being organised with the University of Milan Bicocca.

For invited speakers, see the attached list.

### **ON SITE VISIT**

On January 28 and 29, 2016 a Panel of external reviewers (P. Alves, Lisbona, M. Bentivoglio, Verona, M. Celio, Fribourg) visited NICO. After evaluating activities and researcher of NICO during the first five years, they issued a report which is available on the NICO website.

### **BRAIN IMAGING CENTER**

The Foundation Cavalieri Ottolenghi ha cofunded in the past the project for the Brain Imaging center of the Interdepartmental Center “Neuroscience Institute of Turin” (NIT), with a grant for the acquisition of a functional MRI Philips Ingenia 3T. During 2017, the Foundation has supported the activities of the Brain Imaging Center by financing a plan of grants for researchers of the University of Turin in the field of fMRI and by paying a salary for an EP technician for the fMRI.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: clinical neurobiology

# 1. LABORATORY DESCRIPTION – PERSONNEL:

## Principal Investigator

Antonio Bertolotto Birthdate (12/02/1952)  
MD Gender: M  
Nationality: Italy Phone: 00 39 011 670 66 00  
Email: [antonio.bertolotto@gmail.com](mailto:antonio.bertolotto@gmail.com)

## Personnel

1. Arianna Sala Birthdate (22/05/1972)  
MSc in Biology Gender: F  
Role: Resident Biologist, Specialist in Clinical Pathology Nationality: Italian  
Expertise: She is principally involved in the diagnostic process of inflammatory diseases of the nervous system and in the development of novel laboratory procedures for the advancement of diagnostic technologies.
2. Marzia Caldano Birthdate (20/07/1975)  
MSc and Board Certification in Clinical and Analytical Biochemistry Gender: F  
Role: Pharmacist Nationality: Italian  
Expertise: relevant experience in drug immunogenicity, cell cultures, gene expression analysis and cerebrospinal fluid analysis. She is in charge of an Italian Service for the detection of anti-Interferon and anti-Natalizumab antibodies in multiple sclerosis patients. Currently her studies is focused on personalization of therapy and identification of new biomarkers to establish the efficacy of treatment.
3. Fabiana Marnetto Birthdate (14/12/1980)  
MSc and Board Certification in Clinical and Analytical Biochemistry Gender: F  
Role: Medical Biologist Nationality: Italian  
Expertise: Detection of antibodies in autoimmune diseases (anti-KIR4.1 antibodies in MS and anti-Aquaporin 4 antibodies in NMO). Investigating the Epstein Barr virus (EBV) involvement in MS pathogenesis. Evaluation of clinical/biological response to different therapies in MS and NMO: biomarkers discovery and validation, assessing the clinical/biological response to different therapies in MS. Experience in performing cerebrospinal fluid evaluation and serological tests for anti- central nervous system antibodies, for diagnosis and management of patients with MS, NMO and other neurological disorders.
4. Serena Martire Birthdate (01/08/1987)  
MSc and Master in *Medical and Genomic Statistics* Gender: F  
Role: Medical Biotechnologist Nationality: Italian

Expertise: Molecular biology, data management, gene expression and genotype data analysis, biostatistics

5. Francesca Montarolo Birthdate (14/05/1983)  
MSc and PhD in Neuroscience Gender: F  
Role: Biologist Nationality: Italian  
Expertise: Technical skills to work “in vivo” with experimental murine model, looking at cognitive behavior tests and at immunohistological and biomolecular aspects in the central nervous system.

6. Simona Perga Birthdate (29/03/1977)  
MSc, PhD in Molecular and Experimental Pathology and Board Certification in Clinical and Analytical Biochemistry Gender: F  
Role: Medical Biotechnologist Nationality: Italian  
Expertise: Previous research activity concerned the investigation of the molecular mechanisms underlying the physiological pathological neuronal aging in “in vitro” primary neuronal and glial cultures and in vivo mice models and disease biomarkers research in biological fluids (cerebrospinal fluid and serum) through the application of proteomics and biochemical techniques. Her current research activity is relates to the molecular mechanisms involved in the pathogenesis of multiple sclerosis (MS). In particular this research is carried on performing gene and protein expression analysis in peripheral blood mononuclear cells or in sub-population isolated from whole blood obtained from patients and healthy controls; immunohistochemically and immunofluorescence analysis in post-mortem MS human brain tissues and in the EAE mouse models of MS.

7. Michela Spadaro Birthdate (10/03/1975)  
MSc and PhD in in Immunology and Cellular Biology Gender: F  
Role Biologist Nationality: Italian  
Expertise: Technical skills to work “in vivo” with experimental murine model and human samples to explore the immune mechanisms underlying multiple sclerosis pathology by flow cytometry and functional assays, molecular biology and data management.

8. Paola Valentino Birthdate (11/08/1981)  
MSc and Board Certification in Clinical and Analytical Biochemistry Gender: F  
Role: Medical Biotechnologist Nationality: Italian  
Expertise: gene expression analysis and evaluation of drug immunogenicity therapies in MS and NMO patients. Evaluation and validation of diagnostic and prognostic tests for the detection of biomarkers for MS and NMO. Cerebrospinal fluid evaluation and serological tests for diagnosis and management of patients with MS, NMO and other neurological disorders

9. Federica Brescia Birthdate (26/03/1984)  
Bachelor's degree in Biomedical Laboratory Technicians Gender: F  
Role Biomedical Laboratory Technicians Nationality: Italian  
Expertise: Cerebrospinal fluid analysis and serological tests, DNA and RNA extraction, databases management, Bio-Bank management, cells culture and CPE test.

10. Alessia Balbo Birthdate (25/02/1992)  
Bachelor's degree in Biomedical Laboratory Technicians Gender: F  
Role: Biomedical Laboratory Technicians Nationality: Italian  
Expertise: Cerebrospinal fluid analysis, RNA extraction, reverse transcription, real time PCR, ELISA and immunofluorescence analysis, databases management.

11. Jessica Bertolo Birthdate (26/12/1994)  
Bachelor's degree in Biomedical Laboratory Technicians Gender: F  
Role: Biomedical Laboratory Technicians Nationality: Italian  
Expertise: RNA extraction, reverse transcription, real time PCR, ELISA and immunofluorescence analysis, databases management, Bio-Bank management.

12. Raffaella Ghiggia Birthdate (13/03/1972)  
MSc in Architecture Gender: F  
Role Secretary Nationality: Italian  
Expertise: compiling databases and acceptance samples.

## PRINCIPAL INVESTIGATOR

H index, 44; Citations 5865

### Relevant discoveries:

Peri-neuronal nets and Extracellular Matrix components in CNS

Identification of subsets of resting microglia in normal CNS

Antibodies against bio-pharmaceutical

Quantification of IFNbeta Biological activity for non-responders identification

Procedure for lumbar puncture reducing pain

Anti-inflammatory molecules involved in Multiple Sclerosis

Auto-Antibodies specific for Multiple Sclerosis and NMOSD

### List of grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2014-2017	national	Comp	FISM	“Ruolo della deubiquitinasi TNFAIP3/TNFAIP3 nell’immuno-patologia della SM”.	FISM code 2014/R/14	100.000	10%
2017-2018	national	PI	Novartis	“Effects of fingolimod and AUY954 on Nuclear Receptor subfamily (NR4As) in multiple sclerosis”	-	67000	10%
2015-2018	National	Comp	FISM	Studio dell’espressione di geni dei virus di Epstein Barr e geni cellulari in pazienti con CIS, SM, recidivante remittente e SM primaria progressiva: ricerca di biomarcatori diagnostici e prognostici		€51950	10%
2016-2018	National	PI	Biogen	Evaluation of biological activity by RNA-sequencing in PEG-Interferon beta 1a and Interferon beta 1a im treated Multiple Sclerosis patients and comparison of genetic expression between different cellular populations		279.000	10%
2015-2017	National	PI	Ministero Salute	«Improving therapeutic appropriateness of Multiple Sclerosis treatments using biomolecular approaches to personalize therapy and save pharmaceutical spending»		381.880	*

\*The financial management of the project was in charge to the administration of AOU San Luigi, but the research was performed mainly at NICO. Scientific instruments funded by the project are located at NICO and are available to all the researcher of NICO.

### List of names of PhDs you have supervised:

Board Certification in Clinical Pathology: Dr Arianna Sala, Dr Letizia Granieri

Board Certification in Clinical and Analytical Biochemistry: Dr. Fabiana Marnetto, Dr Marzia Caldano, Dr Paola Valentino, Dr Simona Perga, Dr Nicole D. Navone

Master in Medical and Genomic Statistics: Dr. Serena Martire

### Please list honours, prizes or awards received

Member of the jury “Neurology Merck Prize 2016”

### **Outreach activities**

- **International collaborative experiences.**

University of Munchen (anti-KIR antibodies);

Muenster (European Bio-bank and LSelectin);

MAGE (European project for antibodies anti-biological drugs);

Tel Aviv (SNPS for prevention of PML in Natalizumab treated patients)

San Francisco – Prof. Oksenberg (Evaluation of responsiveness in Fingolimod treated patients)

- **Invited talk**

3 febbraio 2017, Orbassano (TO), ”Le mille facce della SM”

10 marzo 2017, Torino, ESCO “ESCO ESperienze e CONdivisione nella gestione del paziente con SCLEROSI MULTIPLA”

24 marzo 2017, Gallarate, “Approfondimenti monotematici nella Sclerosi Multipla fattori genetici ed ambientali: esistono strategie di prevenzione?”

3-4 aprile 2017, Boston, Advisory Board Genzyme

13-14 aprile 2017, Caltanissetta, “Sicily Multiple Sclerosis Update: between reality and hope”

3 maggio 2017, Roma, ISHEO

10-11 maggio 2017, Milano, BEMS

18 maggio 2017, Torino, IMUSE

18-20 maggio 2017, Padova, 53° Congresso AINPeNC – 43° Congresso AIRIC

24-25 maggio 2017, Firenze, “Il ruolo dei biomarcatori nella pratica clinica nella SM”

29-31 maggio 2017, Roma, AISM

6 giugno 2017 Torino, “Approfondimenti sulla mindfulness: dalla neurobiologia alle applicazioni cliniche”

8-10 giugno 2017, Baveno, 15° ED. TOP SEMINARS

12-14 giugno 2017, Orbassano (TO), “La gestione quotidiana del paziente con sclerosi multipla”

20-21 settembre 2017, Roma, Let’z Bright

28-29 settembre 2017, Cagliari, “Quarant’anni di Sclerosi Multipla: un party scientifico”

3-4 ottobre 2017, Roma, J5983 Stand Alone

14-17 ottobre 2017, Napoli, SIN

25-28 ottobre 2017, Parigi, ECTRIMS

3 novembre 2017, Torino, “La terapia con Natalizumab nella SM: 10 anni di esperienze a confronto”

10 novembre 2017, Pollenzo, “Management della gravidanza in SM”

15-16 novembre 2017, Milano, “Le urgenze neurologiche”

24-25 novembre 2017, Aosta, V Congresso triregionale SIN SNO

27 novembre 2017, Milano, BOCCONI

4 dicembre 2017, Milano, EPICA

14-15 dicembre 2017, Torino, “PROGETTO BUSSOLA: Patient-oriented nursing experience in MS

- **Editorial duties**

“Multiple Sclerosis International” since 2012

“Progress in Neuroscience” since 2012

“Dataset Papers in Neuroscience” dal 2012

“Journal of Multiple Sclerosis” since 2014

“Neurology and Therapy” since 2014

“Multiple Sclerosis and Demyelinating Disorders” since 2015

Member of the Scientific Committee of AISM 1997-1998; 2010-2013

- **Workshops, Schools or Conferences organized**

3 febbraio 2017, Orbassano (TO), "Le mille facce della SM"

12-14 giugno 2017, Orbassano (TO), "La gestione quotidiana del paziente con Sclerosi Multipla"

10 novembre 2017, Pollenzo, "Management della gravidanza in SM"

14-15 dicembre 2017, Torino, "PROGETTO BUSSOLA: Patient-oriented nursing experience in MS"



## 2. PI's PUBLICATIONS:

Spadaro M, Montarolo F, Perga S, Martire S, Brescia F, Malucchi S, Bertolotto A. Biological activity of glatiramer acetate on Treg and anti-inflammatory monocytes persists for more than 10years in responder multiple sclerosis patients. *Clin Immunol.* 2017 Aug;181:83-88. doi: 10.1016/j.clim.2017.06.006. Epub 2017 Jun 19. IF 3.999. R = 36/161

Marnetto F, Valentino P, Caldano M, Bertolotto A. (2017) Detection of potassium channel KIR4.1 antibodies in Multiple Sclerosis patients. *J Immunol Methods*, 445:53-58. IF: 2.1. R = 91/161

Valentino P, Marnetto F, Granieri L, Capobianco M, Bertolotto A. (2017) Aquaporin-4 antibody titration in NMO patients treated with rituximab: A retrospective study. *Neurol Neuroimmunol Neuroinflamm.* 15;4(2).

Clemente N, Comi C, Raineri D, Cappellano G, Vecchio D, Orilieri E, Gigliotti CL, Boggio E, Dianzani C, Sorosina M, Martinelli-Boneschi F, Caldano M, Bertolotto A, Ambrogio L, Sblattero D, Cena T, Leone M, Dianzani U, Chiocchetti A. Role of Anti-Osteopontin Antibodies in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis. *Front Immunol.* 2017 Mar 23;8:321.IF= 6.429; R= 19/161

Caldano M, Raoul W, Rispens T, Bertolotto A. Drug Efficacy Monitoring in Pharmacotherapy of Multiple Sclerosis With Biological Agents. *Ther Drug Monit.* 2017 Aug;39(4):350-355.IF=2.178; R= 88/230

Amato MP, Bertolotto A, Brunelli R, Cavalla P, Goretti B, Marrosu MG, Patti F, Pozzilli C, Provinciali L, Rizzo N, Strobelt N, Tedeschi G, Trojano M, Comi G.(2017) Correction to: Management of pregnancy-related issues in multiple sclerosis patients: the need for an interdisciplinary approach. *Neurol Sci.* Dec 26 IF=1.74; R=clinical neurology 136/194; R= neurosciences 206/259

Carletto S, Tesio V, Borghi M, Francone D, Scavelli F, Bertino G, Malucchi S, Bertolotto A, Oliva F, Torta R, Ostacoli L. (2017) The Effectiveness of a Body-Affective Mindfulness Intervention for Multiple Sclerosis Patients with Depressive Symptoms: A Randomized Controlled Clinical Trial. *Front Psychol.* Nov 30;8:2083. IF= 2.32; R=33/129

Rocca MA, Sormani MP, Rovaris M, Caputo D, Ghezzi A, Montanari E, Bertolotto A, Laroni A, Bergamaschi R, Martinelli V, Comi G, Filippi M. (2017) Long-term disability progression in primary progressive multiple sclerosis: a 15-year study. *Brain* Nov 1;140(11):2814-2819. IF=10.29; R= clinical neurology 4/194; R= neurosciences 13/259.

Franciotta D, Gastaldi M, Sala A, Andreetta F, Rinaldi E, Ruggieri M, Leante R, Costa G, Biagioli T, Massacesi L, Bazzigaluppi E, Fazio R, Mariotto S, Ferrari S, Galloni E, Perini F, Zardini E, Zuliani L, Zoccarato M, Giometto B, Bertolotto A. (2017). Diagnostics of the neuromyelitis optica spectrum disorders (NMOSD). *Neurol Sci.* 2017 Oct;38(Suppl 2) Review. IF=1.74; R=clinical neurology 136/194; R= neurosciences 206/259.

Gastaldi M, Zardini E, Leante R, Ruggieri M, Costa G, Cocco E, De Luca G, Cataldo I, Biagioli T, Ballerini C, Castellazzi M, Fainardi E, Pettini P, Zaffaroni M, Giunti D, Capello E, Bernardi G, Ciusani E, Giannotta C, Nobile-Orazio E, Bazzigaluppi E, Passerini G, Bedin R, Sola P, Brivio R, Cavaletti G, Sala A, Bertolotto A, Desina G, Leone MA, Mariotto S, Ferrari S, Paternoster A, Giavarina D, Lolli F, Franciotta D. (2017). Cerebrospinal fluid analysis and the determination of oligoclonal bands. *Neurol Sci.* Oct;38(Suppl 2):217-22. Review IF=1.74; R=clinical neurology 136/194; R= neurosciences 206/259.

Ghezzi A, Bianchi A, Baroncini D, Bertolotto A, Malucchi S, Bresciamorra V, Lanzillo R, Milani N, Martinelli V, Patti F, Chisari C, Rottoli M, Simone M, Paolicelli D, Visconti A; FUTURE Study Group. (2017).A multicenter, observational, prospective study of self- and parent-reported quality of life in adolescent multiple sclerosis patients self-administering interferon- $\beta$ 1a using RebiSmart™-the FUTURE study. *Neurol Sci.* Nov;38(11):1999-2005. IF=1.74; R=clinical neurology 136/194; R= neurosciences 206/259.

Amato MP, Bertolotto A, Brunelli R, Cavalla P, Goretti B, Marrosu MG, Patti F, Pozzilli C, Provinciali L, Rizzo N, Strobelt N, Tedeschi G, Trojano M, Comi G. (2017). Management of pregnancy-related issues in multiple sclerosis patients: the need for an interdisciplinary approach. *Neurol Sci.* Oct;38(10) Erratum in: *Neurol Sci.* 2017 Dec 26;: IF=1.74; R=clinical neurology 136/194; R= neurosciences 206/259.

Annovazzi P, Bertolotto A, Brescia Morra V, Gasperini C, Montanari E, Navarra P, Patti F, Sormani MP, Ghezzi A. (2017) A Comprehensive Review on Copemyl®. *Neurol Ther.*Dec;6(2):161-173. Review R=94/138

Melillo F, Di Sapio A, Martire S, Malentacchi M, Matta M, Bertolotto A (2017) Computerized posturography is more sensitive than clinical Romberg Test in detecting postural control impairment in minimally impaired Multiple Sclerosis patients. *Mult Scler Relat Disord.* May;14:51-55. IF=4.84, R=clinical neurology 27/194; R= neurosciences 46/259.

Paintaud G, Passot C, Ternant D, Bertolotto A, Bejan-Angoulvant T, Pascual-Salcedo D, Mulleman D. (2017) Rationale for Therapeutic Drug Monitoring of Biopharmaceuticals in Inflammatory Diseases. *Ther Drug Monit.* Aug;39(4):339-343. IF=2.17; R= toxicology 53/92, R= pharmacology 153/257, R= medical laboratory 15/30

Gils A, Bertolotto A, Mulleman D, Bejan-Angoulvant T, Declerck PJ. (2017) Biopharmaceuticals: Reference Products and Biosimilars to Treat Inflammatory Diseases. *Ther Drug Monit.* Aug;39(4):308-315. IF=2.17; R= toxicology 53/92, R= pharmacology 153/257, R= medical laboratory 15/30

Landi D, De Rossi N, Zagaglia S, Scarpazza C, Prosperini L, Albanese M, Buttari F, Mori F, Marfia GA, Sormani MP, Capra R, Centonze D; Italian PML study group. (2017) No evidence of beneficial effects of plasmapheresis in natalizumab-associated PML. *Neurology.* Mar 21;88(12):1144-1152. IF= 8.32; R= 9/194

Malucchi S, Capobianco M, Lo Re M, Malentacchi M, di Sapio A, Matta M, Sperli F, Bertolotto A. (2017) High-Risk PML Patients Switching from Natalizumab to Alemtuzumab: an Observational Study. *Neurol Ther.* Jun;6(1):145-152. R= 94/138

#### 4. GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2016 -. Please indicate the journal IF, ranking)

Please use the following format throughout:

#### 5. GROUP's additional information:

List of the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2015-2018	National	Marnetto F	FISM	Studio dell'espressione di geni dei virus di Epstein Barr e geni cellulari in pazienti con CIS, SM, recidivante remittente e SM primaria progressiva: ricerca di biomarcatori diagnostici e prognostici		€51950	10%
2014-2017	national	Perga S.	FISM	"Ruolo della deubiquitinasi TNFAIP3/TNFAIP3 nell'immuno-patologia della SM".	FISM code 2014/R/14	100.000	5% <sup>#</sup>

<sup>#</sup>The financial management of the project was in charge to the administration of FISM, but the research was performed mainly at NICO. The project overhead (5%) and scientific instruments funded by the project are located at NICO and are available to all the researcher of NICO.

#### Please list honours, prizes or awards received by other members of the group:

Dr. Montarolo:

Travel grant for the 33th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), Paris (France) 2017

Dr. Martire:

Travel grant for the 33th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), Paris (France) 2017

#### Please list outreach activities of other members of the group:

Dr. Simona Perga:

- Official speaker for "More than neurons" congress, Turin (1-3 December 2017).

Dr. Caldano, Marnetto, Martire, Montarolo, Perga, Sala, Spadaro, Valentino:

- Official speaker for MS patients' care courses at CRESM: "La gestione quotidiana del paziente con Sclerosi Multipla" organized by Biogen Idec (06/2017).

Dr. Paola Valentino:

Official speaker for MS course: "Corso teorico-pratico di aggiornamento in sclerosi Multipla"

Dr. Michela Spadaro:

Immunobiologia della gravidanza, Pollenzo 10 November 2017

## 6. Past Research activity

(Summarize the PI and group research activities in 2017)

### **DIAGNOSTIC ACTIVITY**

The Clinical Neurobiology Laboratory is housed at NICO and is part of SCDO Neurologia- Centro di Riferimento Regionale Sclerosi Multipla CRESM in the San Luigi Gonzaga Hospital; CRESM, directed by PI is the core of a collaborative network with all the neurological divisions and clinics for Multiple Sclerosis (MS) patients in Italy.

The Clinical Neurobiology Laboratory deals with routine Cerebrospinal fluid (CSF) analysis from AOU San Luigi Gonzaga patients and from part of Piedmont neurologies. Even if CSF analysis is no more required for MS diagnosis, it is still important to offer diagnostic and prognostic information and to rule out differential diagnoses. Furthermore, it is important to provide an important research tool. In 2017, 297 medical reports were produced.

Detection of anti-AQP4 and anti-MOG Antibodies on serum samples by immunofluorescence assay and FACS assay respectively were performed for differential diagnosis with NMO disease (616 medical reports for anti-AQP4 Antibodies and for anti-MOG antibodies).

Furthermore, our lab is focused in the evaluation of treatment-response biomarkers in MS treated patients to monitor the biological response to therapy and identify risk factors (see Research activity and future project).

In particular:

- IFN-beta treatment. IFN-Beta treatment can induce the production of binding and neutralizing antibodies (NABS). Our center is the once in Italy deputed to detect NABs in Italy since 2002. The method used for Nabs titration is a Cytopathic effect assay. Correspondingly, biological activity of IFN-Beta is evaluated by measuring mRNA MxA expression in PBMC, by real-time PCR. Patients showing NABS and/or low biological activity are shifted to other treatments. In 2017, 530 medical reports were produced.
- Natalizumab. A small proportion of NTZ treated patients develop persistent anti-drug antibodies, which are associated with an increase in infusion-related adverse events. Our center is the once in Italy deputed to detect anti-NTZ antibodies in Italy: the analysis is performed by using an ELISA assay (567 medical reports).
- Rituximab (RTX). In 2016, we started the diagnostic quantification of anti-RTX antibodies and free circulating RTX to personalize RTX re-treatment in NMOSDs patients. These tests are performed by ELISA assays (in 2017, 105 medical reports).

### **RESEARCH ACTIVITY**

The research activity of the Clinical Neurobiology Lab covers several topics of MS:

1. Immunopathogenesis
2. Biomarkers for Multiple Sclerosis
3. CRESM Bio-Bank

#### **1) IMMUNOPATHOGENESIS**

##### **a. Summary (up to 500 characters)**

MS is an autoimmune disease characterized by inflammation. We showed down-regulation of two potent NF-KB inhibitors (TNFAIP3 and NR4A2) in blood cells from MS patients, which might contribute to autoimmune processes. Thus, we first aimed to elucidate how an altered expression of these genes could influence MS pathogenesis both in immune and brain resident cells.

##### **b-d. Background, rationale and objectives**

TNFAIP3 and NR4A2 are key molecules in inflammation and immunity. Our group demonstrated a TNFAIP3 and NR4A2 down-regulation in whole blood and peripheral PBMCs of MS patients, mainly affecting circulating monocytes. Consistently, macrophages (MO) of MS patients showed increased

activation of NF- $\kappa$ B pathway. Different studies have highlighted an important role of monocytes and monocyte-derived cells such as macrophages and dendritic cells (DCs) in MS pathogenesis. However, the contribution of TNFAIP3 and NR4A2 gene expression in different human cell populations to autoimmune diseases is still unclear.

Since monocytes, MO, DCs and microglia have a central role in inflammation of MS, we planned to examine whether the altered expression of TNFAIP3 and NR4A2 in myeloid and brain resident cells can influence MS inflammation. Furthermore, we were interested in these molecules as potential therapeutic targets.

The aims of our study were:

1. To examine the localization of TNFAIP3 and NR4A2 in control cases (CC) and MS human post-mortem brain. We aimed to compare TNFAIP3 and NR4A2 protein and gene expression in both active (AL) and chronic active lesions (CAL) and normal appearing areas from white (WM) and grey matter (GM) of post-mortem secondary and primary progressive MS. By using double immunofluorescence we intended to verify the expression of these genes in immune and CNS cell populations in human brain tissues.
2. To evaluate the possible effect of FTY720 on NR4A2 gene expression in blood obtained from MS patients.
3. To investigate the impact of reducing TNFAIP3 expression in a mouse model of MS. To this aim we used transgenic mice lacking TNFAIP3 in myeloid-derived cells including microglia or only in mDCs. These mice were immunized with the myelin oligodendrocyte glycoprotein (MOG<sub>35-55</sub>) to induce the EAE, the best model of chronic MS. The role of NR4A2 in the MS murine model has been previously investigated by our group and results are already published.

#### **e. Results (4000 characters)**

1. To unveil the contribution of TNFAIP3 and NR4A2 in the CNS MS pathology, we studied their expression in human post-mortem MS brain tissues, obtained by the United Kingdom MS Tissue Bank at Imperial College of London. In the previous year we demonstrated that TNFAIP3 is present in control human brain tissues in both WM, in few parenchymal astrocytes and in GM, in neuronal cells. In MS brain, we observed a massive TNFAIP3 expression in the WM lesions in both perivascular infiltrates and ramified cells. In particular, in active and pre-active lesions, TNFAIP3 is expressed in the active core of AL, whereas in CAL, TNFAIP3 is mainly expressed on the active-margin. Double immunofluorescence staining unveiled that TNFAIP3 is expressed infiltrating macrophages and by resident astrocytes and a subpopulation of microglial cells. During this year, we observed that TNFAIP3 was also present in the normal appearing (NA) GM (NAGM) of MS patients in the perinuclear area of a sub-population of neurons and by few ramified cells apparently similar to GM astrocytes, as occurs in the NAWM. In active GM lesions, TNFAIP3 was still present in the nuclear area of some neurons but particularly it was massively expressed by a huge number of astrocytes making an intricate network resembling astrocytosis.

In order to characterize the expression of NR4A2 in human brain, immunohistochemistry analysis has been performed in post-mortem brain tissues obtained from CC. NR4A2 immunoreactivity has been detected in morphologically ramified cells in WM parenchyma and near to blood vessel indicating its expression in astrocytes and microglia. Particularly, in GM parenchyma also NR4A2 + ramified and morphologically-resembling neurons are present. To evaluate a direct role of NR4A2 expressing cells in MS, brain tissues obtained from MS patients have been analyzed. In details, NR4A2 staining has been highlighted in SP and PPMS human post-mortem brain tissues in morphologically ramified cells without differences between them. Particularly, NR4A2 immunoreactivity is present in NAWM and NAGM of MS, indicating that also in brain obtained from MS patients, NR4A2 is ubiquitously expressed. Furthermore, we have not found alterations in NR4A2 expression in AL and CAL.

2. In this study, naïve and FTY720-treated RRMS patients (from 8 to 60 months) were evaluated in order to understand both short- and long-term influence of FTY720 on the NR4As gene expression level. Here, we observed for the first time that FTY720 is able to up-regulate the NR4As transcripts in peripheral blood obtained from MS patients. Additionally, it should be kept in mind that this

analysis was restricted only to the cohorts of FTY720 responder patients. In fact, any patient who seems to be a FTY720 non-responder is immediately shifted to another treatment regimen at the CReSM center due to clinical and ethical reasons. Hence, such individuals are not a part of the present study. Based on this, we can suppose that FTY720 induces the increase of NR4As transcript level which might be a necessary, if not sufficient condition, for a clinical response to treatment.

3. The TNFAIP3<sup>lox</sup>::CX3CR1 murine model was generated by crossing the TNFAIP3<sup>lox/lox</sup> mice with the transgenic mice carrying the Cre recombinase under the control of a specific promoter for myeloid cells including microglia (CX3CR1). Since the TNFAIP3<sup>lox</sup>::CX3CR1 mouse has never been generated before, we characterized their phenotype (TNFAIP3<sup>lox/wt</sup>::CX3CR1 heterozygous HT and TNFAIP3<sup>lox/lox</sup>::CX3CR1 homozygous KO) compared to their WT littermates (TNFAIP3<sup>wt/wt</sup>::CX3CR1). Anatomic-pathological studies were performed in order to evaluate the impact of reducing TNFAIP3 in myeloid cells. First, we observed that the HT and the KO mice are viable, but, although the former are fertile, the latter have a mortality rate of 50%. Female KO had a lower weight compared to both HT and WT, starting already from one month of age. The HT transgenic mice displayed a decreased weight compared to WT starting from two months of age. Conversely, we did not observe the same results with male mice. Histological studies revealed some altered features in spleen, liver and glands obtained from 2 months old KO mice. Particularly, a massive hypertrophy of the spleen with expansion of the red pulp showing an increased extramedullary hematopoietic process with various degrees of normal differentiation were highlighted in KO mice. Also, spleen architecture resulted altered by a partial substitution of the white pulp sheaths of lymphoid cells by myeloid cells. Furthermore, liver reported sinusoid dilatation and massive periportal and centro-lobular inflammatory infiltrate. Finally, salivary gland of KO mice showed hyperplasia and prominent inflammatory infiltrate surrounding the intercalated ducts. The phenotypical analysis performed in this murine model suggested that the deficiency of TNFAIP3 expression in myeloid cells is involved in multi-organs alterations.

#### **f. Advancement in the field (1000 characters)**

The final purpose of this study is to determine whether a TNFAIP3 and NR4A2 altered expression in immune cells is involved in the pathogenesis of MS and to identify molecules able to modulate their gene expression. As a whole, results of the present study might lead to a better understanding of the mechanism by which TNFAIP3 and NR4A2 influence inflammation and autoimmunity. This might result, in turn, in a better definition of treatment strategies for this condition. The activity of these anti-inflammatory genes or molecules in their pathway could be boosted to tone down the inflammatory component of MS; this may provide the basis for the development of novel therapeutic strategies.

## **2. BIOMARKERS FOR MULTIPLE SCLEROSIS**

### **2.1. Anti-KIR4.1 Antibodies in MS patients**

#### **a. Summary (up to 500 characters)**

KIR4.1 antibodies were specifically found in a subset of MS patients. This could have a significant impact on disease management. The original procedure for isolating pure KIR4.1 and for detecting KIR4.1 antibodies in serum is too complex and too variable to be applied in diagnostic routine. The current phase of this project aims at studying the oligodendrocytic cell line MO3.13 as a putative new source for KIR4.1.

#### **b-d. Background, rationale and objectives**

The presence of KIR4.1 antibodies could have a significant impact on disease management in MS. However, a validation of this claim is lacking due to challenges including isolation of LG-KIR4.1, the putative target antigen of autoantibodies expressed in oligodendrocytes. The first aim of this project was to verify the presence of KIR4.1 antibodies in MS patients. We independently replicated the original procedure and found anti-KIR4.1 antibodies in 28% of MS patients and 5%

of controls (Marnetto F et al, JIM 2017). Nevertheless, this procedure is not suitable for diagnostic routine given its excessive complexity and variability attributable in part to the usage of HEK cell line. We attempted to resolve issues related to the reproducibility of this method by identifying a new reliable source of KIR 4.1. We selected the human oligodendrocyte MO3.13 cell line in this regard. Given that KIR4.1 is an immunogen extensively expressed by oligodendrocytes, MO3.13 could represent a reliable source for its purification. Therefore, our revised aims in this regard were, 1) To characterize MO3.13 cells as oligodendrocytic precursors and their differentiation in mature cells post 7 days in vitro; 2) To study KIR4.1 expression in non-differentiated and differentiated MO3.13 cells; 3) To evaluate the reactivity of the positive and negative sera (as per ELISA test) against MO3.13 cells.

#### **e. Results**

Human MO3.13 cells matured to an oligodendrocytic phenotype upon culturing in serum free medium in the presence of 100nM 4-b-phorbol-12-myristate-13-acetate (PMA) for 7 days. The differentiation of MO3.13 OPCs into more mature oligodendrocytes was evaluated from a morphological and biochemical aspect.

IIF analysis showed that KIR4.1 expression is significantly increased in MO3.13 cells after 7 days of PMA treatment, as detected with both commercial antibody and with mAb20F9 that is specific for immunogen KIR4.1. Western blot analysis confirmed the increased KIR4.1 expression in differentiated cells.

Finally, serum samples from MS patients (positive for KIR4.1 Abs in our ELISA test) showed an intense reactivity against differentiated MO3.13 cells, compared to serum from HCs.

#### **f. Advancement in the field**

1. KIR4.1 Abs are present in a subgroup of MS patients. 2. The ELISA test described by Srivastava and colleagues in 2012 is not suitable for routine diagnostic practice, due to technical problems related to KIR4.1 expression and isolation from HEK cells. 3. Differentiated MO3.13 cell line could represent a new source of the immunogen KIR4.1. 4. Serum samples from MS patients and HC showed a different immunoreactivity on differentiated MO3.13 cells. 5. Further studies are needed to standardize the isolation and purification of the antigen for antibody quantification along with confirmation of true serum reactivity against KIR4.1.

### **2.2. Improving therapeutic appropriateness of Multiple Sclerosis treatments using biomolecular approaches to personalize therapy and save pharmaceutical spending**

#### **a) Summary (500 characters)**

Non-responding patients may undergo irreversible disease progression and potentially severe outcomes during ineffective treatments; the overall cost of failed treatment puts tremendous pressure on public health budgets. Approved and future therapies for Multiple Sclerosis (MS) are very expensive while the National Health Service (NHS) fund is getting slimmer every year. We propose a strategy to improve therapeutic appropriateness by using biological approaches, in particular:

1. early identification of biological non responders (NRs) to different approved treatments
2. better timing of drug administration, based on serum drug levels or specific drug biomarkers
3. study of the relationship, if any, between T-cells adhesion-molecule expression and the risk to develop serious adverse events during treatment.

#### **b) Background (2000 characters) and Rationale**

All drugs for MS treatment are very expensive, ranging from 7000 to 20000 €/year/patient. As over 30,000 patients (pts) are under treatment in Italy (50% of the MS population) the NHS total expense is more than 400 million €/year.

Early identification of NRs to treatment is a milestone to improve appropriateness and save or better allocate a huge amount of money.

1. A well-known biological mechanism that causes lack of response is the development of specific and persistent Anti Drug Antibodies (ADA). Our MS Clinic is the Italian referral center for ADA against IFN $\beta$  and Natalizumab (NAT). A new IFN $\beta$  formulation (IFN $\beta$  PEGylated) [3] will be available next year and its immunogenicity, biological activity and cross-reactivity with old IFN $\beta$ s must be investigated.

2. A strategy to improve appropriateness is to tailor time and dose of infusion for the single patient. This approach can be applied to NAT and Rituximab (and other anti-CD20 drugs) that are infused at fixed schedule. Quantification of blood drug concentration and/or of specific biomarkers allows personalized treatment.

3. CD62L is a potential biomarker for the individual risk of progressive multifocal leukoencephalopathy (PML) in MS pts

**c) Objectives (1500 characters)**

Treatment efficacy depends on drug availability and its biological activity. Dose and timing of infusion can be personalized.

Biological marker can stratify the risk of adverse events. Consequently, quantification of drugs level, ADA, biological activity and biomarkers can early identify non-responders patients, improve treatment efficacy and reduce the risk of adverse events. These biological methods can improve allocation of a large amount of pharmaceutical spending.

- Early detection of non-responders. Detection and titration of Binding/Neutralizing Antibodies (BAbs/NAbs) against NAT and RTX will be performed. Cross reactivity between NABs against IFN $\beta$  and PEG-IFN $\beta$  and between different anti-CD20 monoclonal will be evaluated. The biological activity of PEG-IFN $\beta$  will be tested by analyzing mRNA expression of a specific biomarker. Biological data will be correlated with the responsiveness to the treatment measured by EDSS, MRI and clinical activity. An economic analysis will be performed to evaluate the therapeutic appropriateness.
- Optimization of dose and time of infusion of NAT and RTX. NAT and RTX responders are defined as patients without clinical activity and without new MRI lesions. NAT and RTX serum concentration in samples stored in our bio-bank will be correlated with responsiveness. The frequency of RTX infusion will be decided according to the number of circulating CD19+ and CD27+ B cells detected by FACS and by a new molecular (RT-PCR and Droplet digital PCR) approach set up in our center. This latter method could be used also for the new anti-CD20 drugs. The cost of traditional infusions will be compared with the optimized procedures.
- To analyze L-selectin expression in T-cells to detect the risk to develop Progressive Multifocal Leukoencephalopathy (PML). In fact, the major adverse event of NAT is PML that involves about 1 out of 1000 patients. The risk of PML limits the use of the highly effective NAT and PML early detection and management are very expensive. Recently, Schwab et al. investigated the influence of NAT on adhesion molecules in T-cells and found a very low expression of L-selectin in patients who will develop PML.

**d) Results**

1. Collection of samples from patients treated with IFN beta and Natalizumab: 200 serum and RNA samples from IFN beta treated patients have been collected to evaluate respectively ADA and biological activity; 50 Natalizumab treated patients have been enrolled to collect serum samples every month for 12 months to evaluate ADA and drug
2. Set up of Flow cytometry based assay for the quantification of NAT serum levels: a new FACS assay to evaluate Natalizumab drug level has been developed. The protocol has been set and, currently, the method validation phase is ongoing
3. Collection of samples for Rituximab ADA and drug level. 50 SM patients Rituximab treated has been enrolled and serum sample has been collected. Two different ELISA assays are performed to detect both ADA and drug level: currently, almost 500 serum samples have been evaluated for the drug level and 100 for ADA presence
4. Set up and validation of a droplet digital PCR based assay for the quantification of CD19 mRNA.



Set up: 100 monthly-collected blood samples from 3 NMOSD patients have been analysed to set up the ddPCR assay. Preliminary data from this first phase showed that ddPCR is a more sensitive technique to detect CD19 positive B cells compared to flow cytometry. The second phase of the project consists in the validation of this assay: 150 blood samples from 10 NMOSD patients and 100 blood samples from 10 MS patients treated with RTX have been already collected and will be analysed.

**e) Advancement in the field (1000 characters)**

Results obtained in this project may lead to the set up of biological assays that can improve the efficacy of treatment, selecting the best drug for each patient, and save, or better allocate, enormous amounts of NHS funds.

**2.3 “Evaluation of biological activity by RNA-sequencing in PEG-Interferon beta 1a and Interferon beta 1a in treated Multiple Sclerosis patients and comparison of genetic expression between different cellular populations”**

**a) Summary (500 char)**

IFNbeta has been a preferred first-line therapeutic option for the treatment of the RRMS for over 20 years. Has been demonstrated that the new pegylated form of IFNbeta 1a has the same clinical effect of the non-pegylated form, but the frequency of administration and the adverse events are reduced. However, the evaluation of the whole transcriptome, using the Next Generation Sequencing Technology, is needed to better understand the mechanism of action of the 2 forms of IFNbeta and to identify specific biomarkers able to predict the treatment response.

**b) Background (2000 characters) and Rationale**

Several products containing Interferon Beta (IFNβ) were already approved for multiple sclerosis (MS) treatment. It has been shown that the new pegylated form of Interferon beta 1a (Plegridy) has the same clinical and radiological effects of the Non-Pegylated im one (Avonex) although it is administrated every 2 weeks instead of ones a week. The exact mechanism of action of interferon beta in MS is not completely known. To better understand the mechanism of action of a drug it is necessary to study its biological activity. Biological activity of a drug includes the total effects determined by the interaction of the molecule with its target receptor: a drug without biological activity is not clinical effective. The measurement of IFNβ biological activity in every single patient can allow the identification of the subset of patients who are non-responsive to the drug. Till now, the biological activity of IFNβ has been studied by measuring a number of Interferon Stimulated Genes (ISGs) at protein or mRNA level normally using real-time PCR. The most current and reliable method to evaluate ISGs is the RNA-seq (RNA Sequencing) that uses the capabilities of Next-Generation Sequencing to reveal a snapshot of RNA presence and quantity from a genome at a given moment in time.

This study is a pilot study that aims to evaluate biological activity in two groups of MS patients treated with Plegridy and Avonex respectively at different time points using the NGS technology.

**c) Objectives (1500 characters)**

Primary:

The primary objective of the study is the comparison of biological activity between Plegridy and Avonex in RRMS patients naïve to treatment.

Additional:

The additional objectives of this study are as follows:

1. The comparison of biological activity between Plegridy and Avonex in subsets of cellular populations
2. The evaluation of pharmacological biomarker
3. The identification of biomarker(s) for the evaluation of biological activity and the treatment adherence.

If the comparison will show differences in T-cells and monocytes gene expression, the level of expression of IFNAR1 on surface of the two cell types will be determined to identify or rule out this as a potential reason for differences.

#### **d) Results**

21 patients have been enrolled and 160 samples has been collected. Currently, 70 samples have been analyzed using NGS Technology.

#### **e) Advancement in the field (1000 characters)**

Data obtained from this pilot study can help to better understand IFN $\beta$  pharmacodynamic, which cellular subset is most influenced by treatment and the efficacy of treatment for every single patient.

### **2.4 “Side effects of long-term rituximab treatment in NMOSD patients: hypogammaglobulinemia and impairment of specific humoral immunity.”**

#### **a. Summary (up to 500 characters)**

Rituximab (RTX) is widely used in the treatment of many systemic autoimmune diseases. It is an effective therapy in Neuromyelitis Optica Spectrum Disorders (NMOSD) as well. Patients of several hematological and rheumatological diseases demonstrate major side effects including hypogammaglobulinemia after a prolonged treatment with RTX. There are no such studies with long and detailed follow up in patients of neurological disorders treated with RTX. We evaluated total IgG, IgA and IgM, AQP4 antibodies titers and levels of 3 pathogen-specific antibodies in 15 NMOSD patients and in 6 controls. Long-term RTX treatment is associated with the risk of Hypo-Ig and reduction of vaccine protection in NMOSD patients.

#### **b-d. Background, rationale and objectives**

RTX is used in the treatment of many systemic autoimmune diseases including NMOSD. Major side effects such as hypogammaglobulinemia (Hypo-Ig) have been reported after prolonged RTX treatment. However, till date there are no studies with long and detailed follow up of RTX treated NMOSD patients. We sought to characterize the qualitative and quantitative changes in humoral immunity in NMOSD patients during a sustained RTX therapy through the evaluation of total IgG, IgA and IgM levels and on levels of 3 pathogen-specific antibodies. To our knowledge, this is a first study of its kind involving RTX in NMOSD field with a long follow-up period, systematic measurements and relatively large number of patients under study.

#### **e. Results.**

RTX reduced total Ig levels in patients treated for a long term (annual % of reduction: 4.7% for IgG, 4.4% for IgA and IgM). Hypo-IgG (IgG<7g/l) developed in 11/15 patients and 46% of tested samples. One patient developed infective complications (IgG=3.7 g/l).

A strong correlation was found between IgG levels and AQP4-Ab titers ( $p < 0.0001$ ).

The levels of anti-tetanus, anti-VZV and anti-EBNA IgG in RTX treated patients were significantly lower than the ones measured in HC. No significant differences were observed when comparing patients and controls on the basis of the temporal modification of these specific IgG levels. Anti-tetanus IgG was found to be reduced below the protection threshold in 1/16 patients during RTX treatment and anti-tetanus IgG half-life showed an important reduction trend.

#### **f. Advancement in the field**

Long-term RTX treatment is associated with the risk of Hypo-Ig and reduction of vaccine protection in NMOSD patients. Anti-CD20 therapy requires evaluation of the level of total and specific Igs prior to and during treatment to prevent Hypo-Ig related complications and ensure better overall outcomes. In addition, data obtained in this project could give important information about treatment with anti-CD20 drugs in other autoimmune neurological disorders such as Multiple Sclerosis.

## **2.5 “Combined analysis of EBV and cellular gene expression in clinically isolated syndrome, relapsing-remitting and primary progressive multiple sclerosis for the identification of diagnostic and prognostic biomarkers”**

### **a. Summary (up to 500 characters)**

The hunt for biomarkers to predict MS disease evolution and identify patient subsets that may benefit from specific therapeutic regimens is a continuous effort in MS research. Because there is increasing evidence that EBV infection is strongly associated with MS and that the immune response to EBV differs both qualitatively and quantitatively in MS patients compared to healthy controls, a reasonable hypothesis to test is that EBV deregulation might be implicated in the dysimmune process that damages the CNS. From this it follows that combining the study of EBV infection status with that of the host's immune system might help shed light into disease relevant virus-host interactions and identify biomarkers with predictive value.

### **b-d. Background, rationale and objectives**

In a previous FISM funded multicenter study that involved the same partners of this project (PI and 3 MS centers at S. Luigi Gonzaga Hospital/Orbassano, Florence and Cagliari Universities) we have used for the first time pre-amplification real-time RT-PCR (Taqman technology) to investigate EBV infection status and immune activation in paired CSF cell and PBMC samples from therapy-free patients with relapsing remitting MS (RRMS) (n = 31) and have correlated the transcriptional profiles with demographic, clinical, laboratory and MRI data. The main results of this study are: 1) establishment of the methodology for quantification of 7 EBV and 41 cellular transcripts; 2) detection of EBV transcripts in 16% of RRMS patients, with 9.7% of CSF samples (3/31) and 6.9% (2/29) of PBMC samples showing EBV deregulation (disruption of EBV latency or productive infection); 3) identification of an immune signature associated with peripheral EBV reactivation in relapsing MS; 4) poor correlation between systemic and intrathecal transcriptional profiles; 5) identification of a set of genes associated with innate immunity (type-I IFN, pro-inflammatory macrophages) that are differentially expressed in CSF and discriminate with high accuracy a group of remitting patients (n = 6; all females devoid of detectable EBV signals in CSF and PBMC, 5 of 6 MRI inactive) and a larger group (n = 24) comprising male/female, relapsing/remitting, MRI active/inactive, and EBV+/EBV- patients. This project builds on the established collaboration, pre-Amp real time RT-PCR methodology and encouraging results obtained in the preliminary study to perform a combined analysis of viral and cellular transcriptional profiles in CSF and PBMC samples obtained at diagnosis from a larger MS cohort including patients with RRMS (n = 60), primary progressive MS (n = 20) and clinically isolated syndrome (n = 30). All patients will undergo a complete diagnostic work-up and follow-up (3 years) with serial clinical/MRI examinations and PBMC gene expression analysis. The main goal of this study is to identify transcriptional signatures that may be useful to discriminate patient subgroups early in the course of MS, predict disease evolution or response to therapy.

### **e. Results.**

The Project is still ongoing. In 2017, our Center finished the enrollment of patients (40 patients enrolled). In addition, RNA extraction from PBMCs and CSF cells obtained from our patients was performed in our center. Finally, we performed real time PCR analysis of viral transcripts of EBV on all samples collected from all the 3 centers involved in the project.

### **f. Advancement in the field**

By investigating simultaneously EBV and immune response-associated cellular transcripts in the CSF and blood of MS patients, this project will help understand whether abnormalities in persistent EBV infection (i.e., disruption of viral latency and productive infection) drive immune activation and unravel possible associations with disease activity and clinical course.

## **3. CRESM BIOBANK**

### **a. Summary (500 characters)**

Despite considerable investment in biological clinical research, very few laboratory results are transformed in drugs, due to the poor reproducibility of published data, to the lack of rigor in the collection of biological

samples, to the insufficient validation of the methods according to the instructions of FDA and to the limited sharing of data.

This project aims to address these problems by joining the activity of the bio-bank and of the Clinical Neurobiology Lab (CNB) of CRESM that overlooks 1800 patients with MS, of which 250 PPMS patients. The main aim of this project is the creation of a structured Biobank able to collect, store and distribute data and samples obtained from MS patients to other researchers, following rigorous ethical and technical guidelines.

#### **b. Background and Rationale**

Biological research suffers from poor reproducibility of published data, even in prestigious journals, because of the lack of rigor in the collection of biological samples, the insufficient validation of the methods according to the instructions of FDA and limited sharing of data. In a chronic disease such as PPMS there are two further obstacles: the small number of patients makes it difficult to collect biological samples, and the patients need careful follow-ups through the collection of clinical, biological, MRI, neuro-cognitive and neurophysiological data. This project aims to address these problems by joining the activity of the bio-bank and of the Clinical Neurobiology Lab (CNB) of CRESM that overlooks 1800 patients with MS, of which 250 PPMS patients.

#### **c. Objectives**

The project aims to expand the biobank which is already in operation at the CRESM, through the collection of biological samples (serum, plasma, cerebrospinal fluid (CSF), urine, cells from blood and CSF for DNA and RNA,) of PPMS patients, other types of MS and various controls, according to strict criteria and recorded in a database. Moreover, the biobank project aims to distribute samples to projects funded by FISM or other institutions. The CNB Lab will offer technical support for co-validation of methods and will perform quality controls on biological materials stored in the Biobank (i.e. evaluation of the influence of pre-analytical variables, time and temperature, on blood samples used in gene expression studies). The last aim of the project is the cooperation with other biobanks in the future network of biobanks dedicated to MS research.

#### **d. Results:**

To allow homogeneous samples collection, we established rigorous procedures regarding the selection of patients, the ideal time points for blood collection during their follow-up and the standardization of sample collection and handling. Blood samples are handled within 3 hours from collection; CSF samples are stored at -80°C within 2 hours from lumbar puncture. A robust biobanking system including cryovials with barcode, many aliquots of the same biological material, anonymization of samples etc has been created. Samples from patients treated with all types of approved MS drugs are collected, thereby providing the researchers with a wealth of samples. The blood samples from treatment naïve MS patients are also included in the biobank. The development of new lumbar puncture technique (Bertolotto et al, Cephalgia 2016) results in a reduction of the headache post-lumbar puncture and allows the collection of approximately 20 ml CSF and so multiple aliquots are available from the same collection.

All the patients willing to participate were informed of the project and the related procedures in detail.

The collaboration with “Sant’Anna” Obstetric and Gynaecologic Hospital in Turin allowed the collection of blood samples from pregnant women, both from MS patients and healthy donors.

Samples stored in the biobank have been used in 8 projects of the Principal Investigator (PI) research group and in 6 external projects so far; 5 additional projects are under review. These projects focused on the MS pathogenesis, the evaluation of treatment response and the identification of diagnostic and/or prognostic biomarkers.

Given the importance of sample integrity, an ongoing study of RNA stability is being designed to check for systematic changes due to handling and storage conditions over the lifetime of the project. In PBMCs isolated from EDTA tubes, RNA expression of specific target genes is affected by the different pre-analytical conditions analysed, as RNA levels of other genes are stable across the different pre-analytical conditions studied. Different degree of variation in RNA expression was observed, according to the housekeeping gene used in the analysis. No variation in RNA expression was observed using Tempus RNA tubes.

The biobank collaborates with other laboratories for cross-validation of biological methods. The PI research group has been included in the project “Monitoring of monoclonal Antibodies Group in Europe (MAGE) for inflammatory diseases – Le Studium” that involves 7 other European research groups.

A lot of work has been done to institutionalize the biobank and careful attention has been paid to ensure implementation of best practices. After a collaboration with a bioethicist, the Ethic Committee of San Luigi Hospital approved a revised Informed Consent for adults and we are working on the creation of an Informed Consent for minors. The distribution of samples to other research groups needs to be regulated by a rigorous procedure: for this purpose, the revision of the procedures allowing to obtain biological material from the bio-bank and the creation of a specific Material Transfer Agreement has been made.

**f. Advancement in the field**

Thanks to this project, what began as a collection of samples of patients with MS and healthy subjects is becoming an increasingly structured bio-research bank (informed consent, standardization of procedures for collecting and storing biological material and associated data). The growing interest in biobank research by researchers around the world is a sign that projects like this are a vital resource for the entire scientific community and beyond.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### **BIOMARKERS FOR TREATMENT RESPONSE IN MS**

#### **1) “Effects of Fingolimod and AUY954 on Nuclear Receptor 4 A subfamily (NR4As) in Multiple Sclerosis (MS) patients”**

**a. Summary (up to 2000 characters):**

Through this Novartis funded project we plan to identify a modulation of NR4A2 expression in FTY720 treated patients, in order to evaluate a possible drug effect through the NR4A2 pathway.

**b. Background and Significance (up to 4000 characters):**

Fingolimod (FTY720) is an oral drug for the treatment of relapsing-remitting MS (RRMS). This agent binds to sphingosin-1-phosphate (S1P) receptors and, renders lymphocytes unresponsive to physiological S1P stimulation, which is important in immune-cell trafficking. Therefore, FTY720 exerts its beneficial effects on RRMS by sequestering lymphocytes within the lymph node. However, there is increasing evidence suggesting that FTY720 also affects the function of various cell types in the CNS. A recent work reports that FTY720 accumulates in the brain, including the hippocampus, and enhances histone acetylation and gene expression programs associated with memory and learning. This process involves an up-regulation of the transcription factor NR4A2. During the past year we demonstrated that MS patients treated with FTY720 for more than two years showed an increased peripheral blood gene expression of NR4A2 in comparison to untreated patients.

**c. General aim and integration with mission of the Institute (up to 1000 characters)**

Assuming that FTY720 induces a direct effect on NR4A2 gene expression and that this drug exerts several functions on immune system and CNS, we are interested to evaluate a possible FTY720 effect on human MS patients through the activation of the NR4A2 pathway, and to evaluate the effect of a next-generation S1P receptor modulator, AUY954, that selectively target S1P1.

**d. Specific objectives and strategies (up to 4000 characters):**

To evaluate the ex-vivo effects of FTY720 and AUY954 on NR4As expression in human CD3+ lymphocytes and CD14+ monocytes. We will set-up functional studies on CD3+ lymphocytes and CD14+ monocytes isolated from buffy coat obtained from HC treated ex vivo with FTY720 and AUY954 to evaluate possible drug effects on NR4A2 gene expression level. We selected these population because are the most involved in MS and in the NR4A2 down-regulation. Gene expression analysis will be performed using Real Time PCR technique. Interesting results will be corroborated by protein expression analysis using western blot. This objective allows us to clarify the drugs effect on NR4A2 in more specific subcellular population. Furthermore, after treatments, the production of anti- or pro-inflammatory molecules will be evaluated using RT Real Time PCR technique. Considering the great sample amount necessary to perform functional studies we will characterize the effect of FTY720 and AUY954 on NR4A2 starting from HC buffy coat.

**e. Unique features of the project research (up to 2500 characters):**

To achieve these aims we take advantages directly from human MS sample obtained from CReSM BB (FISM 2014/PMS/1; Ethic Committee of AOU San Luigi 7777-2013). Samples are already stored in our laboratory.

#### **2) Titration of Anti-AQP4 Antibodies in NMOSD patients**

**a. Summary (up to 2000 characters):**

Titration of AQP4-Abs could be useful in the clinical management of NMO patients. With this study we aim to compare IIF assay with different FACS assays to titrate AQP4-Abs in serum from NMOSD patients

**b. Background and Significance (up to 4000 characters):**

We previously demonstrated that titration of AQP4-Abs could be useful in the clinical management of NMO patients treated with RTX providing information about responsiveness to RTX. IIF assay is considered the gold standard assay for AQP4-Ab detection, because it demonstrated the best sensitivity and specificity. However, this technique is not the ideal assay for antibody titration. Other assays, as FACS assay, could represent a good strategy for antibody titration.

**c. General aim and integration with mission of the Institute (up to 1000 characters)**

With this study, we aim to identify a strategy to monitor NMOSD patients based on AQP4-ab levels.

**d. Specific objectives and strategies (up to 4000 characters):**

We aim to compare IIF assay with different FACS assays to identify the better one for titration of AQP4-Abs in serum from NMOSD patients. This study will be performed in collaboration with the MS research laboratory of prof. Bernhard Hemmer.

**e. Unique features of the project research (up to 2500 characters):**

NMOSD samples are already available in CReSM BB (FISM 2014/PMS/1; Ethic Committee of AOU San Luigi 7777-2013). This study will take advantage from collaboration with the German laboratory directed by Prof. Hemmer.

**3) Identification of CSF markers of disease progression in Primary Progressive MS**

**a. Summary (up to 2000 characters):**

MS is characterized by a very heterogeneous and unpredictable disease course. To date biomarkers of disease progression are not available yet. With this study we aim at characterizing cerebrospinal fluid of primary progressive MS patients to identify molecules associated with disease progression

**b. Background and Significance (up to 4000 characters):**

MS is a chronic inflammatory disease of the CNS. The Primary Progressive (PP) form of MS is the rarest but most severe, as in a few years patients reach a degree of disabling disability. CSF is a well-studied biological sample for the search of biological markers associated with the disease. Till now, there are no markers associated with MS diagnosis or prognosis. PPMS represent the ideal model for the study of disease progression, because it reflects natural history of the disease.

**c. General aim and integration with mission of the Institute (up to 1000 characters)**

With this study we aim to identify one or more CSF biological parameters that could be correlated with disease progression in PPMS patients.

**d. Specific objectives and strategies (up to 4000 characters):** 1. Characterization of the CSF profile of samples obtained from SMPP patients; 2. Identification of possible CSF markers associated with disease progression.

**e. Unique features of the project research (up to 2500 characters):**

CSF samples from PPMS patients are already available in CReSM BB (FISM 2014/PMS/1; Ethic Committee of AOU San Luigi 7777-2013). Clinical and biological data of patients are also available.





*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: **Adult neurogenesis**



## 1. LABORATORY DESCRIPTION – PERSONNEL (Year 2017):

NOTE: Since the birth of NICO (in 2010), the group **Adult Neurogenesis** was created from two independent research groups (already working and collaborating in Turin since 1994), which joined their expertise on structural plasticity and neurogenesis. Since then, the group at NICO has been organized with two PI coordinating independent but complementary research lines. Besides the two PI, the group involves two researchers with a permanent position in the University, which progressively have opened independent research lines within the same field. The most important keywords of this group are reciprocal cooperation on complementary research topics related to structural plasticity and neurogenesis.

### Principal Investigator 1

Paolo Peretto Birthdate: (18/09/63)  
Full professor Gender: M  
Nationality: Italian Phone: 011 6706605  
Email: [paolo.peretto@unito.it](mailto:paolo.peretto@unito.it)

### Principal Investigator 2

Luca Bonfanti Birthdate : (19/05/1962)  
Associate professor Gender: M  
Nationality: Italian Phone: 011 6706606  
Email: [luca.bonfanti@unito.it](mailto:luca.bonfanti@unito.it)

### Researchers (permanent position at the University of Turin)

1. First name: Silvia De Marchis Birthdate (14/09/66)  
Associate professor Gender: F  
Role: Senior researcher Nationality: Italian  
Expertise: in vivo and in vitro molecular and cellular analyses
2. First name: Federico Luzzati Birthdate (20/10/1974)  
Assistant professor Gender: M  
Role: Senior researcher Nationality: Italian  
Expertise: in vivo morphological analyses and 3D reconstructions

### Researchers (Postdoc and PhD students)

3. First name: Sara Trova Birthdate (25/04/89)  
PhD student (fourth year) Gender: F  
Role: young researcher Nationality: Italian

Expertise: behavioural aspects of AN in the olfactory system

4. First name: Chiara La Rosa Birthdate (01/07/88)

PhD student Gender: F

Role: young researcher (second year) Nationality: Italian

Expertise: comparative analyses of immature neurons in domestic and wild mammals

5. First name: Isabella Crisci Birthdate (17/12/89)

PhD student (first year) Gender: F

Role: young researcher Nationality: Italian

Expertise: cellular and molecular analyses of AN in the hippocampus

## 2. PRINCIPAL INVESTIGATOR

### 2a. PRINCIPAL INVESTIGATOR 1 (Paolo Peretto)

### 2b. PRINCIPAL INVESTIGATOR 2 (Luca Bonfanti)

h index (LB): 30 Total citations: 3498 (source: Scopus)

h index (PP): 26 Total citations: 2544 (source: Scopus)

#### Relevant recent discoveries:

- Evidence that opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis
- Evidence that “immature” non-newlyborn neurons are more abundant in gyrencephalic, large-brained, long-living mammals with respect to laboratory rodents.

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
Jan 2017 - Jan 2018	National	Local Unit Coordinator (LB)	PRIN - MIUR (bando 2015)	A new non-invasive approach to the investigation of cerebral activity in domestic animals using functional near-infrared spectroscopy	2015Y5W9YP	€ 10.000	8%
2017	Local reseach (UNITO)	Coordinator (LB)	University of Turin	Popolazioni di neuroni immaturi nell'amigdala e capsula esterna della pecora adulta	BONL_R1 LO_16_01	€ 4.000	8%
2016-17	Local reseach (UNITO)	Coordinator (PP)	University of Turin	Regolazione della neurogenesi adulta nell'interazione con l'ambiente	PERP_RILO_16_01	€ 4.665	8%
2017	International	Coordinator (LB)	National Geographic Foundation	Identification of brain structural plasticity in mammals: is there a trend?	243181	Pending \$ 25.000	

#### Invited talks

- “*Plasticità cerebrale: cellule che si muovono ma non sempre fanno ciò che vogliamo*” Laurea in Neurobiologia e dottorato di ricerca, Università di Pavia, Pavia (LB)
- “*Brain structural plasticity: bad news and good news*” CIBIO e Università di Trento, Trento (LB)
- “*Brain structural plasticity: bad news and good news*” Institute of Anatomy and Cell Biology Università Cattolica del Sacro Cuore, Roma (LB)
- “*Neurogenic versus non-neurogenic plasticity in mammals*” Joint UZI-UZF congress, Turin, September 2017 (LB)
- “*Adult neurogenesis and its role in the control of opposite sex attraction in male mice*” 63° Convegno Gruppo Embriologico Italiano- Roma 12-15 Giugno. 2017 (PP)

#### PhD supervised (PIs):

- Chiara La Rosa (second year) (LB)
- Sara Trova (fourth year) (PP)

#### PhD supervised (PIs):

- Isabella Crisci (first year) (SDM)

#### International collaborative experiences (PIs):

- Dr. Paolo Giacobini, Jean-Pierre Aubert Research Center, School of Medicine, Lille (France) - *Interplay between adult neurogenesis and endocrine system* (PP)
- Dr. Livio Oboti, Children's National Health System, Center for Neuroscience Research, Washington, DC, (USA) - *Adult neurogenesis in the olfactory bulb* (PP)

- CNRS, UMR6175, F-37380 University of Tours, Nouzilly, F (Dr. Frederic Levy) (LB)
- Institute of Anatomy, University of Zurich, CH (Dr Irmgard Amrein) - *Comparative aspects of brain structural plasticity in mammals* (LB)
- University of Washington – Laboratory of anthropology, Dr Chet Sherwood (USA) - *Comparative aspects of brain structural plasticity in primates* (LB)
- Juan Nacher, University of Valencia (Spain) – *Neurochemical characterization of immature cortical neurons* (LB)

- **Editorial duties**

***In the Editorial Board of international journals:***

- *Frontiers in Neuroscience, Neurogenesis* (Frontiers journal series, CH), as Editor-in-Chief (LB)
- *Frontiers in Neuroscience, Neurogenesis* (Frontiers journal series, CH), as Associate editor (PP)

***Guest Editor for Special Issues:***

- Adult neurogenesis: beyond rats and mice (2017) *Front Neurosci* (with I. Amrein) (LB)

**Please list your organizational activities:**

- Speakers invited:
  - Irmgard Amrein (University of Zurich, CH) september 2017 (LB)

### 3. PI's PUBLICATIONS:

(Please list below your publications in 2017 -. Please indicate the journal IF, ranking).

For each publication, please indicate:

\* if you contributed equally to the first-author, as stated in the published article

Parolisi R, Cozzi B, **Bonfanti L** (2017) Non-neurogenic SVZ-like niche in dolphins, mammals devoid of olfaction. *Brain Struct Funct*, 222: 2625-2639.  
IF= 4,698; R= 1/21

Piumatti M, Palazzo O, La Rosa C, Crociara P, Parolisi R, Luzzati F, Lévy F, **Bonfanti L** (2017) Non-newly generated, "immature" neurons in the sheep brain are not restricted to cerebral cortex. *J Neurosci*, 38: 826-842. E-pub doi: 10.1523/JNEUROSCI.1781-17.2017  
IF= 5,988; R= 30/259

Oboti L, Trova S, Schellino R, Marraudino M, Harris NR, Abiona OM, Stampar M, Lin W, **Peretto P.** (2017) Activity Dependent Modulation of Granule Cell Survival in the Accessory Olfactory Bulb at Puberty. *Front Neuroanat* 2017 11:44. doi: 10.3389/fnana.2017.00044.  
IF= 3,267; R= 2/21

Pierucci F, Garcia-Gil M, Frati A, Bini F, Martinesi M, Vannini E, Mainardi M, Luzzati F, **Peretto P**, Caleo M, Meacci E (2017) Vitamin D3 protects against A $\beta$  peptide cytotoxicity in differentiated human neuroblastoma SH- SY5Y cells: A role for S1P1/p38MAPK/ATF4 axis. *Neuropharmacology* 2017 Apr;116:328-342. doi: 10.1016/j.neuropharm.2017.01.003. Epub 2017  
IF= 5,012; R= 44/259

### 4.GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2017 -. Please indicate the journal IF, ranking)

Pierucci F, Garcia-Gil M, Frati A, Bini F, Martinesi M, Vannini E, Mainardi M, **Luzzati F**, Peretto P, Caleo M, Meacci E (2017) Vitamin D3 protects against A $\beta$  peptide cytotoxicity in differentiated human neuroblastoma SH- SY5Y cells: A role for S1P1/p38MAPK/ATF4 axis. *Neuropharmacology* 116:328-342. doi: 10.1016/j.neuropharm.2017.01.003. Epub 2017 Jan 7  
IF= 5,012; R= 44/259

Piumatti M, Palazzo O, La Rosa C, Crociara P, Parolisi R, **Luzzati F**, Lévy F, **Bonfanti L** (2018) Non-newly generated, "immature" neurons in the sheep brain are not restricted to cerebral cortex. *J Neurosci*, 38: 826-842.  
IF= 5,988; R= 30/259

Jongbloets BC, Lemstra S, Schellino R, Broekhoven MH, Parkash J, Hellemons AJ, Mao T, Giacobini P, van Praag H, **De Marchis S**, Ramakers GM, Pasterkamp RJ. Stage-specific functions of Semaphorin7A during adult hippocampal neurogenesis rely on distinct receptors. *Nat Commun* 2017 8:14666. doi: 10.1038/ncomms14666.  
IF= 12,124; R= 3/64

Bonzano S., De Marchis S. (2017) Detecting Neuronal Differentiation Markers in Newborn Cells of the Adult Brain. In: Pellicciari C., Biggiogera M. (eds) *Histochemistry of Single Molecules. Methods in Molecular Biology*, vol 1560. Humana Press, New York, NY (book chapter)

Malloul H, Bennis M, Bonzano S, Gambarotta G, Perroteau I, De Marchis S and Ba-M'hamed S (2018) Decreased Hippocampal Neuroplasticity and Behavioral Impairment in an Animal Model of Inhalant Abuse. *Front Neurosci* 12:35. doi: 10.3389/fnins.2018.00035  
IF= 3,566; R= 83/259

## 5. GROUP's additional information:

Please list the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2016-17	Local reseach (UNITO)	Coordinator (De Marchis)	University of Turin	Ruolo di CUPF1 nello sviluppo cerebrale	DEMS_RILO_16_01	€ 6.166	8%
2016-17	Local reseach (UNITO)	Coordinator (Luzzati)	University of Turin	Neurogenesi indotta con attivazione di astrociti striatali	LUZF_RILO_16_01	€ 2.848	8%
2017	National	Coordinator (Luzzati)	MIUR (Finanziamento delle attività base di ricerca)			€ 3.000	8%

### S. De Marchis

#### *Ongoing international collaboration:*

- Dr. Michèle Studer, INSERM U636, Nice Sophia Antipolis, France - Exploring the role of COUP-TFI function on adult hippocampal neurogenesis.
- Prof. Jeroen Pasterkamp, Utrecht University, The Netherlands – Study on the role of Sema7A function on adult neurogenesis.
- Prof. Saadia Bamhamed, Cadi Ayyad University Marrakech, Morocco – Study on the role of abused inhalants on adult hippocampal neurogenesis.
- S. De Marchis is part of an Erasmus Neuron Line Strategic Partnership (2015-1-FR01-KA203-015298)

#### *Scientific Meeting organization:*

2nd International NeuronLine Conference: Frontiers in Neuroscience Teaching & Research – University of Turin, November 24, 2017.

### Federico Luzzati

#### *Ongoing international collaboration:*

From April 1 to September 30/2016; Visiting scientist in Neurology at Johns Hopkins University, Baltimore. During my visit I've been hosted in the Hongjun Song lab to perform single cell RNAseq of neurogenic activated striatal astrocytes.

Benedikt Berninger, University of Mainz – Analysis of integration capacity of newborn striatal neurons through monosynaptic retrograde tracing with rabies virus and electrophysiological recordings.

#### *Invited talks:*

Neurogenic potential of striatal astrocytes in health and disease. 2nd International NeuronLine Conference: Frontiers in Neuroscience Teaching & Research – University of Turin, November 24, 2017.

---

>> *The Adult Neurogenesis group has been deeply involved in the **dissemination of science and public engagement** to promote the image of the NICO Institute within the society. Here are listed the more relevant activities:*

#### LUCA BONFANTI

- Organization and scientific supervision of *UNISTEM DAY 2017* (Torino, marzo 2017), Aula Magna del Rettorato Cavallerizza reale (with 400 students of the secondary school)
- Conferences on brain plasticity and communication of science in 5 Secondary schools

- Conference on Brain plasticity in “ABITARE IL CERVELLO”, CLE, Turin (in the context of “Brain week”)
- Conference “Plasticità del cervello” at HYPATIAE FESTIVAL, Pavia, settembre 2017
- Conferenza at Liceo Pascal di Romentino (NO) “*L'affascinante mondo della ricerca sulla plasticità cerebrale*”
- Conference “*La plasticità cerebrale: dalle cellule staminali alla vita di tutti i giorni*” for “SeralMente”, Grugliasco, aula magna AgroVet (with Paolo Peretto)
- Book presentation “LA SCIENZA FA BENE” at book shop “*Il bardotto*”
- “*Dalla chimica dei neuroni alla chimica delle galassie*” Conference at Fondazione Ferrero, for Liceo Cocito di Alba
- “*La scienza fa bene. Ma come?*” Conference at “*Ben-Essere*” organized by “Associazione per la tutela dei diritti del malato”, Novara
- Conference and discussion with Prof Roberto Mantovani (Humanitas) in the event: “Staminali e vaccini, tra mito e scienza” Cuneo

#### PAOLO PERETTO

- UNISTEM DAY 2017- Corsa, staminali neurali e Memoria. TORINO 17 /03/2017
- ANCHE LA COMUNICAZIONE SCIENTIFICA E' UNA SCIENZA- Attrazione sessuale? Un semplice meccanismo biologico. PISA 6/6/2017

#### SILVIA DE MARCHIS

- Organization of the National Prize Aldo Fasolo 2017 (for the PhD school in neuroscience – in collaboration with NICO) - <http://dott-neuroscienze.campusnet.unito.it>
- Member of the jury of the Fame lab competition 2017 organized by CentroScienza Onlus – March 2017

#### THE GROUP

- Contribution to “*Porte aperte al NICO*”, stand “Cellule staminali: il sogno di rifarsi un cervello”
- Contribution to “*Notte della ricerca*”, stand NICO

## 6. Past Research activity

(Summarize the PI and group research activities in 2017)

In 2017 the research group has been focused on understanding how social stimuli can influence the reproductive behavior through an interplay between adult neurogenesis and gonadal hormones. Moreover, we defined new molecular players in the control of adult DG neurogenesis and defined how abuse of inhalants affects adult DG neurogenesis and cognitive behavior in mice. In parallel, we closed a three-year project aimed at defining the occurrence/degree of neurogenesis in dolphins (mammals devoid of olfaction), showing that adult neurogenesis is absent in these aquatic mammals and thus confirming that neurogenic plasticity is highly heterogeneous through phylogeny, with an evident decrease in large-brained, long-living mammals (e.g., humans). In parallel we showed that “immature” non-newly generated neurons are abundant in sheep (large-brained, long-living species with respect to rodents). We have also analyzed the mechanisms and dynamics of the acquisition of a neurogenic competence in striatal astrocytes, subsequent lineage progression and the integration of newborn striatal neurons.

## 7. Future Projects (Next year)

### Summary (up to 2000 characters):

Future projects are committed to move forward on the characterization of the neurogenic processes taking place in the adult brain and determining their impact on brain function in physiologic and pathologic states. To achieve this goal we will combine multiple, complementary approaches that are well established in our laboratory, together with cutting-edge technologies including two photon microscopy and high-throughput technologies (i.e. genomics and transcriptomics).

The projects are articulated into 4 main research lines:

- I. ***Molecular mechanisms of AN***: we will focus on molecular factors (i.e. COUP-TFI) involved in the control of OB neurogenesis to get deeper insights on their role in AN in the DG of the hippocampus;
- II. ***AN and reproduction***: we will investigate the interplay occurring between AN and the endocrine system to address the role of AN in the reproductive function;
- III. ***AN and neurodegeneration***: the analysis of the potential reparative or “restorative” role played by the quiescent striatal astroglial progenitors in diverse models of striatal neurodegeneration;
- IV. ***Comparative aspects of AN***: definition of common and divergent traits in the process of AN in mammals through extensive comparative analyses and with particular reference to the novel category of “immature neurons”.

Overall, our projects are aimed at enlightening the real impact AN plays in the normal and pathologic mammalian brain, through understanding the neurogenic potential of different brain regions/species, as well as the key extrinsic/intrinsic mechanisms/factors whose modulation can be used to foster adult brain plasticity/repair. Only by knowing the roles of AN and other forms of plasticity in brain homeostasis and dysregulation we could expect to use this biological process for translational purposes (novel therapeutic approaches for neurodegenerative diseases and preventive approaches for optimal brain function/plasticity; both goals ultimately in line with the NICO Mission).





*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: **Physiopathology of stem cells**

## 1. LABORATORY DESCRIPTION – PERSONNEL:

### Principal Investigator

Annalisa Buffo Birthdate (25/12/1967)  
Degree PhD Gender F  
Nationality Italian Phone: 00 39 011 6706614  
Email: annalisa.buffo@unito.it

### Personnel

1. Daniela Carulli Birthdate (17/04/1973)  
Degree PhD Gender F  
Role Assistant Professor Nationality Italian  
Expertise: Extracellular matrix, perineuronal nets  
Currently on sabbatical leave (from August 2015)

2. Enrica Boda Birthdate (08/05/1981)  
Degree PhD Gender F  
Role Assistant Professor in tenure track (RTD-B) Nationality Italian  
Expertise Oligodendroglial heterogeneity and physiopathology  
Lead responsible of research on oligodendroglial physiopathology

3. Valentina Cerrato Birthdate (21/07/1988)  
Degree MSc in Biotechnology Gender F  
Role PostDoc Nationality Italian  
Expertise Generation of astroglial heterogeneity

4. Roberta Parolisi Birthdate (23/01/1985)  
Degree PhD Gender F  
Role PostDoc Nationality Italian  
Expertise Microglia-oligodendrocyte crosstalk, electron microscopy

5. Giulia Nato Birthdate (08/05/1986)  
Degree PhD Gender F  
Role PostDoc Nationality Italian  
Expertise Astrocyte reactivity and neurogenic activation, brain tumors

## 2. PRINCIPAL INVESTIGATOR

H index, 22; Citations: 2210 (Scopus)

### Relevant discoveries:

- We showed in a model of genetic cerebellar ataxia that preventive motor training increases the survival of cerebellar neurons with a positive impact on the motor phenotype and attenuation of aberrant autophagy. This therapeutic approach was proved more effective than perinatal transplant (Fucà et al., Neurobiol Dis 2017)

- We contributed to identify in the HSPG family members syndecan-3 and syndecan-4 as functional receptors for Nogo-A- $\Delta$ 20 and showed that Nogo-A- $\Delta$ 20 promotion of neuroblast migration of neuroblasts (Rolando et al., J Neurosci 2012) occurs via HSPGs but not S1PR2 (Kempf et al., 2017 Dev Cell).

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2016-2018	International	PI	Merck Serono/Grant for Multiple Sclerosis Innovation 2015	Driving microglia metabolism toward remyelination and restoration of brain damage in MS	na	60000	10%
2015-2018	National	Component (Enrica Boda PI)	Fondazione Cariplo	Characterization of a novel microRNA involved in myelination: a new potential pathogenetic mechanisms in multiple sclerosis	ID: 2014-1207)	80000	8%
2014-2018	International	Team Component PI: A Vercelli	FP7 European Union	Neurostemcellrepair		400000	8%
2017	International	Team Component PI: A Vercelli	Internal call of the Neurostem cellrepair consortium	TRACING CONNECTIONS OF HUMAN CELL GRAFTS IN THE HD MODEL WITH VIRAL VECTOR-BASED TOOLS		12000	8%
2016-2017	Local	PI	Local funds of the University of Turin	Ontogenesi, specificazione e funzioni dell'eterogeneità degli astrociti cerebellari		7000	8%
2017	International	PI Coordinator: De Castro	H2020	ETN ID 814058 Mirage		Pending 522000	

		Fernando, Cajal Institute, madrid					
2018	International	Subcontractor Coordinator: Thomaidou, Dimitra Hellenic Pasteur Institute Athens	<b>E-Rare Joint Transnational Call for Proposals 2017</b>	Global gene expression, epigenetic and proteomic changes in Huntington's Disease progression and treatment		Pending 40000	
2017	National		Ministero dell'Istruzione – Finanziamento di base alla ricerca			3000	8%

As external collaborator of the project we are supported by the Telethon grant GGP14164 (2014-2017) to prof Lorenzo Magrassi, University of Pavia

Please list the name of PhDs you have supervised.

Valentina Cerrato, PhD student in Neuroscience

Martina Lorenzati, PhD student in Neuroscience (co-supervised with Alessandro Vercelli)

Please list honours, prizes or awards received, if applicable.

na

Please list your outreach activities

- describe your international collaborative experiences
  - Prof Martin Schwab and dr. Anissa Kempf, Brain Institute Zurich, Switzerland: identification of NogoA receptors
  - Prof. Laura Lopez-Mascaraque (Cajal Institute, Madrid, Spain) and dr. Marion Betizeu (: clonal approaches for astroglialogenesis and neurogenesis.
  - Prof M Parmar, University of Lund, rabies virus based tracing of human medium spiny neuron transplantation into the rat damaged striatum
- Invited talks
  - XIII Euroglia Meeting on Glial Cells in health and Disease, July 2017, Edinburgh, 'Are OPCs all born equal? Exploring the molecular and functional heterogeneity of OPCs in the developing and adult brain', Symposium: Transcriptional states within the oligodendrocyte lineage
  - Institute of Neurobiology- UNAM Queértaro, Mexico, April 2017, Development of the rodent cerebellum: unfolding lineages, maturation and interconnections between interneurons and astrocytes (visiting professorship April 25-29).

*Science communication*

CONVEGNO: PICCOLISSIMI AL NIDO. Relatore su la plasticità cerebrale nel primo periodo di vita 28 novembre 2017, Gruppo Nidi e Infanzia – Gruppo Territoriale Piemonte organizza insieme con Nidi d'Infanzia 0-3, Giunti Scuola e il patrocinio della Città di Torino Torino

- Editorial duties
  - Guest reviewer for the following journals: The Journal of Neuroscience, Glia, Journal of Neurochemistry, PlosOne, Frontiers in Neuroscience, Glia, Cerebellum.

Agencies: French National Research Agency (ANR), ARSEP, DAAD, FISM (Federazione Italiana Sclerosi Multipla), Israel Science Foundation, ANEP, Medical Research Council (GB).

Topic Editor: Engineering Adult Neurogenesis and Gliogenesis (Frontiers in Neuroscience)  
Editorial Board of [Neuroglia](#)

Please list your organizational activities:

Organization of Open days at NICO

Invited to join the Task Force for Cerebellar Ataxia

- Speakers invited

Arthur Butt, Biomedical Science University of Portsmouth, Why White Matter Matters: Keeping the Brain Wired, November 30 2017

Marion Betizeau, Brain Research Institute - University of Zurich, Higher diversity of primate cortical precursors revealed by unsupervised lineage-based classification, October 27 2017

Elena Chierito, Paris Descartes University, Novel models of stretch-induced injury in mouse oligodendrocytes and organotypic culture of cerebellar slices: study of pathophysiological mechanisms, September 22 2017

- Workshops, Schools or Conferences organized

na

Please list your technology transfer achievements (patents, etc.), if applicable

na

AB is Co-funder of the Start-up S & P Brain (<http://www.spbrain.com/>)

### 3. PI's PUBLICATIONS:

(Please list below your publications in 2017 -. Please indicate the journal IF, ranking).

For each publication, please indicate:

\* if you contributed equally to the first-author, as stated in the published article

Kempf A, Boda E, Kwok JCF, Fritz R, Grande V, Kaelin AM, Ristic Z, Schmandke A, Schmandke A, Tews B, Fawcett JW, Pertz O, Buffo A, Schwab ME. (2017) Control of Cell Shape, Neurite Outgrowth, and Migration by a Nogo-A/HSPG Interaction. *Dev Cell*. 43(1):24-34.e5. doi: 10.1016/j.devcel.2017.08.014  
IF=9.174 R= 2/41 (Dev Biology, JCR)

Fucà E, Guglielmotto M, Boda E, Rossi F, Leto K, Buffo A. (2017) Preventive motor training but not progenitor grafting ameliorates cerebellar ataxia and deregulated autophagy in tambaleante mice. *Neurobiol Dis*. 102:49-59. doi: 10.1016/j.nbd.2017.02.005.  
IF=5.020 R= 43/259(Neuroscience, JCR)

Boda E, Nato G, Buffo A. (2017) Emerging pharmacological approaches to promote neurogenesis from endogenous glial cells. *Biochem Pharmacol*. 141:23-41. doi: 10.1016/j.bcp.2017.06.129.  
IF=4.581 R= 30/257 (Pharmacology and Pharmacy, JCR)

Azzalin A, Nato G, Parmigiani E, Garelo F, Buffo A, Magrassi L. (2017) Inhibitors of GLUT/SLC2A Enhance the Action of BCNU and Temozolomide against High-Grade Gliomas. *Neoplasia*. 19(4):364-373. doi: 10.1016/j.neo.2017.02.009.  
IF= 5 R= 47/217 (Oncology, JCR)

Garelo F, Pagoto A, Arena F, Buffo A, Blasi F, Alberti D, Terreno E. (2017) MRI visualization of neuroinflammation using VCAM-1 targeted paramagnetic micelles. *Nanomedicine*. 2017 Nov 8. pii: S1549-9634(17)30181-8. doi: 10.1016/j.nano.2017.10.002.  
IF=4.727 R=21/160 (Biotechnology and applied microbiology, JCR)

Joshi P, Gabrielli M, Ponzoni L, Pelucchi S, Stravalaci M, Beeg M, Mazzitelli S, Braidà D, Sala M, Boda E, Buffo A, Gobbi M, Gardoni F, Matteoli M, Marcello E, Verderio C. (2017) Fingolimod Limits Acute A $\beta$  Neurotoxicity and Promotes Synaptic Versus Extrasynaptic NMDA Receptor Functionality in Hippocampal Neurons. *Sci Rep*. 7:41734. doi: 10.1038/srep41734.  
IF=4.259 R=10/64 (Multidisciplinary science, JCR)

#### **4.GROUP's PUBLICATIONS:**

(Please list below up to ten most relevant publications of the other members of the group in 2016 -. Please indicate the journal IF, ranking)

Parolisi, R., Cozzi, B., Bonfanti, L. (2017) Non-neurogenic SVZ-like niche in two aquatic mammals devoid of olfaction. *Brain Struct Funct*, doi:10.1007/s00429-016-1361-3.  
IF= 4.698; R=1/21 (Anatomy); 54/259 (Neuroscience, JCR)

## 5. GROUP's additional information:

Please list the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2015-2018	National	Enrica Boda (PI of partner unit)	Fondazione Cariplo	Characterization of a novel microRNA involved in myelination: a new potential pathogenetic mechanisms in multiple sclerosis	ID: 2014-1207)	80000	8%
2017	National	Enrica Boda (PI)	Ministero dell'Istruzione – Finanziamento di base alla ricerca			3000	8%
2018	National	Enrica Boda (PI)	Aviva Community Fund	Bambini pretermine oggi, adulti in salute domani	ID: 30-180	Pending 15000	
2018	National	Roberta Parolisi (PI of partner unit)	Fondazione Cariplo	The role of Particulate Matter exposure in Sudden Infant Death Syndrome (SIDS) through orexinergic system deregulation		Pending 71000	

Please list honours, prizes or awards received by other members of the group If applicable.

Please list outreach activities of other members of the group:

- Describe your international collaborative experiences.
- Invited talks
- Editorial duties

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group

Please list your technology transfer achievements (patents, etc.), if applicable



## Enrica Boda

- International collaborative experiences

Prof. Verdon Taylor, Dr. Chiara Rolando (Embryology and Stem Cell Biology, Dept. of Biomedicine, University of Basel, Basel, Switzerland); Prof. Martin Schwab (University of Zurich, Zurich, Switzerland); Dr. Anissa Kempf (Dept. of Physiology, Anatomy and Genetics, University of Oxford, UK).

- Invited talks

Oral communication: Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model. Oral session “Glia and neurovascular unit in health and disease”, XXVII Convegno Nazionale Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.), 30 november- 1 december 2017, Bologna, Italy

- Editorial duties

Ad hoc reviewer for Neurochemical Research (3 in 2017), Plos One (1 in 2017), Biochemical Pharmacology (1 in 2017).

- Speakers invited

Irene Appolloni, University of Genoa, Tumor immunoescape during glioma progression. 11 September 2017

- Organizational activities

- Member of the Organizing and Scientific Committee of the BraYn (*Brainstorming Research Assembly for Young Neuroscientists*) Conference, 29-30 June 2018, Genoa, Italy.

<https://www.braynconference.com/>

- Organization of the NICO Progress Report series: 20 seminars in 2017 (oral communications held by NICO junior members)

## Roberta Parolisi

- International collaborative experiences

- Merck-Serono Grant Consortium (Pierre Gressens, Claudia Verderio) to study microglia-derived extracellular vesicles
- Neurostemcellrepair (NSCR) project (coordinated by Prof. Elena Cattaneo), related to NSC transplantation in a model of Huntington’s disease.

- Invited talks

Oral presentation: Parolisi R, Lombardi M, Fumagalli M, Bonfanti E, Verderio C, Buffo A. *Microglia-derived extracellular vesicles regulate proliferation and differentiation of oligodendrocyte precursor cells*. Oral session “Glia and neurovascular unit in health and disease”, XXVII Convegno Nazionale Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.), 30 november- 1 december 2017, Bologna, Italy

- Editorial duties

Ad hoc reviewer for Journal of the Neurological Sciences and Neurochemical Research.

- Speakers invited

Maria Talmon, University of Piemonte Orientale (Novara), Vortioxetine exerts anti-inflammatory and immunomodulatory effects on human monocytes/macrophages. 20th October 2017

- Organizational activities

- May 2017. Organizing member of the “Beautiful mind” sessions for national festival ‘Pint of Science 2017’. Turin, May 15<sup>th</sup>-16<sup>th</sup>, 2017.
- September 2017. organizing member of NICO stand at the European Researchers Night, Turin, September 29<sup>th</sup>, 2017.

## **Valentina Cerrato**

- International collaborative experiences:

- Prof Laura López-Mascaraque, Instituto Cajal, Madrid, clonal analysis of astroglia in the cerebellum;
- Prof Magdalena Gotz, LM, Munich, mechanisms of Bergmann Glia proliferation.

- Invited talks

- Seminar for the teaching course of Developmental Biology at the Master's degree in Molecular and Cellular Biology, entitled "Generation of astroglial diversity in the CNS", April 11th 2017.

- Editorial duties

Ad hoc reviewer for Journal of the Neurological Sciences and Neurochemical Research.

- Organizational activities

- May 2017. Organizing member of the "Beautiful mind" sessions for "Pint of Science 2017", Turin, May 15th-17th.

## **Giulia Nato**

- International collaborative experiences

Benedikt Berninger, Institute of Physiological Chemistry, Mainz, Germany: electrophysiological profiling of lesion induced newborn neuroblasts produced by striatal parenchymal astrocytes

- Awards

December 2017 "Post-Doctoral Fellowship - year 2018 Fondazione Umberto Veronesi" has been awarded on the project: 'Investigating the role of SOX2 in the acquisition of stem cell potential and neurorestorative states by parenchymal astrocytes after brain damage.

- Invited talks

Seminar for the teaching course of Developmental Biology at the Master's degree in Molecular and Cellular Biology, entitled "Neurodegeneration and neurorepair", 06 June 2017.

**All members** contributed to (Organizational activities, Science Communication):

- Alternanza Scuola-lavoro: 10-days- long stages (12-22/06/2017) for high school students (tutoring and laboratory activities, formulation and validation of scientific hypothesis, data collection, interpretation and discussion of results).
- NICO porte aperte: Open day at the Neuroscience Institute Cavalieri Ottolenghi.

## **6. Past Research activity**

(Summarize the PI and group research activities in 2017)

### **a. Summary (500 characters)**

Cell replacement and training protocols were applied to promote functional recovery in neurodegenerative models. We found that preventive rehabilitative motor training is effective in both promoting the survival of neurons and in ameliorating the motor phenotypes in Ataxia mutants. In parallel project aimed at disclosing novel mechanisms mediating the effects of NogoA, we found that heparansulfate proteoglycans act as functional receptors for Nogo-A- $\Delta$ 20 thereby supporting repulsive mechanisms that sustain neuroblast migration.

## **b. Background (2000 characters)**

In 2017 we have investigated mechanisms regulating or promoting neural plasticity in view of helping the development of therapies for neurological diseases based on promotion of circuit plasticity and adaptive cell replacement.

Among modulators of neural plasticity, Nogo-A, which we study in collaboration with M Schwab since many years, is a well-known inhibitor of neurite outgrowth and regrowth, and a regulator of synaptic plasticity and adult neurogenesis. Nogo-A exerts most of its action by acting as a repulsive signal during cell-to-cell interaction. These actions are partly mediated by canonical Nogo receptors. However, the extracellular 180 amino acid Nogo-A region (Nogo-A-D20) acts independently of Nogo receptors. The molecular mechanisms through which this domain acts have remained unknown for a long time. Recently, the Sphingosine-1-Phosphate receptor 2 (S1PR2) has been shown to be a Nogo-A-D20 receptor. Yet, evidence suggested the existence of additional mediators for Nogo-A-D20.

Treatment options for neurodegenerative are currently very limited. During disease progression it is difficult to halt ongoing neuronal death, and functional deficits are often only partially reversible or even irreversible due to limited plasticity of the circuits. Therapeutic strategies aimed at replacing and/or preserving damaged neurons may represent promising options. However, approaches based on replacement of lost neurons at the symptomatic stages of disease have been challenged by the low receptiveness for full integration of grafted cells of the mature degenerating nervous tissue. Further, among non-invasive treatments, motor exercise has been demonstrated to trigger neuroprotection and stimulate circuit plasticity so to alleviate symptoms. However, the extent of this amelioration, as well as its endurance, become more limited with the progression of the pathology. Preventive transplantation and rehabilitation approaches in genetic neurodegenerative pathologies may exert more efficacious and lasting therapeutic effects.

## **c. Rationale (2000 characters)**

The capability of the adult mammalian CNS to regenerate or repair after damage is very limited as a consequence of the postnatal decline of neurogenesis and gliogenesis, and of the upregulation of molecules inhibiting circuit remodelling such as Nogo-A. However, it retains significant levels of structural plasticity that, if fostered by pharmacological approaches or non-invasive treatment such as motor training or enriched environment, might promote brain repair.

On these bases, we sought to investigate molecular mechanisms that regulate key components of CNS structural plasticity in view of defining new targets to implement functional and anatomic repair in developmental diseases and neurodegenerative pathologies. In detail, searched for new receptors mediating the action of the extracellular D20 domain of Nogo-A, known to act as a potent inhibitor of neurite outgrowth in the adult CNS. Based on evidence that the cell-surface heparan sulfate proteoglycans (HSPGs) are highly expressed in the mammalian nervous system and mediate functions similar to those of Nogo-A-D20, in collaboration with M Schwab, we investigated the role of HSPG in mediating Nogo-A-D20 functions.

In a parallel study, we compared the outcomes of fostering brain repair in a rodent model of genetic cerebellar Ataxia, for which no effective cure exists. We reasoned that hereditary cerebellar ataxias, whose familiar transmission facilitates an early diagnosis, may allow to start preventive treatments before the onset of the neurodegeneration and appearance of first symptoms. We therefore tested the therapeutic actions of two potential preventive therapeutic strategies, provided by cell transplantation of Purkinje neurons to replace lost and damaged neurons, or motor training.

## **d. Objectives (1500 characters)**

Main goal of our work is to elucidate the fundamental processes of CNS structural plasticity, including developmental and repair processes. This knowledge is crucial to understand pathogenic mechanisms of neuro-developmental disorders and to define efficient therapeutic approaches for a broad spectrum of CNS diseases, including neurodegeneration, developmental disorders and vascular or traumatic damage.

During 2017, we specifically aimed at:

- a) identify new molecular mechanisms mediating the repulsive effects of the D20 extracellular domain of the plasticity inhibitor Nogo-A;
- b) define effective strategies based on preventive cell replacement or motor rehabilitative training in rodent models of Ataxia and HD;

**e. Results (4000 characters)**

a) We showed that Nogo-A-D20, binds to heparin and brain-derived heparan sulfate glycosaminoglycans but not to the closely related chondroitin sulfate glycosaminoglycans. Moreover, HSPGs are required for Nogo-A-D20-induced inhibition of adhesion, cell spreading, and neurite outgrowth, as well as for RhoA activation. Data also indicate that HSPG not only act as co-receptors that promote the binding of a ligand to its obligate receptor

through their glycosaminoglycan chains but do also act as signal-transducing receptors themselves. We also show that Nogo-A-D20 can act via HSPGs independently of its receptor, S1PR2, as specifically evident for repulsive actions supporting migration of neuronal progenitors. Moreover, the HSPG family members syndecan-3 and syndecan-4 are identified as functional receptors for Nogo-A-D20. Thus, more than one receptor for the active Nogo-A-D20 region exists and Nogo-A-D20-induced inhibitory effects are regulated in a cell-type-specific manner.

b) We compared the efficacy of two different preventive strategies in tambaleante (tbl) mice, a model for progressive ataxia caused by overactivation of autophagy leading to selective loss of Purkinje neurons. We tested the effects of preventive transplantation of healthy fetal Purkinje neurons into early postnatal tbl cerebella, in terms of PC replacement and functional preservation. On the other hand, we investigated the effects of motor training on diseased Purkinje cell survival, cerebellar circuitry and associated behavioural correlates. We showed that, despite a good survival rate and integration of grafted PCs, preventive transplantation does not alleviate the ataxic symptoms in tbl mice, whereas preventive motor training increases Purkinje neuron survival with positive consequences on both cerebellar circuit alterations and the motor phenotype. Of note, physical exercise activated protective mechanisms in mutant mice, promoted plasticity molecules and, remarkably, reduced dysregulated autophagy. These results therefore enforce the use of preventive motor exercise in inherited ataxia to promote a delay in the progression of the disease and indicate that this approach could be a promising step toward the amelioration of patients' quality of life. IN addition, evidence gained with these studies provide the background knowledge to address the efficacy of the potential combination of cell replacement approaches and rehabilitative training in rodent models of Ataxia or of other neurodegenerative diseases.

**f. Advancement in the field (1000 characters)**

- Transmembrane HSPG (syndecan-3 and -4) are new receptors for Nogo-A-D20 and regulate adhesion, cell spreading, neurite outgrowth, and migration of neuronal cells;

- Preventive motor exercise in inherited ataxia promotes a delay in the progression of the disease, indicating that this approach could be a promising step toward the amelioration of patients' quality of life.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### **a. Summary (up to 2000 characters):**

Our research will be focused on the role of glia and progenitor cells in brain development and repair, and on the identification of mechanisms whose manipulation may promote functional recovery in CNS diseases.

We believe that specific issues regarding glia and neural progenitors as well as their reciprocal relationships are particularly promising to unveil new keys to the understanding of physiology, disease and repair. As for astrocytes, very little is known on how distinct astroglial subtypes are specified and how much it impacts the shaping of the circuits. We will address this issue by studying the specification of astroglial subtypes and their morphogenetic programs in the cerebellum. Oligodendrocyte progenitors self-maintain but have limited capability to repair myelin. Understanding their biology and complex interplay with surrounding glial elements may help fostering myelin regeneration.

### **b. Background and Significance (up to 4000 characters):**

Issues on glia biology considered most promising to unveil how glia contributes to CNS physiopathology and may promote brain repair include: i) the understanding of how the various astrocyte phenotypes are generated and specified; ii) the identification of mechanisms regulating the functions of oligodendrocyte progenitor cells (OPC) and mediating their vulnerability to insults and full differentiation into myelinating cells. In our studies we will address these aspects.

- Astrocytes comprise extremely heterogeneous phenotypes. In the intact parenchyma, astrocytes participate in neuronal activity and are increasingly implicated in neurodevelopment and disease. However, how astroglial heterogeneity is achieved developmentally and how it differentially impacts on CNS functions is unknown. One limitation to the achievement of these goals is the lack of systematic characterization of astroglial diversity in most brain regions. We will circumvent this problem by addressing the ontogenesis of astrocyte heterogeneity in the cerebellum, where astrocyte diversity is well-established on the basis of distinct cell morphologies and layering. This will confer to our analysis a unique resolution power. Understanding these aspects may produce new knowledge to exploit glial cells and/or their progenitors for the treatment of neurologic and psychiatric disorders such as Ataxia and Autism Spectrum Disorders.
- OPC are the major population of proliferative progenitors in the mature CNS, where they are the source of myelinating cells under basal and injury conditions. We have shown that during the adult life OPC sustain both self-renewal and oligodendrogenesis by undergoing asymmetric divisions. They also display significant levels of phenotypic and functional heterogeneity. We are interested in unveiling: i) the cellular and molecular substrates of OPC heterogeneity and whether their diversity translates into potential distinct responses to pathological insults; ii) intrinsic and extrinsic factors promoting remyelination. These studies aim at disclosing novel aspects of oligodendrocyte biology in view of fostering their capability to regenerate myelin. Further, a specific focus will be devoted to microglia-oligodendrocyte interactions in view of evidence showing that the functional statuses of this glial types critically impact on oligodendrocyte myelination in vivo.

### **c. General aim and integration with mission of the Institute (up to 1000 characters)**

In 2017 we will toward these main aims:

- understanding glial/glia progenitor heterogeneity and physiology at the molecular, cellular and functional levels and clarify how such features impact on CNS pathophysiology in order to exploit adult glia and their progenitors as therapeutic actors to treat disease;
- developing therapies for neurological diseases where regenerative events may be sustained by implemented endogenous sources.

The contribution of our group will be to deliver innovative evidence and expand knowledge on fundamental processes of neural progenitor/glia cell physiopathology. Knowledge on these processes may lead to identify

mechanisms to be fostered or manipulated in view of proposing preclinical therapeutic approaches for CNS diseases.

**d. Specific objectives and strategies (up to 4000 characters):**

We will focus on glia physiopathology and neural progenitor plasticity in brain physiology and pathology. The cerebellum will be used as the major model for investigations, together with myelin lesions.

**To unravel mechanisms of astrocyte specification and plasticity we will:**

**-understand phenotypic specification of cerebellar astrocytes and their role in neuronal functions.**

Through *in vivo* clonal analyses, we found that distinct embryonic lineages with diverse degrees of heterogeneity produce the 4 types of cerebellar astrocytes (Cerrato, in preparation). Moreover, preliminary data suggest that Sox2 is required for the proper development and maintenance of the Bergmann glia phenotype (Cerrato, in preparation), whilst deletion of Sox2 in the whole cerebellum leads to ataxic-like behaviors. We will investigate developmental rules driving cerebellar astroglialogenesis during both embryonic and postnatal development and study the role of Sox2 in this process as well as its impact on the formation and functions of the cerebellar circuits. As an expansion of these studies we shall investigate the role of Sox2 in astrocyte reactivity and neurogenic conversion in collaboration with Federico Luzzati (NICO).

Coll: S Nicolis, Univ Bicocca, Milan.

**To understand oligodendroglia physiopathology, we will:**

**- investigate oligodendrocyte heterogeneity in Citron K KO mice**

Deletion of Citron K in oligodendroglial cells determines cell death of dorsal oligodendrocytes and senescence of ventral cells. These distinct responses are caused by different ROS production and activation of antioxidant mechanisms (Boda, in preparation). These data represent one of the first examples of functional heterogeneity of oligodendrocytes derived from distinct embryonic sources. We shall finalize the study of the molecular mechanisms driving pathological responses in OPCs upon Citron K deletion.

Coll: F. di Cunto, NICO.

**- identify strategies to promote OPC remyelination**

a) miR-125a is upregulated during OPC differentiation and affects their maturation *in vitro* (Lecca, Sci Reports 2016). We have found that its manipulation *in vivo* during development and in toxic demyelination affects oligodendrocyte maturation (Boda, in preparation). Underlying cellular and molecular mechanisms will be investigated.

Coll: D Lecca, MP Abbracchio, Univ of Milan.

b) Extracellular vesicles released by alternatively activated/mesenchymal stem cells exposed-microglia promote myelination *in vitro* and affect remyelination *in vivo* after toxic demyelination (Parolisi, Verderio, Buffo, in preparation). Underlying cellular and molecular mechanisms will be investigated. As an expansion of this study we will also address the effect of extracellular vesicles released by proinflammatory microglia on healthy hippocampal neurons *in vivo*, in order to validate *in vitro* data showing a negative impact on formation of excitatory synapse formation and spinogenesis (Verderio, unpublished).

Coll: C Verderio, CNR, Milan, A Uccelli, Univ of Genoa.

**e. Unique features of the project research:**

Several of the addressed questions (eg understanding the emergence of astrocyte heterogeneity, identifying molecular substrates of oligodendrocyte diversity) are fundamental questions essentially unanswered. Our studies will therefore provide unique insight to this evolving field. Our approaches (eg technology for clonal analyses *in vivo* and data elaboration, gene expression analyses on select glial cell populations isolated from the CNS) represent cutting edge techniques whose integration confers methodological originalities to our studies. Developed mutant lines constitute unique experimental models and focus on the cerebellum provides a specific advantage in the field of astrocyte diversity which, at difference with other mouse CNS areas, is well established for this territory.

**f. Methodology: please fill-out this section only in the case of innovative technologies**

In collaboration with Filippo Molinari (Politecnico di Torino) we will develop a refined informatic tool for the automatized analysis and interpretation of confocal images suitable to produce unbiased data and perform quantitative analyses for clonal studies or dispersion of cells in the nervous tissue. We aim at developing a

versatile tool to be applied for different mapping-related purposes and released as an open source tool in 2018.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Embryonic Neurogenesis



## 1. LABORATORY DESCRIPTION – PERSONNEL:

### Principal Investigator

Ferdinando Di Cunto Birthdate (20/12/1969)  
Degree: MD, PhD Gender: M  
Nationality: Italian Phone: 0116706616  
Email:ferdinando.dicunto@unito.it

### Personnel

1. Gaia Elena Berto Birthdate (19/05/1979)  
Degree: PhD Gender: F  
Role: Post Doctoral Fellow Nationality: Italian  
Expertise: Molecular and cellular biology, neuronal and neural progenitor cultures, analysis of genetically modified mouse models, laboratory management.

2. Federico Tommaso Bianchi Birthdate (27/11/1980)  
Degree: PhD Gender: M  
Role: Post Doctoral Fellow Nationality: Italian  
Expertise: Molecular and cellular biology, neuronal and neural progenitor cultures, analysis of cortical development, analysis of genetically modified mouse models.

3. Alessandra Maria Adelaide Chiotto Birthdate (05/02/1989)  
Degree: MoS in Molecular Biotechnology Gender: F  
Role: PhD student Nationality: Italian  
Expertise: Molecular and cellular biology, neuronal and neural progenitor cultures, analysis of genetically modified mouse models.

4. Gianmarco Pallavicini Birthdate (10/10/1991)  
Degree: MoS in Molecular Biotechnology Gender: M  
Role: PhD student Nationality: Italian  
Expertise: Molecular and cellular biology, analysis of genetically modified mouse models

## 2. PRINCIPAL INVESTIGATOR

H index, 23; Citations: 2310 (Scopus)

### Relevant discoveries:

The main discovery published by my group in 2017 was that the absence or inactivation of the microcephaly gene MCPH17 (CITK) leads to apoptosis by increasing the accumulation of DNA double strand breaks. Since it was first assumed that the apoptotic phenotype is caused by cytokinesis failure, this finding opened a new perspective on the common molecular and cellular mechanisms leading to microcephaly

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
31/12/15 01/01/19	National	PI	AIRC	Validation of Citron kinase as a therapeutic target for medulloblastoma.	IG 17527	322000	170000 (8%)
01/08/13 31/08/17	National	Component	Telethon Foundation	Relevance of the axonal SMN protein (a-SMN) for spinal muscular atrophy: novel cell models, transgenic mice and therapeutic approaches.	GGP13081	130000	0
01/01/16 31/12/18	National	Component	CNR, Epigen flagship project.	Disruption of circadian rhythms and epigenetic modifications in <i>D. melanogaster</i> .		53000	8%
01/08/16 31/07/17	International	Component	LouLou Foundation : CDKL5 pilot program	Exploiting computational biology for target identification and drug repositioning in CDKL5 disorder		30000	8%
01/04/17 31/03/18	National	PI	Fondo ricerca locale Ex-60%	Validazione di interattori molecolari di Citron Kinase implicati nella riparazione del danno al DNA.		5879	8%
01/06/2017	International	PI	Fondation	Identification		40000	10%

31/05/2019	ional		Jerome Lejeune	and initial validation of new possible treatments for intellectual disability in Down syndrome through drug repositioning.			
------------	-------	--	----------------	--	--	--	--

### PhDs supervised in 2017:

- Alessandra Maria Adelaide Chiotto
- Gianmarco Pallavicini

### Outreach activities

- Scientific societies

October 2017: elected member of the governing board of the Italian Society for Neuroscience (SINS)

- International collaborative experiences

The group is collaborating with many distinguished Scientists working abroad, who are recognized experts in the fields of reference of our research projects.

The most relevant collaborators are:

- **Prof. Wieland B. Huttner**, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

- **Prof. Pierre Gressens**, Inserm, U1141, Paris, France; Univ Paris Diderot, Sorbonne Paris Cité, UMRS, 1141, Paris, France.

- **Prof. Joseph Gleeson**, Laboratory for Pediatric Brain Disease, Howard Hughes Medical Institute, Department of Neurosciences, University of California, San Diego, La Jolla, California, USA

- **Dr. Silvia Cappello**, Max Planck Institute of Psychiatry, Developmental Neurobiology Laboratory, Munich, Germany.

- **Dr. Yohann Couté**, Laboratoire Biologie à Grande Echelle, Biosciences and Biotechnology Institute of Grenoble, France

- **Invited talks**

08/09/2017 Annual meeting of the 'Associazione dei Genetisti Italiani (AGI), Cortona, invited speaker, plenary lecture, title of the talk : Think globally, act locally: facing the data deluge in biological sciences

03/11/2017 Meeting 2nd Congress of joint European Neonatal Societies (jENS), Venezia, invited speaker, Zika congenital encephalopathies

- **Editorial duties**

- Associate Editor of PLoS ONE

- Associate Editor BMC Research Notes

### **3. PI's PUBLICATIONS:**

Bianchi, F.T., Tocco, C., Pallavicini, G., Liu, Y., Verni, F., Merigliano, C., Bonaccorsi, S., El-Assawy, N., Priano, L., Gai, M., et al. (2017a). Citron Kinase Deficiency Leads to Chromosomal Instability and TP53-Sensitive Microcephaly. *Cell Rep.* 18, 1674–1686.

IF= 8; R= 26/190

Bianchi, F.T., Gai, M., Berto, G.E., and Di Cunto, F. (2017b). Of rings and spines: The multiple facets of Citron proteins in neural development. *Small GTPases* 1–9.

Impact factor not yet released

Bosia, C., Sgrò, F., Conti, L., Baldassi, C., Brusa, D., Cavallo, F., Cunto, F.D., Turco, E., Pagnani, A., and Zecchina, R. (2017). RNAs competing for microRNAs mutually influence their fluctuations in a highly non-linear microRNA-dependent manner in single cells. *Genome Biol.* 18, 37.

IF= 11,9 ; R= 4/160

Gai, M., and Di Cunto, F. (2017). Citron kinase in spindle orientation and primary microcephaly. *Cell Cycle Georget. Tex* 16, 245–246.

IF= 3,53; R= 88/190

### **4.GROUP's PUBLICATIONS:**

## 5. GROUP's additional information:

Outreach activities of other members of the group:

**Federico Bianchi**, XIX Telethon Scientific Convention, 15-03-2017, Citron Kinase deficiency leads to chromosomal instability and TP53-sensitive microcephaly

## 6 .Past Research activity

### a. Summary

We study the genetic and molecular mechanisms that control neuron generation, survival and differentiation during normal brain development and how the alteration of these processes may lead to neurodevelopmental disorders, such as microcephaly and Down syndrome. To this aim, we currently use a combination of experimental and computational methods, including bioinformatic analysis of gene expression data, biochemistry, molecular biology, advanced microscopy to analyze in vitro and in vivo models of these disorders.

### g. Background

The human brain is composed of approximately 90 billion neurons, which are generated during embryonic life starting from many different types of neural stem cells, whose proliferation is extremely well organized in space and time. Indeed, stem cells' proliferation is very intense during early brain development, but chases almost completely in post-natal life. If too few neurons are produced or too many neurons die during development, the brain volume can be very compromised, a condition commonly known as microcephaly. Although a significantly reduced brain volume can be compatible with normal brain function and intelligence, microcephaly is frequently associated with strongly invalidating symptoms, such as intellectual disability, epilepsy and cerebral palsy. Microcephaly can be the result of rare genetic disorders, mostly characterized by autosomal recessive inheritance. Even more frequently, it is produced by environmental factors, such as hypoxia, drugs and alcohol exposure or infectious agents, such as Rubella, Toxoplasmosis, Cytomegalovirus or Zika virus. Research conducted in the last decade has shown that all these conditions may affect common molecular pathways, regulating genome stability, cell proliferation, cell survival and determination of cell identity.

### h. Rationale

The main focus of our group is to understand the molecular events activated by genetic and non genetic conditions leading to normal neuronal numbers and neuronal differentiation. In particular, on the genetic side we have been studying for many years the neurological syndrome produced in mammals by CITK inactivation, characterized by microcephaly, ataxia and epilepsy. This syndrome has recently been identified in humans with the name of MCPH17. Neural progenitors of humans or mice carrying CITK mutations fail to divide and undergo programmed cell death, leading to strong reduction of the final neuron number. During the last few years we have dedicated much effort to clarify the causal relationship between these events and the other mechanisms classically associated with microcephaly, such as asymmetric cell division of neural precursors and DNA damage. We also study the role played in Down syndrome by TTC3, which is one of the candidates belonging to the Down Critical Region (DCR), is overexpressed in other forms of intellectual disability and is known to interact with Citron proteins. On the non-genetic side, we have been studying the molecular events produced in neuronal progenitors by the flavivirus Zika, which has recently been linked to severe congenital microcephaly. In particular, we tested the hypothesis that Zika may act through some of the mechanisms which are known to contribute to genetic microcephaly. Finally, considering the strong involvement of CITK in proliferation, we are addressing the hypothesis that it may be required also by brain tumor cells, in particular those that characterize the pediatric tumor medulloblastoma. If this possibility should be confirmed, CITK could be an excellent target for the development of new drugs for these devastating tumors.

### e. Objectives

Specifically, our research aims at clarifying:

1. how mutations in Citron kinase lead to microcephaly;
2. what are the molecular consequences of CITK loss;
3. neuronal alterations in Down syndrome;
4. mechanisms of microcephaly induction by Zika virus;
5. CITK as a possible target for cancer therapy.

#### **f. Results**

1. We have discovered that, besides impairing cytokinesis, CITK may lead to microcephaly by two additional mechanisms. The first is through an alteration of the cell division plan, which may affect cell the rate of exit from the cell cycle and therefore reduce the number of neurons possibly generated by neural progenitors. An interaction between CITK and the prominent microcephaly protein ASPM is essential for this function.

The second mechanism is by directly regulating genomic stability, independently from its role in cytokinesis. Indeed, we found that cells lacking CITK display increased DNA damage early in the cell cycle. An alteration of DNA repair mechanisms may be the leading cause of this phenotype. Increased DNA damage leads to P53 activation, which is the main cause of apoptosis in CITK null models. Indeed, we found that the inactivation of P53 in CITK knockout animals leads to a disappearance of apoptosis, and strongly improves the overall neurological phenotype. The latter discovery could pave the way to the identification of new possible therapeutic strategies for apoptosis-related microcephaly.

2. CITK was originally identified as a protein important for remodeling the actin cytoskeleton. We have discovered that it may be even more important to regulate the stability of microtubules, and that this function is crucial both for completing cytokinesis and for spindle orientation.

3. Down syndrome (DS) is a multi-genic disorder produced by trisomy of Chromosome (Chr.) 21 and principally characterized by intellectual disability (ID), which also represents the most invalidating manifestation of the disease. However, the causative events that alter neuronal circuitry within the cortex remain unknown. During the last few years we used the Ts65Dn mouse model of Down syndrome to address the consequences of trisomy in the developing cortex and in cortical neurons in primary culture. Using these models, we found that the alteration of dendritic arborizations induced by trisomy are not neuron-intrinsic, because they are not present in cultures. In contrast, the characteristic defects in dendritic spines are visible both in cultures and in vivo. Moreover, trisomic neurons may be characterized by delay of cell migration. We are now focusing our attention on the role played in these phenotype TTC3, a gene located in the region of Chr. 21 believed to play the strongest role in determining intellectual disability.

4. Zika virus, a mosquito-borne flavivirus originally identified in Uganda in 1947 is the latest addition to the list of infectious agents that may lead to microcephaly. Since 2015, the spreading of ZIKV infection in Brazil and throughout Latin America has been associated with a sharp increase of the incidence of severe microcephaly, leading to the declaration of a 'Global Emergency' by the World Health Organization. We are studying the molecular mechanism by which Zika leads to microcephaly and in particular how these mechanisms may be related with those responsible for genetic microcephaly. We found that, as it has been described in the case of many microcephaly genes, including CITK, Zika infection leads to genotoxic stress and P53 activation, which may be the main event leading to apoptosis.

5. We are addressing the possibility that the function of CITK may be essential for proliferation in medulloblastomas, devastating brain tumors of the infancy that urgently require the development of new therapies. To do so, we have produced a conditional model for deleting CITK in medulloblastomas arising in mutant mice.

#### **g. Advancement in the field**

The results which we obtained have contributed important advances in the field of microcephaly studies, as testified by the publication of the results summarized above in important international journals.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### a. Summary

During the next three years, we plan first of all to continue the development of the current research lines and in particular:

1. We will continue to dissect the molecular mechanisms by which Citron kinase loss leads to microcephaly. To this regard, we plan to study the mechanisms by which CITK loss alters microtubule nucleation and stability. Moreover, we will investigate how CITK mutation or inactivation leads to DNA damage. Finally, we will address the possible causal relationships between these events and will investigate how they relate to the other genes so far involved in primary microcephaly and, possibly, in medulloblastoma progression.
2. We will continue to study the mouse model of Down syndrome Ts65Dn. In particular, we will investigate the role of the trisomic gene TTC3 in the generation of intellectual disability-related phenotypes in these mice. Moreover, we plan to use our computational biology skills to identify FDA approved molecules capable to improve the cellular and behavioural phenotypes of these mice.
3. We will continue to study the mechanisms by which the microcephaly virus Zika alters the proliferation and differentiation of neural progenitors. In particular, we will try to understand which are the causes of the genotoxic stress leading to P53 activation. Moreover, we will continue to investigate how the events activated by viral infection are related to the mechanisms of genetic microcephalies.

In addition to these research lines, we will work on the establishment of a collaborative network between NICO, Regina Margherita Hospital (Proff. B. Vitiello and G.B. Ferrero) and with the Regional Reference Centre for Madical Genetics (Prof. A. Brusco), aimed at the study of Neurodevelopmental Disorders, with particular emphasis on Autism and Intellectual disability.

### b. Background and Significance:

#### **Neurodevelopmental disorders and intellectual disability.**

Neurodevelopmental disorders (NDD) comprise a heterogeneous group of clinical diagnoses, including autism-spectrum disorders, intellectual disability (ID), attention deficit/hyperactivity and epilepsy. Although these syndromes are usually presented as distinct entities in the fifth edition of the Diagnostic Statistical Manual of mental disorders (DSM5) NDD have the tendency to co-occur in the context of complex clinical syndromes, often characterized by recurrence in families. NDD are frequently very invalidating and possess an enormous social impact, because they affect up to 3% of children. Modern molecular genetics technologies, based on massively parallel sequencing platforms, have allowed to identify many genetic alterations significantly associated to NDD. In few cases, the identification of the underlying mutations has rapidly allowed to identify a possible therapy. Despite these advances, the diagnostic and therapeutic approach to NDD is still very critical, especially because of their extremely heterogeneous and multifactorial origin.

#### **Microcephaly**

Congenital microcephaly (CM) is a heterogeneous group of disorders characterized by reduced head circumference at birth, to at least 3 standard deviations below the mean. CM can be the result of non-genetic conditions, such as viral infections and toxic exposure, or it can be generated by rare genetic disorders, with mostly autosomal recessive inheritance. Under More than 450 loci associated with microcephaly are known in the OMIM database. Primary hereditary microcephaly (MCPH) is the simplest form of genetic CM, in which brain size reduction is accompanied by grossly normal brain architecture and mild to moderate intellectual disability. Pure MCPH is a rare condition, since genetic CM is more often associated with syndromic features and co-morbidities, including structural brain abnormalities, seizures, palsy, ataxia, short stature, skeletal abnormalities and cancer predisposition. Although these conditions are usually classified as separate clinical entities, the elucidation of their genetic, molecular and cellular basis is revealing a high

degree of overlap. Our studies are aimed at significantly extending the current knowledge on these disorders, and to identify possible therapeutic strategies.

### **Down syndrome**

Down syndrome (DS) is the most frequent form of intellectual disability (ID) and is characterized by dosage imbalance of dozens of genes, which in turn affect the expression and may impact on function of hundreds of non-Hsa21 genes. The current focus of efforts directed at providing pharmacological treatments for DS is on the improvement of cognitive impairment. The development of suitable mouse preclinical models, especially of the Ts65Dn, was the first milestone achievement in this direction.

Systems Biology (SB) approaches are increasingly proposed, to move the search for ID-active drugs out of classical reductionism. SB methods could allow the identification of new drugable targets, which may potentially affect many different forms of ID. Even more importantly, the same methods may lead to the indication that some drugs, already in clinical usage for other disorders, have the potential of being useful for ID treatment. The latter approach, commonly referred to as 'drug repositioning', is especially interesting because it does not require the huge financial resources necessary to perform phase-one and phase-two clinical trials on new molecules and would therefore allow to move directly from pre-clinical models to patients. We have previously developed a novel SB-inspired method, based on the identification of Anticoexpressed Gene Clusters (CAGCs), to obtain strong drug repositioning hypotheses for rare genetic diseases.

### **c. General aim and integration with mission of the Institute**

The general aim of our group is to significantly advance our knowledge on neurodevelopmental disorders, in particular microcephaly and Down syndrome. In the next three years, we plan to extend our studies to selected forms of autism spectrum disorders. Since intellectual disability and behavioural abnormalities are the most important clinical consequences of these conditions, we think that our research is fully consistent with the mission of the Foundation and of the Institute.

### **d. Specific objectives and strategies:**

#### **1. Validation of new potential CITK partners and substrates identified through proteomics.**

To identify CITK physical interactors and substrates, we performed a proteomics screen. We identified many proteins capable of forming complexes with CITK independently of kinase activity, but also 34 proteins specifically co-purified by the catalytically inactive CITK bait. Importantly, the latter list contains many tubulins and tubulin-related molecules, suggesting that kinase activity is crucial for regulating CITK-microtubules interactions. We will work to validate the most interesting proteins in this list.

#### **2. Hypothesis-driven investigation of the molecular mechanisms through which CITK regulates microtubule dynamics.**

We found that CITK controls cytokinesis and spindle orientation by altering microtubule dynamics, a scenario supported by the results of our proteomics screen. These functions involve at least in part the capability of CITK to modulate TUBB3 phosphorylation through CK2a recruitment. Moreover KIF14, whose loss leads to microcephaly in mice and humans, is a partner of CITK in regulation of midbody stability. Since kinesins play established roles in microtubule dynamics and CK2a has been involved in kinesins' regulation, we will set out to obtain more information about the interplay between all these molecules.

#### **3. Hypothesis-driven investigation of the molecular mechanisms through which CITK prevents DSBs accumulation.**

An important question raised by our studies is how CITK protects cells from accumulation of DNA damage, independently of its role in cytokinesis. Therefore, we plan to investigate in detail the mechanisms by which CITK may affect RAD51, which shows reduced recruitment to foci. Moreover, we need to address whether other repair pathways, in particular the non-homologous end joining (NHEJ), are also compromised by CITK loss. A final question is whether the activity of CITK on microtubule dynamics and its role in genome stability are independent or related phenomena.

#### **4. Implementation of new mouse and human MCPH17 pre-clinical models.**

We aim at translating our mechanistic findings to experimental models directly relevant for the human disease. Since most MCPH17 patients carry kinase dead mutations, we have undertaken the production a



new mouse model, characterized by a similar alteration. We would also like to explore the potential usefulness of neural progenitor cells derived from MCPH17 patients as a possible platform for drug screening and validation, by transferring to these cells our discovery that the effects of CITK loss can be alleviated by P53 inactivation.

**5. Computational identification and experimental validation of new potential drugs for DS-related ID.**

We plan to obtain drug-repositioning hypothesis by analyzing recent and public DS gene expression datasets. Our assumption is that, although DS is caused by increased dosage of Hsa21 genes, the indirect down-modulation of these genes could play an important role in the overall phenotype. To identify the genes that display the strongest transcriptional anti-correlation with DS genes, we will resort to a previously described CNS-specific human anti-correlation network. This analysis is expected to produce a high number of potential target genes. We plan to validate a short list of the possible candidate drugs for their capability to revert the phenotypic abnormalities of primary neurons cultured from Ts65Dn mice.

**6. Identification of new genes involved in NDD.**

We will work with our collaborators to identify NDD patients who may carry novel genetic alterations. In particular, we will use our computational skills to analyze the copy number variation data and the exome sequencing data produced by our collaborators, to identify the variants most likely causing the disorders. We plan to validate the most interesting alterations using neural stem cell culture and also human brain organoids, derived from patient-specific induced pluripotent stem cells.

**i. Unique features of the project research:**

The most peculiar aspect of our group is our capability to combine different approaches, including computational biology, biochemistry, molecular biology and experimental analysis in cultured and in vivo models for approaching sophisticated biological questions related to brain development and brain disorders.

**j. Methodology: please fill-out this section only in the case of innovative technologies**

The most innovative aspects of our research will be:

1. the use of human brain organoids, derived from induced pluripotent stem cells. These sophisticated cultures are ideally suited to reproduce in culture the fundamental cellular events that characterize the first stages of brain embryonic development, especially those that are specific of humans and cannot be therefore mimicked by mouse models. We plan to setup this system at NICO, and to use it both for our studies on microcephaly and for functionally characterize the new NDD genes which we should identify with our collaborators.
2. the extensive use of computational biology/bioinformatics techniques, with the aim of directing and optimizing the experimental work.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Neuropsychopharmacology

## **1. LABORATORY DESCRIPTION – PERSONNEL:**

- **Principal Investigator**

Carola Eugenia Eva Birthdate (21/07/1957)  
PhD Female  
Italy Phone:+390116706608  
Email:carola.eva@unito.it

- **Personnel**

1. Alessandra Oberto Birthdate (24/10/1967)  
PhD in pharmacology Female  
Research Associate Italy  
Biotechnology, behavioral analysis, immunohistochemistry

2. Ilaria Bertocchi Birthdate (13/04/1982)  
PhD in pharmacology Female  
Research contract Italy

Biotechnology, behavior analysis, immunohistochemistry

3. Angela Longo Birthdate (19/03/1982)  
PhD in pharmacology Female  
Postdoctoral fellow Italy  
Behavioral analysis, immunohistochemistry

4. Paolo Mele Birthdate (22/06/1973)  
PhD in pharmacology Male  
Postdoctoral fellow Italy  
Expertise Behavioral analysis, immunohistochemistry

5. Mattia Ghigo Birthdate (12/08/1989)  
Master in Psychology Male  
PhD Student Italy  
Learning behavioral analysis and immunohistochemistry

## 2. PRINCIPAL INVESTIGATOR

H index 20, citations 1492 (Scopus)

### Relevant discoveries:

Carola Eva is internationally recognized for her studies on NPY e Npy1r receptor that were performed by using biomolecular, histochemical, image analysis, behavioural and pharmacological techniques.

The research group coordinated by Carola Eva has generated conditional knockout mice for the murine Npy1r gene that represent innovative models to study the effect of perinatal conditions on susceptibility to psychiatric diseases, behavioural inflexibility in OCD and energy homeostasis after menopause. The results of Dr. Eva's studies are published in 69 publications, 54 listed in PUB Med (1141 citations, H-index 19, mean IF 5,03).

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2014-2017	National	PI	Cariplo Foundation	A novel hypothesis on the development of metabolic syndrome in women	2013-0786	100.000	8%
2015-2017	National	PI	Compagnia di San Paolo Foundation	Influence of maternal behaviour on the expression of brain plasticity brakes: a role in the susceptibility to anxiety?		97.918,38	8%

Please list the name of PhDs you have supervised.

Ilaria Bertocchi

Please list honours, prizes or awards received, if applicable.

Please list your outreach activities

- describe your international collaborative experiences.
- Dr. Rolf Sprengel (Max Plank Institute for Medical Research, Heidelberg, Germany) we have started a collaboration to study the localization of fear memory engram and we will collaborate for behavioural characterization of conditional ko mice.
- We have sent our Npy1r floxed mice to Dr. Gavin Bewick (Division of Diabetes & Nutritional Sciences, School of Biomedical sciences, King's College London, London, UK). They will develop

an adult Npy1r beta cell specific knockout mouse to understand the importance of signalling at this receptor on beta-cell function.

- We have sent our Npy1r floxed mice to Dr. Roland Schuele (Department of Urology, Center for Clinical Research, University Freiburg Medical Center, Freiburg, Germany). They will induce cell-selective deletion of Npy1r in metabolic tissues such as liver and muscle to investigate whether histone demethylase LSD1 and Npyr1 might interplay to control LSD1-regulated gene activity.
- Collaborations with Dr Jessica Kwok (Faculty of Biological Sciences, University of Leeds, UK), Dr. Ralf Rütcher (Parque tecnológico de San Sebastián, San Sebastian, Spain) and with Dr. Stefano Vicini (Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington DC, USA).
- We have sent our Npy1r floxed mice to Prof. Martyn Goulding (Molecular Neurobiology Laboratory The Salk Institute, La Jolla, CA,USA)

- Editorial duties

Reviewer of manuscripts for

Eating and Weight Disorders, Endocrinology, Plos One, European Neuropsychopharmacology

Please list your organizational activities:

- Speakers invited
- 
- Workshops, Schools or Conferences organized

Please list your technology transfer achievements (patents, etc.), if applicable

Founding member and President of the spinoff S&P BRAIN

S&P BRAIN 2017 activities

- Mentoring program of the Camera di Commercio di Torino (Mentor: Dr. A.Pellacani, Scientific Director of Menarini in 2017)
- ISO 9001:2015 certification starting January 2018
- Meet in Italy participation (Torino, 2017)
- Enterprise Europe Network registration
- Bioindustry Park association
- Business partnership with the CRO Accelera (Nerviano, MI)

### **3. PI's PUBLICATIONS:**

Longo A, Fadda M, Brasso , Mele P1, Palanza P, Nanavaty I, Bertocchi I, Oberto A, Eva C. (2018) *Neuropharmacology* 133:12-22. Conditional inactivation of Npy1r gene in mice induces behavioural inflexibility and orbitofrontal cortex hyperactivity that are reversed by escitalopram. IF=5.012; R=Q1 44/259.

Statello R, Carnevali L, Paterlini S, Gioiosa L, Bertocchi I, Oberto A, Eva C, Palanza P, Sgoifo A. *Physiol Behav.* (2017) 172:31-39. Reduced NPY Y1 receptor hippocampal expression and signs of decreased vagal modulation of heart rate in mice. IF= 2.34; R= Q2 6/14

Nuzzo AM, Giuffrida D, Masturzo B, Mele P, Piccoli E, Eva C, Todros T, Rolfo A. (2017) Altered expression of G1/S phase cell cycle regulators in placental mesenchymal stromal cells derived from preeclamptic pregnancies with fetal-placental compromise. *Cell Cycle.* 16(2):200-212. IF=3.53; R=Q2 88/190

### **4.GROUP's PUBLICATIONS:**

Lissek T1 et al. (2017) Building Bridges through Science. *Neuron.* 96(4):730-735. IF=14.02; R=Q1 6/259

### **5. GROUP's additional information:**

## 6 .Past Research activity

### a. Summary

We use two different conditional mutant mice to investigate the role of NPY-Y1R transmission in

- 1) resilience to psychopathologies (anxiety disorders and OCD endophenotypic traits)
- 2) sex-related differences in the brain control of energy homeostasis *Npy1rrfb* mice
- 3) susceptibility to Metabolic syndrome (MetS) after menopause
- 4) cognitive functions
- 5) structural plasticity

### b. Background

1) NPY plays an important role in the response to stress and in psychiatric disorders. In humans, NPY haploinsufficiency is correlated with characteristic brain responses to emotional and stress challenges and with trait anxiety. In rodents, NPY reduces both anxiety and stress-related behavior, an effect that is primarily mediated by *Npy1r* expressed in amygdala, hippocampus, and locus. Early life experience, such as maternal environment, has a central role in the susceptibility to psychopathology in adulthood. Anxiety and emotionality are influenced by exposures to stress in a pattern consistent with gene–environment interaction. These observations point to the importance to identify stress-vulnerability associated genes.

2) In addition to its crucial role in emotional behavior, NPY is the prototype hormone to stimulate feeding, reduce energy expenditure and induce obesity via the activation of hypothalamic *Npy1r*. NPY-signaling in the hypothalamus is strongly influenced by the nutritional status, and estrogen receptors activate *Npy1r* gene transcription, strongly suggesting that brain *Npy1r* represents a key metabolic target gene through which estrogens modulate energy metabolism in relation to reproductive activity.

3) NPY also plays a role in the regulation of learning and memory, but the underlying mechanisms are far from clear. During spatial memory training, hippocampal NPY gene expression is increased and NPY knock-out mice display impaired spatial memory.

4) NPY regulates cognition and emotional behaviour via the activation of Y1 and Y5R receptors. In rodents, *Npy1r* and *Npy5r* have an overlapping role in regulating anxiety and co-localize in several forebrain regions belonging to circuits of cognitive and emotional functions.

Rationale (2000 characters)

### c. Rationale

1) *Y1Rrfb* male mice display increased anxiety, reduced body weight, and increased HPA axis activity. Moreover, differences in phenotypes between *Npy1rrfb* and floxed (control) mice became apparent when both genotypes were raised by dams with high levels of maternal care, suggesting that *Npy1r* represents one of the targets of maternal care-induced programming of anxiety resilience (Bertocchi et al., 2011). Given the role that perineuronal nets (PNN) development during juvenile development retain in regulating synaptic activity and structural stability and that impaired prefrontal cortex (PFC) plasticity is thought to be a core pathological feature of several neuropsychiatric disorders, we investigated whether limbic *Npy1r* plays a role in maternal environment modulation of PNN in PFC.

2) The incidence of MetS increases significantly after menopause suggesting the potential involvement of ovarian steroids. In the arcuate nucleus, estrogen increases activity of anorexigenic POMC neurons and represses synthesis of orexigenic AgRP and NPY. Given that *Npy1rrfb* male but not female mice show an increased vulnerability to metabolic challenges in adulthood, we investigated whether limbic *Npy1r* represents a key target gene through which estrogens in brain modulate energy. metabolism in relation to reproductive functions.

3) PNNs play crucial role in learning and memory. In the amygdala PNNs protect fear memories from erasure, and disruption of the PNNs in the hippocampus or the mPFC impairs long-term fear memory. Moreover, degradation of PNNs in the cortex chondroitinase enzymatic digestion enhances object recognition memory. To elucidate whether *Npy1r* plays a role in learning and memory and affects PNNs, we tested spatial memory of *Npy1rrfb* and we analyzed PNN expression and distribution in the dorsal hippocampus,

4) *Npy1rY5R<sup>-/-</sup>* mice show an anxious phenotype that might be related to inactivation of the Y1R in the BLA. Additionally, *Npy1rY5R<sup>-/-</sup>* mice display increased spatial reference memory, suggesting an inflexible-perseverative phenotype and habit learning (Longo et al, 2014; 2015).

**c. Objectives (1500 characters)**

The aims of our past research were:

- 1) “*Vulnerability to psychopathologies*”: to uncover the extent of involvement of NPY and its cognate *Npy1r* in modulating inter-individual variation in emotion and stress resiliency, with specific attention to the role of NPY-*Npy1r* system in permanent effects of maternal care on behavioral and PNN.
- 2) “*Gender difference in vulnerability to metabolic challenges*”: to determine the effect of a moderate to high fat, high-energy diet on *Npy1r* gene expression in the hypothalamus of male and female mice.
- 3) “*Neuropeptide Y pathways in learning and memory*”: to examine whether selective ablation of *Npyr* in forebrain excitatory neurons may affect learning and memory and PNN expression.
- 4) “*Npy1r and Behavioural inflexibility*”: to uncover whether the targeted disruption of *Npy1r* gene in Y5R containing neurons affects cognitive flexibility and neuronal activity in the OFC and whether treatment with the SSRI escitalopram normalizes OFC neuronal activity and restores behavioural flexibility in *Npy1rY5R<sup>-/-</sup>* mice.

**d. Results (4000 characters)**

- 1) We have demonstrated that the lower expression levels of *Npy1r* in the limbic system in adult mice, induced by low levels of maternal care or by postnatal inactivation of *Npy1r* in excitatory neurons in *Npy1rrfb* mice, is coupled with a deficit of prefrontal cortex driven cognitive abilities in the puzzle box test, a problem-solving test with increasing difficulty. that is associated with increased density of PNN in the frontal cortex (Bertocchi et al., in preparation).
- 2) We demonstrated that the postnatal inactivation of *Npy1r* in excitatory neurons of *Npy1rrfb* mice induce a different metabolism-related phenotype in male and in female mice. *Npy1rrfb* male display a lower growth rate, decreased leptin serum levels, and lower weight of visceral, s.c. and epididymal white adipose tissue (WAT) compared their control littermates. Conversely, conditional mutant female mice showed no changes in growth rate and WAT weight but increased leptin serum levels. Of interest, *Npy1rrfb* female, but not male, mice show a significant decrease in *Npy1r* mRNA in the paraventricular nucleus. *Npy1rrfb* female mice also display resilience to metabolic challenges, compared with their control littermates that is no longer observed in ovariectomized (ovx) *Npy1rrfb* female mice (a condition mimicking menopause).
- 3) *Npy1rrfb* mice show a delayed spatial learning in both the Morris water maze and the Barnes maze compared and a significant increase of the number of strongly stained PNNs in the CA1 region, compared to control mice. When we restricted our analysis of PNN intensity to PV+ neurons in the CA1, the intensity of WFA signal of *Npy1rrfb* mice was significantly stronger than that of control animals, in both the stratum pyramidale and the stratum oriens. Bilateral injection in the CA1, of chondroitinase ABC reverts the learning impairment of *Npy1rrfb* mice during the early phase of the MWM (Mele et al, in preparation)
- 4) Cognitive flexibility is the ability to rapidly adapt established patterns of behaviour in the face of changing circumstance and depends critically on the orbitofrontal cortex (OFC). Impaired flexibility also results from altered serotonin transmission in the OFC. The Y1 (Y1R) and Y5 (Y5R) receptors for neuropeptide Y (NPY) colocalize in several brain regions and have overlapping functions in regulating cognition and emotional behaviour. The targeted disruption of gene encoding Y1R (*Npy1r* gene) in Y5R containing neurons (*Npy1rY5R<sup>-/-</sup>* mice) increases anxiety-like behaviour and spatial reference memory. Here we used the same conditional system to analyse whether the coordinated expression of the Y1R and Y5R might be required for behavioural flexibility in reversal learning tasks, OFC serotonergic tone and OFC neural activity, as detected by immunohistochemical quantification of the immediate-early gene, c-Fos. In addition, we investigated whether the acute treatment of *Npy1rY5R<sup>-/-</sup>* mice with the selective serotonin reuptake inhibitor escitalopram affected behavioural flexibility and OFC c-Fos expression. *Npy1rY5R<sup>-/-</sup>* male mice exhibit an impairment in performing the reversal task of the Morris water maze and the water T-maze but normal spatial learning, working memory and sociability, compared to their control siblings. Furthermore, *Npy1rY5R<sup>-/-</sup>* male mice display decreased 5-hydroxytryptamine (5-HT) positive fibres and increased baseline neural activity in OFC. Importantly, escitalopram normalizes OFC neural activity and restores behavioural flexibility of *Npy1rY5R<sup>-/-</sup>* male mice.



**Advancement in the field (1000 characters)**

- 1) We provided the first experimental genetic evidence that NPY/Npy1r pathways in the limbic system are key targets of maternal care-induced programming of cognitive abilities.
- 2) We have shown, for the first time, a sexual dimorphism of Npy1r expression in the PVN that might underline the interaction between the Npy1r signal and estrogens in the modulation of energy metabolism in relation to reproductive functions. We also demonstrated that ko mice may display sex related phenotypes.
- 3) We demonstrated that local enzymatic digestion of PNNs reverses the learning deficits of Y1 knock-out mice, highlighting a previously unknown link between NPY-Y1 function and PNN regulation.
- 4) We demonstrated that the inactivation of Y1R in Y5R containing neurons increases pyramidal neuron activity and dysregulates serotonergic tone in OFC, whereby contributing to reversal learning impairment

## 7. Future Projects (Next 2 years)

### a. Summary (up to 2000 characters):

In the next two years we will investigate the role of limbic NPY-Npy1r signal in the resilience to psychopathologies and in the sex differences (and effect of chronic stress) in susceptibility to metabolic syndrome. We will therefore develop two main projects that are described below in details: “Vulnerability to psychopathologies” and “Gender difference in vulnerability to metabolic challenges”. In parallel, we will also focus on the role of Npy1r in behavioral inflexibility of Npy1rY5R<sup>-/-</sup> mice, by analyzing the effect of selective deletion of Npy1r in the OFC on the reversal task of the MWM and c-fos immunoreactivity (*Npy1r and Behavioural inflexibility*).

### b. Background and Significance (up to 4000 characters):

#### 1) “Vulnerability to psychopathologies”

We previously demonstrated that lower expression levels of Npy1r in the limbic system of adult mice, induced by low levels of maternal care or by postnatal inactivation of Npy1r in excitatory neurons, increases anxiety, HPA axis activity and PFC driven cognition skills. .

Given the role that PNN development and myelin maturation during juvenile development retain in regulating synaptic activity and structural stability and given that impaired PFC function and plasticity is thought to be a core pathological feature of several neuropsychiatric disorders, our main goal is to evaluate whether: i) a rearrangement of PNNs (in terms of thickness, molecular composition, sulfation pattern, physical properties) and myelin development in limbic areas is involved in maternal care programming of anxiety-like phenotype; ii) Enzymatic disruption of the PNNs in the PFC can restore cognitive performance of Npy1rrfb mice, highlighting a previously unknown link between NPY-Y1R transmission and PNNs expression in the limbic system. Development of the present project will be based on the synergic collaboration among groups working at NICO (A. Buffo and D. Carulli) and international groups characterized by diverse, but strongly complementary, scientific expertise. By connecting molecular interactions and matrix properties to behavior, we expect that this collaborative research will offer mechanistic insight into the role of plasticity modulators in inducing anxiety in early-life stressed mice.

#### 2) “Gender difference in vulnerability to metabolic challenges”

Prior studies demonstrated that estrogen, not progesterone, is responsible for the control of energy homeostasis. In mammals ovaries and liver are the main targets of estrogen action and the liver estrogen receptor (the isoform ER $\alpha$ ) is essential for the regulation of lipid metabolism. Previous results obtained by A. Maggi’s laboratory (University of Milano) suggest that the liver is a key element at organismal level for the co-ordination of the hormetic response. The aim of the research, that will take advantage from previous collaboration among NICO, the University of Milano (A. Maggi, E. Nisoli) and of Parma (P. Palanza) is to demonstrate the mechanistic and biological consequences of liver ER $\alpha$  stimulation on organs other than liver and relevant for energy homeostasis (fat, muscle and brain) in mice fertile or with surgical menopause. The study will be done in the presence/absence of hormetic stimuli (e.g. overnutrition, exercise). In particular our laboratory will investigate physiological and neurochemical mechanisms underlying the cross talk between estrogen and brain Npy1r in the control of energy homeostasis and, most importantly, whether such interaction could involve bidirectional signals between liver and brain.

### c. General aim and integration with mission of the Institute (up to 1000 characters)

The mission of Cavalieri Ottolenghi Foundation is “to study in depth the current knowledges on the interconnections between chemical-physical condition of the human body and psychological symptoms namely, on causes and cure of mental disorders”.

Our projects well integrate with the mission since:

-project 1 will be focused on the understanding of neurobiological, biochemical, physical and neurophysiological mechanisms underlying structural neuronal plasticity and, in turn, a wide range of psychopathological disorders, characterized by unbalanced excitatory and inhibitory systems.

-project 2 will be focused on the understanding of brain molecular and neurochemical mechanisms underlying the gender related differences in vulnerability to the pathogenesis of obesity and MetS and their comorbidity with stress related disorders.

-project 3 will be focused on the understanding of the fronto-striatal neuro-circuitry underlying the reversal behaviour deficit of OCD.

-all projects aim to reveal new targets for therapeutic interventions in stress and anxiety-related disorders.

**d. Specific objectives and strategies (up to 4000 characters):**

1) *“Vulnerability to psychopathologies”*

In the limbic system of mice exposed to high/low maternal care we will analyze:

- thickness and number of PNNs around specific populations of GABAergic neurons in adult mice.
- myelin/PNN appearance during juvenile development. We expect that the long term effect of maternal behavior on anxiety and stress susceptibility may be mediated by a precocious development of plasticity brakes;
- alterations of myelin structure and myelin plasticity inhibitors.
- remodeling of GABAergic, glutamatergic and NPYergic innervation and modifications of dendritic branching and dendritic spines.

To investigate the role of Npy1r in PNN formation, the same analysis will be run in wild type and Npy1rrfb mice.

To prove that PNN in the limbic system is implicated in the maternal care-induced programming of anxiety, we will examine the effect of maternal environment on PNNs, neuritic modifications and behavior in: i) mice after PNN digestion by chondroitinase in specific limbic regions; and ii) mice exposed to environmental enrichment.

Moreover:

a) in collaboration with Dr. J. Kwok (Faculty of Biological Sciences, University of Leeds, UK), we will investigate whether PNNs bind different molecules upon low maternal care or conditional deletion of Npy1r gene, which may be important for the development of anxiety. We will focus on: analysis of the molecular composition of myelin and of different nets in the limbic system ; detection of changes in myelin and PNN sulfation pattern in the limbic system and proteomic analysis to detect binding partners of differently composed PNNs and of myelin.

b) in collaboration with Dr R.Richter (University of San Sebastian, Spain), we will assess mechanical features of myelin and PNNs in the different experimental conditions, such as elastic compressibility (i.e. stiffness or Young's modulus), viscosity and threshold forces required for irreversible deformation.

2) *“Gender difference in vulnerability to metabolic challenges”*

Female floxed and Npy1r mice exposed to normal diet, diet enriched in lipids and exercise will be analysed for

- body weight, food consumption, glucose and insulin tolerance tests, locomotor activity, blood pressure
- Npy1r, NPY and CRH mRNA expression in limbic system and hypothalamus
- histochemical analysis of  $\alpha$ MSH and CART (anorexigenic peptides) and NPY and AgRP (orexigenic peptides) in the hypothalamus.
- in liver, fatty acids, FGF21, Sirt1, lipid metabolism, mitochondrial biogenesis, activity and respiration;
- ii) in serum, IGF-1, GH, leptin, tryglicerides, FFA, LDL, HDL, cholesterol, FGF21 Glucose, Insulin; iii) in WAT, BAT, muscle and brain, mitochondrial biogenesis, UCP1, mitochondrial analysis by electron and confocal microscopy, ATP and O<sub>2</sub> consumption, glucose concentration, lipid metabolism, and insulin sensitivity, Sirt1; in WAT and BAT, white adipose fat and sympathetic parameters.

In addition:

- to investigate the physiological relevance of the liver-CNS axis for the control of energy metabolism, in collaboration with Dr. A. Maggi (University of Milano) we will analyze the effect of the different diets in combination with ovx on NPY signaling in limbic and hypothalamic areas of wild type and liver specific ER $\alpha$  ko mice.

**e. Unique features of the project research (up to 2500 characters):**

*“Vulnerability to psychopathologies”*

Epidemiologic evidence suggests a strong association between poor postnatal environments and the development of psychiatric disorders in adult life. The neuronal plasticity associated to brain development during early infancy might be considered a possible risk factor for psychopathology but

also a potent mechanism for compensation. In this project we will combine several innovative approaches, including the study of the glyco-profile and mechanical features of PNNs/myelin, and optogenetic manipulation of neuronal activity, to address the role of plasticity-regulatory molecules in early-life stress-induced anxiety from entirely novel angles, spanning different levels of complexity, from molecules to the living organism. We expect to gain further insight into the complex and fine-tuned mechanisms underlying developmental programming. Moreover, we expect to elucidate whether pathological behaviours can be reverted to normal by enriched experience and/or pharmacological treatment in adulthood. The knowledge that will originate from this application has a tremendous potential in view of finding novel therapeutic approaches and early intervention strategies for the cure and possibly prevention of mental disorders, such as anxiety and stress-related disorders that have a strong relationship with early life adversities.

*“Gender difference in vulnerability to metabolic challenges”*

This is an innovative study focused on novel pathogenetic mechanisms that may lead to the age-related disorders in females. It is based on the integration of the knowledge of multiple disciplines (molecular biology, cell biology, behavioral science and metabolic phenotyping), as well as on the synergic collaboration between groups characterized by diverse, but strongly complementary, scientific expertise. It is expected that this collaborative research will allow: i.) to demonstrate the extent by which changes of ovarian estrogens and liver ERs are responsible for the increased incidence of MetS during menopause; ii.) to identify the potential mechanisms involved in this phenomenon, including chronic social stress; iii.) to evaluate the effects of specific dietary regimens in females with impaired ovarian functions. In case we were able to demonstrate a major involvement of liver ER in the dysmetabolisms consequent to the cessation of ovarian functions, our study will open the way to the generation of an entire class of novel Estrogen Receptor Modulators to be used for the therapy of MetS, thus allowing a major step forward in the development of therapies for a disorder that so far cannot be satisfactorily treated.

**f. Methodology (up to 2000 characters): please fill-out this section only in the case of innovative technologies**

To generate OFC-specific *Npy1r* gene knockout mice, we will apply combinatorial genetic approach using viruses for conditional Cre recombinase (*Cre*) gene expression in genetically engineered mice (*Npy1r*<sup>2lox</sup>) in which exons of the *Npy1r* gene were flanked with *loxP* sites. We will use two recombinant adeno-associated viruses (rAAVs), which are equipped with the doxycycline (Dox)-controlled genetic switches. The first virus (*rAAV-hSYN-rtTA*) allows expression of reverse tetracycline transactivator (*rtTA*) gene under control of the human synapsin promoter (*hSYN, Pro*). The second virus (*rAAV-P<sub>tet</sub>bi-Cre/tdTOM*) is equipped with a bidirectional tet promoter (*P<sub>tet</sub>bi*) to simultaneously express two different genes encoding for the Cre recombinase protein and a red fluorescent protein variant, the tdTomato (tdTOM), for visualizing virus-targeted neurons. The *Cre* and the *tdTOM* genes are expressed when rtTA binds to *P<sub>tet</sub>bi* in the presence of Dox. To increase the spread of virus in the OFC by hyperosmolarity, D-mannitol/virus mixture will be injected in the brain and D-mannitol was also delivered into mice systemically by intraperitoneal injection. Viruses will be injected in the OCD of age-matched wild-type littermates and *Npy1r*<sup>2lox</sup> mice. tdTOM and Cre expression in the OFC will be analysed three weeks after virus injection. Virus (v)-delivered Cre expression in neurons enabled, by Cre-loxP-mediated gene recombination, the generation of highly specific *Npy1r* gene knockout mice, *Npy1r-vΔ*<sup>OFC</sup>, in which the *Npy1r* gene will be selectively deleted ( $\Delta$ ) in the OFC. The virus-injected, age-matched wild-type littermates will serve as controls (*Contr-v*<sup>OFC</sup>).



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Peripheral Nerve Regeneration Unit

## **1. LABORATORY DESCRIPTION – PERSONNEL:**

### **Principal Investigator**

Stefano Geuna Birthdate: 25/09/1965  
Degree: Graduated in Medicine and Surgery, University of Turin Gender: Male  
Nationality: Italian  
Phone: +39 011/6705433  
Email: stefano.geuna@unito.it

### **Personnel**

1. Stefania Raimondo Birthdate: 25/02/1977  
Degree: Biological Sciences, University of Turin Gender: Female  
Role: Researcher Nationality: Italian  
Expertise:

- Light, confocal and electron microscopy
- Stereological and morpho-quantitative analysis
- Retro-transcriptase polymerase-chain-reaction (RT/PCR) and Western Blot
- Functional tests for motor recovery (grasping test)
- Cell and tissue transplantation
- Cell cultures

2. Giulia Ronchi Birthdate 27/11/1982  
Degree: Master degree in Neurobiology, University of Turin Gender: Female  
Role: Post-doctoral fellowship recipient Nationality: Italian  
Expertise:

- Light, laser confocal and electron microscopy.
- Immunohistochemistry and Western blot.
- RT-PCR and quantitative Real Time PCR
- Cell and tissue (DRG explants) culture.
- Surgical procedures to induce peripheral nerve damages.
- Stereological and morpho-quantitative analysis
- Functional tests for motor recovery (grasping test)

3. Federica Fregnan Birthdate: 02/07/1976  
Degree: biological sciences, University of Turin Gender: Female  
Role: Post-doctoral fellowship recipient Nationality: Italian  
Expertise:

- Optical microscopy and confocal analysis of histological and cytological specimens prepared by histological techniques, histochemical and immunofluorescence.
- Electron microscopy and ultrastructural analysis.
- Analysis of recovery of motor function in rats and mice using behavioral tests.

- Quantitative morphological analysis of the regeneration of nerve fibers by stereological methods.
- Extraction and culture of primary lines of ganglion sensory neurons.
- Analysis of protein expression (western blotting) and mrna (qualitative rt-pcr and real time quantitative rt-pcr).
- Cell culture, transient and stable transfection, proliferation assays, migration assays, time lapse.
- Recombinant techniques of molecular biology (cloning, production of fusion proteins with gfp or flag, preparation of constructs in plasmids, adenoviral and lentiviral vectors).
- Validation of microarray analysis.

4. Luisa Muratori Birthdate 02/05/1984

Degree: Master degree in Neurobiology, University of Turin

Gender: Female

Role: PhD Student

Nationality: Italian

Expertise:

- Light and laser confocal microscopy.
- Immunohistochemistry and Western blot.
- RT-PCR and quantitative Real Time PCR
- Cell and tissue (DRG explants) culture.

5. Benedetta Elena Fornasari Birthdate: 11/07/1989

Degree: Master degree in Molecular and Cellular Biology, University of Turin

Gender: Female

Role: PhD Student

Nationality: Italian

Expertise:

- BIOMOLECULAR TECHNIQUES: DNA, RNA and protein extraction, quantitative Real-time PCR, primers study and design, Western blot.
- CELLULAR BIOLOGY: cell culture, primary culture of Schwann cells and dorsal root ganglia, cell transfection, cell migration assays, proliferation and viability assays, time lapse assays.
- MORPHOLOGICAL ANALYSIS: immunofluorescence, resin embedding procedures
- ANIMAL CARE
- BIOMATERIAL PRODUCTION: production of fibres through electrospinning technique

6. Michela Morano Birthdate: 29/10/1988

Degree: Master degree in Molecular and Cellular Biology, University of Turin

Gender: Female

Role: PhD Student

Nationality: Italian

Expertise:

- Manual ability with different type of cell cultures (cell lines and also primary cultures)

- Molecular and cellular biology techniques known and used: PCR, Real-Time PCR, cloning plasmids, western blot, cell transfection, transwell migration assay, immunofluorescence, ELISA, MTT survival assay.
- Informatics competences: well knowledge of programs with biological application (Annhyb, GraphPad, ImageJ, PlasmaDNA, ) graphic software (Photoshop, Prezi, Inkscape), Microsoft Office (Excel, Word, PowerPoint) and editing software (Mendelev, Endnote).

7. Loredana Grasso

Birthdate: 13/08/1982

Degree: Master degree in biological science

Gender: Female

Role: Fellowship recipient

Nationality: Italian

Expertise:

- Cell (blood cell) cultures.
- DNA extraction from whole blood by Phenol / Chloroform
- Surgical procedures to induce muscle damages and muscle's extraction.
- Paraffin embedding
- Histological staining
- Western blotting
- Immunohistochemistry



## 2. PRINCIPAL INVESTIGATOR CURRICULUM VITAE (two pages)

H index, 40, citation 4457 (Scopus)

### Relevant discoveries:

The results of our recent research, in the context of the European Project funded (Biohybrid), allowed to put on the market Reaxon<sup>®</sup> Nerve Guide, a chitosan conduit design for the repair of peripheral nerves. Reaxon<sup>®</sup> Nerve Guide got the CE mark in January 2014.

Please list your grants according to the table below (current and pending).

Startin g- end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Direc tly Avail able to NICO
2016-2017	National	PI	Università degli Studi di Torino	NeuroLink - Esercizio fisico e malattie del sistema nervoso: focus sulle cellule della glia	Fondo per la Ricerca Locale	€ 5776,63	8%

### Please list the name of PhDs you have supervised.

- Morano Michela
- Muratori Luisa

### Please list honours, prizes or awards received, If applicable.

None

### Please list your outreach activities

- International collaborative experiences.

2010-present: President of the European Microsurgical Research Association.

2014-present: general Secretary of the European Society for Peripheral Nerve Repair and Regeneration, Bruxelles.

- Invited talks

TERMIS European Chapter Meeting 2017 - 26 - 30 June, 2017, Davos, Switzerland

6<sup>th</sup> Vienna Symposium on Surgery of Peripheral Nerves - March 17-19, 2017 Vienna, Austria.

- Editorial duties

Member of Editorial Board of *Microsurgery*

**Please list your organizational activities:**

- Speakers invited

Prof. Yuki Shirozaki, Fukuoka University, Japan - "Introduction of Kyutech & inorganic-organic hybrids for bone and nerve regeneration".

- Workshops, Schools or Conferences organized

4th International Symposium on Peripheral Nerve Regeneration July 06th - 08th, 2017 - Barcelona, Spain (Scientific Committee member)

**Please list your technology transfer achievements (patents, etc.), if applicable**

In 2016, a patent about the clinical use of chitosan membranes for protection of peri-prostatic nerve plexus has been issued. (Application reference: 102016000070911).

**3. PI's PUBLICATIONS:**

Ronchi G, Cillino M, Gambarotta G, Fornasari BE, Raimondo S, Pugliese P, Tos P, Cordova A, Moschella F, Geuna S. Irreversible changes occurring in long-term denervated Schwann cells affect delayed nerve repair. *J Neurosurg.* 2017 Oct;127(4):843-856. doi: 10.3171/2016.9.JNS16140. Epub 2017 Jan 6. PubMed PMID: 28059646.

Ribeiro J, Caseiro AR, Pereira T, Armada-da-Silva PA, Pires I, Prada J, Amorim I, Leal Reis I, Amado S, Santos JD, Bompasso S, Raimondo S, Varejão AS, Geuna S, Luís AL, Maurício AC. Evaluation of PVA biodegradable electric conductive membranes for nerve regeneration in axonotmesis injuries: the rat sciatic nerve animal model. *J Biomed Mater Res A.* 2017 May;105(5):1267-1280. doi: 10.1002/jbm.a.35998. Epub 2017 Feb 13. PubMed PMID: 28078802.

Carriel V, Campos A, Alaminos M, Raimondo S, Geuna S. Staining Methods for Normal and Regenerative Myelin in the Nervous System. *Methods Mol Biol.* 2017;1560:207-218. doi: 10.1007/978-1-4939-6788-9\_15. PubMed PMID: 28155156.

Morano M, Angotti C, Tullio F, Gambarotta G, Penna C, Pagliaro P, Geuna S. Myocardial ischemia/reperfusion upregulates the transcription of the Neuregulin1 receptor ErbB3, but only postconditioning preserves protein translation: Role in oxidative stress. *Int J Cardiol.* 2017 Apr 15;233:73-79. doi: 10.1016/j.ijcard.2017.01.122. Epub 2017 Jan 31. PubMed PMID: 28162790.

Onger ME, Kaplan S, Deniz ÖG, Altun G, Altunkaynak BZ, Balcı K, Raimondo S, Geuna S. Possible promoting effects of melatonin, leptin and alcar on regeneration of the sciatic nerve. *J Chem Neuroanat.* 2017 Apr;81:34-41. doi: 10.1016/j.jchemneu.2017.02.003. Epub 2017 Feb 3. PubMed PMID: 28163216.

Mazzara PG, Massimino L, Pellegatta M, Ronchi G, Ricca A, Iannielli A, Giannelli SG, Cursi M, Cancellieri C, Sessa A, Del Carro U, Quattrini A, Geuna S, Gritti A, Taveggia C, Broccoli V. Two factor-based reprogramming of rodent and human fibroblasts into Schwann cells. *Nat Commun.* 2017 Feb 7;8:14088. doi: 10.1038/ncomms14088. PubMed PMID: 28169300; PubMed Central PMCID: PMC5309703.

Luganini A, Cavaletto N, Raimondo S, Geuna S, Gribaudo G. Loss of the Human Cytomegalovirus US16 Protein Abrogates Virus Entry into Endothelial and Epithelial Cells by Reducing the Virion Content of the Pentamer. *J Virol.* 2017 May 12;91(11). pii: e00205-17. doi: 10.1128/JVI.00205-17. Print 2017 Jun 1. PubMed PMID: 28331097; PubMed Central PMCID: PMC5432857.

Reano S, Angelino E, Ferrara M, Malacarne V, Sustova H, Sabry O, Agosti E, Clerici S, Ruozi G, Zentilin L, Prodam F, Geuna S, Giacca M, Graziani A, Filigheddu N. Unacylated Ghrelin Enhances Satellite Cell Function and Relieves the Dystrophic Phenotype in Duchenne Muscular Dystrophy mdx Model. *Stem Cells.* 2017 Jul;35(7):1733-1746. doi: 10.1002/stem.2632. Epub 2017 May 7. PubMed PMID: 28436144.

Cherubino M, Pellegatta I, Crosio A, Valdatta L, Geuna S, Gornati R, Tos P. Use of human fat grafting in the prevention of perineural adherence: Experimental study in athymic mouse. *PLoS One.* 2017 Apr 26;12(4):e0176393. doi: 10.1371/journal.pone.0176393. eCollection 2017. PubMed PMID: 28445518; PubMed Central PMCID: PMC5406022.

Geuna S, Papalia I, Ronchi G, d'Alcontres FS, Natsis K, Papadopulos NA, Colonna MR. The reasons for end-to-side coaptation: how does lateral axon sprouting work? *Neural Regen Res.* 2017 Apr;12(4):529-533. doi: 10.4103/1673-5374.205081. Review. PubMed PMID: 28553322; PubMed Central PMCID: PMC5436340.

Onger ME, Kaplan S, Geuna S, Türkmen AP, Muratori L, Altun G, Altunkaynak BZ. Possible effects of some agents on the injured nerve in obese rats: A stereological and electron microscopic study. *J Craniomaxillofac Surg.* 2017 Aug;45(8):1258-1267. doi: 10.1016/j.jcms.2017.05.004. Epub 2017 May 11. PubMed PMID: 28601299.

Diogo CC, Camassa JA, Pereira JE, Costa LMD, Filipe V, Couto PA, Geuna S, Maurício AC, Varejão AS. The use of sheep as a model for studying peripheral nerve regeneration following nerve injury: review of the literature. *Neurol Res.* 2017 Oct;39(10):926-939. doi: 10.1080/01616412.2017.1331873. Epub 2017 Jun 11. PubMed PMID: 28604272.

Porpiglia F, Bertolo R, Fiori C, Manfredi M, De Cillis S, Geuna S. Chitosan membranes applied on the prostatic neurovascular bundles after nerve-sparing robot-assisted radical prostatectomy: a phase II study. *BJU Int.* 2017 Jul 15. doi: 10.1111/bju.13959. [Epub ahead of print] PubMed PMID: 28710845.

Stenberg L, Stößel M, Ronchi G, Geuna S, Yin Y, Mommert S, Mårtensson L, Metzen J, Grothe C, Dahlin LB, Haastert-Talini K. Regeneration of long-distance peripheral nerve defects after delayed reconstruction in healthy and diabetic rats is supported by immunomodulatory chitosan nerve guides. *BMC Neurosci.* 2017 Jul 18;18(1):53. doi: 10.1186/s12868-017-0374-z. PubMed PMID: 28720074; PubMed Central PMCID: PMC5516317.

Diogo CC, Costa LMD, Pereira JE, Filipe V, Couto PA, Magalhães LG, Geuna S, Armada-da-Silva PA, Maurício AC, Varejão AS. Dynamic feet distance: A new functional assessment during treadmill locomotion in normal and thoracic spinal cord injured rats. *Behav Brain Res.* 2017 Sep 29;335:132-135. doi:10.1016/j.bbr.2017.08.016. Epub 2017 Aug 10. PubMed PMID: 28803852.

Fornasari BE, Ronchi G, Pascal D, Visigalli D, Capodivento G, Nobbio L, Perroteau I, Schenone A, Geuna S, Gambarotta G. Soluble Neuregulin1 is strongly up-regulated in the rat model of Charcot-

Marie-Tooth 1A disease. *Exp Biol Med* (Maywood). 2018 Feb;243(4):370-374. doi: 10.1177/1535370218754492. Epub 2018 Jan 19. PubMed PMID: 29350067.

#### 4.GROUP's PUBLICATIONS:

Ronchi G, Raimondo S. Chronically denervated distal nerve stump inhibits peripheral nerve regeneration. *Neural Regen Res*. 2017 May;12(5):739-740.

#### 5. GROUP's additional information:

Please list the grants of the other members of the group in the last 5 years -2012/2017- according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2015_2017	National	S. Raimondo PI	Compagnia di San Paolo	Moving Again: Integrated Therapies to Cure Post-Traumatic Paralysis	Progetti di Ricerca di Ateneo/ CSP 2014	€ 84.660	8%
2016- 2017	National	S. Raimondo PI	Università degli Studi di Torino	Ricerca scientifica finanziata dall'universita' di torino - anni 2016 e 2017	Fondo per la Ricerca Locale	€ 5.776	8%
2016_2018	National	S. Raimondo PI	CRT	Raccolta e preparazione di nervi periferici umani per la creazione di una banca del nervo	CRT - Richieste Ordinarie 2016 II tornata	€ 35.000	8%

**Please list honours, prizes or awards received by other members of the group If applicable.**

**Please list outreach activities of other members of the group:**

- Describe your international collaborative experiences.

Dr. Stefania Raimondo: 2015-2016: stage in IINS Bordeaux

- Invited talks

none

- Editorial duties

- Dr. Stefania Raimondo: Reviewer for Scientific International Journal (Acta Biomaterialia, International Review of Neurobiology, BioMed Research International).
- Dr. Giulia Ronchi Reviewer for Medical Science Monitor, Neural Regeneration Research, Neuroscience

## **6. Past Research activity**

(Summarize the PI and group research activities in the last 10 years)

### **a. Summary (500 characters)**

The research activities of Geuna's group have been focused on the study of peripheral nerve repair and regeneration. Different aspects have been studied: i) biological processes that occurs during peripheral nerve regeneration, ii) surgical techniques for nerve repair after different type of injuries, iii) biomaterials compatibility for nerve prosthesis constitution, iv) growth factors delivery strategies for the improvement of nerve regeneration or for the prevention of muscle atrophy. The main activities have been realized in the context of the European Project "Biohybrid" and the local project "Biconerve".

### **b. Background (2000 characters)**

Paralysis after peripheral nerve injury is a common condition and, although peripheral nerve fibres retain a considerable regeneration potential also in the adulthood, recovery is usually rather poor, especially in case of large nerve defects.

The increasing number of patients receiving nerve surgery will represent an enormous stimulus for more research in peripheral nerve regeneration and, most of all, for defining innovative strategies for improving functional recovery of repaired nerves.

Transected peripheral nerve fibers, unlike those of the central nervous system, are able to regenerate and lead to functional recovery provided that an appropriate milieu and guide is available. Thanks to this property, surgeons can obtain good functional recovery in patients who suffered a trauma that discontinued one or more nerve trunks by re-aligning and suturing the two stumps of the severed nerves. Unfortunately, severe traumas (especially at limb level) often cause substance loss in severed nerves so that direct repair is not possible, and a graft is required to bridge the proximal and distal stumps of the severed nerve(s). Transacted fibers can thus regenerate inside the graft and reach their optimal milieu represented by the distal nerve trunk, which will eventually guide them towards their original peripheral target. Although autologous sensory nerve segments have proved to be an excellent graft material for bridging severed nerve trunks and have been widely used in the clinical practice, their employment implies the withdrawal of a healthy nerve, requires additional surgical incisions in adjacent areas and causes sensory residual deficits. Therefore, alternative non-nervous graft materials, both biologic and synthetic, have been devised and successfully employed in the clinical practice.

### **c. Rationale (2000 characters)**

Lesions of the nerve structure result in a decreased or a complete loss of sensitivity and/or motor activity in correspondence of the innervated territory. Since the clinical outcome after nerve lesions is far from being satisfactory and functional recovery is almost never complete, more research is needed in peripheral nerve trauma recovery field.

The poor outcome can be attributed to many factors, including (i) the lesion site, ii) the interval of time between the injury and the surgical repair, iii) the inability of denervated muscle to accept reinnervation and to recover from muscle atrophy, (iv) the reduced ability of injured axons to regenerate after a long axotomy and (v) the loss of the Schwann cell (SC) capability to support regeneration.

Such research brings together different disciplines which might contribute, not only to increase knowledge about the biological mechanisms that underlie the complex sequence of events which follows nerve damage, but also to define the best strategies for optimizing posttraumatic nerve regeneration and, eventually, the full recovery of the patient's motor and sensory function.

A complete rehabilitation after a peripheral nerve injury should follow three general phases: i) regeneration of the axons; ii) reinnervation of the targets; iii) recovery of function.

#### **d. Objectives (1500 characters)**

The objectives of Geuna's group activities were to better understand biological process implicated in nerve regeneration and to study how improving functional recovery after peripheral nerve injuries, acting on peripheral nerve regeneration improvement and on prevention of denervated-muscle atrophy.

These goals have been reached: i) investigating new bioengineered and biomimetic graft materials for the repair of segmental nerve defects, as a powerful alternative to autographs, ii) developing new bio- and micro/nano-delivery systems of biomolecules stimulating nerve fiber regeneration with the effect to reduce the lag time before muscle reinnervation and inhibiting denervation-induced muscular atrophy until the nerve regeneration process has been completed and iii) analysing the changes in genes/proteins expression levels during the process of nerve injury-regeneration and muscle denervation-reinnervation.

Both in vitro and in vivo analysis have been conducted to describe the biological process implicated in peripheral nerve regeneration and to investigate new strategies for the repair of severe nerve lesions and to prevent muscle atrophy.

In vitro analysis has been useful to choose biomaterials that can be used for the constitution of the nerve prosthesis and to choose growth factors that act improving nerve regeneration and muscle tropism. The use of in vivo experimental models has been useful for the study of the basic biological processes and for the final pre-clinical testing of new strategies for improving peripheral nerve repair and regeneration.

#### **e. Results (4000 characters)**

Different activities have been carried out to reach the objectives. Both in vitro and in vivo analysis on animal models have been performed. All activities are summarized below.

##### Peripheral nerve regeneration study

In vitro and in vivo analysis have been performed in order to study the role on NRG/ErbB system during peripheral nerve injury and repair. The expression of NRG/ErbB genes and proteins have been analyzed in different type of nerve injury/repair (crush, end-to-end, tubulization) and at different time point after repair (early and late regeneration). Our results demonstrate that the components of the NRG1/ErbB system are differently regulated in the different phases a peripheral nerve undergoes after injury with the aim to regenerate. The precise regulation of this system indicates that each molecule is crucially involved in successful peripheral nerve regeneration and could be a target for pre-clinical evaluation of regeneration promoting factors. Moreover, also in vitro analysis allowed to evaluate the potential role of these molecules in the improvement of peripheral nerve regeneration and in the prevention of muscle atrophy.

### Peripheral nerve repair with conduits

Main results about techniques of nerve repair have been obtained in the context of “Biohybrid” and “Biconerve” projects. Different types of chitosan conduits have been analyzed.

Basically, chitosan-based materials were used in in vitro and in vivo studies to evaluate the most efficient formulation in the context of nerve regeneration and to select the most promising types for the more complex approaches. We have demonstrated that fine-tuned chitosan conduits, with a degree of acetylation of ~5%, allow functional and structural regeneration across a 10-mm sciatic nerve gap in rats to a similar extent as autologous nerve grafts. These chitosan nerve conduits (Reaxon® Nerve Guide) got the CE mark in January 2014, market entry of Reaxon® Nerve Guide was performed in June 2014.

After that, the selected hollow chitosan tubes were modified with luminal fillers, biomatrices, and/or (genetically modified) cells and analysed in in vitro and in vivo studies, in order to then further support functional recovery especially in advanced animal models, across long gaps or even after delayed repair (45 days after nerve transection injury).

### Study of muscle response to denervation/reinnervation

The regulation of NRG/ErbB system has also been investigated in skeletal muscles after different types of nerve injury/repair (crush injury, end-to-end repair, tubulization) at different time point (early and late regeneration). Results revealed a time-related modulation of both ErbB receptors and Nrg-1 suggesting that each molecule is crucially involved in processes related to muscle atrophy associated to denervation. Moreover, in vitro experiments with C2C12 cells stimulated with Nrg-1 were also performed to understand the involvement of this system during muscle atrophy. Our results, indeed, suggested that the system is deeply involved in this process and could be a target for new clinical therapies

#### **f. Advancement in the field (1000 characters)**

Results of our research, in the context of the European Project funded (Biohybrid), allowed to put on the market Reaxon® Nerve Guide, a chitosan conduit design for the repair of peripheral nerves.

Regarding the translational work Reaxon® Nerve Guide got the CE mark in January 2014. The product has already been implanted in patients with peripheral nerve defects at several German clinics. Moreover, FDA submission is under preparation. In addition, finalising of the translational work for clinical application of the hollow tube (ethic applications) and continuation of translational work for complex nerve conduits are ongoing activities. The preparation of a multicentre clinical trial on Reaxon® Nerve Guide in median and ulnar nerves is already completed.

### **7. Future Projects (Next 3 years)**

Please describe the following information relevant to the research that you are planning to do.

#### **a. Summary (up to 2000 characters):**

In the last years Geuna's group worked mainly on peripheral nerve regeneration, studying, among the main aspects, the role of NRG/ErbB system in different type of nerve repair after lesion and evaluating if this system can be used to improve the regeneration.

In order to increase the integration of the group with the mission of the Institute the objectives of future activities will be twofold.

The first goal will be to realize an integrated therapy to improve the patients' outcome after peripheral nerve damage acting simultaneously at multiple levels over the entire neuromuscular system i) by potentiating axonal regeneration, ii) by preventing and recovering muscle atrophy, iii) by acting on central nervous system plasticity in order to facilitate functional recovery.

The second goal will be to study the role of NRG1/ErbB system in the central nervous system, indeed they are genes implicated in neuronal migration and deficits in neuronal migration during development that may contribute to psychiatric diseases. Experiments on this field started in the last year in collaboration with another group of NICO Institute.

The third goal will be the study and the clinical application of a chitosan membrane in patients undergoing radical prostatectomy in order to enhance the functional recovery. Experiments in this fields started in the last years in collaboration with Professor Porpiglia the head of the Department of Urology in San Luigi Gonzaga Hospital.

## **b. Background and Significance (up to 4000 characters):**

### 1.a Potentiation of axonal regeneration

Although the PNS has an intrinsic capability to regenerate after trauma, functional recovery is often incomplete and unsatisfactory. A need therefore exists for devising new strategies for promoting the outcome after nerve trauma, especially in cases of severe nerve lesions such as brachial plexus avulsions.

### 1.b Prevention of skeletal muscle atrophy

Trauma to the mixed peripheral nerves induces a denervation-related atrophy of the distal target skeletal muscles. Muscle atrophy is progressive and ends up with the disappearance of muscle fibers. In this case, even if the motor axons regenerated and reach again the target muscles, motor functional recovery cannot occur. A need therefore exists for devising new strategies for promoting complete skeletal muscle atrophy during posttraumatic peripheral nerve regeneration.

### 1.c Promote plasticity of central nervous system

External stimuli, which comprise sensory inflow, motor activity, cognitive elaboration, or social interaction, are crucial for functional recovery after peripheral nerve damage. These phenomena depend on the capability of neurons to modify their functional properties and/or their connections, generally defined as “plasticity”. A need therefore exists for devising new strategies for manipulating CNS plasticity to improve functional recovery after nerve trauma.

### 2.a NRG/ErbB system role in neuron migration

Neuronal migration represents a critical step in the development of the central nervous system, where neuronal progenitors migrate from their birth site to their target and their final destination. The tyrosine kinase receptor ErbB4 and one of its ligands, the neuregulin1 (NRG1), are involved in the migration of neuronal progenitors from the medial ganglionic eminence (MGE) to the cortex during the development and from the subventricular zone (SVZ) to the olfactory bulb (OB) during the development and in the adult life and it has been demonstrated that the conditional deletion of ErbB4 interferes with correct neuronal migration.

A need therefore exists for better understand the role played by this system both in physiological and pathological (schizophrenia) conditions.

### 3.a Strategies to improve the functional recovery after radical prostatectomy

Prostatic cancer is the most frequent cancer in males. Whereas the progress in early cancer detection and surgical removal has made significant improvement in patient survival, erectile dysfunction often results after radical prostatectomy due to damage of the peri-prostatic nerve bundles.

The aim of this study is to assess the regenerative and anti-cancer properties of a biomedical device consisting of chitosan (CS), a derivative of chitin, that is achieving resounding interest both in basic research and in clinical settings exerting pro-regenerative action on nerves. The BIOHYBRID



project already succeeded to bring to the clinic a medical device for the treatment of injuries to somatic nerves.

In the context of prostate cancer, an increasing percentage of young men have an early prostate cancer diagnosis. Erectile dysfunction caused by radical prostatectomy is associated with distress and impaired quality of life. The application of new techniques and new materials in the field of peripheral nerve regeneration would result in minor inconvenience for patients and allow to extend the treatment also for applications in oncology.

#### 4.a Effects of neurodynamic treatments on peripheral nervous system

Reating the disorders of the PNS and CNS is a main part of clinical practice of the physiotherapist, the knowledge of the biomechanical properties of these systems is essential to manage their damage or alteration. Neurodynamic Treatment (NMT) is a type of intervention used by the physiotherapist to treat these conditions and the diseases of the musculoskeletal system. It is not known what biological mechanism the NMT can enable on the SNP cells.

The Aim of this work is to evaluate the effects of NMT *in vitro* on cell morphology, to assess the extent of neuroplasticity/cell growth phenomena, the biological and chemical behavior, compare two different protocols for intensity and assess any adverse effects.

It is hypothesized that it does not generate negative effects and that it is possible to define an optimal protocol for treatment intensity. It is both useful and difficult to hypothesize the efficacy of the biological effects or neuroplasticity / cell growth of the SNP given by the NMT and their relationship with the patient's symptomatology and disability. Considering the strong impact that low back pain and nec pain have on the health of the population, this project is expected to be relevant for clinical and economic relapse.

#### **c. General aim and integration with mission of the Institute (up to 1000 characters)**

The general aim of our group is to explore innovative solutions for improving functional recovery after nerve trauma. Nerve trauma represent one of the major cause of neuronal disability with significant influences on the patient quality of live, including the psychosocial and relational level. Significant advancements in the treatment of these patients requires an integrated approach which brings together bot CNS and PNS scientists in line with the mission of the NICO.

In addition, our research group aims to investigate the role of NRG1/ErbB system on neuronal migration in the perspective of better understanding some psychiatric diseases which might related to neuronal migration disorders.

#### **d. Specific objectives and strategies (up to 4000 characters):**

The first specific objectives are:

i) Potentiating axonal regeneration after traumatic lesion. This objective will be pursued by investigating innovative strategies of tissue engineering of the peripheral nerve. These include the construction of nanostructured scaffolds, cell transplantation, gene therapy, and physical stimulation of tissue repair.

ii) Preventing denervation-related muscle atrophy. This objective will be pursued by investigating innovative strategies for the local release of myotrophic molecules such as ghrelin and neuregulin-1.

iii) Modulating central nervous system plasticity after nerve trauma. This objective will be pursued by investigating innovative strategies for improving functional recovery by means of rehabilitation protocols directed at facilitating the CNS adaptation to the new PNS conditions.

iv) Investigating the role of NRG1/ErbB system in the central nervous system. This objective will be pursued by investigating how the NRG1/ErbB system is implicated in neuronal migration and in the deficits in neuronal migration during development and how those deficits may contribute to psychiatric diseases.

v) Developing a medical device represented by chitosan for its application in the urological field. This objective will be pursued investigating the suitable neuro-regenerative properties of chitosan in autonomic nerves.

vi) Developing a protocol of neurodynamic treatments with impact on motor impairment and rehabilitation and also on acute and chronic pain.

**e. Unique features of the project research (up to 2500 characters):**

The unique features of our project research are the following.

1) The project research represents one of the most innovative approaches in Europe focused on the study of peripheral nerve repair and regeneration.

2) The research group brings together interdisciplinary competencies and skills.

3) The project research is carried out under good laboratory practice (GLP)-inspired procedures

4) the research group focus on the translational approach, i.e. on the applicability of the research results for developing new therapeutic strategies that could successfully be translated to the clinical practice.

5) The project research has also a potential for industrial spin off of the results, as demonstrated by the recent introduction on the market of the Reaxon© nerve guides the patent on iron-conjugated neurogulin-1.

**f. Methodology (up to 2000 characters): please fill-out this section only in the case of innovative technologies**

One of the main technologies adopted by our group is the employment of modern unbiased stereological techniques for the morphoquantitative estimation of the nerve tissue (both in CNS and PNS). The PI has long term experience in this field and organizes stereological courses in different countries (Italy, Germany, Portugal, Turkey, Tanzania).



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Neuroendocrinology

## 1. LABORATORY DESCRIPTION – PERSONNEL:

### Principal Investigator

GianCarlo Panzica Birthdate (17/08/1949)  
Degree PhD Gender male  
Nationality Italian Phone: 011 670 6607  
Email: giancarlo.panzica@unito.it

### Personnel

1. Stefano Gotti Birthdate (17/06/1971)  
Degree PhD Gender male  
Role Researcher RTI Nationality Italian  
Expertise Co-PI

2. Giovanna Ponti Birthdate (05/04/1975)  
Degree PhD Gender female  
Role Researcher RTD Nationality Italian  
Expertise Neurogenesis, phytoestrogens

3. Alice Farinetti Birthdate (23/12/1981)  
Degree PhD Gender female  
Role Post-Doc Nationality Italian  
Expertise Neurogenesis, Gonadal hormones, eating disorders models

4. Marilena Marraudino Birthdate (08/06/1988)  
Degree Master Degree Gender female  
Role PhD Student Nationality Italian  
Expertise Control of reproduction, endocrine disruptors

5. Brigitta Bonaldo Birthdate (30/01/1992)  
Degree Master Degree Gender female  
Role PhD Student Nationality Italian  
Expertise Neurodegenerative disorders models, endocrine disruptors

6. Godstime Stephen K. Morgan Birthdate (27/06/1993)  
Degree Master Degree Gender male  
Role PhD Student Nationality Ghana  
Expertise eating disorders models

## 2. PRINCIPAL INVESTIGATOR

H index, 37; Citations: 4661 (WoS)

Relevant discoveries:

Dr. Panzica started his independent career as full-time researcher (permanent position) in 1980, and his main interest was the study of hypothalamic circuits related to the control of different behaviors, in particular the reproductive behavior. During the period 1970-1980, many people started to investigate sex differences in brain structures, mainly in rodents (and in humans), but only a few studies were dedicated to this topic in other vertebrates. Dr. Panzica, in cooperation with dr. Balthazart (Liege) and dr. Viglietti (Torino), was the first to discover a sexually dimorphic nucleus within the preoptic area of the Japanese Quail (Viglietti-Panzica et al., 1986). Several studies have been performed in our and other laboratories about the cellular populations, the presence of the enzyme aromatase, and the role played by this nucleus in the control of male copulatory behavior. The medial preoptic nucleus of the Japanese Quail is still considered the best model to link neural circuits, aromatase action and the control of a sexually dimorphic behavior. These discoveries were summarized in a review in the journal "Frontiers in Neuroendocrinology" (Panzica et al., 1996). This paper is still discussed in several classes of master courses in Behavioral Neuroendocrinology.

Following this research line, dr. Panzica and his co-workers tried to discover specific pathways particularly linked to the control of sexual behavior. They identified the parvocellular sexually dimorphic vasotocin system in the limbic system of the Japanese quail (Panzica et al., 1998). This paper was the first demonstration of a clear relationship among male sexual behavior and a neurochemically defined circuit in birds (but also in all vertebrates) and gave a clean experimental model also in comparison with the several problems arising from the study of a similar system in mammals (De Vries and Panzica, 2006).

More recently, dr. Panzica and his team started to study the effects of endocrine disruptors over the neural circuits controlling food intake and energy metabolism. The current focus on the etiology of obesity remains on imbalance between food intake and energy expenditure, and the role of hypothalamic circuits in this process has been underestimated. Our team demonstrated for the first time a direct effect of one important obesogenic molecule (tributyltin, TBT) over hypothalamic circuits controlling feeding behavior and energy metabolism in mice, by using the c-fos technique (Bo et al., 2011). This study was the first morphological evidence that obesogenic compounds may act not only at the periphery stimulating the increase of fat tissue, but also at the level of the hypothalamic circuits. This study is opening a new field of studies for the action of the so-called "metabolic disruptors" (Heindel et al., 2015, 2017): the alteration of the brain circuits dedicated to the control of food intake and energy metabolism.

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2013-2015	National	PI	UNITO-ex 60% 2012	Effetti cerebrali degli obesogeni		3.053	8%
2012-2014	National (Spain)	C	Ministerio de Ciencia e Innovación (Spain)	Participación del óxido nítrico en el control neurohormonal de la ingesta		60.000	8%
2014-2016	National	PI	UNITO-ex 60% 2013	Effetti cerebrali degli obesogeni: azione della TBT sul circuito a NPY		2.127	8%
2013-2016	International Foundation		Postfinasteride Foundation	Effects of finasteride on behavior and neurosteroids		14.656	10%
2015-	National	PI	UNITO-ex	Distruttori		2.343	8%

2017			60% 2014	endocrini e circuiti che regolano l'assunzione di cibo ed il metabolismo energetico			
2016-2019	National (Spain)	C	Ministerio de Ciencia e Innovación (Spain)	Involvement of estradiol on feeding neurohormonal circuit programming in the rat		70.000	8%
2017-2019	National	PI	UNITO-ex 60% 2016-17	Effetti dell'esposizione e a distruttori metabolici sui circuiti ipotalamici che controllano l'assunzione di cibo ed il metabolismo energetico		7.095	8%

Please list the name of PhDs you have supervised.

Please list honours, prizes or awards received, if applicable.

Please list your outreach activities

- *International collaborative experiences.*

Dr. Panzica had several international cooperations, and many of them are still active. They are chronologically listed here:

1981-1990 Cooperation with dr. H. Korf (University of Giessen, Germany) on the hypothalamic magnocellular system in birds and its relations with osmoregulation.

1982-today Cooperation with dr. J. Balthazart (University of Liege, Belgium) on the definition of neural circuits controlling male copulatory behavior in the Japanese Quail.

1985-90 Cooperation with dr. R. Foster (University of Bristol, UK) on the photoperiodic control of the GnRH system in the Japanese Quail.

1990-today Cooperation with dr. M.A. Ottinger (University of Maryland, College Park, USA) on the effects of aging on vasotocin and GnRH systems in the Japanese Quail, and on the effects of endocrine disruptors on neural circuits and behaviors in birds.

1990-1995 Cooperation with dr. H. Vaudry (Rouen, France) on the distribution of the NPY system in birds.

1991-1996 Cooperation with dr. F. Sanchez and dr. J.R. Alonso (University of Salamanca) on the distribution of NADPH-diaphorase (nNOS) in the avian brain

1992-1999 Cooperation with dr. R. Grossman (Institute for Animal Science, Mariensee, Germany) on the sexually dimorphic vasotocin system in birds

2000-today Cooperation with dr. N. Harada (Kyoto University, Japan) on aromatase function in mammals, using a murine model knock-out for the aromatase gene.

2000-today Cooperation with dr. J. Bakker (University of Liege, Belgium) on sexually dimorphic vasopressin system in mice: effects of gonadal hormones in the differentiation and functioning of the system.

2001-today Cooperation with dr. P. Collado (UNED, Madrid, Spain) on the role of gonadal hormones in the regulation of nNOS expression, Vasopressin expression, and neural circuits controlling food intake and energy metabolism.

2005-2008 Cooperation with dr. K. Hallidin (Karoliska Institute, Stockholm, Sweden) on the effects of endocrine disruptors on sexual behavior and neural circuits in birds

2009-today Cooperation with L.M. Garcia Segura (Cajal Institute, Madrid, Spain) on the regulation of the expression of vasopressin in the paraventricular nucleus and in cell cultures in various experimental conditions

2009-today Cooperation with M. Keller (INRA, Tours, France) on the effects of endocrine disruptors on the kisspeptin system in mice

All these cooperations were supported by international grants from NATO, European training program, USDA, National Science Foundation, European Commission, France government, Spain Ministry of Science, CNR, FNRS.

### NETWORKING

At present, we are also involved in two European networks belonging to COST action.

*COST ACTION: A systematic elucidation of differences of sex development (DSDnet)*

[http://www.cost.eu/COST\\_Actions/bmbs/BM1303](http://www.cost.eu/COST_Actions/bmbs/BM1303)

To study Differences or Disorders of Sex Development (DSD) that constitute a complex group of rare diseases caused by chromosomal, genetic and endocrine metabolic disturbances that affect the endocrine-reproductive system, thereby modulating the sexual phenotype of a given person.

### *COST ACTION: GnRH NETWORK*

[http://www.gnrhnetwork.eu/hhn\\_home/hhn-cost/hhn-costorganization/hhn-wg3basicssciences.htm](http://www.gnrhnetwork.eu/hhn_home/hhn-cost/hhn-costorganization/hhn-wg3basicssciences.htm)

Devoted to developing an international network of clinicians and investigators in the fields of reproductive medicine and neuroscience. Specifically, we aim to explore the causes of GnRH deficiency, including Kallmann syndrome.

Dr. Panzica is also member of EDCs EU-ES TASK FORCE

<https://www.endocrine.org/advocacy/endocrine-insider/2017/september-28-2017>

It is a group of European and US scientists, working in the field of endocrine disruption, dedicated to meet with EU policymakers on the issue of endocrine-disrupting chemicals. This group is part of a strategic initiative to ensure endocrine principles are incorporated into global EDC regulatory policies, the Endocrine Society established its EU EDC Task Force to inform and advocate with members of the European Parliament and officials within the EU Directorates General in charge of chemicals laws and regulations.

### **Invited talks (last ten years)**

- Sexual dimorphism and diergism of nitric oxide producing systems in the mammalian central nervous system (2<sup>nd</sup> World Congress on Gender-specific Medicine and Aging, Rome, March 2007)
- Effects of Xenoestrogens on the Differentiation of Behavioral Relevant Neural Circuits (24th Conference of European Comparative Endocrinologists, Genova, September 2008)
- Organizational Effects of Bisphenol-A on Kisspeptin Expression in the Hypothalamus of CD1 Mouse, 1st Kisspeptin World Conference, Cordoba, October 2008)
- Role of Androgens in the Differentiation of Rodent Arginine-Vasopressin System (XXXIII Congress of the Italian Society of Histochemistry, Rome, June 2009)
- Neuropeptidergic systems - Targets for the Action of Endocrine Disrupting Chemicals in the Vertebrate Brain (Int. Symposium on Disturbance of Cerebral Function Induced by Food and Water Contaminants, Valencia, March 2010)
- Neuropeptidergic systems - Targets for the Action of Xenoestrogens or Xenoandrogens in the Vertebrate Brain (Symposium on Neuroendocrine effects of Endocrine Disruptors - Rouen, July 2010)
- Avian vasotocin system: a model for the study of xenoestrogens' effects on brain circuits and behavior (International Conference of Neuroendocrinology, Rouen, July 2010)
- Environment and brain sexual differentiation: what role for endocrine disruptors? (Congresso Società Italiana di Anatomia, Padova, Settembre 2011)
- Hypothalamic NPY Expression in Adult Male Mice is Influenced by Adult Exposure to Environmental Endocrine Disruptors (The Obese Species, Erice, Italy, October 2011)

- Environment and brain sexual differentiation: what role for endocrine disrupters? (SiNAPSA Neuroscience Conference, Ljubljana, Slovenia, September 2011)
- Endocrine disruption of hypothalamic circuits controlling energy balance (Gordon Research Conference on Environmental Endocrine, Mount Snow, VT, USA, June 2012)
- Ambiente e Cervello: Come Nascono le Differenze tra i Sessi (Settimana del Cervello, Torino, Marzo 2012)
- Differenziamento Cerebrale e Ambiente - Quale ruolo per i distruttori endocrini? (10° Congresso Società Italiana Andrologia e Medicina Sessuale, Lecce, November 2012)
- Ambiente e Cervello: Come Nascono le Differenze tra i Sessi (Infinitamente, Festival di Scienza, Verona, Marzo 2013)
- Sistema a Kisspeptina e Interferenti Endocrini (1° Incontro Network Ipogonadismo Centrale, NICE, Milano, Novembre 2013)
- Sexually dimorphic effects of endocrine disruptors on brain and behavior (8th Int. Conf. on Hormones Brain and Behavior, Liege, Belgium, June 2014)
- Distruttori endocrini e circuiti ipotalamici che controllano il metabolismo energetico (4i, Incontri Italiani Ipotalamo Ipofisari, Milano, February 2014)
- Impact of endocrine disrupters on neuroendocrine circuits controlling food intake (8th Copenhagen Workshop on Endocrine Disrupters, Copenhagen, April 2015)
- The sexually dimorphic vasotocin system as target for neuroendocrine disruption in birds (North American Society for Comparative Endocrinology, NASCE, Ottawa, Canada, June 2015)
- Interferenti endocrini e circuiti neuroendocrini che controllano l'assunzione del cibo e il metabolismo energetico (INN: Prospettive di neuroscienze, Verona, June 2016)
- Metabolic disrupting chemicals and peptidergic pathways controlling food intake and energy metabolism (Symposium Neuroactive steroids and metabolic axis, Torino, February 2017)
- Interferenti endocrini e circuiti neuroendocrini (1° Convegno di Endocrinologia ambientale (Roma, June 2017).
- Environmental modulation of neural circuits: how genistein or other endocrine disruptors may interfere with the neuroendocrine brain and related behaviors (2nd International Congress of Psychobiology, Avila, Spain, June 2017)

#### **Editorial duties.**

##### *Member of the Editorial board of:*

- Domestic Animal Endocrinology (2007-2010)
- European Journal of Anatomy (1995-2010)
- Cell and Tissue Research (1996-today)
- Hormones and Behavior (2000-2015)
- Journal of Chemical Neuroanatomy (2010-2015)
- Frontiers in Endocrinology (2015-today)

##### *Guest editor of the following special issues:*

- Neuropeptides and neuronal circuitries (by G. Filogamo and G.C. **Panzica**), *Special Issue*, Basic and Applied Histochemistry, vol. 32/1, 1988, pp 1-192.
- Hormones, Brain, and Behavior (by G.C.**Panzica** and J.Balthazart), *Special Issue*, Brain Research Bulletin, vol.44/4, 1997, pp.319-557.
- The Role of Nitric Oxide (by G.C.**Panzica**). *Special Issue*, Eur. J. Anatomy, vol.2/1, 1998, pp.25-76.
- Neuroactive steroids in the third millenium (by R.C.Melcangi and G.C. **Panzica**), *Special Issue*, Brain Research Reviews, vol. 37 (1-3), 2001, pp. 1-384.
- Steroids and The Nervous System. (by R.C.Melcangi and G.C.**Panzica**), Annals of the New York Academy of Sciences, 2003, Vol. 1007, pp.1-406.
- Action of environmental estrogens on neural circuits and behavior (by G.C. **Panzica** and M.A.Ottinger), *Special Issue*, Brain Research Bulletin, vol. 65, 2005, pp. 185-275.
- Neuroactive steroids: Old players in a new game (by R.C. Melcangi and G.C. **Panzica**), *Special Issue*, Neuroscience, vol.138(3), 2006, pp.733-1048.



- The endocrine nervous system: source and target for neuroactive steroids (by R.C. Melcangi and G.C. **Panzica**), *Special Issue*, Brain Res. Reviews, vol. 57, 2008, pp. 271-605.
- Neuroactive steroids: effects and mechanisms of action (by R.C. Melcangi and G.C. **Panzica**), *Special Issue*, Psychoneuroendocrinology, vol. 34 (Suppl. 1) 2009, pp. 1-286 - ISSN.0306-4530.
- Neuroactive steroids: Focus on human brain (by R.C. Melcangi, L.M. Garcia-Segura and G.C. **Panzica**), *Special Issue*, Neuroscience, vol. 191, 2011, pp. 1-158 – ISSN.0306-4522.
- Recent advances in peptides and neurotransmitters (by G.C. **Panzica** and L. D'Este), *Special Issue*, J. Chem. Neuroanatomy, vol. 42, 2011, pp. 221-340 – ISSN.0891-0618.
- Steroids and the nervous system (by G.C. **Panzica** and R.C. Melcangi), *Special Issue*, Journal of Neuroendocrinology, vol. 24, 2012, pp. 1-248 - ISSN 0953–8194.
- Steroids and the nervous system (by R.C. Melcangi and G.C. **Panzica**), *Special Issue*, Journal of Neuroendocrinology, vol. 25, 2013, pp. 957-1238 - ISSN 0953–8194.
- Allopregnanolone: State of the art (by R.C. Melcangi and G.C. **Panzica**) *Special Issue*, Progr. Neurobiol, vol. 113, 2014, pp 1-136, ISSN: 0301-0082
- Gender influence on neurodegenerative and psychiatric disorders (by R.C. Melcangi and G.C. **Panzica**) *Special Issue* Neuroscience & Biobehavioral Reviews vol. 67, 2016, pp 1-146 ISSN: 0149-7634.
- Steroids & Nervous System (by R.C. Melcangi and G.C. **Panzica**) *Special Issue* The Journal of Steroid Biochemistry and Molecular Biology, 2016, vol. 160, pp 1-226 ISSN: 0960-0760.

Please list your organizational activities:

- **Speakers invited**

In addition to the speakers invited for the conferences and symposia, in the last 5 years we invited the following speakers for seminars at the Department of Neuroscience and/or NICO:

Jacques Balthazart (Liege, Belgium), Luis Miguel Garcia Segura (Madrid, Spain), Guy Mensah-Nyagam (Strasbourg, France), Manuel Tena Sempere (Cordoba, Spain)

- **Workshops, Schools or Conferences organized**

**Conferences**

- VI International Conference on Hormones, Brain and Behavior, Torino, August 1996
- VII International Conference on Hormones, Brain and Behavior, Torino, February 2009
- International Meeting Steroids and Nervous System, Torino. A series of conferences organized each two years in Torino, starting from 2001: 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, and 2017 (9<sup>th</sup> edition).

**Satellite Symposia-**

- Satellite symposium: Behavior as a biomarker of the effects of estrogenic pollutants in higher vertebrates, Torino, September 2001
- Satellite symposium: Action of environmental estrogens on behaviorally relevant neural circuits, Torino, February 2003
- Satellite Symposium: Steroid hormone regulation of NPY system, Torino, February 2005.
- Satellite Symposium: Selective Estrogen Receptors Modulators and the Brain, Torino, February 2007
- Satellite Symposium: Neuroactive steroids: Focus on human brain, Torino, February 2011
- Satellite Symposium: Allopregnanolone: State of Art, Torino, February 2013
- Satellite Symposium: Gender Differences on Neurodegenerative and Psychiatric Disorders, Torino February 2015
- Satellite Symposium: Neuroactive steroids and metabolic axis, Torino, February 2017)

**Workshops**

- Image analysis on neurohistology - Technical Workshop of ENA meeting, Torino, 1989
- Metodi e problematiche della neurobiologia comparata - Workshop for the Meeting of the UZI, Torino, Italy, 1993
- Il Ruolo del NO - Workshop for the Meeting of the Società Italiana di Anatomia, Torino, 1997

- Avian models for studying xenoestrogens action on brain and behavior - Workshop for the XXIX Ethological Conference, Budapest, August 2005

Please list your technology transfer achievements (patents, etc.), if applicable

### 3. PI's PUBLICATIONS:

(Please list below your publications in 2017 -. Please indicate the journal IF, ranking).

For each publication, please indicate:

\* if you contributed equally to the first-author, as stated in the published article

Melcangi RC, **Panzica GC**. (2017) Neuroactive steroids and metabolic axis. *Front Neuroendocrinol*. Nov 13. pii: S0091-3022(17)30069-9.

IF= 9.425; R = 1/24 endocrine and autonomic system

Tetel MJ, de Vries GJ, Melcangi RC, **Panzica G**, O'Mahony SM. (2017) Steroids, Stress, and the Gut Microbiome-Brain Axis. *J Neuroendocrinol*. Oct 12.

IF= 3.470; R = 45/205 medicine, endocrinology

Marraudino M, Miceli D, Farinetti A, Ponti G, **Panzica G**, Gotti S. (2017) Kisspeptin innervation of the hypothalamic paraventricular nucleus: sexual dimorphism and effect of estrous cycle in female mice. *J Anat*. Jun;230(6):775-786.

IF= 2.182; R = 10/37 medicine, anatomy

Ponti G, Rodriguez-Gomez A, Farinetti A, Marraudino M, Filice F, Foglio B, Sciacca G, **Panzica GC**, Gotti S. (2017) Early postnatal genistein administration permanently affects nitergic and vasopressinergic systems in a sex-specific way. *Neuroscience*. Mar 27;346:203-215.

IF= 3.277; R = 22/101 general neuroscience

Grassi D, Lagunas N, Pinos H, **Panzica G**, Garcia-Segura LM, Collado P. (2017) NADPH-Diaphorase Colocalizes with GPER and Is Modulated by the GPER Agonist G1 in the Supraoptic and Paraventricular Nuclei of Ovariectomized Female Rats. *Neuroendocrinology*. 104(1):94-104.

IF= 3.608; R = 61/205 medicine, endocrinology

Heindel JJ, Blumberg B, Cave M, Machtiger R, Mantovani A, Mendez MA, Nadal A, Palanza P, **Panzica G**, Sargis R, Vandenberg LN, Vom Saal F. (2017) Metabolism disrupting chemicals and metabolic disorders. *Reprod Toxicol*. Mar;68:3-33.

IF= 2.341; R = 27/108 toxicology

#### 4.GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2017 -. Please indicate the journal IF, ranking)

Romano F, Perotto S, Cricenti L, **Gotti S**, Aimetti M. (2017) Epithelial Inclusions Following a Bilaminar Root Coverage Procedure with a Subepithelial Connective Tissue Graft: A Histologic and Clinical Study. *Int J Periodontics Restorative Dent.* Sep/Oct;37(5):e245-e252.  
IF= 1.113; R = 8/11 Dentistry

Perotto S, Romano F, Cricenti L, **Gotti S**, Aimetti M. (2017) Vascularization and Innervation of Connective Tissue Grafts in the Treatment of Gingival Recessions: A Histologic and Immunohistochemical Study. *Int J Periodontics Restorative Dent.* Jul/Aug;37(4):551-558.  
IF= 1.113; R = 8/11 Dentistry

Oboti L, Trova S, Schellino R, **Marraudino M**, Harris NR, Abiona OM, Stampar M, Lin W, Peretto P. (2017) Activity Dependent Modulation of Granule Cell Survival in the Accessory Olfactory Bulb at Puberty. *Front Neuroanat.* 2017 May 23;11:44.  
IF= 3.267; R = 5/37 Medicine, Anatomy

## 5. GROUP's additional information:

Please list the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
01/01/2013-31/12/2018	National	Gotti S; PI	UNITO-ex 60% 2012	Neurosteroidi e modulazione della neurogenesi nell'ippocampo di ratto		2.787	8%
01/10/2015-31/10/2018	National	Gotti S; PI	UNITO-ex 60% 2015	The Kisspeptin neuronal system: study of the distribution and of the pubertal development		1.669	8%
28/06/2016-30/06/2018	National	Gotti S; Co-PI	UNITO-ex 60% 2016-progetti traslazionali	Anorexia nervosa, physical activity, hormones and morphological alteration in hippocampus. A translational study		27206	8%
22/12/2015-31/07/2018	National	Gotti S; PI	CRT, richieste ordinarie	Ruolo dei neuropeptide ipofisari e degli ormoni gonadici nel disturbo da deficit di attenzione/iperattività		28000	8%
20/02/2017-20/02/2019	National	Gotti S; PI	UNITO-ex 60% 2016-2017	Effetti della somministrazione prenatale di chlorpyrifos nei circuiti ipotalamici del topo		4755	8%
1/1/2016-31/01/2017	National	Ponti G, Co-PI	UNITO-ex 60% 2015	Pattern di espressione genica nel differenziamento mammario della specie bovina		11984	8%
1/01/2017-31/01/2018	National	Ponti G, Co-PI	UNITO-ex 60% 2016	Valutazione innovativa del benessere animale e della resilienza attraverso biosensori e biomarcatori nei ruminanti		14.256	8%
1/01/2017-31/01/2018	National	Ponti G, Co-PI	UNITO-ex 60% 2017	Valutazione multifattoriale del benessere animale in avicoltura		10120	8%

## **Gotti S**

Please list outreach activities of other members of the group:

- Describe your international collaborative experiences.

- Prof. Guy Mensah-Nyagan, Equipe Stéroïdes, Neuromodulateurs et Neuropathologies, University of Strasbourg, France

- Dr. Paloma Collado (UNED, Madrid, Spain)

since 2016 participate to the University of Turin working group in the "HackUNITO for aging"

- Invited talks

Maternal separation in anorexic rats: a sexually dimorphic effect. (2nd International Congress of Psychobiology, Avila, Spagna, June 2017)

- Editorial duties

Since 2006 guest referee for these international scientific journals:

Brain Research, Journal of, Chemical Neuroanatomy, Cell and Tissue Research, Physiology and Behavior, Neurological Science, Histology and Histopathology

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group

Member of the Local Organizing Committee of the International Meeting Steroids and Nervous System (Turin, 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, 2017) and co-editors of the scientific abstracts issue.

## **Ponti G.**

Please list outreach activities of other members of the group:

- Describe your international collaborative experiences.

- 2-2-2017 Has achieved the Academic Qualification as Lector in Life Sciences from the Agència per a la Qualitat del Sistema Universitari de Catalunya (AQU)
- 2013 has achieved the National Academic Qualification as Associate Professor in Veterinary Anatomy
- 2009-2012 Marie Curie Outgoing International Fellowship: "*Imaging of the neural stem cell origin, proliferation, and fate within the stem cell niches of the mammalian brain*", University of California San Francisco, Department of Neurosurgery (Prof. Arturo Alvarez-Buylla lab.) in collaboration with the University of Turin, Italy, Department of Veterinary Morphophysiology (230669 €)

2012-2014 National member of the COST ACTION: NANONET

since 2016 participate to the University of Turin working group for the EUROPEAN TECHNOLOGY PLATFORM "FOOD FOR LIFE" working group nutrition and health

since 2016 participate to the University of Turin working group in the "HackUNITO for aging"

since 2017 participate to the University of Turin working group for the KIC EIT Food"

UNITO "contact person" in EIT Education project (MOOC) "Fork2Farm: Sustainable agriculture in a changing environment" coordinated by Queen University of Belfast

Prof. Arturo Alvarez-Buylla, UCSF, San Francisco, California, USA Prof. Elly Hol (University of Utrecht)  
Prof. Roy Quinlan (University of Newcastle) Prof. J.C.V.M (Sjef) Copray (University of Groningen)

- Editorial duties

Since 22-01-2018	Reviewer for Free Radical Research
Since 05-10-2017	Reviewer for Journal Molecular and cellular Neuroscience
Since 11-09-2017	Reviewer for National Science Center, Poland
Since 07-6-16	Reviewer for Endocrine
Since 23-05-2017	Reviewer for Stem Cells and Development
Since 07-12-2014	Referee for Neuroscience
Since 01-07-2015	Review panel member for FISM-AISM (Federazione Italiana Sclerosi Multipla)
Since 15-01-2015	Expert Reviewer Italiani per Progetti Europei JPND
Since 27-02-2014	Review panel member for Frontiers in Neuroscience
Since 01-01-2013	Euraxess expert
Since 2009	Associate Faculty Member of F1000

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group

Member of the Local Organizing Committee of the International Meeting Steroids and Nervous System (Turin, 2013, 2015, 2017)

## 6 .Past Research activity

(Summarize the PI and group research activities in 2017)

### **g. Summary (500 characters)**

The central focus of our researches has been the study of the interactions among steroids and nervous circuits. Moreover, we studied the effect of endocrine disrupting chemicals (EDCs) in the derangement of the circuit involved in the control of energetic metabolism.

### **h. Background (2000 characters)**

Gonadal hormones play key roles in the development of primary and secondary sex characteristics in higher vertebrates, including several steroid dependent behaviors. After the demonstration that both nuclear estrogen receptors (ER $\alpha$  and ER  $\beta$ ) and the membrane receptor (GPER-1) are expressed in many brain areas during ontogeny, it was soon realized that estrogens may modulate neuronal differentiation, notably by influencing cell migration, survival and death, and synaptic plasticity of neurons.

Appropriate levels of gonadal hormones are essential for normal development and sexual differentiation of the central nervous system (CNS), and of the reproductive behavior. Disturbing this developmental milieu, via exogenous estrogen treatment or gonadectomy, during critical periods of the pre- and/or postnatal development, may induce irreversible changes in the organization of the central nervous system and determine behavioral alterations in many species.

Due to the fact that many EDCs are xenoestrogens (XEs) or xenoandrogens (XAs), they could, even in very low concentrations, deeply influence the development and the function of gonadal hormones-dependent neural circuits and related behaviors. Behavioral responses represent the culmination of several integrated systems, therefore, even small changes of neural or neuroendocrine components are likely to disrupt or modify behavior. Importantly, disturbances in normal behavior may influence the individual fitness and, therefore, assume a real biological significance in both animal and human ecosystems.

Moreover, considering our interest in gender differences, critical periods and alteration of food intake circuits last year we started a collaboration with clinicians in a project focused on Anorexia Nervosa, an eating disorder that typically affects women; in order to elucidate the neurobiological mechanisms that may play a role in this disorder, we studied an animals model for activity-based anorexia (ABA) and the relations with the maternal separation in both sexes.

### **i. Rationale (2000 characters)**

EDCs acting at low levels can exert subtle effects by interfering with gene expression and other cellular activities, which can cause transient activational responses, or permanent impairment. Thus, the impact of EDCs will vary depending upon a variety of factors, including when exposure occurs in the life-cycle of an organism, as well as the duration and amount of the exposure. During the life-cycle of an organism, developmental stages are typically far more vulnerable to signal disruption than adult stages and the consequences of fetal exposure may be drastically different from those of adult exposure. This is thought to occur for several reasons, including the absence of fully developed protective enzyme systems and higher metabolic rates. Most importantly, however, the events underway in development involve a series of organizational alternatives that are (largely) irreversible once the “choice” in development is determined.

### **j. Objectives (1500 characters)**

We have investigated, in turn, the role played by neurosteroids in many disease, and the effects of endocrine disruptors in some hypothalamic circuits involved in the control of food intake.

### **k. Results (4000 characters)**

#### **Gonadal hormones.**

Gonadal hormones are involved in the regulation of several brain system including the nitrergic system. Nitric oxide carries out a wide range of functions by acting as a neurotransmitter-like molecule. We have previously demonstrated that estradiol, via classical estrogen receptors (ERs), regulates Nitric Oxide Synthase activity in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus,



acting through both ER $\alpha$  and ER $\beta$ . In this study, we have assessed whether the G protein-coupled estrogen receptors (GPER) is also involved in the regulation of nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase in the SON and PVN. Our results have highlighted that the activation of GPER in the SON and PVN inhibits the phosphorylation of ERK 1/2, which induces a decrease in NADPH-diaphorase expression (**Grassi et al., 2017**).

#### **Role played by neurosteroids (Translational research).**

Due to the large diffusion of metabolic diseases the study of the interaction between metabolic axis and nervous function represents a really hot topic in biomedical research. For this reason, we have collected in a special issue some reviews exploring the relationships among neuroactive steroids and the metabolic axis (**Melcangi and Panzica, 2017**) that originated from the lectures presented at a satellite symposium of the 9th International meeting "Steroids and Nervous System" (February 2017, Torino, Italy)

It is becoming well established that the gut microbiome has a profound impact on human health and disease. We have highlighted how steroids can influence the gut microbiota, and in turn how the gut microbiota can influence hormone levels. Finally, we present some of the challenges and important questions that need to be addressed by future research in this exciting new area at the intersection of steroids, stress, gut-brain axis and human health (**Tetel et al., 2017**).

#### **Endocrine disruptors.**

Genistein (GEN) is a natural xenoestrogen (isoflavonoid) that may interfere with the development of estrogen-sensitive neural circuits. Due to the large and increasing use of soy-based formulas for babies (characterized by a high content of GEN), there are some concerns that this could result in an impairment of some estrogen-sensitive neural circuits and behaviors. In the present study, we have used an experimental protocol mimicking, in mice, the direct precocious exposure to GEN. Once these animals were adults we analyzed the Nitric oxide synthase and Vasopressin neural systems (**Ponti et al., 2017**). Moreover, we reviewed findings related to the role of environmental chemicals, referred to as "metabolic disruptors", in obesity and metabolic syndrome with special attention to recent discoveries from animal model and epidemiology studies. We propose broadening the definition of obesogens to that of metabolic disruptors, to encompass chemicals that play a role in altered susceptibility to obesity, diabetes and related metabolic disorders including metabolic syndrome (**Heindel et al., 2017**).

#### **Puberty control and metabolism.**

The hypothalamic paraventricular nucleus (PVN) is the major autonomic output area of the hypothalamus and a critical regulatory center for energy homeostasis. Several studies have suggested a relationship among neural circuits controlling energy homeostasis and the kisspeptin peptide. In the present study, we investigated the distribution of the kisspeptin fibers within the PVN of adult CD1 mice. We observed a significant kisspeptin sexual dimorphism for AVPV and ARC, as well as for the PVN innervation. Using co-localization of several marker we suggested that the majority of the kisspeptin fibers found in the PVN might arise directly from AVPV. Moreover, we investigated relationships among Kiss positive fibers and various systems located in the PVN, in particular the AVP, OXT, NOS and TH cells (**Marraudino et al., 2017**).

#### **Anorexia nervosa (a translational study).**

We have analyzed male and female adolescent rats and tested the effect of emotional deprivation, induced by the maternal separation in a model of Anorexia nervosa. Our results indicate that the maternal separation induces in the females a greater hyperactive behavior than in males (**Farinetti et al., submitted**).

### **I. Advancement in the field (1000 characters)**

The health problems related to endocrine disruptors (in particular, those related to obesity) gained more attention in these years. Our studies, as well as those performed in other laboratories, established some new end-points to determine the EDCs activity, in particular the behavior and the brain circuits. In

addition, these studies pointed to the importance of "windows of activity" to determine the effects of these molecules.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### a. Summary (up to 2000 characters):

Our long-term cooperation with the laboratory of dr. Collado in Madrid, is dedicated to the study of the involvement of gonadal hormones (chiefly estradiol) on neuroendocrine circuits programming feeding in rodents. In particular, we will investigate if estradiol during first stage of development participates in the programming/organization of these circuits as well as in the expression of the feeding behavior and the receptor pathways that are involved.

All these research lines will include groups of animals treated with several EDCs, in order to see how the exposure to these compounds will impact on these steroid hormone-dependent neuronal systems and behaviors.

Finally, we will expand our cooperation with our colleagues in Psychiatry to test the effects of oxytocin administration in our newly developed ABA model. Moreover, we started this year a PhD project that will study a mouse model for multiple sclerosis and the possible involvement of Endocrine Disruptors in the onset of this disease.

### b. Background and Significance (up to 4000 characters):

Steroid hormones, which are synthesized in adrenal glands, gonads or placenta, exert a large array of biological effects on the nervous system. These hormones play important roles in the development, growth, maturation, differentiation and protection of the central (CNS) and peripheral nervous system (PNS). In addition, the nervous system itself is capable of metabolize or de novo synthesize active steroids (*neurosteroids*) which may control the activity and survival of nerve cells. In the living animal, there are mechanisms (i.e. the alpha-fetoprotein in rodents or the sex hormone binding globulin in primates) that protect the brain from the circulating gonadal hormones, in order to prevent “mistakes” in the differentiation of gonadal hormone-dependent circuits during specific windows of activity (critical period) that occurs in the pre- or/and postnatal development.

Endocrine disrupting chemicals (EDCs) are compounds that are biologically active and mimic endogenous hormones (often estrogens or androgens), thereby altering hormone-modulated responses. They are not blocked by protective mechanisms as the alpha-fetoprotein, therefore they have been shown to disrupt embryonic development, sexual differentiation, reproduction, immune function, behavior, and responses mediated by hormones. The issue of EDCs has gained increasing attention as it has become clear that these environmental contaminants have endocrine activity in humans, as well as in wildlife and domestic animal species. Some of these chemicals, most notably the plant phytoestrogens largely present in the food, may play an important role in the reproductive cycles of small rodents as well as have positive (or negative) effects in other animals including humans.

A range of EDC effects has now been documented in a number of animal species, both in laboratory studies and in wild populations, demonstrating that xenoestrogenic or xenoandrogenic compounds may exert deleterious effects, even long time after exposure. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. Experimental data in galliforms and rodents showed that EDCs exposure, though nonlethal, left the individual impaired or even incapable of reproducing (Panzica et al., 2007). A recent acquisition is the concept of metabolic disruptors, i.e. substances that are able to induce profound alterations of the metabolism and inducing diseases like the diabetes (Heindel et al., 2015).

Many studies on EDCs have a toxicological approach and are performed in vitro. Among those done in vivo, only a few studies have considered the brain as a major target. In this field, our laboratory is very active and we will continue our researches covering several research lines dealing with different aspects of the interactions gonadal hormones-nervous system.

One research line is strictly related to the control of reproduction and the interaction with the metabolic state of the animals. It appears from our preliminary studies that the hypothalamic paraventricular nucleus could be a privileged site for these interactions. In fact, we have observed a large supply of kisspeptin

fibers ending in this region that could potentially interact with the other peptidergic and neurotransmitter systems. Kisspeptin system is particularly vulnerable to EDCs and part of the metabolic effects of these compounds could be explained through the action over the kisspeptin system.

Another research line involves the study of the effects of estrogens over the orexigenic and anorexigenic neuroendocrine circuits. The anorexigenic effect of estradiol in the adult life is well known. We want to investigate its effects during early development and those of EDCs administered both in adult and in early life.

**c. General aim and integration with mission of the Institute (up to 1000 characters)**

Our research lines are covering different important physiological activities and our aim is to understand how the gonadal hormones may interact and regulate the neural circuits that are involved in these functions, with particular consideration of gender differences. This is particularly related to the topic of neuroendocrine basis of some neurodegenerative diseases in which it is present a significant sex dimorphism. The approach to cure these diseases should always consider that some basic mechanisms could be sexually differentiated. In addition, in some cases it appears that environmental factors may have a role in the development of these diseases, therefore EDCs, that may interact with gonadal hormones receptors, are good candidates for this environmental action. The elucidation of how these compounds interact with nervous circuits will open a new frontier in our knowledge. For this reason we have planned to collaborate with clinician groups in proposing research project trying to correlate Parkinson Disease and Multiple Sclerosis with EDCs/environmental factors.

**d. Specific objectives and strategies (up to 4000 characters):**

**Effect of xenoestrogens EDCs.** We will investigate the effects of exposure to xenoestrogens in different ways:

- the effect of GEN in *in vitro* study: we know that estradiol promotes neuritic processes' outgrowth of different neuronal types, so we will analyze if GEN, like estradiol, could affect neuritic outgrowth (neuritogenesis) in hypothalamic neurons in sexually characterized cultures and what estrogen receptor is likely to be implicated.

- the effects of early postnatal GEN administration, at doses similar to that of infant formulas, to understand if may interfere with the development of specific steroid-sensitive neuronal circuits, causing irreversible alterations in adults.

- the effects of a chronic treatment in adult mice with some xenoestrogens (bisphenol A, BPA; diethylstilbestrol, DES) on hypothalamic circuits (arcuate, ventromedial, dorsomedial and paraventricular nuclei) involved in food intake (Neuropeptide Y, that increase the food intake and decrease energy expenditures; Pro-opiomelanocortin that decrease food intake and increase energy expenditure).

**Effect of xenoandrogens EDCs.** In this case we will analyze the effect of a chronic treatment with another EDCs, the Tributyltin (TBT) that is considered a metabolic disruptor and has an anti-androgenic activity; we want to know if TBT may interfere with the anorexigenic system in hypothalamic areas (arcuate, dorsomedial, ventromedial, paraventricular nuclei) involved in the control of food intake.

**Effect of steroids. Kisspeptin system.** We know that in physiological conditions, gonadal hormones modulate the kisspeptin system with expression changes according to different phases of the estrous cycle. We want to study these hormonal fluctuations in gonadectomized female mice, investigating possible changes in the kisspeptin system (arcuate, RP3V and paraventricular nuclei) by administration of progesterone (P) or estradiol (E<sub>2</sub>), alone or together.

**Translational studies.** Finally, we will expand our cooperation with our colleagues in Psychiatry to test the effects of oxytocin administration in our newly developed ABA model. Moreover, we started this year a PhD project that will study a mouse model for multiple sclerosis and the possible involvement of Endocrine Disruptors in the onset of this disease.

**e. Unique features of the project research (up to 2500 characters):**

Our research unit is devoted to the study of the interactions among steroid hormones and the nervous tissue, using as main physiological end point the behavior. Due to the large distribution of steroid hormone receptors within the brain and the importance that steroid hormones have for neuronal and glial

differentiation, survival and protection, a better understanding of the relationships steroids-nervous system seems to be of crucial importance.

In addition to this, the importance of gender medicine is increasing, and the interactions among gonadal hormones and the nervous system can partly explain gender differences in both physiological and pathological conditions (Panzica and Melcangi, 2016 for a review).

This is the general context, but, in last two decades the problem of how the environment can interact with human and animal physiology to induce pathologies became an important topic for the biomedical sciences. It is not surprising that a large number of synthetic substances may interact with hormone receptors and therefore induce endocrine unbalance and diseases. However, for many years the neuroendocrine effects were underestimated and the nervous tissue was not the main target of studies as well as, more importantly, it was not considered as an important end-point to be included to develop toxicological tests for the regulations of the EDCs use. Our researches, coupled with the lobbying activity to the members of the European parliament, will induce, hopefully, major attention to the dangers that EDCs may have mainly at the level of the central nervous system.

In summary, we believe that our researches can improve our understanding of gender differences in the healthy brain, as well as in several neural pathologies, and the complex interactions among the neural circuits, behavior, and environmental contaminants.

**d. Methodology (up to 2000 characters): please fill-out this section only in the case of innovative technologies**



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Electrophysiology of neurodegenerative diseases

## 1. LABORATORY DESCRIPTION – PERSONNEL:

### Principal Investigator

First name and surname	Birthdate (dd/mm/yyyy)
FILIPPO TEMPIA	20/08/1960
Degree: MD, PhD	Gender: MALE
Nationality: ITALIAN	Phone: +39-011-670-6609
Email: filippo.tempia@unito.it	

### Personnel

1. First name and surname:	Birthdate (dd/mm/yyyy)
ERIOLOA HOXHA	26/01/1981
Degree: PHD	Gender: FEMALE
Role: POSTDOC	Nationality: ITALIAN, ALBANIAN
Expertise: PATCH-CLAMP, BEHAVIOR, IMMUNOHISTOCHEMISTRY, MOLECULAR BIOLOGY,	

2. First name and surname	Birthdate (dd/mm/yyyy)
ILARIA BALBO	06/05/1993
Degree MS	Gender: FEMALE
Role: PHD STUDENT	Nationality: ITALIAN
Expertise BEHAVIOR, IMMUNOHISTOCHEMISTRY, MOLECULAR BIOLOGY	

## 2. PRINCIPAL INVESTIGATOR

H index 23, citations 1504 (scopus)

### Relevant discoveries:

- structural and functional alterations in animal models of hereditary ataxia
- cognitive impairment of mice with genetic deletion of the gene *Fgf14*
- mechanisms of spreading of pathological lesions in Alzheimer's disease models, by means of transplantation
- expression and physiological roles of voltage-dependent potassium currents ( $I_A$ ,  $Kv3$ ,  $erg$ ) and resurgent sodium current in cerebellar Purkinje cells
- cerebellar synaptic plasticity induced by fear conditioning
- synaptic physiology of deep cerebellar nuclei
- synaptic integration and maturation of electrophysiological properties of synaptic currents of Purkinje cells from embryonic cerebellum grafts
- electrophysiological alterations of Purkinje cells in an animal model of the human genetic disease ataxia telangiectasia
- postsynaptic currents due to metabotropic glutamate receptors in Purkinje cells

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2017-2020	National	PI	Ricerca Locale	Cellular and molecular mechanisms of spino-cerebellar ataxia type 38 (SCA38)	n.a.	€ 7.153	8%
2015-2018	National	PI	Ricerca Locale	Role of neuronal excitability in the prefrontal cortex of mice model of psychiatric disorders	n.a.	€ 2.605	8%
2014-2017	National	PI	Ricerca Locale	Cerebellar symptoms and cellular mechanisms in a murine model of spino-cerebellar ataxia type 38 (SCA38)	n.a.	€ 2.400	8%
2014-2018	National	co-PI	Telethon-Italy	Translating molecular pathology into a therapeutic	GGP14225	€ 86,400	8%



				strategy in SCA38, a newly identified form of spinocerebellar ataxia			
--	--	--	--	--	--	--	--

Please list your outreach activities

- describe your international collaborative experiences.
  - Collaboration with prof. F. Laezza of the University of Texas Medical Branch at Galveston (TX, USA)
  - Collaboration with Dr. Horton and Dr. Moon, University of Texas Southwestern Medical Center, Dallas (TX, USA)
  - Collaboration with Dr. M.C. Miniaci, University of Naples Federico II (Italy)
  - Collaborations for the SCA38 project: Prof. Borroni, University of Brescia; Prof. Caruso and Prof. Mitro, University of Milano; Dr. Brusco, University of Turin.
  - Collaboration with Dr. Chiara VerPELLI, CNR Institute, Milano
- Invited talks
  - Pontifical Council for Culture. Plenary Assembly "The future of humanity-new challenges to anthropology". Plenary lecture: "The human person, the brain and the soul".
- Editorial duties
  - Frontiers in Aging Neuroscience (Associate Editor)
  - Frontiers in Synaptic Neuroscience (Associate Editor)
  - Frontiers in Dementia (Review Editor)
  - The American Journal of Alzheimer's disease

Please list your organizational activities:

- Speakers invited
  - Prof. Chiara VerPELLI, CNR Institute, Milano

### 3. PI's PUBLICATIONS:

(Please list below your publications in 2017 -. Please indicate the journal IF, ranking).

For each publication, please indicate:

\* if you contributed equally to the first-author, as stated in the published article

Please use the following format throughout:

Manca, A., Capsoni, S., Di Luzio, A., Vignone D, Malerba F, Paoletti F, Brandi R, Arisi I, Cattaneo A, Levi-Montalcini R. (2012). Nerve growth factor regulates axial rotation during early stages of chick embryo development. *Proc Natl Acad Sci U S A.*, 109(6):2009-14  
IF= 9.67; R = 4/57

1. Hoxha E, Lippiello P, Scelfo B, **Tempia F**, Ghirardi M, Miniaci MC. (2017) Maturation and refinement of cerebellar cortical circuits in normal development and in murine models of autism. *Neural Plasticity*, Article ID 2017:6595740, 14 pages. Review. doi: 10.1155/2017/6595740.  
IF = 3.054; R(neuroscience) = 115/259
2. Manes M, Alberici A, Di Gregorio E, Boccone L, Premi E, Mitro N, Pasolini MP, Pani C, Paghera B, Perani D, Orsi L, Costanzi C, Ferrero M, Zoppo A, **Tempia F**, Caruso D, Grassi M, Padovani A, Brusco A, Borroni B (2017) DHA is a beneficial replacement treatment for Spinocerebellar Ataxia 38 (SCA38). *Ann Neurol.* 2017 Oct 4. doi: 10.1002/ana.25059.  
IF = 9.890; R(neuroscience) = 14/259; R(clinical neurology) = 6/194
3. Hoxha E\*, Gabriele RMC, Balbo I, Ravera F, Masante L, Zambelli V, Albergo C, Mitro N, Caruso D, Di Gregorio E, Brusco A, Borroni B and **Tempia F** (2017) Motor Deficits and Cerebellar Atrophy in Elov15 Knock Out Mice. *Front. Cell. Neurosci.* 11:343. doi: 10.3389/fncel.2017.00343. \*corresponding author.  
IF = 4.555; R(neuroscience) = 55/259

#### 4.GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2016 -. Please indicate the journal IF, ranking)

Please use the following format throughout:

Manca, A., Capsoni, S., Di Luzio, A., Vignone D, Malerba F, Paoletti F, Brandi R, Arisi I, Cattaneo A, Levi-Montalcini R. (2012). Nerve growth factor regulates axial rotation during early stages of chick embryo development. *Proc Natl Acad Sci U S A.*, 109(6):2009-14  
IF= 9.67; R = 4/57

1. **Hoxha E**, Lippiello P, Scelfo B, Tempia F, Ghirardi M, Miniaci MC. (2017) Maturation and refinement of cerebellar cortical circuits in normal development and in murine models of autism. *Neural Plasticity*, Article ID 2017:6595740, 14 pages. Review. doi: 10.1155/2017/6595740.
2. **Hoxha E\***, Gabriele RMC, **Balbo I**, Ravera F, Masante L, Zambelli V, Albergo C, Mitro N, Caruso D, Di Gregorio E, Brusco A, Borroni B and Tempia F (2017) Motor Deficits and Cerebellar Atrophy in Elov15 Knock Out Mice. *Front. Cell. Neurosci.* 11:343. doi: 10.3389/fncel.2017.00343. \*corresponding author.

## 5. GROUP's additional information:

Please list the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
	National vs International	PI vs. Component	MIUR, ERC, ecc...			€	€

Please list honours, prizes or awards received by other members of the group If applicable.

Please list outreach activities of other members of the group:

- Describe your international collaborative experiences.
- Invited talks
- Editorial duties

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group

Please list your technology transfer achievements (patents, etc.), if applicable

## 6 .Past Research activity

(Summarize the PI and group research activities in 2017)

### a. Summary (500 characters)

The main project of the year 2017 was the validation of the Elov15 knock-out mouse as an animal model of spino-cerebellar ataxia type 38 (SCA38), in which we found motor deficits similar to those of patients. In addition, Elov15 knock-out mice displayed cerebellar atrophy with a significant reduction of the dendritic tree of cerebellar Purkinje cells. These findings validate the animal model and will be the basis of the search for the functional alterations responsible for this neurological disease. We also took part in the first clinical trial on SCA38 patients, showing beneficial effects.

### b. Background (2000 characters)

Spinocerebellar ataxias (SCAs) are autosomal dominant neurological disorders characterized by gait ataxia, incoordination of eye movements, speech, and hand movements, and usually associated with cerebellar atrophy. In a large collaborative study, we recently identified a novel form of spinocerebellar ataxia (SCA38) due to missense mutations in the gene *ELongase of Very Long chain fatty acids 5, ELOVL5*. The pathophysiological hypotheses are a loss of function effect of mutations, or a toxic gain of function due to accumulation of misfolded mutated ELOVL5 protein.

### c. Rationale (2000 characters)

Dr. Horton and Dr. Moon, University of Texas Southwestern Medical Center, Dallas (TX, USA), recently developed an Elov15 knock-out mouse, in which they studied the unbalance of lipids in the liver in response to a high-fat diet. They agreed to collaborate with us and sent these mice to NICO. Since Elov15 knock-out mice completely lack Elov15 activity, they are a model of the loss of function hypothesis. If Elov15 knock-out mice reproduce the main features of SCA38, this will be a first evidence in favour of the loss of function hypothesis.

### d. Objectives (1500 characters)

The aim of the study was to probe the main signs and symptoms of SCA38 in Elov15 knock-out mice. A first aspect regards motor symptoms, including balance instability, which is present in the first stage of SCA38; and hyposmia, which is also present in SCA38 patients. The second aspect concerns pathological findings, consisting in cerebellar atrophy. To this aim we planned to perform an overall assessment of the cerebellum, followed by a more detailed analysis at the cellular level.

### e. Results (4000 characters)

Elov15 knock-out mice showed a significant motor deficit in the balance beam test starting from the first time point analyzed of 3 months of age. Hyposmia was also present, but started at 12 months of age. Thus, the two main clinical features of patients with SCA38 are reproduced by Elov15 knock-out mice. We proceeded with the study of cerebellar morphology, in which we found a global reduction in size already at 6 months of age, with shorter Purkinje cell layer length, smaller white matter area and a thinner molecular layer. At 18 months also the granular layer became significantly thinner. Since most forms of ataxia are associated with Purkinje cell lesions, we focused on their dendritic tree, which is contained in the thinner molecular layer. We showed a reduced extension of Purkinje cell dendrites, but with preserved spine density. These results suggest that symptoms appear before structural alterations, and they are followed by a reduction of the principal components of the cerebellar cortex. The molecular layer becomes thinner, at least in part, because of hypotrophy of Purkinje cell dendrites, suggesting a functional impairment in this cell compartment, which might trigger further functional and structural alterations as the disease progresses.

### f. Advancement in the field (1000 characters)

The pathogenesis of spino-cerebellar ataxias is very heterogeneous, but at present no therapy is available for any type of SCA. Our study on Elov15 knock-out mice might be a first milestone in the understanding of SCA38 disease mechanism. The validated animal model will be the basis of studies aimed at identifying mechanisms, which can be targeted to stop or revert the disease. In an associated study by the Neurology

Unit of the University of Brescia, we contributed in showing that a replacement therapy with the main molecules downstream ELOVL5 significantly ameliorates symptoms in SCA38 patients.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### a. Summary (up to 2000 characters):

Starting from our results that the dendritic tree of PCs is altered in *Elovl5* knockout mice, we plan to identify the pathogenic mechanism linking the loss of this elongase enzyme to SCA38 ataxia. We expect functional deficits of PCs. Moreover, we aim at finding novel functions of Elovl5. Finally, we plan to try different rescue strategies to revert the ataxic phenotype of *Elovl5* knockout mice.

The cerebellum of *Shank3* mutant mice, model of the Phelan McDermid syndrome, will be studied in collaboration with Dr. Verpelli (CNR, Milan). Synaptic transmission mediated by AMPA and mGlu receptors will be assessed to uncover molecular mechanisms that might be targeted to cure this syndrome or other forms of autism spectrum disorders.

### b. Background and Significance (up to 4000 characters):

Aim 1. Spinocerebellar ataxias (SCAs) are autosomal dominant neurological disorders characterized by gait ataxia, incoordination of eye movements, speech, and hand movements, and usually associated with cerebellar atrophy. In a large collaborative study, we recently identified a novel form of spinocerebellar ataxia (SCA38) due to missense mutations in the gene *ELongase of Very Long chain fatty acids 5, ELOVL5*. The molecular pathogenesis of SCA38 has not been studied yet. We have recently demonstrated that the deletion of *Elovl5* in mice causes symptoms that recapitulate SCA38, suggesting that human mutations found in patients act by a loss-of-function mechanism. The most abundant brain long chain PUFAs are the omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the omega-6 arachidonic acid. These molecules are substrates for the production of a huge variety of active substances, including prostaglandins, protectins and recoverins, involved in induction and resolution of inflammation. Since the actions of these lipidic mediators in the brain is largely unknown, it is likely that important roles in physiology and in reaction to pathology are yet to be discovered. The *Elovl5* knock mouse is an excellent model to discover new molecular mechanisms, in addition to allowing studies about the pathogenic mechanism of SCA38.

Aim 2. Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders affecting around 1% of the population worldwide. ASDs are characterized by abnormal social interaction, deficits in verbal and nonverbal communication, repetitive behaviors and restricted interests. The cerebellum is frequently altered in ASD patients, but the mechanism linking this structure to autism is not known. We recently demonstrated that, in mice, a selective deletion of *Pten* in PCs is sufficient to cause autistic-like deficits. In the next year we plan to extend this finding to another disease frequently associated with autism, the Phelan McDermid syndrome (PMS), due to mutations of the *SHANK3* gene. PMS is characterized by significant mental retardation, intellectual disability, hypotonia, craniofacial dysmorphisms, epilepsy and autistic features. Moreover, *SHANK3* mutations have been identified in patients with autistic spectrum disorders (ASD). Shank proteins interact with more than 30 synaptic proteins, including ionotropic and metabotropic glutamate receptors. By interaction with Homer, Shank3 stabilizes mGlu1 and mGlu5 receptors at excitatory synapses and regulates their transduction pathway. Since alterations in Homer1 and mGlu5 have been identified as a risk factor for ASD, we hypothesize that such interaction between Homer and mGlu receptors in the cerebellar cortex is involved in the pathogenesis of autism. The aim of this project is to investigate the functional alterations of calcium signaling related to the mGlu1-Homer pathway in cerebellar Purkinje cells in *Shank3* mutant mice.

### a. General aim and integration with mission of the Institute (up to 1000 characters)

The majority of our projects are aimed at finding the molecular and neural mechanisms of diseases caused by cerebellar dysfunction. In fact, the final aim of our project on SCA38 is to understand the pathogenic mechanism and to design a specific therapy. Regarding autism, we want to provide a mechanistic explanation of the role of the cerebellum in this disorder, so that therapeutic interventions can be envisaged. The mission of the Institute is exactly the same as ours, namely to advance scientific knowledge regarding brain disorders, including neurologic and psychiatric diseases.

**a. Specific objectives and strategies (up to 4000 characters):**

**Aim 1: Mechanisms of SCA38 ataxia**

Starting from our demonstration of ataxic signs and of alterations in the dendritic tree of PCs in *Elovl5* knockout mice, we plan to investigate the functional alterations of PCs, due to the lack of this enzyme. PCs of *Elovl5* knockout mice will be patch-clamp recorded in acute cerebellar slices. Intrinsic excitability of PCs and the function of synapses impinging on them will be analyzed. We expect that functional PC deficits are correlated with the onset and progression of ataxic deficits. More specifically, we hypothesize that *Elovl5* activity in PCs is required for the synthesis of lipid mediators that play a major role in signal processing in the cerebellar cortex. First, we plan to find which PC functions are altered in *Elovl5* knockout mice. Then such functions will be studied in detail with rescue experiments based on supplementation of different molecules downstream to *Elovl5*. Finally, in wild-type mice, after strong and prolonged activation of the *Elovl5*-dependent function, the slice perfusate will be analyzed in collaboration with a laboratory of the University of Milano, in order to find the lipid molecules whose production is triggered by the stimulation.

**Aim 2: Role of cerebellum in autism spectrum disorders**

Previous studies have shown that *Shank3* mutant mice present defects in mGlu1 and mGlu5 transduction pathways. A first specific aim is to investigate, by the patch-clamp technique in cerebellar slices, Purkinje cell responses mediated by mGlu1. Such responses consist of release of calcium ions from endoplasmic reticulum and generation of a slow postsynaptic current due to TRPC3 channel opening. We plan to search for alterations of the mGlu1-mediated postsynaptic currents. A second specific aim is to study alterations of calcium signaling along the mGlu1-TRPC3 pathway. A third specific aim is to study alterations of calcium signals mediated by the Homer-mGlu1 pathway.

**a. Methodology (up to 2000 characters): please fill-out this section only in the case of innovative technologies**

The recent acquisition of a two photon microscope, which is now located at NICO, will allow us to complement patch-clamp studies with *in vitro* and *in vivo* imaging of calcium to measure neuronal activity in large networks of neurons of the cerebellar cortex.





*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2017**

Laboratory name: Brain development and disease

#### 4. LABORATORY DESCRIPTION – PERSONNEL:

##### Principal Investigator

Alessandro Vercelli 09/07/1961  
MD PhD Male  
Italian Phone: 011/6706617  
Email: alessandro.vercelli@unito.it

##### Personnel

1. Adriano Ceccarelli 28/10/1957  
MD PhD Male  
Role Associate Professor Nationality Italian  
Expertise Molecular Biology
  
2. Elena Tamagno 14/07/1967  
PhD Female  
Role Associate Professor Nationality Italian  
Expertise Alzheimer's disease
  
3. Marina Maria Boido 06/09/1980  
PhD Gender Female  
Role Assistant Professor RTD Nationality Italian  
Expertise Spinal cord injury, motor neuron diseases, Huntington disease, stem cells
  
4. Michela Guglielmotto 28/02/1977  
PhD Female  
Role Post-doc Nationality Italian  
Expertise Alzheimer's disease
  
5. Debora Monteleone 06/02/1986  
PhD Female  
Role Post-doc Nationality Italian  
Expertise Alzheimer's disease
  
6. Roberta Schellino 11/02/1985  
PhD Female  
Role Post-doc Nationality Italian

Expertise Neurogenesis, motoneuron diseases, Huntington disease, behavior

- |  |                     |
|--|---------------------|
| 7. Giusi Manassero                                     | 01/11/1979          |
| PhD  | Female              |
| Role Post-doc  | Nationality Italian |
| Expertise Alzheimer's disease, neuropathic pain        |                     |
| 8. Ivan Enrico Repetto                                 | 11/09/1986          |
| Biotechnology degree                                   | Male                |
| Role doctorate student                                 | Nationality Italian |
| Expertise neuronal death, models of stroke             |                     |
| 9. Matilde Ghibaudi                                    | 19/09/1988          |
| Biotechnology degree                                   | Female              |
| Role doctorate student                                 | Nationality Italian |
| Expertise Spinal cord injury, miRNA, molecular biology |                     |
| 10. Elena Signorino                                    | 06/10/1976          |
| Biology degree   | Female              |
| Role technician  | Nationality Italian |
| Expertise molecular biology                            |                     |

## 5. PRINCIPAL INVESTIGATOR

H index 28, citations 3561

Relevant discoveries:

- We showed that human embryonic stem-derived striatal progenitors can be transplanted in a Huntington's disease rat models and can differentiate and integrate into the host, extending fibers over a long distance (Faedo et al., PNAS 2017).
- We contributed to clarify the mechanisms of motor neuron death in Spinal Muscular Atrophy (SMA): autophagy is dysregulated in SMA and its inhibition can significantly delay motor neuron degeneration and extend murine lifespan (Piras et al., Cell Death Dis., 2017); moreover we observed that apoptosis and autophagy pathways are intricately intertwined, and that the pharmacological inhibition of apoptosis can represent an effective therapeutic strategy in SMA (unpublished).

Please list your grants according to the table below (current and pending).

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2016-19	International	Coordinator	Horizon 2020	My-AHA		395000	8%
2014-17	International	PI (with A.Buffo)	FP7	NSCR		400000	8%
2017-20	National	PI	PRIN (MIUR)	Generation of functional striatal neurons for brain repair in Huntington Disease	2015AY 9AYB_002	75530	8%
2016-18	Local	PI	CRT			30000	10%
2017	Local	PI	Smarathon			14000	10%
2017	Local	PI	Atrofia spinale onlus			25000	10%

Please list the name of PhDs you have supervised.

Matilde Ghibaudi  
Martina Lorenzati

Please list honours, prizes or awards received, if applicable.

Please list your outreach activities

- describe your international collaborative experiences.
- My-AHA project, started in 2016, which we are coordinating, is performed by a Consortium of 16 partners (Universities, Research Centers and SMAs) in Europe (Austria, Germany, Great Britain, Nederland, Portugal, Spain) and Extra-EU (Australia, Japan and South Korea);
- NSCR project involves several European partners, in particular in Great Britain, Germany and Sweden;
- We have collaborations with Switzerland (University Lausanne, mithophagy in the CNS), and UK (miRNAs in spinal cord injury).

- Invited talks

12 May 2017 Terapie con cellule staminali nelle patologie della retina, Torino

20 September My-AHA, InformaEduca, Torino

22 September 2017 An ICT platform to detect frailty risk and propose intervention, Softcom 2017 Split (Symposium organized by L.Patrono)

2 October 2017 Integration and behavioral outcomes of hMSN progenitors in the striatum of a model of HD, XVII Congress of the Italian Society for Neuroscience, Ischia (Symposium organized by A.Vercelli)

21 November 2017 myAHA project : a tool for prevention and early diagnosis of neurodegenerative diseases, 2017 International Symposium on mHealth, Seul

- Editorial duties

Member of the Board of Editors of the Journal Digitcult

Please list your organizational activities:

- Speakers invited

Corrado Cali

Gabriella Viero

Michael Courtney

Michele Simonato

- Workshops, Schools or Conferences organized

Brain Awareness Week, Torino

Italian Society for Neuroscience, Ischia (2 October 2017), Symposium

Meetings of the My-AHA Consortium (Vienna, June 2017 and Naples, December 2017).

## 6. PI's PUBLICATIONS:

Piras, A., Schiaffino, L., Boido, M., Valsecchi, V., Guglielmotto, M., De Amicis, E., Puyal, J., Garcera, A., Tamagno, E., Soler, R.M., Vercelli, A. (2017). Inhibition of autophagy delays motoneuron degeneration and extends lifespan in a mouse model of spinal muscular atrophy. *Cell Death Dis.*, 8(12):3223.

IF = 5.965; R = 39/190

Ghibaudi, M., Boido, M., Vercelli, A. (2017). Functional integration of complex miRNA networks in central and peripheral lesion and axonal regeneration. *Prog Neurobiol.*, 158:69-93.

IF = 13.217; R = 7/259

Faedo, A., Laporta, A., Segnali, A., Galimberti, M., Besusso, D., Cesana, E., Belloli, S., Moresco, R.M., Tropiano, M., Fucà, E., Wild, S., Bosio, A., Vercelli, A.E., Biella, G., Cattaneo, E. (2017). Differentiation of human telencephalic progenitor cells into MSNs by inducible expression of Gsx2 and Ebf1. *Proc Natl Acad Sci U S A.*, 114(7):E1234-42.

IF = 9.661; R = 4/64

Vercelli, A. Rainero, I., De Rosario, H., Summers, M., Wieching, R., Aumayr, G., Bandelow, S., Ciferri, L., Bazzani, M. (2017) My-active and healthy ageing (My-AHA): An ICT platform to detect frailty risk and propose intervention. SoftCOM Article number 8115505.

Vercelli A, Rainero I, Ciferri L., Boido, M, Pirri F. (2017) Robots in Elderly Care. Digitcult Special Issue.

Summers M, Madl T, Vercelli A., Aumayr G., Bleier DM, Ciferri L., Deep Machine Learning Application to the detection of Preclinical Neurodegenerative Diseases of Ageing. Digitcult Special Issue.

#### 4.GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2016 -. Please indicate the journal IF, ranking)

Filippi, M., Boido, M., Terreno, E. (2017). Imaging of MSC transplantation in neuroscience. *Oncotarget.*, 8(7):10781-82.  
IF = 5.168; R = 44/2017

Manassero, G., Guglielmotto, M., Monteleone, D., Vasciaveo, V., Butenko, O., Tamagno, E., Arancio, O., Tabaton, M.. (2017). Dual Mechanism of Toxicity for Extracellular Injection of Tau Oligomers versus Monomers in Human Tau Mice. *JAlzheimers Dis.*, 59(2):743-51.  
IF = 3.731; R = 77/259

Guglielmotto, M., Monteleone, D., Vasciaveo, V., Repetto, I.E., Manassero, G., Tabaton, M., Tamagno, E. (2017). The Decrease of Uch-L1 Activity Is a Common Mechanism Responsible for A $\beta$  42 Accumulation in Alzheimer's and Vascular Disease. *Front Aging Neurosci.*, 9:320.  
IF = 4.504; R = 7/49

Tamagno, E., Guglielmotto, M., Monteleone, D., Manassero, G., Vasciaveo, V., Tabaton, M (2017).. The Unexpected Role of A $\beta$ 1-42 Monomers in the Pathogenesis of Alzheimer's Disease. *JAlzheimers Dis.* 2017 Oct 30.  
IF = 3.731; R = 77/259

## 5. GROUP's additional information:

Please list the grants (current and pending) of the other members of the group - according to the table below:

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
25/02/16 - 31/12/17	National	M. Boido (Component)	Ricerca Locale 2015	Nurr1: un nuovo possibile target terapeutico nella SLA		2.012,24 €	8%
25/02/16 - 31/12/17	National	E. Tamagno (Component)	Ricerca Locale 2015	The role of proteasome and autophagy impairment in the pathogenesis of Alzheimer's disease.		2751,94€	8%
Febbraio 2017- Febbraio 2019	National	E. Tamagno (Component)	Ricerca Locale 2016-2017	Gli ormoni sessuali mediano effetti differenti sulla conformazione di Tau mediata dalla $\beta$ amiloide		7080,45€	8%
Febbraio 2017- Febbraio 2019	National	M. Boido (Component)	Ricerca Locale 2016-2017	Nurr1, un promettente target terapeutico per la SLA		7150,16 €	8%
2016-2022	National	M. Boido (Component)	Ricerca autofinanziata			2292,77€	8%
2017-2020 (fondo disponibile dal 2018)	National	M. Boido (Component)	CRT	I mitocondri nell'Atrofia Muscolare Spinale: disfunzioni e mitofagia	2017.2052	28.000 €	8%
2017	National	M. Boido (Component)	Progetto Traslaazionale Dipartimento	Spinal muscular atrophy: can mitochondria represent both innovative		pending 25.000 €	



				targets and predictive biomarkers?			
2017	National	M. Boido (Component)	Roche per la ricerca	MRI in vivo dei linfociti: da killer spietati a efficaci carrier di farmaci nella sclerosi multipla		pending 100.000€	
2017	National	M. Boido	Ministero dell'Istruzione – Finanziamento di base alla ricerca			3000	8%

Please list honours, prizes or awards received by other members of the group If applicable.

Please list outreach activities of other members of the group:

- Describe your international collaborative experiences.  
Boido M. - Collaborations with Switzerland (Julien Puyal, University Lausanne; Pharmafox company, Möhlin, Aargau), UK (Tamas Dalmay, University of East Anglia) and Spain (Rosa Soler, University of Lleida-IRBLLEIDA).
- Invited talks
  - Boido M. Autofagia e apoptosi nella SMA: due facce della stessa medaglia? Fondazione I.R.C.C.S. Istituto Neurologico “Carlo Besta”, Sede Bicocca, Milano, 27/02/17.
  - Boido M. Spinal cord: for better or for worse. Giornate di Approfondimento in Neuroscienze, Giornate di Formazione per il Dottorato di Ricerca. Torino, 20/04/2017.
  - Boido M, Schellino R, Piras A, Valsecchi V, Vercelli A Self-eating and self-killing: alternative or cooperating pathways in SMA? XVII Congresso Nazionale SINS (Società Italiana di Neuroscienze), Ischia, 1-4/10/2017.
- Editorial duties

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group

Please list your technology transfer achievements (patents, etc.), if applicable

Boido M./Tamagno E.: Founders of the academic spin-off S&P BRAIN SRL

## **6. Past Research activity**

### **a. Summary (500 characters)**

We study CNS development (from the embryo to the aged) and the common neurobiological mechanisms and molecular pathways leading to normal development and to neurodegeneration. We are interested in neuronal cell death pathways, which we study in development and in experimental models of transient/permanent cerebral ischemia, acute/chronic glaucoma, epilepsy, SMA and AD. Finally, we are studying cell therapy in preclinical experimental models of ALS, SCI and HD.

### **b. Background (2000 characters)**

The study of the CNS represents a great challenge to the scientist of the 21st century, and neurodevelopmental and neurodegenerative disorders provide major insights in the understanding of its anatomy, physiology and pathology and the design of new therapies. Many cellular events and mechanisms occurring during development may have profound influences on the adult nervous system, and healthy aging may be considered as the last phase of neural development.

Recently, Europe with the Human Brain Project and USA with the Connectome project, together with similar projects launched by other countries such as Japan and China, targeted the micro-, meso- and macro-connectome from a normal and pathological point of view. In the meanwhile, collaborative projects such as the Joint Program for Neurodegenerative Diseases and ERA-NET Neuron in Europe aim to investigate the basic mechanism underlying neurodegenerative diseases, with a translational aim to design new diagnostic and therapeutic measures. Networks from genes to miRNAs and molecules, from neurons to brain areas represent the building blocks of neural function. On the one hand, they may represent pathways for spreading of neurodegenerative diseases and, on the other, some nodes in the network (such as the “hubs”) may be more liable to disease. Therefore, only an holistic approach, from molecules to brain areas, from development to disease and from a multidisciplinary point of view can provide new insights and concept on brain function, disease and repair.

### **c. Rationale (2000 characters)**

Understanding the development of the CNS, and how neurons can form axonal connections participating in anatomical and functional networks is fundamental to the comprehension of brain function and disease, and to design new therapeutic strategies. To this aim we take advantage of the study of normal brains and of the brains of transgenic mice, in which specific molecules are knocked down to investigate their function. On the other hand, we have developed through the years several cellular and animal models of neurodegenerative diseases, in which to study the molecular mechanisms involved and to target them with stem cell therapy or specific inhibitors to prevent disease and promote brain repair at cellular, network and behavioral levels. Finally, we maintain a close connection with clinicians in order not only to favor a translation from bench to bedside but also to have a continuous feedback on the clinical needs.

The advancement of science does not only consist of new ideas, concepts and mechanisms to be understood but also of new tools which allow to investigate the nervous system from new points of view. To this aim, we are spending part of our time and economical efforts to technological improvement and to apply other disciplines to neuroscience, believing that only the contamination among different forms of knowledge may provide breakthrough innovation in the field. The collection of increasing amount of data with IoT (Internet of things) and big data pose new challenges to Neuroscience and we would like to participate to this new era.

### **d. Objectives (1500 characters)**

We aim to understand the structural/functional building blocks of the cerebral cortex and their circuitry, as substrate for brain activities and entities which may be disrupted in several congenital and degenerative diseases. In particular, we study pyramidal neurons, the organization of their dendritic bundles and axonal projections. We aim to study neural networks and connectivity, and how they are disrupted in disease.

We study the mechanisms of neuronal death during development and disease, such as excitotoxicity, apoptosis, autophagy and oxidative stress induced in different models of human disease, to prevent them. We have addressed the role of a MAP-kinase (JNK) in neuronal death and using specific inhibitors we have obtained substantial prevention of neuronal death in models of cerebral ischemia, SMA and AD.

In several neurodegenerative diseases, the pathology is not cell-autonomous, i.e. pathogenesis involves other cells in addition to neurons. Therefore, we study neuroinflammation in stroke and ALS and how to prevent it to delay the onset/progression of disease.

Stem cells are a growing field of research related to normal development, disease and cancer. We study the integration of hES-derived striatal progenitors grafted into the striatum (in HD). Moreover, we use neural and/or mesenchymal stem cells to treat neurodegenerative/traumatic diseases (in ALS and SCI), to provide trophic and immunomodulatory substances to host neurons.

#### **e. Results (4000 characters)**

##### *Development of cerebral cortex*

We study the development of corticofugal axons. With A. Buffo we study the axon/oligodendrocyte (OL) relationship, and the role of OL activity: we have identified a MAP-kinase, JNK, as a key molecule in OL development and axon myelination, both in vitro and in vivo.

##### *Mechanisms of neuronal death and neuroinflammation in ALS, SMA and AD*

We are investigating role/interaction of two major types of programmed cell death, autophagy and apoptosis. The injection of an autophagy inhibitor can improve motor performance, extend the lifespan and delay motor neuron death in SMA (Piras et al., 2017). The JNK-signaling pathway is involved in the SMA pathogenesis: the administration of a JNK-inhibitor peptide (DJNKI) exerted positive effects on motor neuron survival and muscular atrophy (*in preparation*).

Re ALS, we are evaluating the role of Nurr1, a nuclear receptor implicated both in neuroprotection and immunomodulation in PD and MS: in our ALS mouse model, Nurr1 activation can modulate neuroinflammation and protect motor neurons, at least at the onset of disease.

Re AD, we investigated the role ubiquitin C-terminal hydrolase L1 (Uch-L1), a key neuronal enzyme involved in the elimination of misfolded proteins, in ischemic injury as well as in AD. Uch-L1 inhibition induces BACE1 up-regulation and increases neuronal and apoptotic cell death in control as well as in transgenic AD mouse model subjected to Bengal Rose, a light-sensitive dye that induces a cortical infarction through photo-activation. Thus, the restoration of Uch-L1 was able to completely prevent both the increase in BACE1 protein levels and the amount of cell death. The Uch-L1-mediated BACE1 up-regulation could be an important mechanism responsible for A $\beta$  peptides accumulation in vascular injury and indicate that the modulation of the activity of this enzyme could provide new therapeutic strategies in AD.

##### *Stem cell therapy in HD*

With E. Cattaneo and A. Buffo, we are exploring the potential of human embryonic stem (hES) cells in an experimental model of HD, with good results in terms of cell replacement and establishment of new connections. We showed that hES-derived striatal progenitors can be transplanted, differentiate and integrate into the host, extending fibers over a long distance (Faedo et al., 2017).

##### *Spinal cord injury*

We previously demonstrated the therapeutic effects of stem cells (NPs and MSCs) in SCI. To improve the graft success (in terms of cell survival, uncontrolled proliferation), with Dr. Tonda-Turo (Polito), we are developing and testing biomimetic injectable hydrogels (chitosan) in which stem cells can be encapsulated. The in vitro results are encouraging and preliminary in vivo approaches are ongoing.

Finally, with Prof. Dalmy and De Pietri Tonelli, we performed a profiling of miRNA expression in a mouse model of SCI: we identified miR-7b-3p as a key-miRNA involved in the regulation of axon growth. In vitro experiments are still running to confirm such hypothesis, in order to find a possible new therapeutic target for SCI.

##### *Active and Healthy Ageing*

We are coordinating a Horizon 2020 project entitled My-AHA (Active and Healthy Ageing).

Stemming from a holistic view of interrelated frailties, cognitive decline, physical frailty, depression and anxiety, social isolation and poor sleep quality, My-AHA proposes an ICT platform for early detection of pre-frailty and intervention to sustain active and healthy ageing and slowing or reversing further decline. The main aim of My-AHA is to reduce frailty risk by improving physical activity and cognitive function, psychological state, social resources, nutrition, sleep and overall well-being in older adults. After a pilot study on a limited number of subjects, a randomized controlled study (RCT), 300 subjects receiving intervention and 300 controls from many EU and non-EU countries, is ongoing to evaluate intercultural aspects, is ongoing in order to evaluate efficacy of the my-AHA platform.

#### **f. Advancement in the field (1000 characters)**

Our group is actually working in several hot topics in Neuroscience, such as axonal development and growth in the normal brain and disease, neuronal cell death and stem cell therapy. It is also involved in the study of anatomical and functional connectivity of the human brain, and how it is altered in disease. Some of our contributions to the field were very relevant, and we are collaborating with other groups worldwide. Also, we are involved in the technical revolution about microscopy to investigate micro- and meso-connectome, with the use of confocal microscopy and, more recently, 2 photon microscopy. On the other hand, we are collaborating with groups working on fMRI aiming to macroconnectome, and the PI is involved in the Brain Imaging center of the University of Torino. We are also involved in several studies to identify and test new drugs for neurodegenerative diseases, and new biomaterials to support brain repair. Moreover, we are involved in studies using Internet of Things, Medical Devices and Artificial Intelligence to support Active and Healthy Ageing, i.e. to empower the elderly in their everyday life and to improve early detection and personalized prevention of disease.

## 7. Future Projects (Next year)

Please describe the following information relevant to the research that you are planning to do

### a. Summary (up to 2000 characters):

We intend to exploit our previous research on i) axonal growth in the CNS, ii) molecular and cellular mechanisms of neuronal death in neurodegenerative diseases, iii) network analysis at multiscale level, iv) stem cell therapy. We want to identify some new therapeutic targets (such as autophagy for neurodegenerative diseases, JNK-related molecules for neuronal death, miRNAs for neural development, neuronal cell death and axonal growth). Recently, there is a growing interest on mitochondria in neurodegenerative diseases: we have therefore started a new line of research. We also intend, in collaboration with internal and external groups, to import in the institute new techniques, such as the organoids for in vitro analysis of brain development and disease modeling, 2 photon microscopy for in vivo analysis and 3D electron microscopy. We will continue to collaborate with the Brain Imaging center to study human brain morphology (voxel-based morphometry and tractography) and functional networks (fMRI). Moreover, the PI is the coordinator of a 4-year Horizon 2020 grant, my-AHA (my Active and Healthy Aging), from January 2016. His task is mostly related to the overall organization of the activities, to be performed on human subjects and not involving directly the structures of NICO. Nevertheless, being the scientific coordination acted by A. Vercelli, this will allow the NICO to receive overheads. In particular we are preparing applications for the next EC calls on Ageing to improve early detection of age-related frailty in the individual by use of Artificial Intelligence (machine and deep learning). On the same subjects, in collaboration with F. Cauda (Dept. Psychology) and I.Rainero (Dept. Neurosci) we will perform morphometric and functional analysis of the brain and networks.

### b. Background and Significance (up to 4000 characters):

There is a growing interest in studying the development and disease of the CNS in terms of networks: genes, miRNAs and molecular networks at a ultramicroscopic level of magnitude, synaptic networks at the microscale, and anatomical and functional networks at the meso- and macroscale. Perturbances in the networks at the different scale levels may result in developmental or neurodegenerative disorders. To this extent, one may refer to “damage networks”: is it possible that some brain areas are more vulnerable than others to damage, or maybe more relevant than others for the onset of disease and of functional disorders? Such perturbances may be responsible for developmental disorders, such as schizophrenia, autism, epilepsy where there is an altered connectivity in terms of synapses and axonal connections, and of excitability. Also, neural networks may underlie the spread of neurodegenerative diseases in the CNS, such as for the Braak hypothesis of the molecular and cellular damage. Understanding the mechanisms of the onset and establishment of neural disorders at different scale levels is dramatically relevant to design neuroprotective and repair strategies to prevent and modify disease progression. These strategies therefore may be at a genetic, molecular, cellular and behavioral level, and must be considered in a holistic strategy. To this aim we will collaborate with F. Di Cunto (molecular biologist at NICO) and F. Cauda (fMRI) to investigate the existence of damage networks in some neurodegenerative and psychiatric diseases. We are strongly connected to clinicians working within the field of neurodegenerative diseases, such as A. Chiò (Turin, ALS), I. Rainero (Turin, AD), Tabaton (Genoa, AD), G. Viero (Trento, SMA), P. Rocca (Turin, Schizophrenia) and T. Mongini (Turin, SMA): we intend to continue and implement this kind of collaborations in order to have a continuous exchange of ideas, data and therapeutic strategies to favor a back and forth flow of information and bidirectional translation to find innovative therapeutic solutions.

### c. General aim and integration with mission of the Institute (up to 1000 characters)

Our research aims to understand some basic mechanisms of neural development, whose alterations may be involved in the onset of neuropsychiatric diseases, and of neuronal cell death in neurodegenerative diseases.

We are also interested in investigating the micro-, meso- and macro-scale of the CNS as the fundamental principles of brain function and disease.

Our findings are finally aimed to develop new therapeutic strategies to prevent neurodegenerative diseases and to brain repair. Therefore, we believe that our research is perfectly fitted to study “the interdependence between physico-chemical state of the human body and the expression of the psyche”, and fully integrated with the mission of the Neuroscience Institute.

#### **d. Specific objectives and strategies (up to 4000 characters):**

*Axonal growth.* A fundamental issue in the building of neural connections and in their conductive properties consists in axon formation and maintenance during brain development, disease and repair. Therefore, we will study, in collaboration with A. Buffo, the relationship between cortical axons and OLs and their precursors, and some molecules which may affect this interaction in the normal brain and in experimental models of disease, such as schizophrenia.

For SCI treatment, we intend to employ nanocarriers, trackable by MRI, attracted by inflammatory sites, and able to deliver in situ molecules/drugs inducing axonal growth/sprouting.

Moreover, miRNAs, a novel class of regulatory molecules, seem to be implicated in several neurological diseases such as neurodegenerative/psychiatric disorders and traumatic CNS injury: based on preliminary results obtained in the last year, and in collaboration with the IIT, we will study miRNA networks in SCI, in order to identify promising therapeutic targets.

*Stem cell therapy.* We will continue previous work on stem cell transplantation in experimental models of neurodegenerative diseases and SCI. On one hand, we will dissect the neuroprotective and immunomodulatory properties of stem cells, in particular of mesenchymal stem cells, as reservoirs to deliver molecules to the diseased/injured CNS (also in combination with biomaterials).

On the other hand, we will aim at cell replacement, using iPSCs in HD, PD and ALS models as paradigms. In collaboration with E. Cattaneo, we also intend to transplant organoids/striatoids into HD rats, hypothesizing that whole-mini-tissue can better survive and integrate into a host environment, compared to standard conditions (dissociated cells). To this aim we are involved, together with A. Buffo, in Italian and European Consortia.

*Molecular mechanisms of cell death.* We will continue to investigate the role of specific genes and molecules in neuronal cell death in neurodegenerative diseases.

In ALS, we have identified in Nurr1 a powerful nuclear receptor able to positively modulate neuroinflammation: we intend now to pharmacologically stimulate the Nurr1 activation in ALS mice, in order to early counteract inflammation. We also intend to evaluate gender-related Nurr1 expression in ALS, in order to clarify its mechanisms of neuroprotection in this pathology.

Moreover, starting from our results concerning autophagy in SMA, we will also study mitophagy, i.e. the selective removal of damaged mitochondria by autophagosomes, to specifically target a key organelle in neuronal death. More generally, we aim to unravel the mitochondria alterations: indeed these organelles could be therapeutically targeted and, based on the obtained results, this approach could be translated to other pathologies. Additionally, to fully understand the functions of SMN, in collaboration with G. Viero (CNR, Trento), we intend to verify the presence of translation defects in our murine model of SMA: by analyzing both transcriptome and translome in delta7 SMA mice, we will evaluate the translation efficiency in different stages of disease. We also intend to correlate such alterations with the mitochondrial abnormalities.

As concerns AD, in 2016 we discovered that A $\beta$ 42 monomers, but not oligomers: i) produce PHF-like conformation of Tau protein, ii) induce two phosphorylated epitopes not present in normal Tau (Ser396 and Ser422) through the activation of GSK3 $\beta$ , JNK and ERK 1/2 kinases. Our preliminary data revealed that A $\beta$ 42 monomers are not able to produce the conformational changes of Tau protein in female mice. We will further explore gender differences on Tau pathology. Thus, the main objective of the project will be to investigate if the different sex-related effect of A $\beta$ 42 monomers on pathological Tau conformational change depends on estrogens decline as well as oxidative stress.

**e. Unique features of the project research (up to 2500 characters):**

Some of our i) research topics, ii) methodologies employed and iii) external collaborations with top institutes, scientists and biotech companies, allow us to be involved in hot topics of research. Our studies on axonal thickness and its plasticity depending on the neuronal pattern of origin/projection, and on activity represent a new field which may have very important significance not only for normal development but also for disease.

Our experience on some molecular pathways related to neuronal death, such as JNK and those related to autophagy is a specific competence which allowed us to design new therapeutic drugs.

The emergence of new concepts in brain function and disease in terms of networks and damage networks may be fundamental for investigating the onset of disease and eventually prevent its full development. Also, our group is one of the major groups working with stem cell therapy at a preclinical level in Italy and Europe.

**f. Methodology (up to 2000 characters): please fill-out this section only in the case of innovative technologies**

We are collaborating with IIT (Italian Institute of Technology, D. De Pietri Tonelli) in the study of miRNA networks, and with T. Dalmay (University of East Anglia) to develop new techniques to exploit miRNA analysis from small samples of cells.

In collaboration with groups of the Polytechnic of Turin, we intend to develop three-dimensional cellularized constructs by bioprinting technique for SCI treatment: with this innovative approach, we will encapsulate stem cells in new 3D materials “printed” to recreate the longitudinal course of the nerve fibers of the spinal cord, and improve their ability to fill the lesion gap.

We also plan to exploit genome editing technology (Crispr/Cas9 system) to potentially treat autosomal dominant disorders (as fALS or SCA), in order to improve the disease prognosis.

Finally, to deeply analyze the mitochondrial alterations in SMA, we will exploit i) the 2PM microscope available at NICO to in vivo study the mitochondrial trafficking; ii) the multiSEM (3D electron microscope, available at the POLITO, c.o. prof. Pirri) to evaluate the ultrastructural morphology, structure and distribution of SMA mitochondria. Moreover we intend to use/implement a protocol developed by Prof. Pinton’s group (Univ. Ferrara) to isolate mitochondrial fractions from different tissues (spinal cord, brain, heart, muscles, spleen) and then perform proteomic analysis.



20/12

**Modulation of mGlu5 receptors to rescue neuronal dysfunctions underlying Phelan - McDermid Syndrome**

**Chiara Verpelli** - CNR Istituto di Neuroscienze, Milano

15/12

**Neuroanatomical basis of brain energy metabolism in the mammalian brain.**

**Corrado Cali, PhD** - King Abdullah University of Science and Technology, Kingdom of Saudi Arabia

30/11

**Why White Matter Matters: Keeping the Brain Wired**

**Arthur Butt** - Institute of Biomedical and Biomolecular Sciences School of Pharmacy and Biomedical Science University of Portsmouth

9/11

**When scientific space missions meet biology**

**Sabrina Corpino** - Department of Mechanical and Aerospace Engineering, Politecnico di Torino  
Space for Science: challenges and opportunities

27/10 – con la Scuola di Dottorato in Scienze della Vita e della Salute

Dottorato in Neuroscienze, Università di Torino

**Higher diversity of primate cortical precursors revealed by unsupervised lineage-based classification**

**Marion Betizeau** - Brain Research Institute, University of Zurich - Switzerland

20/10

**Vortioxetine exerts anti-inflammatory and immunomodulatory effects on human monocytes/macrophages**

**Maria Talmon, PhD** - Lab of pharmacology, Department of Health Sciences - School of Medicine  
University of Piemonte Orientale – Novara

6/10

**On the unexpected role of SMN protein in controlling translation: implications for Spinal Muscular Atrophy**

**Gabriella Viero** - Institute of Biophysics, CNR Unit, Trento

22/9

**Novel models of stretch-induced injury in mouse oligodendrocytes and organotypic culture of cerebellar slices: study of pathophysiological mechanisms**

**Elena Chierto, PhD** - Paris Descartes University



12/9

**Structure-function relations of the hippocampal formation**

**Irmgard Amrein** - Institute of Anatomy, University of Zurich (UZH/ETH Zurich, Switzerland)

8/9

**Tumor immunoescape during glioma progression**

**Dr. Irene Appolloni** - Laboratory of Gene Transfer - IRCCS AOU S. Martino-IST, Genova (Italy)

26/5

**Neuronal signalling pathway responsiveness to temporally-encoded inputs - new insights and new tools**

**Michael Courtney** - Turku Centre for Biotechnology, University of Turku and Åbo Academy University, Finland

11/5 con il NIT

**Gene Regulation of Functional Brain Networks**

**Michele Caselle** - Università di Torino, Dipartimento di Fisica e INFN

16/3

**How stem cells signal to host immune**

**Stefano Pluchino, MD, PhD** - Dept of Clinical Neurosciences, Wellcome Trust-Medical Research Council Stem Cell Institute University of Cambridge

16/3

**Meta-analysis of microRNAs dysregulated in the hippocampal dentate gyrus of animal models of epilepsy**

**Michele Simonato, MD, PhD** - University of Ferrara – Department of Medical Sciences – Section of Pharmacology – Ferrara | Sant'Anna Hospital – Clinical Pharmacology Unit – Ferrara | University Vita-Salute San Raffaele - Division of Neuroscience – Milano

gennaio - dicembre 2017

## REPORT COMUNICAZIONE | UFFICIO STAMPA | EVENTI

Responsabile: dr.ssa Barbara Magnani

### SITO WEB

[www.nico.ottolenghi.unito.it](http://www.nico.ottolenghi.unito.it)

Il sito è costantemente aggiornato con eventi (seminari, convegni, ecc.), news e pubblicazioni. Particolarmente significativi gli aggiornamenti della **sezione RICERCA**, suddivisa in:

- **NEWS RICERCA**: è la vetrina delle pubblicazioni scientifiche, selezionate e adattate con un linguaggio divulgativo. Include inoltre news sulla partecipazione dei ricercatori del NICO a convegni scientifici e (dove i temi sono collegati a quelli di ricerca) commenti su leggi e regolamenti.
- **GRUPPI DI RICERCA** comprende le sottosezioni dedicate ai 9 gruppi di ricerca
- **INVECCHIARE IN SALUTE** presenta i progetti dedicati all'invecchiamento.

Aumentano i contatti registrati dalla **newsletter NICO**, il periodico aggiornamento su eventi e ricerche, che conta **700 iscritti** (con un aumento di circa 100 in un anno).

Nel maggio 2017 è stata attivata la possibilità di effettuare direttamente dal sito **donazioni tramite PayPal**, dalle pagine dei progetti, dei gruppi di ricerca e nella sezione **SOSTIENI IL NICO**.

---

### PAGINA FACEBOOK

[www.facebook.com/NeuroscienceInstituteCavalieriOttolenghi](http://www.facebook.com/NeuroscienceInstituteCavalieriOttolenghi)

La pagina NICO ha **raggiunto i 2271 followers** (crescendo di oltre 1000 in un anno).

I post hanno una media di 1500 - 2000 visualizzazioni, con picchi che superano le 3-4000.

La pagina è una vetrina degli eventi e soprattutto delle news di ricerca dell'Istituto (con rimando alle pagine dedicate del sito).

---

### UFFICIO STAMPA

#### COMUNICATI STAMPA

[http://www.nico.ottolenghi.unito.it/ita/Press/Comunicati-stampa/\(year\)/2017](http://www.nico.ottolenghi.unito.it/ita/Press/Comunicati-stampa/(year)/2017)

I comunicati stampa (16 in totale nel 2017) sono tutti disponibili nella sezione dedicata del sito.

I comunicati dedicati alle pubblicazioni scientifiche sono spesso ripresi anche da **LeScienze.it**

2/3/2017 - **ATTRAZIONE SESSUALE? QUESTIONE DI BIOLOGIA** [ [link](#) ]

10/4/2017 - **CAPIRE LA PLASTICITA' CEREBRALE STUDIANDO IL DELFINO** [ [link](#) ]

12/10/2017 - **UN NUOVO INTERRUTTORE PER METTERE IN MOTO LA PLASTICITÀ CEREBRALE** [ [link](#) ]

21/12/2017 - **MODULARE LA 'PULIZIA' CELLULARE: UNA POSSIBILE CURA PER LA SMA** [ [link](#) ]

## RASSEGNA STAMPA

[www.nico.ottolenghi.unito.it/ita/Press/Rassegna-stampa](http://www.nico.ottolenghi.unito.it/ita/Press/Rassegna-stampa)

Anche la rassegna stampa/web è continuamente aggiornata e disponibile nella sezione dedicata del sito. Nel **2017** conta **oltre 70 articoli** (su testate nazionali e locali) dedicati a ricerche ed eventi del NICO, tra cui **3 servizi del TG scientifico Leonardo di RAI 3**.

## CONFERENZE STAMPA – EVENTI DI PRESENTAZIONE

21 marzo 2017 - Molecular Biotechnology Centre

**Open Access Lab** [ [link](#) ]

Presentazione del progetto di Microscopia Avanzata dell'Università di Torino

---

## COMUNICATI STAMPA - 2017

21 dicembre 2017

### **MODULARE LA “PULIZIA” CELLULARE: UNA POSSIBILE CURA PER LA SMA**

Su CELL DEATH AND DISEASE (gruppo Nature) - Un team di ricerca internazionale guidato dal prof. Alessandro Vercelli, direttore del NICO di Orbassano (TO) - Università di Torino, ha individuato nell'autofagia uno dei meccanismi responsabili della neurodegenerazione nell'atrofia muscolare spinale.

9 dicembre 2017

### **METTI BENZINA ALLA RICERCA: IL NICO-UNIVERSITÀ DI TORINO TORNA IN PISTA**

2° Rally Day Il Grappolo a San Damiano d'Asti il 9 e 10 dicembre. Il dottor Lorenzo Fabiani - Promosport e Tecnica Bertino – corre di nuovo con i colori del NICO – Istituto di Neuroscienze dell'Università di Torino.

28 novembre 2017

### **LA SCIENZA UNISCE LE CULTURE**

Nasce The Science Bridge: oltre 200 scienziati di tutto il mondo insieme per sviluppare nuove terapie e superare le barriere culturali tra Occidente e Medio Oriente.

12 ottobre 2017

### **UN NUOVO INTERRUETTORE PER METTERE IN MOTO LA PLASTICITÀ CEREBRALE**

Identificato un nuovo recettore della proteina NoGo-A, noto regolatore della plasticità nervosa. La ricerca - pubblicata sulla rivista Developmental Cell - ha la firma del Brain Research Institute di Zurigo, in collaborazione con il team della prof.ssa Annalisa Buffo, NICO di Orbassano (TO) e Dipartimento di Neuroscienze dell'Università di Torino.

8 luglio 2017

### **Metti benzina alla ricerca. Il NICO-Università di Torino alla Cesana-Sestriere 2017**

Corre anche il NICO – Istituto di Neuroscienze dell'Università di Torino sulle strade della rinomata competizione per auto storiche con l'auto del dottor Lorenzo Fabiani - Promosport di Torino e Tecnica Bertino.

8 maggio 2017

### **PorteAperte@NICO - CAPIRE IL CERVELLO PER CURARLO**

Sabato 13 maggio, per Le Settimane della Scienza, i ricercatori del NICO - Università di Torino invitano a visitare i laboratori di Orbassano (TO).

7 maggio 2017

### **OLIMPIADI DELLE NEUROSCIENZE: PIEMONTE AL PRIMO POSTO CON UNA STUDENTESSA DI ALBA**

La campionessa nazionale Bianca Slivinski del Liceo Govone di Alba vola a Washington per la finale internazionale di agosto.

10 aprile 2017

### **CAPIRE LA PLASTICITÀ CEREBRALE STUDIANDO IL DELFINO**

La neurogenesi è legata all'esistenza della funzione olfattiva: lo hanno dimostrato i nostri ricercatori con un lavoro di 4 anni sui delfini, che non hanno olfatto.

21 marzo 2017

### **UN LABORATORIO CONDIVISO DI MICROSCOPIA AVANZATA PER VINCERE LA SFIDA DELLA COMPETITIVITÀ**

L'Università di Torino presenta OPEN ACCESS LAB: tre apparecchiature di microscopia confocale installate all'MBC di Torino e al NICO di Orbassano condivise dai ricercatori di sei dipartimenti

18 marzo 2017

### **OLIMPIADI DELLE NEUROSCIENZE: SUL PODIO 3 STUDENTI DI ALBA E NOVARA**

150 studenti in gara a Torino per la finale regionale. Con oltre 600 studenti di 31 scuole in gara il Piemonte è la regione con più adesioni.

16 marzo 2017

### **17 marzo Unistem Day 2017**

Nona edizione della giornata europea dedicata alla divulgazione sulle cellule staminali

15 marzo 2017

### **Il NICO-Università di Torino alla Convention Telethon 13-15 marzo, Riva del Garda**

I gruppi di ricerca del prof. Di Cunto e del Prof. Tempia del NICO-Università di Torino studiano le cause genetiche di microcefalie e atassie: gli ultimi studi, realizzati grazie al sostegno di Fondazione Telethon e presentati in occasione della convention, aprono la strada a possibili nuovi approcci terapeutici.

10 marzo 2017

### **ATASSIA: TRAINING MOTORIO PREVENTIVO RALLENTA LA DEGENERAZIONE NEURONALE**

Lo studio delle ricercatrici del NICO - Università di Torino, pubblicato su Neurobiology of Disease, apre nuove prospettive per contrastare la morte neuronale nelle patologie neurodegenerative.

2 marzo 2017

### **L'ATTRAZIONE SESSUALE? QUESTIONE DI BIOLOGIA**

Lo studio dei ricercatori del NICO - Università di Torino chiarisce un aspetto della complessa interazione tra geni e ambiente che, modellando i circuiti nervosi, regola il comportamento sessuale.

19 febbraio 2017

### **SCLEROSI MULTIPLA: il CRESM del San Luigi vara un calendario di incontri tra medici e pazienti - Primo incontro giovedì 23 febbraio 2017 alle 15:00**

16 febbraio 2017

**OLIMPIADI DELLE NEUROSCIENZE 2017: IN PIEMONTE 600 STUDENTI DI 31 SCUOLE IN GARA DA TUTTE LE PROVINCE** - In tutta Italia partecipano 5mila studenti di oltre 200 scuole

---

### [Estratto della rassegna stampa/web/TV](#)

13 ottobre 2017 - Luna Nuova

**Ecco la proteina ripara-cervello** [ [pdf](#) ]

12 ottobre 2017 - LaStampa.it

Scoperto un nuovo interruttore della proteina che regola la capacità del cervello di ripararsi  
Ricerca del Brain Research Institute di Zurigo con il Neuroscience Institute Cavalieri Ottolenghi di Orbassano e Dipartimento di Neuroscienze dell'Università di Torino. [ [link](#) ]

12 ottobre 2017 - IlGiornale.it

**Scoperto l'interruttore della plasticità cerebrale**

Gli scienziati hanno identificato un nuovo recettore della proteina NoGo-A, regolatore della plasticità del sistema nervoso centrale, cioè la capacità del cervello di adattarsi e ripararsi in seguito a un danno nervoso [ [link](#) ]

8 luglio 2017 - Ansa Piemonte

**Università Torino corre Cesana-Sestriere**

Obiettivo, sensibilizzare e raccogliere fondi per la ricerca [ [link](#) ]

21 maggio 2017 - LaStampa.it

**Al Salone cercasi volontari che vogliono invecchiare in salute**

Il progetto dell'Università di Torino My-AHA sta reclutando volontari al Salone del Libro: si cercano persone dai 55 anni in su che vogliono aderire all'iniziativa a favore dell'invecchiamento sano e attivo. [ [link al video](#) ]

16 maggio 2017 - TGR Leonardo - RAI 3

**Interferenti e distruttori endocrini: che cosa sono e perché fanno male**

800 sostanze presenti in plastiche, vernici e additivi che inquinano l'ambiente e alterano le funzioni regolate dagli ormoni. Sono gli interferenti endocrini, al momento oggetto di discussione di una normativa europea che dovrebbe ridurre l'utilizzo [ [link al video](#) ]

21 aprile 2017 - Il Venerdì - La Repubblica

**Gli ottocento veleni nascosti dove meno te l'aspetti** [ [link all'articolo](#) ]

17 aprile 2017 - RAI 3 - TGR Leonardo

**Studiare il cervello del delfino per capire la plasticità cerebrale nell'uomo**

[ servizio al minuto 12:00 circa - [link al video](#) ]

10 aprile 2017 - Galileonet.it - Giornale di scienza

**La plasticità cerebrale spiegata dai delfini**

La neurogenesi nel bulbo olfattivo è legata all'esistenza della funzione olfattiva: lo hanno dimostrato i ricercatori del Nico di Torino con un lavoro di 4 anni sui delfini, che non hanno olfatto. [ [link all'articolo](#) ]

10 aprile 2017 - LaStampa.it - Torino

**“La capacità del cervello di generare nuovi neuroni è legata all'olfatto”**

Uno studio dell'Istituto di Neuroscienze dell'Università di Torino analizza la reciprocità tra i delfini, che hanno perso questo senso, e l'uomo, per il quale è diventato meno importante [ [link all'articolo](#) ]

10 aprile 2017 - Ansa.it - Piemonte

Da delfini segreto per riparare cervello  
Esaminate 12mila sezioni di cervello in 10 delfini [ [link all'articolo](#) ]

10 aprile 2017 - Ansa.it - Science & Technology  
Dolphins show how to repair human brains  
Neuron production linked to smell, Turin university team says [ [link all'articolo](#) ]

29 marzo 2017 - TuttoScienze - La Stampa  
"Sport e pensiero: le regole per la mente che sfida il tempo"  
Allenare i neuroni è possibile, ma si deve tenere lontano lo "stress cattivo". Intervista a Luca Bonfanti in occasione di Abitare il Cervello, evento in programma il 30 e 31 marzo al Campus Einaudi dell'Università di Torino [ [pdf](#) ]

24 marzo 2017 - laRepubblica.it - Scienze  
Etero o omosessuale, l'attrazione è questione di biologia  
Uno studio dell'Università di Torino svela un complesso meccanismo con cui geni e ambiente regolano il comportamento sessuale nei topi. E conferma come l'attrazione sessuale sia un fenomeno fondamentalmente biologico, indipendentemente dal sesso da cui si è attratti. [ [link all'articolo](#) ]

20 marzo 2017 - LaStampa.it - Novara  
L'Antonelli di Novara torna sul podio delle Neuroscienze: Alessia Mittino alla finale nazionale  
Il liceo conferma la grande preparazione e torna sul podio della sfida regionale [ [link all'articolo](#) ]

10 marzo 2017 - TGcom24.it  
L'attrazione sessuale? E' tutta questione di cervello  
Romanticismo addio: uno studio dimostra la complessa relazione biologica tra circuiti nervosi e sesso [ [link all'articolo](#) ]

9 marzo 2017 - LaStampa.it - Scienza e Benessere  
L'attrazione sessuale? Un semplice meccanismo biologico  
Chiarito un aspetto della complessa interazione tra geni e ambiente che regola il comportamento sessuale dei mammiferi [ [link all'articolo](#) ]

8 marzo 2017 - TGR Leonardo RAI  
Festa della Donna: la parola alle ricercatrici  
Intervista a Gaia Berto, ricercatrice del NICO - Università di Torino (al min. 03:00 circa) [ [video](#) ]

---

## CONFERENZE ED EVENTI DIVULGATIVI 2017

L'archivio di tutti gli eventi di tipo divulgativo (qui si citano solo i principali) è disponibile sul sito web nelle sezioni dedicate: [PorteAperte@NICO](#), [Notte dei Ricercatori](#) e [News](#)

### Settimana del Cervello 2017 | 13 > 17 marzo | L'IMPOSSIBILITÀ DI ESSERE NORMALI

Organizzato da CentroScienza Onlus in collaborazione anche per questa edizione con il prof. Vercelli per la definizione del programma

13 marzo | Circolo dei Lettori

#### LO SVILUPPO DEL CERVELLO: LA COMPLESSITÀ DELLA NORMALITÀ

Benedetto Vitiello - Clinica neuropsichiatrica infantile dell'Università di Torino

Ferdinando Di Cunto - Neuroscience Institute Cavalieri Ottolenghi, Università di Torino

### sabato 13 maggio | PorteAperte@NICO con Le Settimane della Scienza 2017

Anche in questa occasione evento sold out con **oltre 130 prenotazioni/presenze**.

Con l'obiettivo di migliorare l'esperienza dei visitatori e rendere più chiara la presentazione delle linee di ricerca, il percorso di visita è stato riorganizzato con:

- presentazione dell'Istituto a cura del prof. Vercelli
- presentazione delle linee di ricerca, suddivisa in 3 stand tematici
- visita al laboratorio di Neurofisiologia *COME COMUNICANO I NEURONI*
- visita dei laboratori: alcune ricercatrici, al lavoro al confocale, mostrano dal vivo i diversi approcci sperimentali.

### venerdì 29 settembre | Notte dei ricercatori

lo stand NICO intitolato "**Metti in gioco i tuoi neuroni**" è stato organizzato con diverse attività:

- gioco a quiz **METTI ALLA PROVA I TUOI NEURONI** (in palio una giornata al NICO)
- il **QUIZZETTONE: Sclerosi Multipla**, se ne parla tanto, ma la conosci davvero?
- **LE NOSTRE RICERCHE AL MICROSCOPIO**: partendo dall'analisi dei vetrini, i ricercatori spiegano come lavora il cervello, che cosa succede quando non funziona e le possibili strategie di riparazione
- **LE NOSTRE RICERCHE IN POCHE PAROLE**: i ricercatori si sono alternati nel presentare le linee di ricerca dell'Istituto
- **INVECCHIAMENTO**: un angolo (con poster) dedicato ai progetti sull'invecchiamento

La Notte dei ricercatori è un'ottima occasione per raccogliere iscrizioni alla newsletter (con il modulo **RESTIAMO IN CONTATTO**) e invitare i visitatori al PorteAperte in programma a novembre.

### 8 e 9 luglio | 36° Cesana – Sestriere | METTI BENZINA ALLA RICERCA

"Metti benzina alla ricerca". Con questo slogan il dottor Lorenzo Fabiani - Associazione Promosport

e Tecnica Bertino - ha corso la Cesana-Sestriere, rinomata competizione per macchine d'epoca, portando i colori del NICO. Una piccola delegazione del NICO, presente alla partenza della gara a Cesana, ha distribuito le cartoline dell'Istituto sensibilizzando il pubblico sull'importanza di sostenere la ricerca.

### sabato 18 novembre | PorteAperte@NICO

Un altro appuntamento che ha registrato il tutto esaurito con **oltre 130 ospiti** (prenotati).

Anche in questa occasione il percorso di visita è stato organizzato con:

- presentazione dell'Istituto a cura del prof. Bonfanti
- presentazione delle linee di ricerca, suddivisa in 3 stand tematici
- visita al laboratorio di Neurofisiologia *COME COMUNICANO I NEURONI*

- visita dei laboratori: alcune ricercatrici, al lavoro al confocale, mostrano dal vivo i diversi approcci sperimentali.

### **GiovedìScienza | vendita biglietti lotteria Girotondo Onlus**

In occasione di alcune conferenze del ciclo GiovedìScienza 2017 i ricercatori del NICO hanno promosso la vendita dei biglietti della lotteria benefica di Girotondo Onlus, l'associazione che sostiene la ricerca sull'atrofia muscolare spinale (anche del NICO, con una borsa di ricerca annuale).

---

## **SCUOLE**

L'archivio di tutti gli eventi e delle attività dedicate agli studenti è disponibile sul sito web nella sezione [Scuole](#): [Olimpiadi delle Neuroscienze](#), [UnistemDay](#) e [Alternanza Scuola-lavoro](#).

### **Olimpiadi delle Neuroscienze 2017 VIII edizione | Piemonte**

Organizzazione delle selezioni regionali (coordinatrice regionale dottoressa Marina Boido). **Record di partecipazione:** con **oltre 600 studenti di 31 scuole** da tutte le province in gara, il Piemonte è la regione con più adesioni ed è nuovamente campione d'Italia (dopo il 2014 e 2015) con una studentessa di Alba (CN).

### **17 marzo | UniStemDay 2017 | Università Torino, Aula Magna della Cavallerizza**

[Il lungo e affascinante viaggio della ricerca sulle cellule staminali](#)

Oltre **400 studenti da tutto il Piemonte** per l'evento coordinato da Luca Bonfanti e organizzato dal NICO in collaborazione con Agorà Scienza (con il sostegno di SMAT e Centrale del Latte di Torino).