



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

*Annual Report 2018*

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# NICO 2018 by the numbers



**9**

Research Groups  
65 Scientists  
44 Graduating Students



**76**

Peer-Reviewed  
Publications



**62**

Collaborative Initiatives  
with  
International  
Research Groups



**60**

On-going/Granted  
Research Projects



**8**

Scientific  
Conferences/workshops  
organized  
by NICO members



**14**

Invited speakers



**1**

Spin-off Company



**1**

Biobank



**91**

Outreach Activities  
46 Invited Talks  
45 Science Dissemination Initiatives



**14**

trained  
PhD students



**2652**

Facebook Followers

## **1. OVERVIEW OF THE INSTITUTE**

### **1.1 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 a call of interest, 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).

### **1.2 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 Scuola Lavoro). 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.

### **1.3 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. 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 can be accessed 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.

#### **1.4 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 currently supervise 10 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 28 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, 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.

#### **1.5 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 2019 edition will be organized by the Ottolenghi Foundation as well. Since 2018, NICO gives its patronage to BraYn (Brainstorming Research Assembly for Young Neuroscientists), an international meeting devoted to under-40 scientists that is held every year (Dr. Enrica Boda is member of the organizing committee). The first edition of the meeting (2018) had 330 participants and more than 500 presented abstracts. 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. An agreement of collaboration with the IEMEST (Istituto EuroMediterraneo di Scienza e Tecnologia) in Palermo is under preparation. An agreement of collaboration is under preparation as well with the CNR of Naples, starting from the collaboration in a Telethon grant between E. Di Schiavi and A. Vercelli. Also, a collaboration with the Department of Neurology of the University of Texas Medical Branch has been established, which includes exchange of researchers.

### **1.6 THE NICO SPINOFF**

Some NICO researchers (prof. Eva, Panzica, Buffo, Boido and Tamagno) collaborated in preparing the application for an academic spinoff (S&P Brain) of the University of Torino, to offer proof of efficacy of new drugs or pilot in vivo studies to researchers, institution and companies. The spinoff was approved by the technology transfer committee of the University of Torino and by the Academic Senate and Council of Advisors of the University and constituted in 2016. S&P Brain will allow to provide an income to the NICO, and also to participate in cooperative grant applications as a company.

## **2. ORGANISATION AND ACTIVITIES OF THE NEUROSCIENCE INSTITUTE CAVALIERI OTTOLENGHI**

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

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

**Scientific Director of the Foundation Cavalieri Ottolenghi** is prof. Alessandro Vercelli (appointed March 2014, renewed for further three years from June 2018). In addition to the scientific direction he performs also the function of Administrative Director. From November 2018, prof. Annalisa Buffo was appointed vice-Director for the activities at the NICO.

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)

Physiopathology of stem cells (PI Annalisa Buffo)

Neuroendocrinology (PI Giancarlo Panzica)

Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)

Neuropsychopharmacology (PI Carola Eva)

## 2.1 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 Dyrmishi).

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:** 7 full professors, 6 associate professors, 11 university research assistants, 14 post-docs and 10 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.

## 2.2 Labs and Equipment

Several members of the NICO are part of the Department of Neuroscience of the University of Torino, which in 2018 was included in the list of the Departments of Excellence by the Ministry of University and Research. Since in the project which was approved by the Ministry there was a consistent part of acquisition of new instrumentation, a series of upgrades and new microscopes is under analysis. In particular, light-sheet microscopy, automated scanning confocal microscopes for 3D reconstructions are included. Also, new -80° freezers will be acquired.

### 2.2.1 *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.

### 2.2.2 *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 and in utero electroporation. 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 BSL2 procedures are available for viral based studies in vivo.

### 2.2.3 *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, an electroporator for bacteria or ES, as well as many other instruments as a standard laboratory for extraction and analysis of DNA, RNA and proteins.

#### **2.2.4 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.

#### **2.2.5 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).

#### **2.2.6 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.

### **3. 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** - 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).

Also, NICO is participating to the Festival dell'Innovazione and to the activities of the UNITRE (University of the Third Age) of Rivoli.

### **3.1 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.

For invited speakers, see the attached list.

### **3.2 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.



#### **4. OTHER ACTIVITIES OF THE CAVALIERI OTTOLENGHI FOUNDATION IN TORINO: THE CENTER FOR BRAIN IMAGING OF NIT**

In addition to support the activities of the NICO by maintaining the infrastructure and giving room and administrative support to researchers, the Foundation promoted and played a fundamental role in the creation of the Center for Brain Imaging of the University of Torino, in the Interdepartmental Center for Neuroscience (Neuroscience Institute of Turin, NIT).

In fact, it has in the past cofinanced, together the Piedmont Region, the acquisition of the NMR 3T Philips Ingenia, which was donated to the University of Torino, Interdepartmental Center of Torino and located at the “Città della Salute e della Scienza” Hospital. Thanks to this support, the Center for Brain Imaging was created. From its creation, 21 studies (one in Basic Neuroscience, 10 Cognitive Neuroscience, 5 Clinical Neuroscience), lead by 13 different principal investigators were performed in the Center. From these studies, 16 papers were published or are in preparation for publication on international peer reviewed journals. A total of 624 subjects and patients were analysed in these studies.

In 2018, the Foundation also funded a one-year position for a research technician in support of the researchers using the NMR of this Centre. This position was recently extended for another year. Finally, the Foundation launched two calls to support six projects in Brain Imaging.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2018**

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 are 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 and Biostatistician 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 related 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 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

### Relevant discoveries:

H index, 46; Citations 6036

### 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
2014-2018	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	*
2018-2019	National	PI	Novartis	Effects of siponimod on Nuclear Receptor subfamily (NR4As) in human blood and brain derived cells	pending	60.000	

\*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:

PhD: Dr Francesca Gilli

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

**Please list honours, prizes or awards received**

2003: member of “Therapeutics and Technology Assessment subcommittee of the American Academy of Neurology” for anti-IFN $\beta$  antibodies  
Since 2004 Coordinatore della Commissione Regionale SM dell’Assessorato alla Sanità  
2009: member of Accademia di Medicina di Torino  
2012: idoneità a Professore Universitario di Prima Fascia, Settore Neurologia  
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, Workshops, Schools or Conferences organized**

26/02 SDA Bocconi, Milano, "MS Expert Meeting: Ruolo delle cure intermedie e dei modelli di transizione per la Sclerosi Multipla"  
2/03 Torino, "Punti Cardinali e nuove rotte SM Linee Guida e update 2018"  
17/03 Orbassano, AOU S. Luigi Gonzaga, Convegno "Disposizioni anticipate di trattamento"  
20/03 Padova, Lettura nell'ambito dei seminari "Martedì della clinica neurologica"  
23/03 Gallarate, "Corso XXI approfondimenti monotematici sulla SM: l'impatto delle nuove terapie"  
27/03 Torino, Corso Formativo "Progetto Improve 2.0"  
10/04 Milano, Advisory Board Biogen "Induction versus escalation Therapy"  
18/04 Torino, "La terapia orale nel paziente SM: dalla ricerca alla pratica clinica"  
23-27/04 AAN Los Angeles  
10-11/05 BEMS  
16/05 Moncrivello, "SM, dalla fisiopatologia alla riabilitazione"  
18/05 "Convegno Liguria SM SIN", Lerici  
24/05 Torino, "Spider"  
28-30/05 FISM Roma  
7-8/06 Roma, Stand Alone Aubagio  
11-13/06 Scuola di SM, Orbassano  
21-22/06 Bari, "Brain your time"  
5/07 Advisory Board Genzyme Milano  
21/09 Torino, "La gestione multidisciplinare del paziente con SM ad elevata attività di malattia"  
24-26/09 Stage Novartis, Corso teorico-pratico di aggiornamento in SM, Orbassano  
27-28/09 Stand Alone Alemtuzumab, Milano  
4-5/10 "Highlights on new therapeutic options for the MS from clinical to pharmacoeconomics aspects"  
18/10 EPICA Firenze  
19/10 Torino Cognitività Malattie Neurologiche  
27-30/10 SIN, Roma

- **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



## 2.PI's PUBLICATIONS:

Scarpazza C, Signori A, Prosperini L, Sormani MP, Cosottini M, Capra R, Gerevini S. Italian PML Group. Early diagnosis of progressive multifocal leucoencephalopathy: longitudinal lesion evolution. 2018 ; J Neurol Neurosurg Psychiatry. 2018 Nov 2. IF 7.14 R=36/336

Prosperini L, Annovazzi P, Boffa L, Buscarinu MC, Gallo A, Matta M, Moiola L, Musu L, Perini P, Avolio C, Barcella V, Bianco A, Farina D, Ferraro E, Pontecorvo S, Granella F, Grimaldi LME, Laroni A, Lus G, Patti F, Pucci E, Pasca M, Sarchielli P; Italian Alemtuzumab Study Group. No evidence of disease activity (NEDA-3) and disability improvement after alemtuzumab treatment for multiple sclerosis: a 36-month real-world study. J Neurol. 2018 Sep 26. IF=3.78

Marcinnò A, Marnetto F, Valentino P, Martire S, Balbo A, Drago A, Leto M, Capobianco M, Panzica G, Bertolotto A. Rituximab-induced hypogammaglobulinemia in patients with neuromyelitis optica spectrum disorders. Neurol Neuroimmunol Neuroinflamm. 2018 Sep 13;5(6) IF= 6.429; R= 36/336

Bo M, Charrier L, Bartalini S, Benvenuti M, Oggero A, Ulivelli M, Gabbrielli M, Bertolotto A, Gianino MM. Access to social security benefits among multiple sclerosis patients in Italy: A cross-sectional study Mult Scler Relat Disord. 2018 Aug; 24:107-112. IF=4.84, R=clinical neurology 91/336

Mallucci G, Annovazzi P, Mianze S, Torri-Clerici V, Matta M, La Gioia S, Cavarretta R, Mantero V, Costantini G, D'Ambrosio V, Zaffaroni M, Ghezzi A, Perini P, Rossi S, Bertolotto A, Rottoli MR, Rovaris M, Balgera R, Cavalla P, Montomoli C, Bergamaschi R. Two-year real-life efficacy, tolerability and safety of dimethyl fumarate in an Italian multicentre study. J Neurol. 2018 Aug; 265(8):1850-1859. IF= 3.78

Rosato R, Testa S, Bertolotto A, Scavelli F, Giovannetti AM, Confalonieri P, Patti F, Chisari CG, Lugaesi A, Pietrolongo E, Grasso MG, Rossi I, Toscano A, Loera B, Giordano A, Solari A. eMSQOL-29: Prospective validation of the abbreviated, electronic version of MSQOL-54. Mult Scler. 2018 May IF= 5.28 R=69/336

Perga S, Martire S, Montarolo F, Giordani I, Spadaro M, Bono G, Corvisieri S, Messuti I, Panzica G, Orlandi F, Bertolotto A. The Footprints of Poly-Autoimmunity: Evidence for Common Biological Factors Involved in Multiple Sclerosis and Hashimoto's Thyroiditis. Front Immunol. 2018 Feb 20; 9:311. IF= 6.429; R= 24/194

Carletto S, Borghi M, Scavelli F, Francone D, Perucchini ML, Cavallo M, Pagnini F, Bertolotto A, Oliva F, Ostacoli L. Prevalence of Posttraumatic Stress Disorder in Patients With Multiple Sclerosis. J Nerv Ment Dis. 2018 Feb;206(2):149-151. IF= 1.94 R= 167/487

Long-term follow-up of pediatric MS patients starting treatment with injectable first-line agents: A Baroncini D, Zaffaroni M, Moiola L, Loreface L, Fenu G, Iaffaldano P, Simone M, Fanelli F, Patti F, D'Amico E, Capobianco M, Bertolotto A, Gallo P, Margoni M, Mianze S, Milani N, Amato MP, Righini I, Bellantonio P, Scandellari C, Costantino G, Scarpini E, Bergamaschi R, Mallucci G, Comi G, Ghezzi A; iMED Registry and Gruppo di Studio SM-Società Italiana di Neurologia. multicentre, Italian, retrospective, observational study. Mult Scler. 2018 Jan 1 IF=5.28 R=69/336

Correction to: Management of pregnancy-related issues in multiple sclerosis patients: the need for an interdisciplinary approach. 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. Neurol Sci. 2018 Feb;39(2):395 IF=1.74; R=clinical neurology 169/336

#### 4.GROUP's PUBLICATIONS:

[Symptomatic cerebrospinal fluid HIV-1 escape with no resistance-associated mutations following low-level plasma viremia.](#) Trunfio M, Joseph SB, Ghisetti V, Romito A, Martini C, Cusato J, Audagnotto S, Pesci Kincer L, Ferrara M, Sala A, Imperiale D, Di Perri G, Calcagno A. J Neurovirol. 2018 Feb;24(1):132-136 1 IF=3.22 R= 58/336

#### 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%
2014-2018	National	Perga S.	FISM	"Ruolo della deubiquitinasi TNFAIP3/TNFAIP3 nell'immuno-patologia della SM".	FISM code 2014/R/14	100.000	5%#
2018-2019	National	Spadaro M.	FISM	Pregnancy: a powerful transient immunosuppressive phenomenon in Multiple Sclerosis women	FISM code 2017/R/26	30.000	-

#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. Martire:

Travel grant for the XXVII AINI Congress, Trieste 2018

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

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 (06/2018).

Dr. Paola Valentino:

Official speaker for MS course: "Corso teorico-pratico di aggiornamento in sclerosi Multipla" (09/2018)

## 6. Past Research activity

### 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. Till now in 2018, 238 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 (710 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 2018, 444 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 (540 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 2018, 40 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-kB 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 normal appearing areas from white (WM) and grey matter (GM), in lesions in WM classified as active (AL) and chronic active lesions (CAL) and in 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 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.
3. To evaluate a possible effect of Fingolimod (FTY720), a disease modifying treatment for MS, and of AUY954, a next-generation S1P receptor modulator, on the NR4A2 pathway in human blood cells.

#### **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 by immunohistochemistry that TNFAIP3 is present in control human brain tissues in both WM, in few parenchymal astrocytes and in GM, in neuronal cells and rare astrocytes. In MS brain, TNFAIP3 showed a similar distribution in both NAWM and NAGM. Also we highlighted a massive TNFAIP3 expression in the all WM lesions in both perivascular infiltrates and ramified cells. In particular, in AL and pre-AL, TNFAIP3 is expressed in the active core of the lesions, whereas in CAL TNFAIP3 is mainly expressed on the active-margin. Double immunofluorescence staining unveiled that TNFAIP3 is expressed by infiltrating macrophages and by resident astrocytes and a subpopulation of microglial cells. In addition we showed an over expression of TNFAIP3 in the majority of the active lesions in GM, in particular in astrocytes. These data have been confirmed by qRT-PCR.

In order to characterize the expression of NR4A2 in human brain, qRT-PCR analysis has been performed in post-mortem brain tissues obtained from CC and MS patients. We observed a modest down-regulation of NR4A2 expression in GM active lesions in comparison to the adjacent areas of NAGM.

2. We aimed to evaluate the impact of reducing A20 in specific myeloid cell population on the EAE onset and progression. We obtained the A20lox/lox mice from the no-profit RIKEN BioResource Center (Japan), deposited from Dr. H. Honda. In parallel, we purchased from the Jackson laboratory (USA) the transgenic mice carrying the Cre recombinase under the control of a specific promoter for myeloid cells including microglia (Cx3Cr1-cre) and dendritic cells (Cd11c-cre) to generate A20lox::Cx3cr1-Cre and A20lox::cd11c-Cre mice, respectively.

The TNFAIP3 lox-CD11c conditional mouse model has been previously characterize and actually we have performed the EAE experiments on the homozygous KO mice. We reported an increased mortality after EAE induction in KO in comparison to their EAE wild-type littermates. Considering that the KO mice do not reach the chronic EAE phase, we are spending time in the neuropathological analysis of the pre-symptomatic phases.

Conversely, the A20lox::Cx3Cr1 transgenic mouse has never been generated before. For this reason, we decided to characterize in detail the phenotype of this mouse model (conditional heterozygous HT and homozygous KO) in physiological condition before inducing the EAE. We first observed that both the HT and the KO mice are viable, but, although the former are fertile, the seconds have a mortality rate of 50% between the first and second month of life. The weight of mice is recorded every month.

To date, we observed that both female and male KO mice have a lower weight compared to both the littermates HT and wild-type (WT), starting already from one month of age. Conversely, the HT mice at the same age do not show any weight difference compared to the WT littermates. We performed cytofluorimetric and histologic analysis to characterize the phenotype of KO and HT mice compared to their WT littermates. The cytofluorimetric analysis performed on spleens obtained from 3-months old WT, HT and KO mice highlighted that monocyte, macrophages, dendritic cells and B cells are reduced in KO mice in comparison to WT littermates mice. No differences emerged in HT mice. However, analyzing the spleen from 6-months old mice also HT reported a reduction in percentage of macrophages. Considering the mortality rate of the KO mice, we didn't performed the analysis in 6-months old KO mice.

The histological analysis, performed in collaboration with Dr. Manuela Iezzi (Department of Medicine and Aging Sciences, Università di Chieti-Pescara, Italia), highlighted that the A20 loss in myeloid cells induces the hypertrophy of the spleen obtained from 3 months old KO mice. No differences emerged in lung, kidney, liver and heart.

3. To evaluate the ex-vivo effects of FTY720 and AUY954 on NR4As expression in human blood cells, we set-up functional studies on PBMCs isolated from buffy coat obtained from HC treated ex vivo with FTY720 and AUY954. Gene expression analysis revealed that the two drugs upregulate the expression of NR4A2.

#### **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. *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: 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 collected during diagnostic procedures.

**1. Blood sampling and storage**

Blood was drawn every month both in Tempus™ blood RNA Tubes (Life Technologies, Monza, Italy) and immediately stored at -80 °C for mRNA analysis.

**2. RNA extraction**

Total RNA from whole blood was obtained using the “Maxwell R 16 LEV simply RNA Tissue Kit” (Promega, Madison, USA) on the Maxwell 16 instrument (Promega, Madison, USA), following a protocol adapted by the manufacturer to Tempus™ tubes. RNA concentrations were quantified using the Nanodrop® ND-1000 spectrophotometer (Celbio, Milano, Italy).

**3. CD19 mRNA expression by droplet digital PCR (ddPCR) (collaboration with Dr. Giulia Siravegna, IRCC Candiolo)**

- Synthesis of cDNA

1  $\mu$ g of RNA from each sample was used to synthesize cDNA, in a final volume of 15  $\mu$ l. Reverse transcription to cDNA from the RNA extracted was carried out using the High Capacity Reverse Transcription kit (LifeTechnologies, Monza, Italy), according to the manufacturer's instructions.

- ddPCR and Data Analysis

60 µL reaction mixture containing template (12 µL), Assay Probes and QX200™ddPCR™ Supermix (Bio-Rad: 186–4034) were divided in 3 wells on a plate for ddPCR. The ddPCR plate was sealed with a foil heat seal and the PX1™ PCR Plate Sealer (Bio-Rad - 181–4000). Droplet generation and transfer of emulsified samples to PCR plates was performed according to manufacturer's instructions (Instruction Manual, QX200™ Droplet Generator – Bio-Rad). The cycling protocol for amplification was the following: 95 °C enzyme activation step for 5 minutes followed by 40 cycles of a two-step cycling protocol (95 °C for 30 seconds and 55 °C for 1 minute).

After PCR, read-out of positive versus negative droplets was performed with the droplet reader.

The Ratio between the concentration (copies/µL) of the target gene CD19 and the housekeeping gene TBP was calculated using QuantaSoft, BioRad (v.1.7.4). Finally, the data were exported to Microsoft EXCEL for further statistical analysis.

The validation procedure will be performed through the following steps: 1. Definition of control samples for each plate (High positive, low positive, negative, NTC); 2. Definition of the Limit of Blank; 3. Definition of the Limit of Detection; 4. Definition of the Limit of Quantitation; 5. Definition of Precision.

#### 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.

### **2.2 “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”**

#### a) **Summary**

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 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**

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**

Whole transcriptome sequencing was performed on peripheral blood mononuclear cells collected at different time points: before treatment injection (T0) and at 3, 24 hours and 4, 7, 8, 11, 14 days after the injection (including additional injection at day 7 for Avonex). Differential expression analysis was done with EdgeR Bioconductor package (ANOVA-like) using as reference T0 for each drug. The functional class enrichment of the genes was investigated using EnrichR (<http://amp.pharm.mssm.edu/Enrichr/>). 250 differentially expressed genes were detected in PEG-IFN $\beta$  treated samples compared to T0, 201 of them are in common with those differentially expressed in Avonex (311). Interferon receptor expression was not affected during the time course. The kinetics of interferon response was different between the two drugs. PEG-IFN $\beta$  induced, as Avonex, an increase in genes expression at 3 and 24 hours after injection. However, the intensity of interferon responsive genes remained higher compared to that observed for IFN $\beta$ . Specifically, upon PEG-IFN $\beta$  treatment, 13 type I interferon related genes were still overexpressed compared to T0 even after 11 days from the injection. This response was exhausted after 4 days in Avonex.

Data from this study showed that PEG-IFN $\beta$  elicits a long lasting interferon response which leads to an overexpression of interferon related genes till 11 days after injection. Data from this study provides a useful basis to set up an assay for the monitoring of biological activity of PEG-IFN $\beta$ .

#### **e) Advancement in the field**

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. Moreover, data from this study may lead to the identification of a biomarker for the biological monitoring of Peg-IFN treated MS patients.

### **2.3 “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**

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**

EBV infection is strongly associated with MS and increasing evidence indicates that EBV deregulation might be implicated in the dysimmune process that targets the brain and spinal cord. Some studies reported that EBV DNA load is higher in peripheral blood mononuclear cells (PBMC) of patients with MS compared to control subjects. To investigate this issue further, droplet digital PCR and pre-amplification real time RT-PCR were used to quantify EBV DNA and RNA, respectively, in PBMC from healthy donors (HD; n = 28), therapy-free patients with relapsing-remitting MS (RRMS; n = 61), and CIS patients (n = 25). Patients included in the study were enrolled at the MS centers of San Luigi Gonzaga



Hospital/Orbassano, Florence University and Cagliari University; PBMC samples from HD were obtained from CRESM/San Luigi Gonzaga Hospital.

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

EBV DNA was detected in PBMC from 46% of therapy-free RRMS patients, 36% of CIS patients and 39% of HD, without significant differences in EBV DNA frequency or amount between patients and controls. EBV transcripts were detected in PBMC from 22/61 RRMS patients (36%), 6/25 CIS patients (24%), and 7/28 (25%) HD. However, the EBV transcriptional profile markedly differed between patients and HD. Six out of 7 EBV+ samples from the 28 HD contained only EBER1 (latency 0) and only one sample displayed very low amounts of both EBER1 and LMP1 (latency II program).

Interestingly, EBV transcripts associated with EBV latency activation (EBNA3A, LMP1, LMP2A: latency III and latency II programs) and lytic cycle (BZLF1, gp350/220) were detected in 18/22 (82%) and in 3/6 (50%) EBV+ RNA PBMC samples from RRMS and CIS patients, respectively. EBV transcripts were also quantified in CSF cell samples (n=76) matched to PBMC from CIS and RRMS patients. Despite the percentage of patient CSF samples with detectable EBV RNA was very low (7%, 5/76 samples), transcripts associated with EBV latency disruption and/or entry into the lytic cycle were present in all 5 EBV RNA+ CSF cell samples.

This is the first study to show EBV RNA alterations in peripheral blood of CIS/MS patients compared to HD, strengthening the idea that altered control of EBV infection is associated with the disease. These data also suggest that RNA markers of EBV deregulation can be monitored in the peripheral blood during therapy and could provide useful information on the relationship between drug efficacy and changes in EBV-immune system interaction.

#### **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.

### **2.4 Evaluation of the effects of pre-analytical factors on blood samples used in gene expression studies**

#### **a. Summary**

Biobanking of samples has a crucial role in research of chronic diseases. The evaluation of the quality of samples collected or exchanged between centres is essential. Pre-analytical phase represents a crucial step for the whole testing process. Identification of factors influencing RNA stability is mandatory to ensure the accuracy, reliability and reproducibility of the results. This study was designed to evaluate the influence of pre-analytical variables, time and temperature, on blood samples used in gene expression studies. Our study showed that RNA levels from EDTA tubes were influenced in a gene-, housekeeping-, and individual- related manner. Tempus Tubes assure RNA stabilization.

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

Biobanking of samples has a crucial role in research of chronic diseases. The evaluation of the quality of samples collected or exchanged between centres is essential. The same applies to widely used procedures such as gene expression profiling in peripheral blood mononuclear cells (PBMC) wherein the pre-analytical phase represents a crucial step for the whole testing process. Thus, identification of factors influencing RNA stability is mandatory to ensure the accuracy, reliability and reproducibility of the results.

This study was designed to evaluate the influence of pre-analytical variables, time and temperature, on blood samples used in gene expression studies. We focused on samples collected in EDTA tubes. TEMPUS tubes were also tested, representing the gold standard for RNA stabilization. RNA yield and purity along with gene expression levels were considered as endpoints to characterize RNA stability.

Blood specimens collected in EDTA tubes (n=10 participants) were processed for PBMCs isolation either immediately after blood drawn (T0) or following 2, 4, 6 hours and overnight (ON)/24hours incubation either at RT and 4°C. Blood samples collected in Tempus Tubes (n=5 participants) were processed at T0 and following 5 days incubation, either at RT or at 4°C.

The resultant RNA yield and quality were determined in all of experimental conditions. Gene expression analysis was performed by Real Time PCR on 8 candidate genes (CD14, IL10, TNF, TNFAIP3, NR4A2, CD20, CD19, MxA) and 4 housekeeping (HK) genes (GAPDH, HPRT1, B2M, CASC3).

**e. Results.**

1) RNA from Tempus Tubes was stable across all the experimental conditions tested. 2) RNA yield and purity from EDTA tubes were marginally reduced after ON incubation. 3) RNA expression was affected in EDTA sample tubes depending upon the HK-gene and the specific target gene: genes such as TNFAIP3 and NR4A2 demonstrated higher instability, whereas CD19, CD20, MxA were the more stable genes. B2M and CASC3 were the optimal HK-genes that reduced the variability observed in the present study. Variation in RNA levels was stronger after 6 hours of ex-vivo incubation. 4) Inter-individual variation in EDTA tubes was also observed throughout the study.

**f. Advancement in the field**

This study demonstrates the importance of evaluating effects of pre-analytical processes in gene expression studies. In the present study, RNA levels from EDTA tubes were influenced in a gene-, housekeeping-, and patient- related manner. Therefore, when generic blood collection devices are used, time-course studies are mandatory to evaluate the stability of genes of interest, and identify the optimal the HK-gene for particular individual study. Our results confirm that Tempus Tubes are more capable of assuring RNA stabilization. Thus, the usage of these tubes or special analogue devices is recommended for gene expression studies and for biobanking of samples to ensure accurate results.

**3. CRESM BIOBANK**

**a. Summary**

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 funded by FISM (Italian Foundation for Multiple Sclerosis) aims to address these problems by joining the activity of a 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. Disease-oriented Biobanks represent a possible solution for these challenges: they are recognized as a key resource for biomarker discovery and are closely related to the Health care system.

Biobanking has developed at a rapid pace over the years, driven mainly by the push for personalized medicine, the need for high-quality biological resources and associated data for scientific research, and technological advancement of analytical platforms for molecular and genetic research. These developments have enabled the collection and analysis of large numbers of biospecimens in combination with epidemiological and clinical data collected across populations and involving multiple biobanks.

Several factors must be taken into account when setting up and running a biobank: 1. Institutional commitment. The establishment of a biobank must not rely only on individual action but also requires a clear commitment by the host institution, which also needs to ensure that collections are developed within appropriate legal, ethical, clinical, scientific, and technical guidelines, to provide historical continuity in specimen management and recordkeeping. 2. Ethical, legal, and social issues (ELSI) and governance. 3. Biobank management and staffing.

**c. Objectives**

The main objective of the Biobank Project in 2018 was the institutional establishment of the CRESM Biobank, according to the guidelines from the BBMRI (Biobanking and Biomolecular Resources Research Infrastructure of Italy).

The complex process of institutionalization can be summarized in the following steps:

1. Formal establishment within the Hospital
2. Dedicated space and equipment
3. Use dedicated staff to ensure quality and continuity of services
4. Operate within a certified quality system
5. Document the presence of SOPs for involvement of patients, acceptance, preparation and storage of samples, information management and catalogue, distribution of biological material

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

##### **1. Formal establishment within the Hospital**

The process for the formal commitment of the CRESM Biobank within the AOU S. Luigi Gonzaga is ongoing. A draft of the official Regulation of the CRESM Biobank (including also the Standard Operating Procedures manual, the Material and Data Transfer Agreement form, the Informed Consent form) was presented to the Hospital Direction (General Director, Health Director and Administrative Director) for evaluation.

In June 2018 the AOU S. Luigi required an Audit from BBMRI to evaluate the CRESM Biobank. BBMRI Auditors stated that the CRESM Biobank has the minimal requirements to be considered a Biobank, and to join the Italian Node of BBMRI-ERIC.

Further work is needed to implement the formal institutionalization, starting from the formal commitment within the S. Luigi Hospital.

##### **2. Dedicated space and equipment**

The CRESM Biobank is included into the Neurobiology Lab and it is located at NICO. Equipment and consumables for the Biobank are largely provided by FISM (Deep freezers, cryopreservation system, software for biobanking).

##### **3. Use dedicated staff to ensure quality and continuity of services**

The Biobank governance system is explained in the official regulation. The Biobank management is performed by the Director (Dr. Bertolotto) and by one biologist and one laboratory technician.

##### **4. Operate within a certified quality system**

Ongoing.

##### **5. Document the presence of SOPs for involvement of patients, acceptance, preparation and storage of samples, information management and catalogue, distribution of biological material**

The already existing SOP Manual of the CRESM Biobank has been implemented in the last year, according to the guidelines provided by the BBMRI Italian Node. The previous model for the informed consent has been revised according to the European General Data Protection Regulation (GDPR, 679/2016). Finally, the forms for request and distribution of biological material and data have been created, as well.

#### **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

### **IMMUNOPATHOGENESIS**

#### **Pregnancy: a powerful transient immunosuppressive phenomenon in Multiple Sclerosis women**

**a. Summary:**

The role of immunosuppressive placental exosomes during normal pregnancy is clear: to protect the fetus from the immunological maternal attack. The contribution of exosomes in amelioration of the disease condition in MS pregnant women need a more profound evaluation. The comparison of placental exosomes in healthy and MS pregnant women could help to understand the mechanisms underlying the correction of MS immunological alterations during pregnancy.

**b. Background and Significance:**

Pregnancy is an immunological phenomenon where the semi-allogeneic fetus is not rejected because of the immune tolerance induced toward it. Pregnancy acts as modulator of disease activity in MS. During pregnancy, various modulatory signals are released in different forms including a subpopulation of extracellular vesicles (EV). EV are constitutively released from many cell types including immune cells and placenta tissues. Exosomes are EV of 40–120 nm, which have also been shown to modulate the immune system in activating or inhibitory direction. They contain both cytosolic and transmembrane proteins, mRNA and microRNA and can be viewed as a way of intercellular communication without the need for cell–cell contact, thus conveying a potential biologic activity to more distant targets. Exosomes with immunosuppressant activities were found to be increased in pregnant women, compared with non-pregnant ones. Placenta-derived exosomes express significantly higher levels of the pro-apoptotic molecules.

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

To date, there are no studies on exosomes conducted in MS pregnant women, thus this project aims at:

- Characterizing of the two placental components, a fetal and a maternal one, obtained from 5 healthy control (HC) and 5 MS patients;
- studying the regulation or deregulation effects on activated leukocytes exerted by placental exosomes produced from in vitro placental tissues from HC and MS pregnant women.

To do this, we will characterize the exosomes produced by placenta tissues of 5 HC pregnant women) and 5 MS pregnant patients and we will test their ability to modulate activated lymphocytes and monocytes.

We will evaluate the capacity of placental exosomes to convert monocytes in anti – inflammatory monocytes (M2) cells and improve the Treg cells functionality.

The knowledge derived from the characterization of pregnant MS and HC placenta-derived exosomes and their functional studies will open up possibilities for novel, exosome-based treatments of MS patients.

**d. Specific objectives and strategies:**

In particular, the study will focus on the following specific aims:

1) Characterization of the two placental components, the fetal (syncytiotrophoblast) and the maternal one (decidua basalis). Placenta obtained from 5 HC and 5 MS patients will be separated and cultured in vitro for 24 hours. Exosomes produced in vitro from the two placental tissues will be isolated and analyzed in order to identify possible differences in exosome-specific protein profile by Western blot (WB) and flow cytometry.

2) Investigation of the immune mechanisms in which exosomes are involved and elucidation of the contribution of the two placental components in the immune system regulation. To this purpose, we will evaluate the immunosuppressive effect of placental exosomes on activated leukocytes. In particular, considering our preliminary data showing an increase of anti- inflammatory monocytes

during pregnancy, we will test the capability of exosomes to induce an anti-inflammatory phenotype in healthy control buffy coats CD14 monocytes or to suppress a pro-inflammatory behavior in the LPS pre-activated monocytes. In addition, we will investigate the ability of exosomes to improve the Treg suppressive function.

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

The purpose of this project is to elucidate the role of circulating human placental exosomes in the suppression of the immune system in MS pregnant women. The comparison of exosomes' characterization and function in healthy control and MS pregnant women may highlight the mechanisms by which pregnancy turn off the autoimmune response at the base of MS disease.

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

### **1) "Effects of siponimod on Nuclear Receptor subfamily (NR4As) in human blood and brain derived cells"**

**a. Summary:**

Through this project we plan to identify a modulation of NR4A2 expression in siponimod (BAF312) treated human peripheral blood mononuclear cells obtained from buffy coat and immortalized brain derived cell line.

**b. Background and Significance:**

BAF312 is a next-generation sphingosine-1-phosphate (S1P) receptor modulator. A double blind, randomized, phase 3 study has demonstrated that siponimod slow the progression of disability in secondary progressive MS. This agent selectively targets S1P receptors 1 (S1P1) and 5 (S1P5), while the first-generation S1P receptor modulator fingolimod (FTY720), currently in use for the treatment of relapsing-remitting (RR) MS, targets the S1P1, S1P3, S1P4, and S1P5. S1P receptors are widely expressed in the body, including lymphocytes and brain-resident cells such as oligodendrocytes, neurons, microglia and astrocytes. The internalization of S1P1 renders lymphocytes unresponsive to S1P, depriving them of an obligatory signal to egress from the lymph nodes and subsequently recirculate into the central nervous system (CNS). This mechanism of action has been revealed firstly for FTY720. Evidence from preclinical models has demonstrated that S1P receptor modulator readily crosses the blood-brain barrier to enter the CNS. Thus, the demonstrated direct effect of siponimod in CNS, such as the prevention of synaptic neurodegeneration and the promotion of remyelination, may be independent from its effects on peripheral lymphocytes. Particularly, its neurobiological consequence could be related to the selective modulation of S1P1 on astrocytes and S1P5 on oligodendrocytes. However, the molecular mechanisms of action related to the beneficial effects of siponimod directly in brain-resident cells are still unknown. A recent work reports that S1P receptor modulators (i.e. FTY720) accumulating in the brain, enhances histone acetylation and gene expression programs associated with memory and learning. Particularly, this process involves an up-regulation of the transcription factor NR4A2

**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 BAF312 effect on human blood and brain derived resident cells through the activation of the NR4A2 pathway.

**d. Specific objectives and strategies:**

Evaluate the in vitro effects of siponimod on NR4As in human peripheral blood mononuclear cells (PBMCs). Here, we plan to investigate the in vitro effects of siponimod on NR4As expression in human blood samples. To achieve the aim we will take advantages from the already established agreement with S.C. Centro Produzione e Validazione Emocomponenti (Città della Salute e della Scienza di Torino) for the transfer of blood components (buffy coats) for research purposes. We will set-up well-designed studies on PBMCs isolated from buffy coat obtained from healthy donor treated

in vitro with siponimod to evaluate possible drug effects on NR4As gene expression level. The NR4As gene expression level will be quantified using semi-quantitative RT Real Time PCR technique.

2. Evaluate the in vitro effects of siponimod on NR4As in brain-resident cells lines

Considering that NR4As are ubiquitously expressed in human brain, we propose to set-up well-designed studies on brain-derived immortalized cell line treated in vitro with siponimod to evaluate possible drug effects on NR4As gene expression level. This issue allow us to clarify the drugs effect on NR4As in more specific subcellular population such as neurons, astrocytes, microglia and oligodendrocytes. The NR4As gene expression level will be measured using semi-quantitative RT Real Time PCR technique.

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

In this research proposal we hypothesize that the beneficial effects exerted by the siponimod treatment goes through the NR4As. Particularly, assuming that the S1P receptor modulator induces an effect on NR4A2 gene expression in blood and that NR4A2 is related to clinical course of MS, we are interested to evaluate possible siponimod effect through the activation of the NR4As pathway.

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

**a. Summary:**

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:**

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**

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

**d. Specific objectives and strategies:**

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:**

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**

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:**

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**

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:** 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:**  
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.

**4) CD19 mRNA as a more sensitive biomarker for the monitoring of NMOSD patients treated with Rituximab.**

**a. Summary:**

Monoclonal anti-CD20 antibody Rituximab has been recently used to prevent relapses in NMOSD patients, and its efficacy has been widely proven. Drug reinfusions are usually performed when lymphocyte depletion wears off. Lymphocytic depletion is assessed by the detection of CD19 and CD20 positive lymphocytes through flow cytometry (FC) in peripheral blood samples taken monthly from patients. However, the incidence of disease relapses during B cells depletion is relevant. A factor contributing to its magnitude is likely to be the current monitoring method, which appears lackluster in its capability to detect relapses and B-cell repopulation. The aim of this project is to evaluate the occurrence of acute relapses in CD19+ B-cells monitoring via flow-cytometry in treatment-to-target long term therapy with Rituximab in a cohort of 19 NMOSD patients and to compare it with quantitative CD19 mRNA assessment.

**b. Background and Significance:**

Most centres adopt a treatment-to-target approach to reduce adverse effects and optimize long-term treatment. Reinfusions are usually performed when lymphocyte depletion wears off. Lymphocytic depletion is assessed by the detection of CD19 and CD20 positive lymphocytes through flow cytometry (FC) in peripheral blood samples taken monthly from patients. While this method has offered good results so far, growing concern regarding its efficacy has risen, as there is evidence of relapses occurring during lymphocytic depletion. It is unclear whether these relapses are due to the lack of sensitivity of the monitoring method or whether they underlie non-responding patients. This retrospective study aims at analyze occurrence of depletion relapses in a cohort of NMOSD patients at the “CReSM: Centro di Riferimento Regionale per la Sclerosi Multipla” in Orbassano, Turin, Italy. It also will investigate the use of quantitative mRNA assessment via Digital (DD) and Real Time (RT) PCR as a monitoring method opposed to CD19 assessment through flow cytometry.

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

With this study, we aim to identify a strategy to monitor NMOSD patients based on CD19 mRNA.

**d. Specific objectives and strategies:**

1. To evaluate a cohort of NMOSD patients in terms of relapsing patients and overall relapse occurrence, since the evidence about ARR reduction in single patients is already well-established. In addition, to explore different factors that could be independently associated with the patients' clinical course, such as:
  - i. Age at onset, since it has been described that an older age of onset translates into less disease activity.
  - ii. Previous immune therapy, since it is an aspect that has been studied very little and that it could potentially have an influence on relapse dynamic.
  - iii. Serological status, meaning positivity or negativity to NMOIgG or to anti-MOG.
  - iv. Clinical form, meaning isolated form constituted by either LETM or rON, as opposed to complete NMO.
2. To evaluate the monitoring method during treatment with Rituximab, specifically with CD19 FC, studying repopulation time, reinfusion time, and relapse timing according to the monitored parameter. This also translates into analysing CD19 FC efficacy, in terms of sensitivity, specificity and likelihood (LH) ratio, in detecting disease activity and relapses; and it also means to study if there is effectively an association between CD19 FC and relapses. In addition, it will be explored a possible association between the four elements listed in objective 1 and repopulation timing.



3. To study acute relapses, with a special attention to depletion relapses, potentially finding an association between any of the factors listed previously and the occurrence of those relapses.
4. To evaluate quantitative mRNA assessment as a monitoring parameter, evaluating its capability to detect B-cell repopulation compared to FC, and to evaluate depletion relapses with this new technique.

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

12 patients over a follow-up of 4.99 years (95% CI 3.2-6.8) will be included in this retrospective study. NMOSD samples are already available in the CReSM collection (FISM 2014/PMS/1; Ethic Committee of AOU San Luigi 7777-2013).

**5) Serum NFL as additional measure to NEDA status in patients with Multiple Sclerosis: a possible new biomarker for tailored treatment.**

**a. Summary:**

Early and effective intervention is mandatory for successful clinical management in Multiple sclerosis (MS). No Evidence of Disease Activity (NEDA) is proposed as treatment outcome in Relapsing Remitting (RR) MS. However, ongoing subclinical disease progression in MS cannot be captured accurately and surfaces clinically only over a long observation period.

This project aims at evaluating serum neurofilament light chain (sNFL), the most promising marker of degeneration in MS, as a reliable and easy-to-test marker of disease response and progression in individual RR and Primary Progressive (PP) patient. In an observational retrospective followed by a prospective study sNFL levels will be evaluated longitudinally using the ultrasensitive SIMOA technology. sNFLs values could predict the future clinical outcomes of patients and facilitate the personalization of treatment allowing a best allocation of resources.

**b. Background and Significance:**

Early and effective intervention is mandatory for successful clinical management in Multiple sclerosis (MS). “No Evidence of Disease Activity” (NEDA) is proposed as a treatment outcome in Relapsing Remitting (RR) MS. NEDA definitions aim to capture multidimensional aspects of MS clinical outcomes including disease activity and progression. “No evidence of progression” (NEP) is the corresponding treatment outcome proposed for progressive MS.

However, ongoing subclinical disease progression in MS cannot be captured accurately by these definitions and surfaces clinically only over a long observation period.

Neurofilament light chain (NfL) represents the most promising biomarker of degeneration in MS, reflecting axonal damage in Central Nervous System. Increased NfL levels associated with relapse and formation of lesions on MRI were found in MS irrespective of phenotype. Recently, an ultrasensitive immunoassay for NfL based on Simoa technology has been developed, allowing quantification of serum NfL (sNfL) at subfemtomolar concentrations. Thus, NfL could represent the first reliable and easy-to-test marker of MS disease response and progression. In addition, sNfL has been suggested as a candidate biochemical marker to be included in an expanded or alternative definition of NEDA, after validation with long-term follow-up studies.

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

In this study, we propose to evaluate the role of sNfL levels as a biological measure of disease progression and subclinical activity in RRMS patients treated with Natalizumab (NAT), one of the most effective MS treatments. Additionally, sNfL will be evaluated in Primary Progressive MS (PPMS) patients treated with Ocrelizumab (OCRE), the first therapy recently approved for use in PPMS.

**d. Specific objectives and strategies:**

We aim to verify whether

- 1) normalized levels of sNfL correlate with absence of disability progression, a good response to treatment, and a better prognosis,
- 2) altered levels of sNfL correlate with disease progression even if in absence of clinical and MRI activity and may reveal asymptomatic ongoing axonal injury,
- 3) elevated levels of sNfL correlate with absence of response to treatment.



The NfL assay will be performed using Simoa technology, in collaboration with the team led by Dr. Kuhle, an expert on the subject.

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

The project will be developed in two main phases:

- i) a retrospective study, in which we will longitudinally measure sNFL levels in 240 NAT-treated patients stratified in groups according to clinical outcomes (median follow-up 2.9 years, interquartile range 1.5-5.3 years; ~ 1000 samples collected at baseline and once a year during follow-up), and in 40 OCRE-treated clinically well characterized patients (followed for at least one year; ~120 samples collected at baseline and every 6 months during follow-up). The samples are already available.
- ii) A prospective study will be performed including 20 NAT-treated patients and 20 OCRE-treated patients followed-up for at least two years at CReSM, to validate results obtained from retrospective studies.

We hope that the results obtained in this study will enable an informed decision regarding sNFL as an ideal blood marker to be included in NEDA/NEP assessment for monitoring therapeutic response and progression in MS patients. This might lead to personalization of treatment allowing a better allocation of resources.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2018**

Laboratory name: **Adult neurogenesis**

## 1. LABORATORY DESCRIPTION – PERSONNEL (Year 2018):

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 PIs coordinating independent but complementary research lines. Besides the two PIs, the group involves one Associate Professor, one researcher with a permanent position and since June 2018 a new researcher (RTD-B). 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: <a href="mailto:paolo.peretto@unito.it">paolo.peretto@unito.it</a>	

### Principal Investigator 2

Luca Bonfanti	Birthdate: (19/05/1962)
Associate professor	Gender: M
Nationality: Italian	Phone: 011 6706606
Email: <a href="mailto:luca.bonfanti@unito.it">luca.bonfanti@unito.it</a>	

### Researchers (permanent position or RTD 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: morphological analyses and 3D reconstructions |                        |
- |  |                        |
|--|------------------------|
| 3. First name: Serena Bovetti (since June 2018)                                  | Birthdate (13/09/1977) |
| Assistant Professor (RTD-B)  | Gender: F              |
| Expertise: in vivo two-photon microscopy (functional and morphological analyses) |                        |

#### **Researchers (Postdoc and PhD students)**

4. First name: Chiara La Rosa Birthdate (01/07/88)  
PhD student Gender: F  
Role: young researcher (third 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 (third year) Gender: F  
Role: young researcher Nationality: Italian  
Expertise: cellular and molecular analyses of AN in the hippocampus

6. First name: Sara Bonzano  
Postdoc (Veronesi Fellowship) Birthdate (22/03/1987)  
Gender: F  
Expertise: cellular and molecular analyses of AN in the hippocampus

## 2. PRINCIPAL INVESTIGATOR

**h index (LB):** 30 Total citations: 3673 (source: Scopus)

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

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

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

#### 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%
2018	Local research (UNITO)	Coordinator (LB)	University of Turin	-	BONL_R1 LO_18_01	€ 3.700	8%
2018	Local research (UNITO)	Coordinator (PP)	University of Turin	-	PERP_RILO_18_01	€ 3.298,42	8%
2018	Local	Coordinator (LB)	Donations	Neuroni alternativi	-	€ 6.000	10%
<b>Pending</b>							
Pending	National	National Coordinator	PRIN - MIUR (bando 2017) LB	Pre-natally-generated “immature” neurons as additional source of cells involved in adult brain plasticity	-	€ 170.000 (pending)	8%
Pending	National	National Coordinator (PP)	PRIN - MIUR (bando 2017) PP	Aging and neurogenic decline in vertebrates: a functional study	-	€ 161.640,18 (pending)	8%

#### Invited talks

- “From adult neurogenesis to immature neurons: heterogeneity of mammalian brain plasticity” INRA, University of Tours, Nouzilly (January 2018) (LB)
- “Why non-rodent, domestic and wild animal models are needed for studying brain plasticity” Seminar at the PhD program in Veterinary Sciences (Turin, February 2018) (LB)

#### PhD supervised (PIs):

Chiara La Rosa (third year) (LB)

#### 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)
- NIH-NIMH, Bethesda (USA) – *Availability of human brain tissues* (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 (2017-18) *Front Neurosci* (with I. Amrein) (LB)

**Please list your organizational activities:**

- Speakers invited:

### 3. PI's PUBLICATIONS:

(Please list below your publications in 2018 -. 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** (2018) Humans and dolphins: Decline and fall of adult neurogenesis. *Front Neurosci* 12:497. doi: 10.3389/fnins.2018.00497

IF: 3,9 [Q2 (silver) 77/261 - NEUROSCIENCES]

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: 6 [ Q1 (gold) 30/261 - NEUROSCIENCES ]

La Rosa C, Parolisi R, Palazzo O, Lévy F, Meurisse M, **Bonfanti L** (2018) Clusters of DCX+ cells "trapped" in the subcortical white matter of early postnatal Cetartiodactyla (Tursiops truncatus, Stenella coeruleoalba and Ovis aries). *Brain Struct Funct*. 223(8):3613-3632. doi: 10.1007/s00429-018-1708-z.

IF: 4,2 [ Q1 (platinum) 1/21 - ANATOMY & MORPHOLOGY ]

Palazzo O, La Rosa C, Piumatti M, **Bonfanti L** (2018) Do large brains of long-living mammals prefer non-newly generated, immature neurons? *Neural Reg Res* 13: 633-634.

IF: 2,2 [ Q3 (silver) 183/261 - NEUROSCIENCES ]

**Bonfanti L**, Amrein I (2018) Adult neurogenesis: Beyond rats and mice. *Front Neurosci* Editorial (in press).

IF: 3,9 [Q2 (silver) 77/261 - NEUROSCIENCES]

## 4. GROUP'S PUBLICATIONS:

Bonzano S, Crisci I, Podlesny-Drabiniok A, Rolando C, Krezel W, Studer M, **De Marchis S** (2018). Neuron-astroglia cell fate decision in the adult mouse hippocampal neurogenic niche is cell-intrinsically controlled by COUP-TFI in vivo. *Cell Rep* 10;24(2):329-341.

IF: 8 [ Q1 (gold) 28/190 – CELL BIOLOGY ]

Malloul H, Bennis M, Bonzano S, Gambarotta G, Perroteau I, **De Marchis S\***, Ba-M'hamed S. (2018) Decreased Hippocampal Neuroplasticity and Behavioral Impairment in an Animal Model of Inhalant Abuse. *Front Neurosci* 6;12:35. doi: 10.3389/fnins.2018.00035. eCollection 2018.

IF: 3.9 [ Q2 (silver) 77/261 – NEUROSCIENCES ]

Cerrato V, Parmigiani E, Figueres-Oñate M, Betizeau M, Aprato J, Nanavaty I, Berchialla P, **Luzzati F**, de'Sperati C, López-Mascaraque L, Buffo A. (2018) Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity. *PLoS Biol* 16(9):e2005513.

IF: 9,2 [ Q1 (platinum) 3/85 - BIOLOGY ]

Forli A, Vecchia D, Binini N, **Bovetti S**, Moretti C, Mahn M, Baker C, Bolton ML, Yizhar O and Fellin T (2018) Two-photon bidirectional control and imaging of neuronal excitability with cellular resolution in vivo. *Cell Rep* 13;22:3087-3098.

IF: 8 [ Q1 (gold) 28/190 – CELL BIOLOGY ]

Mariotti L, Losi G, Sessolo M, Melone M, Chiavegato A, Marcon I, **Bovetti S**, Forli A, Fellin F, Conti F, and Carmignoto G. (2018) GABAergic interneuron type-specific signaling to astrocytes in neocortical circuits. *Nat Commun* 9:82.

IF: 12 [ Q1 (platinum) 3/64 – MULTIDISCIPLINARY SCIENCES ]

**Bovetti S**, Moretti C and Fellin T. (2018) Patterned two-photon illumination for high-speed functional imaging of brain networks in vivo. In *Advanced Optical Methods for Brain Imaging - Springer book Ed.*

[Book chapter]

## 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
2017-18	Local research (UNITO)	Coordinator (De Marchis)	University of Turin	Molecular mechanisms involved in adult neurogenesis regulation	DEMS_RILO_18_02	€ 2.853	8%
Pending	National	Co-PI local Unit (De Marchis) coordinator Annalisa Buffo	PRIN - MIUR (bando 2017)	Gene regulation in neural stem cells (radial glia) and Bergmann glia as a basis for the study of human genetic disease		€ 244.944 (pending)	
2017-2018	National	Coordinator (Luzzati)	MIUR (Finanziamento delle attività base di ricerca)			3000	
2018	Local research (UNITO)	Coordinator (Luzzati)	University of Turin	Neurogenesis indotta con attivazione di astrociti striatali	LUZF_RILO_18_02	€ 2800	8%
Pending	National	Coordinator of research Unit	MIUR (Programmi di	Reprogramming astrocyte-derived	160000	Pending	



		(Luzzati)	Ricerca Scientifica di Rilevante Interesse Nazionale)	exosomes to tailor innovative			
Pending	National	Coordinator (Luzzati)	Compagnia di San Paolo (Bando Ex-post 2018)	Imaging cellular dynamics in the central nervous system	50000	Pending	

### 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. Saadia Bamhamed, Cadi Ayyad University Marrakech, Morocco – Study on the role of abused inhalants on adult hippocampal neurogenesis.
- Dr. W. Krezel, University of Strasbourg – LPS model of neuroinflammation and effects on adult neurogenic niches

#### *Invited talks:*

“Cell-intrinsic control of adult neurogenesis: the transcription factor COUP-TFI and its multiple roles”  
Plenary Lecture – VII Olfactory meeting ROE (Asturias, Spain – May 2018)

### 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.

### Serena Bovetti

#### *Invited talks:*

Advanced optical approaches for the functional investigation of the mammalian brain. Workshop: Imaging from basic science to clinical applications. Istituto di ricerche farmacologiche Mario Negri (Milano). February 6th 2018.

Two-photon microscopy for in vivo functional investigation of the mammalian brain. Giornata delle neuroscienze, University of Turin. June 27th 2018.

Advances and applications of two-photon microscopy in neuroscience. ZEISS Academy Workshop on correlative microscopy. University of Turin, November 7th 2018.

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>> *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 2018* (Torino, marzo 2018), Aula Magna del Rettorato Cavallerizza reale (with 400 students of the secondary school)

- Conferences on brain plasticity and communication of science in Secondary schools:  
“La plasticità cerebrale: una roba per giovani?” at Liceo Cocito (Alba, 2018)

*“La plasticità cerebrale: una roba per giovani?”* at Liceo Galileo Ferraris (Turin, 2018) in the context of Settimane della scienza

*“Dalla neurogenesi adulta ai neuroni immaturi: le nuove frontiere della plasticità cerebrale”* Liceo Antonelli (Novara, 2018)

*“La plasticità cerebrale: una roba per giovani?”* at Liceo Majorana (Moncalieri, 2018) in the context of Festival dell’Innovazione di settimo torinese

*“La plasticità cerebrale: una roba per giovani?”* at Liceo Pascal di Romentino (NO, 2018)

- Conferences for university students:

*“La plasticità cerebrale tra dogmi e colpi di scena”* at Collegio Cairoli (*Potenziali d’azione* series), Pavia

*“La plasticità cerebrale: una roba per giovani?”* at Collegio Einaudi (Turin)

- Conference *“Neuroni immaturi una riserva contro l’invecchiamento cerebrale?”* Castello di Mango (CN, 2018)

- Conference *“Quale futuro per il cervello umano?”* at Festa di scienza e filosofia, Foligno (2018)

- Conference *“Quale futuro per il cervello umano?”* at Festival di scienza e cultura *“Saper guardare lontano”* (Mogliano Veneto, 2018)

- *“I neuroni immaturi: una riserva di cellule sempre giovani?”* conference at ICSEM (Turin, 2018)

La Rosa C, Bonfanti L. *Il mistero dei neuroni “immaturi”* Sapere n°4, Agosto 2018, pag. 18-23 (popular science publication)

Bonfanti L. *Prefazione* al libro *“Una nuova geografia del cervello”*, di Bernard Sablonnière, Dedalo

PAOLO PERETTO

-Conference (31 Gennaio 2018) *“I nuovi neuroni”*. Accademia delle Scienze Torino- I Lincei per una nuova didattica dell’istruzione: una rete nazionale Accademia delle Scienze Torino.

-Conference (14 febbraio 2018) *“Sulle orme di Frankenstein”*. Museo Regionale Di Scienze naturali Torino- Darwin non extinction day.

-Conference (26 Febbraio 2018) *“Corsa Staminali neurali e memoria”*. DiSFEB Milano- Cervell.a.mente 2018 - Cervello e Sport.

-Conference (9 marzo 2018) *“Corsa e Cervello”*. Politecnico di Torino- IMAGING E CANCRO Storie di ricerca tra arte e scienza.

-Conference (22 maggio 2018) DBioS Torino- Le sinergie della Scienza, *“Attrazione sessuale? Un semplice meccanismo biologico”*.

THE GROUP

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

## 6. Past Research activity

In 2018 the research group has been focused on understanding GnRH system function on adult neurogenesis. Moreover, we identified new molecular players in the control of neurogenesis versus gliogenesis in the adult hippocampus and defined the molecular bases of how abuse of inhalants affects hippocampal neurogenesis and cognitive behavior in adult mice.

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.

In parallel we showed that “immature” non-newly generated neurons are abundant in sheep (large-brained, long-living species with respect to rodents), thus suggesting that this type of plasticity might be abundant in non-rodent mammals.

## 7. Future Projects (2018)

### Summary:

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 2018**

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 PhD 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 23, citations 2379 (Scopus)

### Relevant discoveries:

- By studying the mouse cerebellum we have revealed that a remarkably orderly developmental program is at the source of astrocyte heterogeneity. This program includes: (i) a time-dependent decline in both clone size and progenitor multipotency, associated with clone allocation first to the hemispheres and then to the vermis; (ii) distinctive clonal relationships among astrocyte types, revealing diverse lineage potentials of embryonic and postnatal progenitors; and (iii) stereotyped clone architectures and recurrent modularities that correlate to layer-specific dynamics of postnatal proliferation/differentiation. In silico simulations indicate that the sole presence of a unique multipotent progenitor at the source of the whole astroglial program is unlikely and rather suggest the involvement of additional committed components.

- We have defined a role for the transcription factor SOX2 in cerebellar functions and development, and identified a functional requirement for SOX2 within postnatal Bergmann glia of potential relevance for ataxia in mouse mutants, and in human patients.

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	8%
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 Neurostemcellrepair consortium	TRACING CONNECTIONS OF HUMAN CELL GRAFTS IN THE HD MODEL WITH VIRAL VECTOR-BASED TOOLS		12000	8%
2017	National	PI, Buffo	Ministero dell'Istruzione – Finanziamento di base alla ricerca			3000	
Pending	National	PI, Buffo	Ministero dell'Istruzione – Finanziamento di base alla ricerca 2017(PRIN)	Gene regulation in neural stem cells (radial glia) and Bergmann glia as a basis for the study of human genetic disease		213854	
Pending	Local	PI, Buffo	University of Turin -San Paolo ex post	ITN -Mirage		50000 pending	

Please list the name of PhDs you have supervised.

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

Please list your outreach activities

- describe your international collaborative experiences
  - Prof. Laura Lopez-Mascaraque (Cajal Institute, Madrid, Spain) and dr. Marion Betizeu (Zurich, Switzerland): clonal approaches for astroglialogenesis and neurogenesis.
  - Prof M Parmar (University of Lund, Sweden): rabies virus based tracing of human medium spiny neuron transplantation into the rat damaged striatum
- Invited talks
  - SIF meeting Glial cells and Therapeutic perspectives: from maladaptive plasticity to neurorestoration, June 1018, Florence, Italy. ‘Astrocyte heterogeneity in health and disease’.

#### *Science communication*

Conference “*Tra geni e esperienza*” for “SeralMente”, Grugliasco, aula magna AgroVet, October 19, 2018

Conference “*Il neurone immortale*”, Festival della Scienza e dell’Innovazione, October 18, 2018

Conference “*Le speranze e le certezze nel campo della rigenerazione midollare*”, Il diritto all’informazione e alla qualità della vita delle persone con lesione midollare (with M. Boido), Torino, November 8, 2018

- Editorial duties
  - Guest reviewer for the following journals: The Journal of Neuroscience, Glia, Frontiers in Neuroscience, Elife.
  - Agencies: French National Research Agency (ANR), FISM (Federazione Italiana Sclerosi Multipla), Israel Science Foundation, Czech Research Agency, Alzheimer Research Foundation.
  - Topic Editor: Engineering Adult Neurogenesis and Gliogenesis, Frontiers in Neuroscience
  - Topic Editor: Pharmacology of neurogenesis, Current Opinion in Pharmacology
  - Editorial Board member of [Neuroglia](#)
  - Editorial board member of Scientific reports

Please list your organizational activities:

Organization of Open days and Scuola Lavoro at NICO

Member of the Task Force for Cerebellar Ataxia

- Speakers invited
  - Jenny Sassone, Synapse transmission as potential target in Juvenile Parkinsonism September 28 Vita-Salute University and San Raffaele Scientific Institute Neuropsychopharmacology Unit, Division of Neuroscience

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

### 3. PI's PUBLICATIONS:

Cerrato V, Parmigiani E, Figueres-Oñate M, Betizeau M, Aprato J, Nanavaty I, Berchiolla P, Luzzati F, de'Sperati C, López-Mascaraque L, Buffo A. (2018) Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity. *PLoS Biol.* 2018 Sep 27;16(9):e2005513.  
IF 9.163, Ranking 9/111 (Neuroscience)

Cerrato V, Mercurio S, Leto K, Fucà E, Hoxha E, Bottes S, Pagin M, Milanese M, Ngan CY, Concina G, Ottolenghi S, Wei CL, Bonanno G, Pavesi G, Tempia F, Buffo A\*, Nicolis SK. (2018) Sox2 conditional mutation in mouse causes ataxic symptoms, cerebellar vermis hypoplasia, and postnatal defects of Bergmann glia. *Glia.* 2018 May 6. doi: 10.1002/glia.23448. (\*co-last author)  
IF 5.846, Ranking 7/85 (Cellular and Molecular Neuroscience)

Bertero A, Liska A, Pagani M, Parolisi R, Masferrer ME, Gritti M, Pedrazzoli M, Galbusera A, Sarica A, Cerasa A, Buffelli M, Tonini R, Buffo A, Gross C, Pasqualetti M, Gozzi A. (2018) Autism-associated 16p11.2 microdeletion impairs prefrontal functional connectivity in mouse and human. *Brain.* 2018 May 2. doi: 10.1093/brain/awy111.  
IF 10.84 Ranking 6/336 (Neurology)

Prada I, Gabrielli M, Turola E, Iorio A, D'Arrigo G, Parolisi R, De Luca M, Pacifici M, Bastoni M, Lombardi M, Legname G, Cojoc D, Buffo A, Furlan R, Peruzzi F, Verderio C. (2018) Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations. *Acta Neuropathol.* 2018 Jan 4. doi: 10.1007/s00401-017-1803-x.  
IF 15.872 Ranking 1/336 (Neurology)



#### 4.GROUP's PUBLICATIONS:

**Parolisi R**, Cozzi B and Bonfanti L. Humans and Dolphins: Decline and Fall of Adult Neurogenesis. (2018) Front. Neurosci. 12:497.  
IF= 3.42; R= 29/154 (Neuroscience (miscellaneous))

La Rosa C, **Parolisi R**, Palazzo O, Lévy F, Meurisse M, Bonfanti L. Clusters of DCX+ cells "trapped" in the subcortical white matter of early postnatal *Cetartiodactyla* (*Tursiops truncatus*, *Stenella coeruleoalba* and *Ovis aries*) (2018). Brain Struct Funct. 223(8):3613-3632.  
IF 4.231; R=3/42 (Anatomy); R= 27/154 (Neuroscience (miscellaneous)).

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(4):826-842.  
IF: 5.97; R= 11/154 (Neuroscience (miscellaneous)).

Mancini C, Hoxha E, Iommarini L, Brussino A, Richter U, Montarolo F, Cagnoli C, **Parolisi R**, Morosini D, Nicolò V, Maltecca F, Muratori L, Ronchi G, Geuna S, Giorgio E, Cavalieri S, Di Gregorio E, Pozzi E, Ferrero M, Riberi E, Altruda F, Turco E, Gasparre G, Battersby BJ, Porcelli A, Brusco A, Tempia F. Mice harboring a SCA28 patient mutation in AFG3L2 develop late-onset ataxia associated with enhanced mitochondrial proteotoxicity. Neurobiology of Disease. Accepted.  
IF: 5.227; R=7/154 (Neurology)

**Parolisi R** and **Boda E**. NG2 Glia: Novel Roles beyond Re-/Myelination. (2018). Neuroglia. 1(1), 151-175.  
(no IF yet)

## 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	
2018	Local	Enrica Boda (PI)	Departmental Funds (Ricerca Locale)	Unveiling oligodendrocyte precursor heterogeneity in CNS physiology and pathology		2713	
Pending	National	Enrica Boda (PI)	Fondazione Italiana Sclerosi Multipla 2018 (FISM)	Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in neuroinflammation and de-/remyelination		37700	5%
Pending	National	Enrica Boda (PI of partner unit)	Ministero dell'Istruzione – Finanziamento di base alla ricerca 2017(PRIN)	A role for Mecp2 in glia: an integrated approach for understanding astrocyte dysfunctions in Mecp2-related disorders	Prot. 2017HH XXZS	181667	8%
Pending	International	Enrica Boda (PI)	Narsad Young Investigator Grant 2018 (Brain and Behavior Research Foundation)	Citron as a novel susceptibility gene in schizophrenia: an integrated study in patients and animal models		70000\$	Not allowed
Pending	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		71000	8%
Pending	National	Enrica Boda, PI	Fondazione Just Italia	The role of Particulate		71000 (Pending)	

				Matter exposure in Sudden Infant Death Syndrome (SIDS) through orexinergic system deregulation			
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### Enrica Boda (maternity leave: May-Sept 2018)

- International collaborative experiences

Prof. Verdon Taylor, Dr. Chiara Rolando (Embryology and Stem Cell Biology, Dept. of Biomedicine, University of Basel, Basel, Switzerland)

- Invited talks

Oral communication: Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model. Oral session “Glia in neurodevelopmental and neuropsychiatric disorders”, II More than Neurons Meeting, 29 november- 1 december 2018, Turin, Italy

- Editorial duties

Review Editor of the Editorial Board of Frontiers in Cellular Neuroscience

Ad hoc reviewer for Frontiers in Neurogenesis (1 in 2018), Frontiers in Cellular Neuroscience (2 in 2018), International Journal of Molecular Sciences (1 in 2018)

- Organizational activities

- Member of the Organizing and Scientific Committee of the 1st BraYn (*Brainstorming Research Assembly for Young Neuroscientists*) Conference, 29-30 June 2018, Genoa, Italy.

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

- Member of the Organizing and Scientific Committee of the 2<sup>nd</sup> BraYn (*Brainstorming Research Assembly for Young Neuroscientists*) Conference, November 2019, Milan, Italy.

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

- Organization of the NICO Progress Report series: 20 seminars in 2018 (oral communications held by NICO junior members)

- Organization of the INN (Istituto Italiano di Neuroscienze) Neuroscience Open Forum 2018 (oral communications held by members of the Dept. Neuroscience, University of Turin, and selected invited speakers)

### Roberta Parolisi

- International collaborative experiences

- Merck-Serono Grant Consortium (Pierre Gressens) 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.

- Editorial duties

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

- Organizational activities

- May 2018. Organizing member of the “Beautiful mind” sessions for national festival ‘Pint of Science 2018’. Turin, May 14th-16th, 2018.

### 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 Physiology within the MD program in Medicine and Surgery, entitled “Astrocytes physiology and pathophysiology”, October 16<sup>th</sup>, 2018.

- Editorial duties

Ad hoc reviewer for International Journal of Developmental Neuroscience and Neurochemical Research.

- Organizational activities

- May 2018. Organizing member of the “Beautiful mind” sessions for the festival “Pint of Science 2018”, Turin, May 14<sup>th</sup>-16<sup>th</sup>, 2018.

### **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

*Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion* More than Neurons Congress. Turin, 29-11/01/12/18

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

- Alternanza Scuola-Lavoro: 10-days-long stage (June 2018) 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

### a. Summary

How astroglial heterogeneity is achieved developmentally, and how it differentially impacts on CNS functions is unknown. More specifically, if and how the gliogenic potential of astroglial progenitors changes during development in terms of lineage composition and size of individual clones has not been explored. Similarly, very little information is available on the transcriptional programs implicated in the differentiation of defined astrocyte phenotypes. We found that an ontogenetic program, tightly regulated in space and time, determines astrocyte heterogeneity and identified a functional requirement for SOX2 in postnatal Bergmann glia differentiation and functions, of potential relevance for ataxia in mouse mutants, and in human patients.

### b. Background

In 2018 we focused on understanding how glia contributes to CNS physiopathology. 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. More specifically, if and how the gliogenic potential of astroglial progenitors changes during development in terms of lineage composition and size of individual clones has not been explored. Moreover, very little information is available on the transcriptional programs implicated in the differentiation of defined astrocyte phenotypes.

One limitation to the understanding of astrocyte heterogeneity is the lack of systematic characterization of astroglial diversity in most brain regions. We circumvented this problem by focusing on the mouse cerebellum, where astrocyte diversity is well-established on the basis of distinct cell morphologies, layering, immunophenotyped and functioning. Among possible regulators of the astrocyte differentiation program, we identified an interesting candidate in the transcriptional regulator SOX2. SOX2 is a key factor implicated in the maintenance of pluripotency and stemcellness. However, it is also ubiquitously expressed in astrocytes, prompting the question of its role(s) during astrocyte differentiation and in the mature state.

### c. Rationale

Although different astrocyte types likely display specialized interactions with distinct neuron categories, the different classes of astrocytes have only partially been unmasked. How astrocyte heterogeneity is ontogenetically achieved remains largely unknown. For instance, it remains to be understood whether distinct ventricular progenitors are specified to generate exclusively defined astrocyte types and whether different astrocyte types are produced sequentially during development, as it occurs for neurons. Similarly, the available knowledge on transcriptional regulators responsible of the acquisition and maintenance of specific astrocyte phenotypes is very limited.

Here we approached these issues by studying the development of the main astrocyte types of the cerebellum, which, at difference with other CNS sites, are well established. This conferred a specific advantage because it allowed to overcome the lack of systematic characterization of astroglial diversity that in most brain regions disguises astrocyte heterogeneity. Thus, we employed clonal analyses combined with proliferation studies and in silico modelling to ask whether distinct astrocyte types are produced by different clonal lineages or, instead, are part of mixed lineages. We also asked whether the astrocyte clones are stereotyped in number of cells and composition or whether astrocyte generation is instead governed by random processes.

SOX2 is a transcription factor active in the nervous system, within different cell types, ranging from radial glia neural stem cells to a few specific types of differentiated cells. Mutations in the human SOX2 transcription factor gene cause various central nervous system abnormalities, involving hippocampus and eye defects, as well as ataxia. In the cerebellum, SOX2 is active from early embryogenesis in the neural progenitors of the cerebellar primordium; SOX2 expression is maintained, postnatally, within astrocytes, raising the possibility that this transcription factors participates in astrocyte maturation and supports their functions. We addressed these questions by examining the effects of SOX2 Cre-mediated ablation in the developing and postnatal mouse cerebellum.

#### **d. Objectives**

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 astrocyte generation/maturation impacts the shaping of the circuits. Main goal of our work is to elucidate how astroglial heterogeneity is achieved developmentally and how it differentially influences CNS functions. 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.

In 2018 we specifically aimed at:

- a) revealing how the ontogenetic process leading to the generation of astrocyte diversity;
- b) unveiling the role of the transcription factor SOX2 in cerebellar morphogenesis, astrocyte differentiation and functions.

#### **e. Results**

a) The reconstruction of developmental lineages in the mouse embryo combined with proliferation studies and computational modeling demonstrate that cerebellar astrocyte types emerge according to an unprecedented and remarkably orderly developmental program. Embryonic progenitor cells produce either only a single astrocyte type or more types. These distinct astrocyte lineages display stereotyped architectures and recurrent modularities. Moreover, the generation of astrocytes follows a well-defined spatiotemporal pattern, defined by a time-dependent allocation of astrocytes to distinct cerebellar territories and an inside-out sequence of differentiation, coupled with a decline over time in both progenitor amplification and capability to produce distinct astrocyte types. These results provide the first evidence that an ontogenetic program, tightly regulated in space and time, determines astrocyte heterogeneity (Cerrato et al., PLoS Biol 2018).

b) By performing Sox2 Cre-mediated ablation in the developing and postnatal mouse cerebellum, we reproduced ataxia features. Wnt1Cre-mediated embryonic Sox2 deletion in the cerebellar primordium leads to reduction of the cerebellar vermis, known to be commonly related to ataxia, preceded by deregulation of Otx2 and Gbx2, critical regulators of vermis development. Postnatally, Bergmann glia undergoes a progressive disorganization, mislocalization, and a reduction in the cell number. Bergmann glia was the only type of astrocytes to be affected by Sox2 deletion. Sox2 postnatal deletion, specifically induced in glia (with the GLAST-CreERT2 line), reproduces the Bergmann glia defect, and causes (milder) ataxic features. These results define a role for Sox2 in cerebellar function and development, and identify a functional requirement for Sox2 in postnatal Bergmann glia differentiation and functions, of potential relevance for ataxia in mouse mutants, and in human patients (Cerrato et al., Glia 2018).

##### **a) Advancement in the field**

- First evidence that an ontogenetic program, tightly regulated in space and time, determines astrocyte heterogeneity: astrocyte heterogeneity emerges from an ontogenetic program, tightly regulated in space and time, comprising (i) a time-dependent decline in both clone size and progenitor multipotency, associated with clone allocation first to the hemispheres and then to the vermis; (ii) distinctive clonal relationships among astrocyte types, revealing diverse lineage potentials of embryonic and postnatal progenitors; and (iii) stereotyped clone architectures and recurrent modularities.
- Identification of a novel role for SOX2 in astrocyte and in the regulation of cerebellar functions: SOX2 is implicated in postnatal Bergmann glia differentiation and regulation of Purkinje neuron activity.

## 7. Future Projects (2018)

### a. Summary:

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, after disclosing fundamental cellular mechanisms implicated in the generation of the diversity of cerebellar astrocytes, we will start addressing the underlying molecular mechanisms and expand on human samples. We will further investigate astrocytes in the striatum, to understand their reactivity and neurogenic activation in defined lesion conditions. In regard to oligodendroglia, based on evidence that oligodendrocyte progenitors self-maintain but have limited capability to repair myelin, we will work to understand their biology and complex interplay with surrounding glial elements, in view of fostering myelin regeneration.

### a. Background and Significance:

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 types are generated and of their relationship with neural stem cells; (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 issues and address the following research topics:

- Astrocytes comprise extremely heterogeneous phenotypes. We have unveiled fundamental cellular mechanisms implicated in the generation of the diversity of cerebellar astrocytes. However, much remains to be understood on the molecular actors of astrocyte type specification. For instance, the knowledge on transcription factors implicated at the cell autonomous level in the generation of defined astrocyte types is very limited. Conversely, it appears that extrinsic environmental signals may be crucial factors for the induction of defined astrocyte types. However, how such extrinsic cues are translated within the cells in a permanent phenotype remains to be understood. As a first step to tackle this broad issue, we will focus again on the mouse cerebellum and take a bioinformatic approach to unveil molecular mechanisms, in combination with mouse studies and inspection of human material.
- Striatal astrocytes will instead offer a model to understand the interrelation between astrocytes and neural stem cells, as well as factors regulating astrocyte reactivity. Upon excitotoxic lesion, subsets of striatal astrocytes undergo a spontaneous neurogenic activation leading to the generation of neuroblasts, thus revealing the existence of a latent neurogenic potential in the adult brain parenchyma. Activation of such stem cell potential may represent a defined astrocyte functional state endowed with specific reparative and regenerative actions. We are working to disclose the molecular mechanisms driving the activation of the neural stem cell potential in astrocytes and to characterize the protective/reparative and inflammatory profile of the cells.

Understanding these aspects may produce new knowledge to exploit glial cells and/or their progenitors for the treatment of neurologic disorders such as Ataxia and Huntington Disease.

- 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.

### a. General aim and integration with mission of the Institute

In 2018 we will work toward these main aims:

- understanding glial/glia progenitor heterogeneity and physiology at the molecular, cellular and functional levels and clarifying 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.

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

We will focus on glia physiopathology and neural progenitor plasticity in brain physiology and pathology.

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

**-investigate molecular mechanisms leading to the generation of distinct astrocyte types in the cerebellum.** With the aim to identify candidate molecular mechanisms responsible for the generation of cerebellar astrocyte types and highlight developmental trajectories specific for different astrocyte lineages, we shall undertake a bioinformatic analysis of publicly available mouse single cell data (10.1016/j.cub.2018.07.062) obtained at distinct developmental times. Candidate molecular targets will be validated by immunohistology or in situ hybridization in view of subsequent functional experiments. Analyses will also expand to human fetal material to highlight consistency or variation between mouse and human development. Heterochronic homotopic transplantations of cerebellar astrocyte progenitors in mouse will also help to disclose the contribution of intrinsic vs extrinsic factors in the generation of the different astrocyte types in the cerebellum.

(coll: prof Luca Marozio, dr Fulvio Borrella, Dip Surgical Sciences, University of Turin; Simona Lodato's team, Humanitas, Milan.

**- investigate the role of SOX2 in the neurogenic activation of striatal astrocytes and examine their inflammatory and pro-regenerative profile.** Preliminary data indicate that (i) the stem cell transcription factor SOX2 is upregulated in striatal astrocytes reacting to excitotoxic lesion; (ii) its deletion abrogates lesion-induced stem cell properties in striatal astrocytes within a defined time window; (iii) SOX2 supports the expression of proregenerative and anti-inflammatory factors. This evidence will be strengthened through molecular and functional characterization of astrocyte reactivity in vivo and in vitro. Further, as a proof of principle, we will test in vitro and in vivo if experimental SOX2 overexpression can revert the neurotoxic states in reactive astrocytes. This study, which is carried on in collaboration with F. Luzzati (NICO), will disclose SOX2-related mechanisms exploitable to foster neurorestorative states, which is of great interest in neurodegeneration, inflammation and aging.

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 and attempt to validate the mouse phenotype in human samples of microcephalic patients. This study is carried on in collaboration with 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, M.P. 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.



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

**a. 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 bioinformatic approaches, gene expression analyses on select glial cell populations isolated from the CNS) represent cutting edge techniques whose integration confers methodological originality 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.

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

In collaboration with Filippo Molinari and Massimo Salvi (Politecnico di Torino) we have developed a refined informatic tool (CrAFT) for the automatized analysis and interpretation of confocal images suitable to produce unbiased data. The manuscript describing the tool was submitted in Neuroinformatics. We are now working on the resubmission. CrAFT will be released as an open source tool.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2018**

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, 25; Citations: 2522

### Relevant discoveries:

The main discovery published by my group in 2018 was that the inactivation of microcephaly gene MCPH17 (CITK) delays the progression of medulloblastoma, a very aggressive brain tumors affecting especially children. The discovery was supported by experiments performed in both in vitro and in vivo models. The results of these studies, which obtained significant media coverage, highlight CITK and more in general primary microcephaly genes as possible targets for development of new therapeutic approaches for brain tumors treatment.

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%)
01/01/16 31/12/18	National	Component	CNR, Epigen flagship project.	Disruption of circadian rhythms and epigenetic modifications in <i>D. melanogaster</i> .		53000	8%
01/06/2017 31/05/2019	International	PI	Fondation Jerome Lejeune	Identification and initial validation of new possible treatments for intellectual disability in Down syndrome through drug repositioning.		40000	10%
01/07/18 31/06/19	National	PI	Fondo ricerca locale Ex-60%	Validazione di interattori molecolari di Citron Kinase implicati nella riparazione del danno al DNA.		2857	8%

### PhDs supervised in 2018:

- Gianmarco Pallavicini

### Outreach activities

- Scientific societies

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**

16/03/2018 Unistem 2018, Aula Cavallerizza Reale, Torino: La genesi del cervello da cellule staminali.

- **Communications selected for oral presentation**

19/09/2018 XV FISV meeting (Rome), parallel symposium Chromosome Biology, Cell Division and Cell Cycle: Citron kinase inactivation inhibits medulloblastoma progression by inducing apoptosis and cell senescence

- **Editorial duties**

- Associated Editor of PLoS ONE
- Associated Editor of Frontiers in Neurogenesis

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

Canosa A, De Marco G, Lomartire A, Rinaudo MT, Di Cunto F, Turco E, Barberis M, Brunetti M, Casale F, Moglia C, Calvo A, Marklund SL, Andersen PM, Mora G, Chiò A. A novel p.Ser108LeufsTer15 SOD1 mutation leading to the formation of a premature stop codon in an apparently sporadic ALS patient: insights into the underlying pathomechanisms. *Neurobiol Aging*. 2018 Dec;72:189.e11-189.e17.

IF= 4,45; R= 8/53

Bianchi FT, Berto GE, Di Cunto F. Impact of DNA repair and stability defects on cortical development. *Cell Mol Life Sci*. 2018 Nov;75(21):3963-3976.

IF= 6,72; R= 34/293

Pallavicini G, Sgrò F, Garelo F, Falcone M, Bitonto V, Berto GE, Bianchi FT, Gai M, Chiotto AMA, Filippi M, Cutrin JC, Ala U, Terreno E, Turco E, Cunto FD. Inactivation of Citron Kinase Inhibits Medulloblastoma Progression by Inducing Apoptosis and Cell Senescence. *Cancer Res*. 2018 Aug 15;78(16):4599-4612.

IF= 9,13; R= 17/223

Dema A, Macaluso F, Sgrò F, Berto GE, Bianchi FT, Chiotto AA, Pallavicini G, Di Cunto F, Gai M. Citron kinase-dependent F-actin maintenance at midbody secondary ingression sites mediates abscission. *J Cell Sci*. 2018 Apr 26;131(8).

IF= 4,4; R= 62/190

Bandini C, Pupuleku A, Spaccarotella E, Pellegrino E, Wang R, Vitale N, Duval C, Cantarella D, Rinaldi A, Provero P, Di Cunto F, Medico E, Bertoni F, Inghirami G, Piva R. IRF4 Mediates the Oncogenic Effects of STAT3 in Anaplastic Large Cell Lymphomas. *Cancers (Basel)*. 2018 Jan 18;10(1).

IF= 5,33; R= 45/223

**4.GROUP's PUBLICATIONS:**

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

## 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.

### c. 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.

### d. 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 harborizations 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. We are also addressing whether the discoveries which we have published for Medulloblastoma may apply to more prevalent brain tumors, such as glioblastomas.

## **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 (2018)

### 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), Regional Reference Centre for Medical Genetics (Prof. A. Brusco) and with Politecnico di Torino (Proff. A. Benso and G. Olmo) 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 of 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.

**e. 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.

**f. 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 2018**

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

## 2. PRINCIPAL INVESTIGATOR

### 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
Pending	National	PI	PRIN 2017	The biological bases of the sex-specific incidence of metabolic and cognitive disorders in ageing	2017PPLWM7	Pending	

Please list the name of PhDs you have supervised.

Paolo Mele  
Angela Longo  
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 Ritzler (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.

## **GROUP's PUBLICATIONS:**

Hasan et al., Fear Memory Engram and its Plasticity in the Hypothalamic Oxytocin System, *Neuron*, under revision.

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

##### Bertocchi grant pending

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
Pending	National	PI	PRIN	Stressful experience during adolescence as a risk factor for autism spectrum disorders in offspring: sex difference in a rat model	201787C2L5	Pending	

##### Oberto grant pending

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
Pending	Local	PI	Fondazione CRT 2018	Effetti permanenti dello stress da genitore a figlio: possibili implicazioni nei Disturbi dello Spettro Autistico (DSA)		Pending	

## 6 .Past Research activity

### a. Summary

We use a conditional mutant mouse line to investigate the role of NPY-Y1R transmission in

- 1) resilience to psychopathologies (anxiety disorders)
- 2) sex-related differences in the NPY-Y1 control of energy homeostasis and susceptibility to Metabolic syndrome (MetS)
- 3) cognitive related functions
- 4) 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.

### c. Rationale

1) Y1R<sup>rflb</sup> male mice display increased anxiety, reduced body weight, and increased HPA axis activity. Moreover, differences in phenotypes between Npy1r<sup>rflb</sup> 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 Npy1r<sup>rflb</sup> 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 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 in learning and memory and affects PNNs, we tested spatial memory of Npy1r<sup>rflb</sup> and we analyzed PNN expression and distribution in the dorsal hippocampus,

### d. Objectives

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) “*Sex-related difference of metabolic phenotype*”: to determine the effect depletion of hypothalamic *Npy1r* gene on the different responses of male and female to metabolic challenges.
- 3) “*Neuropeptide Y pathways in learning and memory*”: to examine whether selective ablation of Npy1r in forebrain excitatory neurons may affect learning and memory and PNN expression.

#### e. Results

1) The reduced 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 increased anxiety in the open field and elevated plus maze tests and with a deficit of prefrontal cortex driven cognitive abilities in the puzzle box test, a problem-solving test with increasing difficulty. The same animals showed a significant increase of the number of strongly stained of PNN and a decrease of parvalbumine positive neurons in the prefrontal cortex.

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, lower weight of visceral, subcutaneous and gonadic white adipose tissue (WAT), decreased leptin and increased corticosterone serum levels compared their control littermates. Conversely, conditional mutant female mice showed no changes in growth rate, increased subcutaneous and gonadic WAT, increased leptin but no changes in corticosterone serum levels. These differences appear to be related to different Npy-Y1R and AgRP hypothalamic transmission since Npy1rrfb female, but not male, mice show a significant decrease in Npy1r mRNA and AgRP immunoreactivity in the paraventricular and arcuate hypothalamic nuclei (Bertocchi et al, in preparation).

Low limbic Y1R expression induced by conditional gene inactivation increases susceptibility to metabolic challenges in adulthood and vulnerability to obesity and associated disorders (Paterlini et al., submitted). Conversely, Npy1rrfb female mice 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). These sex related differences in the metabolic phenotype appear to be related to a different Npy-Y1R and AgRP hypothalamic transmission in since Npy1rrfb female, but not male, mice show a significant decrease in Npy1r mRNA and AgRP immunoreactivity in the paraventricular and arcuate hypothalamic nuclei that is rescued by ovariectomy (Oberto et al, in preparation)

3) Npy1rrfb mice show a delayed spatial learning in both the Morris water maze and the Barnes maze compared and an increased density 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 and the Barnes maze (Mele et al, submitted)

#### Advancement in the field

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 and neuronal plasticity.

2) We have shown, for the first time, a sexual dimorphism of Npy1r expression in the ARC and PVN that might underline the interaction between the Npy1r signal, AgRP and estrogens in the modulation of energy metabolism in relation to reproductive functions.

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.

## 7. Future Projects (2018)

### a. Summary:

In the next two years we will continue to investigate the role of NPY-Npy1r signaling in resilience to psychopathologies and sex differences susceptibility to metabolic syndrome (“Vulnerability to psychopathologies” and “The biological bases of the sex-specific incidence of metabolic and cognitive disorders in ageing”). In parallel, we will investigate the neurobiological bases and putative sex differences in the etiology of autism spectrum disorders (ASD).

### b. Background and Significance:

#### 1) “Vulnerability to psychopathologies”

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 ii) a rearrangement of PNNs and myelin development in PFC is involved in maternal care programming of anxiety-like phenotype; ii) Enzymatic disruption of the PNNs in the PFC restores 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) “The biological bases of the sex-specific incidence of metabolic and cognitive disorders in ageing”

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. This study expected outcome, that will take advantage from previous collaboration among NICO, the University of Torino (M. Collino, R. Granata), the University of Milano (A. Maggi,) and of Parma (P. Palanza) are:

- knowledge of the central and systemic metabolic consequences of the liver ER stimulation
- neurobehavioral and cognitive consequences of estrogen related dysmetabolism
- physiological relevance of the sexual dimorphic brain NPY-Y1R signaling for the control of estrogen-dependent energy metabolism and behavior in female mammals,
- identification of the hepatic molecules involved in liver control of the hormetic response both centrally and in periphery.
- identification of novel early biomarkers of liver functionality and multimorbidity risk for the study of drugs able to restore liver hormetic activity in the absence of estrogen stimulation.

#### 3) “Stressful experience during adolescence as a risk factor for autism spectrum disorders in offspring: sex difference in a rat model”

Preconceptional stressful experiences should be considered as crucial elements in the etiology of autism spectrum disorders (ASD). Social isolation (SI) during adolescence is a model of chronic stress. The offspring of SI rats (SI-O) displays some analogies to core symptoms of ASD in children and in genetic mouse models, such as changes in hypothalamic-pituitary-adrenal axis activity, behavioral flexibility, BDNF and oxytocin (OT). In collaboration with Prof. M. Serra, Department of Experimental Biology, University of Cagliari, we will further characterize the phenotype of this environmentally induced animal model in order to investigate the biological bases and putative sex differences in the etiology of ASD using behavioral, biochemical, electrophysiological, molecular, and epigenetic tools to assess social behavior, conditioned responses, gut microbiota, hypothalamic OT system, epigenetic changes and neuroplasticity.

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

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 and 3 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.
- all projects aim to reveal new targets for therapeutic interventions in stress and anxiety-related disorders.

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

##### 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 behaviour 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) Npy1rrfb mice after PNN digestion by chondroitinase in specific limbic regions or exposed to environmental enrichment; ii) floxed mice after viral driven depletion of Npy1r gene in PFC.

##### 2) “*The biological bases of the sex-specific incidence of metabolic and cognitive disorders in ageing*”

This collaborative study will enable us to exploit the most appropriate behavioral, pharmacological and genetic tools, developed in the teams involved in the project, to verify the extent to which specific hepatic factors, as a consequence of ovarian failure and under specific metabolic, exercise or stress challenges, are able to influence brain, WAT, liver and muscle using methodologies aimed at measuring emotional and cognitive behaviour, molecular and neurochemical signals and thermogenic response.

To demonstrate the relevance of the liver ERs in hormesis response of fertile and ovx mice, we will utilize LERKO mice, in which the expression of ER has been specifically ablated in the liver, and ERA floxed syngenic controls. To demonstrate the relevance of the liver ER on brain activity of fertile and ovx mice (specifically on the NPY-Y1R circuit), we will use Npy1rrfb mutant mice and their control littermates. Control and ovx females will be exposed to hypercaloric diet, psychosocial stress and physical exercise. Male/female comparative study in ageing will be also carried out using LERKO and NPY1rrfb (and their controls) mice at senescence (17-20 month old).

In the entire course of the experiment, mice will be monitored for BW gain, food-intake, in-cage locomotor activity.

At the end of each experiment animals will be analyzed for:

- anxiety, depression-like and cognitive behavior
- blood pressure and heart rate, glucose and insulin tolerance tests, adipose tissue distribution, circadian rhythms.
- biochemical and immunohistochemical studies on: liver, BAT and WAT, muscle and plasma; brain; thyroid, heart analysis.

##### 3) “*Stressful experience during adolescence as a risk factor for autism spectrum disorders in offspring: sex difference in a rat model*”

The goal of this collaborative project will be achieved through the following specific aims:

1. Characterization of the behavioural phenotype of male and female SI-O rats and putative gut microbial contribution factors.
2. Characterization of the hypothalamic oxytocin (OT) system in male and female SI-O rats.
3. Epigenetic characterization of male and female SI-O rats.
4. Characterization of neuronal plasticity biomarkers
5. Characterization of plasticity processes in male and female SI-O rats.

Our contribution to the project will be to investigate if the formation process of plasticity brakes during development is altered in SI-O rats and may therefore be associated with the ASD-like phenotype; we will further assess if differences in plasticity, existing between sexes, also involve the rearrangement of PNNs and myelin. Finally, we will investigate if the phenotype of SI-O rats may be rescued by PNNs digestion with ChABC.

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

*“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, 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.

*“The biological bases of the sex-specific incidence of metabolic and cognitive disorders in ageing”*

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 sex differences in liver ability to coordinate the hormetic response and role of estrogens ii.) To identify the impact of chronic stress, hypercaloric diets and exercise on the vulnerability to obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS) and comorbid behavioral disorders in female mice; iii) in the absence of ovarian activity. 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.

3. *“Stressful experience during adolescence as a risk factor for autism spectrum disorders in offspring: sex difference in a rat model”*

The SI-O may be a useful animal model to further understand the neurobiological bases of ASD, avoiding the adaptations that may occur in other genetic or pharmacologic experimental models of these disorders. Understanding the biological consequences of preconceptional stress exposure may aid to develop care guidelines for the general population and therapeutic interventions for the affected offspring.

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

To generate PFC-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>tetbi</sub>-Cre/tdTOM*) is equipped with a bidirectional tet promoter (*P<sub>tetbi</sub>*) 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>tetbi</sub>* in the presence of Dox. To increase the spread of virus in the PFC 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 PFC of age-matched wild-type littermates and *Npy1r<sup>2lox</sup>* mice. tdTOM and Cre expression in the PFC 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>PFC</sup>*, in which the *Npy1r* gene will be selectively deleted ( $\Delta$ ) in the PFC. The virus-injected, age-matched wild-type littermates will serve as controls (*Contr-v<sup>PFC</sup>*).



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2018**

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: RTDA 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

Nationality: Italian

Gender: Female

Degree: Master degree in Neurobiology, University of Turin

Role: Post-doctoral fellowship recipient

Expertise:

- Light and laser confocal microscopy.
- Immunohistochemistry and Western blot.
- RT-PCR and quantitative Real Time PCR
- DRG and autonomic ganglia explants 3D culture.
- Analysis of recovery of motor function in rats and mice using behavioral tests.
- Extraction and culture of primary sensory and autonomic neurons.
- Quantitative morphological analysis by stereological methods (nerve fibers and sensory neurons).

5. Benedetta Elena Fornasari

Birthdate: 11/07/1989

Nationality: Italian

Gender: Female

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

Role: Post-doctoral fellowship recipient

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. Marwa Mohamed Emadeldin Ali Mahmoud El Soury

Birthdate: 22/04/1991

Nationality: Egyptian.

Gender: Female

Degree: Master Degree in Molecular Biology and Biotechnology, Faculty of Science, Alexandria University

Role: PhD student in the University of Turin, Italy, Dipartimento di Neuroscienze , Scuola di Dottorato in Scienze della Vita e della Salute. Cycle XXXII.

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, cell transfection, cell migration assays, proliferation and viability assay.

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

### Relevant discoveries:

The results of our recent 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. Reaxon® Nerve Guide got the CE mark in January 2014.

Please list your grants according to the table below (last five yrs).

Startin g- end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Direc tly Avail able to NICO
2017- 2018	National	PI	Università degli Studi di Torino	Ricerca scientifica finanziata dall'universita' di torino - anni 2017 e 2018	Fondo per la Ricerca Locale	3.399,03	8%

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

- Giacomo Carta

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

None

### Please list your outreach activities

- International collaborative experiences.

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

2013-present: Member of the Research Commitee of the Department of Clinical and Biological Sciences of the University of Torino

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

2018 – present: Head of the Department of Clinical and Biological Sciences – University of Turin.

2018 – present: Chairman of the Scientific Committee of the Accademic Senate of Univerity of Turin,.

- Invited talks

14° Congress of EFSM: European Federation of Societies for Microsurgery – Belgrado, Serbia 5-8 th may 2018

Editorial duties  
Member of Editorial Board of *Microsurgery*

**Please list your organizational activities:**

- Speakers invited
  
- Workshops, Schools or Conferences organized

**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:

1. Costa D, Diogo CC, Costa LMD, Pereira JE, Filipe V, Couto PA, Geuna S, Armada-Da-Silva PA, Maurício AC, Varejão ASP. Kinematic patterns for hindlimb obstacle avoidance during sheep locomotion. *Neurol Res.* 2018 Aug 14;1-9. doi:10.1080/01616412.2018.1505068. [Epub ahead of print] PubMed PMID: 30106355.  
IF: 1.449, Q4
2. Russo I, Femminò S, Barale C, Tullio F, Geuna S, Cavalot F, Pagliaro P, Penna C. Cardioprotective Properties of Human Platelets Are Lost in Uncontrolled Diabetes Mellitus: A Study in Isolated Rat Hearts. *Front Physiol.* 2018 Jul 10;9:875. doi: 10.3389/fphys.2018.00875. eCollection 2018. PubMed PMID: 30042694; PubMed Central PMCID: PMC6048273.  
IF: 3.394, Q1
3. Geuna S, Muratori L, Fregnan F, Manfredi M, Bertolo R, Porpiglia F. Strategies to improve nerve regeneration after radical prostatectomy: a narrative review. *Minerva Urol Nefrol.* 2018 Jul 23. doi: 10.23736/S0393-2249.18.03157-0. [Epub ahead of print] PubMed PMID: 30037210.  
IF: 1.449, Q3
4. Marchesini A, Raimondo S, Zingaretti N, Riccio V, Battiston B, Provinciali M, Geuna S, Riccio M. The amnion muscle combined graft (AMCG) conduits in nerves repair: an anatomical and experimental study on a rat model. *J Mater Sci Mater Med.* 2018 Jul 21;29(8):120. doi: 10.1007/s10856-018-6126-5. PubMed PMID: 30032327.  
IF: 2.448, Q2
5. Ronchi G, Fornasari BE, Crosio A, Budau CA, Tos P, Perroteau I, Battiston B, Geuna S, Raimondo S, Gambarotta G. Chitosan Tubes Enriched with Fresh Skeletal Muscle Fibers for Primary Nerve Repair. *Biomed Res Int.* 2018 Jun 13;2018:9175248. doi: 10.1155/2018/9175248. eCollection 2018. PubMed PMID: 30009176; PubMed Central PMCID: PMC6020668.  
IF: 2.583, Q2
6. El Soury M, Fornasari BE, Morano M, Grazio E, Ronchi G, Incarnato D, Giacobini M, Geuna S, Provero P, Gambarotta G. Soluble Neuregulin1 Down-Regulates Myelination Genes in Schwann Cells. *Front Mol Neurosci.* 2018 May 14;11:157. doi: 10.3389/fnmol.2018.00157. eCollection 2018. PubMed PMID: 29867349; PubMed Central PMCID: PMC5960709.  
IF: 3.902, Q2
7. Carriel V, Geuna S, Alaminos M. Ex Vivo and In Vivo Stem Cells-Based Tissue Engineering Strategies for Their Use in Regenerative Medicine. *Stem Cells Int.* 2018 Apr 15;2018:7143930. doi: 10.1155/2018/7143930. eCollection 2018. PubMed PMID: 29760733; PubMed Central PMCID: PMC5924978.  
IF: 3.989, Q2
8. Muratori L, Gnavi S, Fregnan F, Mancardi A, Raimondo S, Perroteau I, Geuna S. Evaluation of Vascular Endothelial Growth Factor (VEGF) and Its Family Member Expression After Peripheral Nerve Regeneration and Denervation. *Anat Rec (Hoboken).* 2018 Apr 30. doi: 10.1002/ar.23842. [Epub ahead of print] PubMed PMID: 29710417.  
IF: 1.373, Q3
9. Gnavi S, Morano M, Fornasari BE, Riccobono C, Tonda-Turo C, Zanetti M, Ciardelli G, Gambarotta G, Perroteau I, Geuna S, Raimondo S. Combined Influence of Gelatin Fibre Topography and Growth Factors on Cultured Dorsal Root Ganglia Neurons. *Anat Rec (Hoboken).* 2018 Apr 30. doi: 10.1002/ar.23846. [Epub ahead of print] PubMed PMID: 29710414.  
IF: 1.373, Q3

10. Morano M, Ronchi G, Nicolò V, Fornasari BE, Crosio A, Perroteau I, Geuna S, Gambarotta G, Raimondo S. Modulation of the Neuregulin 1/ErbB system after skeletal muscle denervation and reinnervation. *Sci Rep.* 2018 Mar 22;8(1):5047. doi: 10.1038/s41598-018-23454-8. PubMed PMID: 29568012; PubMed Central PMCID: PMC5864756.  
IF: 4.122, Q1
11. 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.  
IF: 2.413, Q3



#### 4.GROUP's PUBLICATIONS:

Carvalho C. R., Wrobel S, Meyer C, Brandenberger C, Cengiza I. F. López-Cebral R, Silva-Correia J, Ronchi G, Reis R. L., Grothe C. Oliveira J. M., Haastert-Talini K. The Potential of Gellan Gum-based Luminal Fillers for Peripheral Nerve Regeneration. An In Vivo Study in the Rat Sciatic Nerve Repair Model. *Biomaterial Science*. 2018, 6, 1059

Popara J, Accomasso L, Vitale E, Gallina C, Roggio D, Iannuzzi A, Raimondo S, Rastaldo R, Alberto G, Catalano F, Martra G, Turinetto V, Pagliaro P, Giachino C. Silica nanoparticles actively engage with mesenchymal stem cells in improving acute functional cardiac integration. *Nanomedicine (Lond)*. 2018 May;13(10):1121-1138. doi: 10.2217/nnm-2017-0309. Epub 2018 Jun 8.

#### 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%
2017- 2018	National	S. Raimondo PI	Università degli Studi di Torino	Ricerca scientifica finanziata dall'universita' di torino - anni 2017 e 2018	Fondo per la Ricerca Locale	€ 3210,19	8%
2017- 2018	National	G. Ronchi PI	Università degli Studi di Torino	Ricerca scientifica finanziata dall'universita' di	Fondo per la Ricerca Locale	€ 3356,85	8%

				torino - anni 2017 e 2018			
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**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**

Dr. Stefania Raimondo - 5th Seminar for Young Researchers in SAKURA Science 2018 “Establish of Sustainable Network in Biomedical Field and Encouragement of Future Women Leader - Kyushu Institute of Technology, Kitakyushu, Japan, 1<sup>st</sup>-7<sup>th</sup> July 2018.

Dr. Stefania Raimondo – invited teacher IBRO-APRC School on Neural plasticity in health and disease 21 october - 4 november 2018 - Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi.

**Editorial duties**

Dr. Stefania Raimondo Guest Associate Editor for the journal “Frontiers in Cellular Neuroscience”. Research topic: “Peripheral nerve regeneration”

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**

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**

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**

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**

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**

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**

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 year)**

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

### **a. Summary:**

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:**

#### 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.

#### .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.

#### 5.a Study of decellularized nerve: research of the more reliable method

Peripheral nerve injuries are more than 70% of upper limb traumas, in case of large defect end to end suture is not possible and conduit is not enough to obtain a good result, the gold standard is the

autograft, but this solution presents disadvantages: donor site morbidity and longer surgery time. For this reason, allograft could be the correct alternative but nerves from specimen cause immunogenic response. Starting from this evidence the focus of this research topic is to study the correct way to decellularize nerves preserving extracellular matrix and basal lamina to improve nerve regeneration.

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

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:**

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:**

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<sup>©</sup> 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 2018**

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, 38; Citations: 4887 (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, Street et al., 2018): the alteration of the brain circuits dedicated to the control of food intake and energy metabolism. In our studies (Bo et al, 2016, Farinetti et al, 2018) we demonstrated that both NPY and POMC neurons are affected by the exposure to TBT, suggesting that the whole neuroendocrine machinery controlling food intake and energy metabolism is potentially affected by the exposure to metabolic disruptors.

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	Local	PI	UNITO-ex 60% 2013	Effetti cerebrali degli obesogeni: azione della TBT sul circuito a NPY		2.127	8%
2013-2016	International Foundation		Postfinasterid e Foundation	Effects of finasteride on behavior and neurosteroids		14.656	10%
2015-2017	Local	PI	UNITO-ex 60% 2014	Distruttori endocrini e		2.343	8%

				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	Local	PI	UNITO-ex 60% 2016-17	Effetti dell'esposizione a distruttori metabolici sui circuiti ipotalamici che controllano l'assunzione di cibo ed il metabolismo energetico		7.095	8%
2018-2020	Local	PI	UNITO-ex 60% 2018	Neuropeptidi e sviluppo dei disturbi del comportamento nel periodo evolutivo: modelli sperimentali e potenzialità terapeutiche'		2.800	8%

Please list the name of PhDs you have supervised.

- Adriana Paraninfo (1996)
- Nicoletta Aste (1996) joint PhD program with University of Liege (Liege, Belgium)
- Claudia Castagna (1998)
- Laura Plumari (2004)
- Stefano Gotti (2004)
- Monica Sica (2005) joint PhD program with University of Maastricht (Maastricht, Holland)
- Mariangela Martini (2008) joint PhD program with UNED (Madrid, Spain)
- Francesca Allieri (2008) joint PhD program with University of Paris VI (Paris, France)
- Elena Mura (2009)
- Daniela Grassi (2010) joint PhD program with UNED (Madrid, Spain)
- Elisabetta Bo (2011)
- Egidio Caricati (2011) joint PhD program with University of Marseille (Marseille, France)
- Desiree Miceli (2012)
- Alicia Rodriguez-Gomez (2013) joint PhD program with UNED (Madrid, Spain)
- Alice Farinetti (2014)
- Benedetta Foglio (2014)
- Marilena Marraudino (2017)

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

- Angelo Costa Prize (1986) for Anatomical, Embriological and Comparative anatomy studies, of the Faculty of Medicine.
- Prize "Extraordinario de Doctorado" (1994) of the University of Salamanca (Spagna), for the best PhD thesis of the year 1993
- Branemark Osseointegration Center Award for a paper presented at the 1st World Congress of Osseointegration (1994).

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-wg3basicsciences.htm](http://www.gnrhnetwork.eu/hhn_home/hhn-cost/hhn-costorganization/hhn-wg3basicsciences.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)**

- 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 disrupters? (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)
- EDC e regolazione dell'appetito: lezioni da modelli animali (Endocrine Disrupting Chemicals and Developmental Origins of Health and Disease, Reggio Emilia, Febbraio 2018)

- Interferenti endocrini e circuiti nervosi: come l'ambiente può contribuire allo sviluppo di malattie sessualmente dimorfiche (4i-Incontri Italiani Ipotalamo Ipofisari, Siracusa, Marzo 2018)
- Environmental modulation of neural circuits and behavior (Multifaceted interactions between sex and brain, Roma, Novembre 2018)

#### 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)
- Frontiers in Neuroscience (2018-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.
- Neuroactive steroids and metabolic axis (a cura di RC Melcangi, GC **Panzica**) Frontiers in Neuroendocrinology, 48, pp 1-78 Elsevier ISSN: 0091-3022 (2018).
- 9<sup>th</sup> International Meeting Steroids and Nervous System (a cura di GC **Panzica**, RC Melcangi) J. Neuroendocrinology, 30 Wiley ISSN:1365-2826 (2018).

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:

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

1. Marc J.Tetel, Geert J.de Vries, Roberto C. Melcangi, GianCarlo Panzica, Siobhain M.O'Mahony (2018) Steroid, stress and the gut microbiome-brain axis. *J.Neuroendocrinology*, 30: e12548 (1-8)  
IF: 3,392 rank:54/142
2. Roberto C Melcangi, Giancarlo Panzica (2018) Neuroactive steroids and metabolic axis. *Front Neuroendocrinol*, 48: 1-2  
IF: 6,875 rank:11/142
3. Giancarlo Panzica, Roberto C Melcangi (2018) New perspectives for the action of steroids in the brain. *J. Neuroendocrinol*, 30: e2576 (1-2)  
IF: 3,392 rank:54/142
4. Simona Perga, Serena Martire, Francesca Monatolo, Iliaria Giordani, Michela Spadaro, Gabriele Bono, Stefania Corvisieri, Iliaria Messuti, Giancarlo Panzica, Fabio Orlandi and Antonio Bertolotto (2018) The footprints of poly-autoimmunity: a search for common biological factors involved in multiple sclerosis and Hashimoto's thyroiditis. *Front. Immunol.*, 9: 301 (1-12)  
IF:5,511 rank: 30/155
5. Giovanna Ponti, Alice Farinetti, Marilena Marraudino, Giancarlo Panzica and Stefano Gotti (2018) Sex steroids and adult neurogenesis in the ventricular-subventricular zone. *Front. Endocrinol. - Neuroendocrine Science*, 9: 156 (1-8).  
IF: 3,519 rank: 52/142
6. Raffaele Nuzzi, Simona Scalabrin, Alice Becco, Giancarlo Panzica (2018) Gonadal Hormones and Retinal Disorders: a Review. *Front. Endocrinol. - Neuroendocrine Science*, 9: 66 (1-15).  
IF: 3,519 rank: 52/142
7. Marilena Marraudino, Mariangela Martini, Sara Trova, Alice Farinetti, Giovanna Ponti, Stefano Gotti, GianCarlo Panzica (2018) Effects of estradiol and progesterone on regulation of the Kisspeptin system in ovariectomized CD1 mice. *Brain Res.*, 1688: 8-14.  
IF: 3,125 rank: 126/261
8. Matteo Briguglio, Bernardo Dell'Osso, Giancarlo Panzica, Antonio Malgaroli, Giuseppe Banfi, Carlotta Zanaboni Dina, Roberta Galentino and Mauro Porta (2018) Dietary Neurotransmitters: A Narrative Review on Current Knowledge. *Nutrients* 10(5): 591 (1-16); <https://doi.org/10.3390/nu10050591>  
IF: 4,196 rank: 18/83
9. Silvia Giatti, Silvia Diviccaro, Giancarlo Panzica, Robertoc C. Melcangi (2018) Post-finasteride syndrome and post-ssri sexual dysfunction: two sides of the same coin? *Endocrine*, 61: 180-193.  
<http://dx.doi.org/10.1007/s12020-018-1593-5>  
IF: 3,179 rank: 62/142
10. Maria Elisabeth Street, Sabrina Angelini, Sergio Bernasconi, Ernesto Burgio, Alessandra Cassio, Cecilia Catellani, Francesca Cirillo, Annalisa Deodati, Enrica Fabbrizi, Vassilios Fanos, Giancarlo Gargano, Enzo Grossi, Lorenzo Iughetti, Pietro Lazzeroni, Alberto Mantovani, Lucia Migliore, Paola Palanza, Giancarlo Panzica, Anna Maria Papini, Stefano Parmigiani, Barbara Predieri, Chiara Sartori, Gabriele Tridenti, Sergio Amarri (2018). Current Knowledge on Endocrine Disrupting Chemicals (EDCs) from Animal Biology to Humans, from Pregnancy to Adulthood: Highlights from a National Italian Meeting. *International journal of molecular sciences* 19: 6 (1-44).  
<http://dx.doi.org/10.3390/ijms19061647>  
IF: 3,687 rank: 90/293

11. Alice Farinetti, Marilena Marraudino, Giovanna Ponti, GianCarlo Panzica, Stefano Gotti (2018) Chronic treatment with tributyltin induces sexually dimorphic alterations in the hypothalamic POMC system of adult mice. *Cell and Tissue Research*, *in press*. <http://dx.doi.org/10.1007/s00441-018-2896-9>.  
IF: 3,043 107/190
12. Marcinnò A, Marnetto F, Valentino P, Martire S, Balbo A, Drago A, Leto M, Capobianco M, Panzica G, Bertolotto A. (2018) Rituximab-induced hypogammaglobulinemia in patients with neuromyelitis optica spectrum disorders. *Neurol Neuroimmunol Neuroinflamm*. 2018 Sep 13;5(6):e498. doi: 10.1212/NXI.0000000000000498.  
IF: 8,055 rank: 13/197

#### 4.GROUP's PUBLICATIONS:

(Please list below up to ten most relevant publications of the other members of the group in 2018 -. Please indicate the journal IF, ranking)

Giovanna Ponti, Alice Farinetti, Marilena Marraudino, Giancarlo Panzica and Stefano Gotti (2018) Sex steroids and adult neurogenesis in the ventricular-subventricular zone. *Front. Endocrinol. - Neuroendocrine Science*, 9: 156 (1-8).

IF: 3,519 rank: 52/142

Marilena Marraudino, Mariangela Martini, Sara Trova, Alice Farinetti, Giovanna Ponti, Stefano Gotti, GianCarlo Panzica (2018) Effects of estradiol and progesterone on regulation of the Kisspeptin system in ovariectomized CD1 mice. *Brain Res.*, 1688: 8-14.

IF: 3,125 rank: 126/261

Alice Farinetti, Marilena Marraudino, Giovanna Ponti, GianCarlo Panzica, Stefano Gotti (2018) Chronic treatment with tributyltin induces sexually dimorphic alterations in the hypothalamic POMC system of adult mice. *Cell and Tissue Research*, *in press*. <http://dx.doi.org/10.1007/s00441-018-2896-9>.

IF: 3,043 107/190

## 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	Local	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	Local	Gotti S; PI	UNITO-ex 60% 2015	The Kisspeptin neuronal system: study of the distribution and of the pubertal development		1.669	8%
20/02/2017-20/02/2019	Local	Gotti S; PI	UNITO-ex 60% 2016-2017	Effetti della somministrazione prenatale di chlorpyrifos nei circuiti ipotalamici del topo		4755	8%
20/02/2017-20/02/2019	Local	Gotti S; P I	UNITO-ex 60% 2018	Fattori ambientali nella sclerosi multipla: effetti in seguito a esposizione a bisfenoli.		2410	8%
Pending	Local	Gotti S; P I	FISM 2018	Does environmental exposure to bisphenols affect sex features in Multiple Sclerosis?		99689	5%
Pending	National	Gotti S; P I	PRIN 2017	Environmental factors in multiple sclerosis: bisphenols exposure effects.		178.226	8%
1/01/2017-31/01/2018	Local	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	Local	Ponti G, Co-PI	UNITO-ex 60% 2017	Valutazione multifattoriale del benessere animale in avicunicoltura		10120	8%
1/01/2018-31/01/2018	International	Ponti G, Co-PI	EIT FOOD Education project (MOOC)	Fork2Farm:Sustainable agriculture in a changing environment		20.000€	
Pending 36 months)	National	Ponti G, Co-PI	PRIN 2017	Long term effects on reproductive systems of neonatal exposure to Genistein in porcine models:		151.986	

				genomic, proteomic and metilomic comprehensive analyses.			
<b>Pending 24 months</b>	National	Ponti G, Co-PI	CARIPLO 2018	Multiple Approaches to explore NEUro-cell-REnewal in aged/frail males and females (MANEUver)		127.674€	
<b>Pending 36 months</b>	National	Ponti G, PI	CRC Agroalimetrare 4.0 2018	Evaluation of housing system on welfare and development of neural circuits controlling fertility of rabbit		86.023,94	
<b>1/01/2018-31/01/2019</b>	Local	Ponti G, Co-PI	UNITO-ex 60% 2018			7398.33€	

## 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.

- 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)

Dott. Katrina Campbell (QUB)

- 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

- g. Summary** 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**

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** 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**

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

### **k. Results**

#### **Gonadal hormones**

*a) Role of estradiol and progesterone on the modulation of Kisspeptin innervation of the PVN (Marraudino et al., 2018).*

The kisspeptin system is clustered in two main groups of cell bodies (the periventricular region, RP3V and the arcuate nucleus, ARC) that send fibers mainly to the GnRH neurons and in a few other locations, including the paraventricular nucleus, PVN. In physiological conditions, gonadal hormones modulate the kisspeptin system with expression changes according to different phases of the estrous cycle: the highest being in estrus phase in RP3V and PVN (*positive feedback*), and in ARC during the diestrus phase (*negative feedback*). In our work we studied these hormonal fluctuations during the estrous cycle,

investigating the role played by progesterone (P) or estradiol (E<sub>2</sub>), alone or together, on the kisspeptin system. Gonadectomized CD1 female mice were treated with P, E<sub>2</sub> or both (E<sub>2</sub>+P), following a timing of administration that emulates the different phases of estrous cycle, for two cycles of 4 days. As expected, the two cell groups were differentially affected by E<sub>2</sub>; the RP3V group was positively influenced by E<sub>2</sub> (alone or with the P), whereas in the ARC the administration of E<sub>2</sub> did not affect the system. However P (alone) induced a rise in the kisspeptin immunoreactivity. All the treatments significantly affected the kisspeptin innervation of the PVN, with regional differences, suggesting that these fibers arrive from both RP3V and ARC nuclei.

*b) Gonadal hormones and retinal disorders (Nuzzi et al., 2018)*

Gonadal hormones are essential for reproductive function but can act on neural and other organ systems, and are probably the cause of the large majority of known sex differences in function and disease. We have investigated this hypothesis in relation to eye disorders and to retinopathies in particular.

Analysis of the biological basis for a relationship between eye diseases and hormones showed that estrogen, androgen, and progesterone receptors are present throughout the eye and that these steroids are locally produced in ocular tissues. Sex hormones can have a neuroprotective action on the retina and modulate ocular blood flow. There are differences between male and female retina; moreover, sex hormones can influence the development (or not) of certain disorders. For example, exposure to endogenous estrogens, depending on age at menarche and menopause and number of pregnancies, and exposure to exogenous estrogens, as in hormone replacement therapy and use of oral contraceptives, appear to protect against age-related macular degeneration (both drusenoid and neurovascular types), whereas exogenous testosterone therapy is a risk factor for central serous chorioretinopathy. Macular hole is more common among women than men, particularly in postmenopausal women probably owing to the sudden drop in estrogen production in later middle age. Progestin therapy appears to ameliorate the course of retinitis pigmentosa. Diabetic retinopathy, a complication of diabetes, may be more common among men than women. In conclusions, we observed a correlation between many retinopathies and sex, probably as a result of the protective effect some gonadal hormones may exert against the development of certain disorders. This may have ramifications for the use of hormone therapy in the treatment of eye disease, and of retinal disorders in particular.

*c) Sex steroids and adult neurogenesis (Ponti et al., 2018)*

The forebrain ventricular-subventricular zone (V-SVZ) continuously generates new neurons throughout life. Neural stem cells (type B1 cells) along the lateral ventricle become activated, self-renew, and give rise to proliferating precursors which progress along the neurogenic lineage from intermediate progenitors (type C cells) to neuroblasts (type A cells). Neuroblasts proliferate and migrate into the olfactory bulb and differentiate into different interneuronal types. Multiple factors regulate each step of this process. Despite the huge amount of studies on adult neurogenesis in the V-SVZ-OB system, still few data focus on its regulation by steroids. The role of steroids on V-SVZ-OB neurogenesis is highly complex. Generally, neurogenesis is more affected by T in males, while E<sub>2</sub> has a higher influence on females. However, the same hormone may determine a different effect depending on sex, age, strain, brain region, and neurogenic process. It is also possible that the different extent of V-SVZ-OB neurogenesis may reflect behavioral differences described among many strains of mice as observed in other brain regions. Those differences may be genetic or depend on a lack of maternal care during development. Profound differences exist between males and females. Some of them are actively determined by steroids levels in adults, while others are established during development. Moreover, sexual hormone's levels changes along life. Important species-specific differences exist between different rodent models. Despite some similarities, adult neurogenesis is regulated by different factors in the V-SVZ-OB system compared with the SGZ of the hippocampus. Furthermore, different cell populations, or different steps of the neurogenic lineage may be sensible to a specific hormone.

The extent of adult neurogenesis in the V-SVZ-OB changes along with each of the above mentioned parameters. However, it is not clear which features are directly or indirectly involved. It is, thus, important to consider all those parameters altogether.

*d) Steroid, stress and the gut microbiome-brain axis (Tetel et al., 2018).*

It is becoming well established that the gut microbiome has a profound impact on human health and disease. We have, thus, explored how steroids can influence the gut microbiota and, in turn, how the gut



microbiota can influence hormone levels. Within the context of the gut microbiome-brain axis, we investigated how perturbations in the gut microbiota can alter the stress axis and behaviour. In addition, human studies on the possible role of gut microbiota in depression and anxiety were examined. Finally, we presented 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.

An interesting extension of this study was a review about foods as natural sources of substances that may exert crucial effects on the nervous system in humans (**Briguglio et al., 2018**). Some of these substances are the neurotransmitters (NTs) acetylcholine (ACh), the modified amino acids glutamate and  $\gamma$ -aminobutyric acid (GABA), and the biogenic amines dopamine, serotonin (5-HT), and histamine. In neuropsychiatry, progressive integration of dietary approaches in clinical routine made it necessary to discern the more about some of these dietary NTs. Relevant books and literature from PubMed and Scopus databases were searched for data on food sources of ACh, glutamate, GABA, dopamine, 5-HT, and histamine. Different animal foods, fruits, edible plants, roots, and botanicals were reported to contain NTs. These substances can either be naturally present, as part of essential metabolic processes and ecological interactions, or derive from controlled/uncontrolled food technology processes. Ripening time, methods of preservation and cooking, and microbial activity further contributes to NTs. Moreover, gut microbiota are considerable sources of NTs. However, the significance of dietary NTs intake needs to be further investigated as there are no significant data on their bioavailability, neuronal/non neuronal effects, or clinical implications.

### **Neurosteroids and brain functions.**

#### *a) Neuroactive steroids and metabolic axis (Melcangi and Panzica, 2018)*

Neuroactive steroids (i.e., steroids synthesised both in peripheral glands and in nervous system) are important physiological regulators of the nervous functions. In particular, they are involved in the control of many physiological aspects of reproduction, stress response, and metabolism, as well as in several pathologies including depression, obesity, feeding and reproduction. 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 edited a special issue exploring the relationships among neuroactive steroids and the metabolic axis that originated from the lectures presented at a satellite symposium of the 9th International meeting "Steroids and Nervous System" (February 2017, Torino, Italy). The data discussed in this issue support the idea that there is a very close link among neuroactive steroids (including both hormones produced by the endocrine peripheral glands and those synthesized within the central nervous system) and the control of metabolic axis. This opens new perspectives to understand the biological basis of many pathologies based on metabolic alterations, as the metabolic syndrome, obesity or diabetes.

#### *b) Neurosteroids and sexual dysfunctions (Giatti et al., 2018)*

Sexual dysfunction is a clinical condition due to different causes including the iatrogenic origin. For instance, it is well known that sexual dysfunction may occur in patients treated with antidepressants like selective serotonin reuptake inhibitors (SSRI). A similar side effect has been also reported during treatment with finasteride, an inhibitor of the enzyme 5 $\alpha$ - reductase, for androgenetic alopecia. Interestingly, sexual dysfunction persists in both cases after drug discontinuation. These conditions have been named post-SSRI sexual dysfunction (PSSD) and post-finasteride syndrome (PFS). In particular, feeling of a lack of connection between the brain and penis, loss of libido and sex drive, difficulty in achieving an erection and genital paresthesia have been reported by patients of both conditions. It is interesting to note that the incidence of these diseases is probably so far underestimated and their etiopathogenesis is not sufficiently explored. To this aim, we reviewed the state of art of these two different pathologies and discussed, on the basis of the role exerted by three different neuromodulators such as dopamine, serotonin and neuroactive steroids, whether the persistent sexual dysfunction observed could be determined by common mechanisms.

### **Endocrine disruptors and circuits controlling food intake and energy metabolism.**

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 and to their action on brain circuits (**Street et al., 2018**). We have examined in particular the action of Tributyltin (TBT), an antifouling agent found in boat paints and a

common contaminant of marine and freshwater ecosystems. TBT is rapidly absorbed by organic materials and accumulated in many aquatic animals. Human exposure may depend on ingestion of contaminated food or by indirect exposure from household items containing organotin compounds. TBT is defined as an endocrine disruptor compound (EDC) because it binds to androgen receptors. Moreover, it is also included on the list of metabolic disruptors. The brain is a known target of TBT and this compound interferes with the orexigenic system, inducing a strong decrease in NPY expression in the hypothalamus. In this experiment (**Farinetti et al., 2018**), we investigated the effect of a chronic treatment with TBT on the mouse anorexigenic system in both sexes, to look at the pro-opiomelanocortin (POMC) expression in the paraventricular (PVN), dorsomedial (DMN), ventromedial (VMN), and arcuate (ARC) hypothalamic nuclei. The results show a sexually dimorphic effect of TBT on both systems. TBT induced a significant decrease of POMC-positive structures only in female mice in DMN, ARC, and in PVN for both sexes. Apparently, these results show that TBT may interfere with the anorexigenic system in hypothalamic areas involved in the control of food intake, by inhibiting POMC in a sexually dimorphic way. In conclusion, in addition to having a direct effect on fat tissue, the effects of TBT as metabolic disruptor, may be due to gender-specific actions on both orexigenic and anorexigenic hypothalamic systems.

#### **Molecular markers of neurodegenerative diseases (Perga et al., 2018; Marcinnò et al., 2018)**

We started a cooperation with the team of dr. Bertolotto to study some chemical markers of some neurodegenerative diseases.

#### **I. Advancement in the field**

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

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.

We will expand our cooperation with our colleagues in Psychiatry. We started to analyze male and female adolescent rats and test the effect of emotional deprivation, induced by the maternal separation in a model of Anorexia nervosa. Our preliminary results (Gotti et al., 2017) indicate that the maternal separation induces in the females a greater hyperactive behavior than in males.

Finally, 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:

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.

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

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:

**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 steroids.**

The ovarian hormone  $17\beta$ -estradiol is known to regulate the release, expression and immunoreactivity of arginine-vasopressin (AVP) in the supraoptic and paraventricular hypothalamic nuclei of rodents. Previous studies we demonstrated that estrogen receptor  $\alpha$  is involved in the effects of chronic estradiol administration on arginine-vasopressin immunoreactivity in the female rat hypothalamus. In cooperation with the Cajal Institute (Madrid) we will investigate the effect of an acute administration of estradiol or specific agonists for estrogen receptors  $\alpha$ ,  $\beta$  and G protein-coupled estrogen receptor 1 on the immunoreactivity of arginine-vasopressin in the hypothalamus of adult ovariectomized female rats.

**Translational studies.** By using our newly developed ABA model, we have understood that the maternal separation induces in the females a greater hyperactive behavior than in males. To test if neuropeptides are involved in this altered behavior, we will administer oxytocin to experimental animals in order to see if oxytocin administration may change the expression of the behavior and the progression of the disease. In our study on the MS, we want to analyze the effects of the disease on the reward system, for this reason we will study the dopamine neurons distribution in the ventral tegmental area (VTA) and in the pars compacta of the substantia nigra (SNpc) and the serotonin neurons distribution in the dorsal raphe nucleus (DRN). Finally, we started this year a PhD project to study, in a mouse model, the possible involvement of Endocrine Disruptors (EDCs) in the onset of the MS. More in detail, this study will start investigating the effects of perinatal exposure (from mating until weaning) to BPA (4 $\mu$ g/kg BW/day, according to the new European TDI) in one of the most widely used murine model of MS, the Experimental Autoimmune Encephalomyelitis (EAE). We will evaluate, by daily examination, the consequences of BPA exposure on the disease onset and progression (rotarod performance and clinical score) and on some physiological parameters (body weight, food intake, vaginal opening, ano-genital distance). Thereafter, we will focalize our attention to possible involvement of EDCs in the onset and progression of the disease.

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

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 2018**

Laboratory name: Electrophysiology of neurodegenerative diseases

## LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

First name and surname	Birthdate
FILIPPO TEMPIA	20/08/1960
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- **Personnel**

1. First name and surname: Birthdate  
ERIOLA 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

### 3. PRINCIPAL INVESTIGATOR

**H index 24, citations 1801 (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
- electrophysiological alterations of cerebellar circuitry in an animal model of Alzheimer's disease
- 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
	National vs International	PI vs. Component	MIUR, ERC, ecc...			€	€
2019-2020	National	PI	Ricerca Locale, Fellowship	Transduction of molecular pathology in a therapeutic strategy of SCA38, a novel form of spino-cerebellar ataxia	n.n.	about € 18.000	8%
2018-2021	Local	PI	Ricerca Locale	no title	n.a.	€ 2.828	8%
2017-2020	Local	PI	Ricerca Locale	Cellular and molecular mechanisms of spino-cerebellar ataxia type 38 (SCA38)	n.a.	€ 7.153	8%
2015-2018	Local	PI	Ricerca Locale	Role of neuronal excitability in the prefrontal cortex of mice model of psychiatric disorders	n.a.	€ 2.605	8%
2014-2018	National	co-PI	Telethon-Italy	Translating molecular pathology into a therapeutic strategy in SCA38, a newly identified form of spinocerebellar ataxia	GGP14225	€ 86,400	8%

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; Prof. Brusco, University of Turin.
  - Collaboration with Dr. Chiara Verpelli, CNR Institute, Milano
  
- 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
  - Dr. Ferdinando Fiumara, Dept. of Neuroscience, University of Torino
  - Dr. Fernanda Laezza, University of Texas Medical Branch at Galveston (TX, USA)
  - Dr. Sathya Puthanveetil, Department of Neuroscience - The Scripps Research Institute, Florida, USA
  - Dr. Chiara Verpelli, CNR Institute, Milano

#### 4. PI's PUBLICATIONS:

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

Hoxha E, Balbo I, Miniaci MC, Tempia F (2018) Purkinje cell signaling deficits in animal models of ataxia. *Front. Synaptic Neurosci.* 10:6 (17 pages). eCollection 2018. Review. <http://dx.doi.org/10.3389/fnsyn.2018.00006>. IF= n.a. R=34/85 (40%)

Cerrato V, Mercurio S, Leto K, Fucà E, Hoxha E, Bottes S, Pagin M, Milanese M, Ngan C-Y, Concina G, Ottolenghi S, Wei C-L, Bonanno G, Pavesi G, Tempia F, Buffo A, Nicolis SK (2018) Sox2 conditional mutation in mouse causes ataxic symptoms, cerebellar vermis hypoplasia, and postnatal defects of Bergmann glia. *Glia* 66:1929-1946. <http://dx.doi.org/10.1002/glia.23448>. IF= 5.846; R = 5/142 (3%)

Eriola Hoxha\*, Andrea Marcinnò\*, Francesca Montarolo, Linda Masante, Iliaria Balbo, Fernanda Laezza, Filippo Tempia (2018) Emerging roles of Fgf14 in behavioral control. *Behavioral Brain Research* 356: 257-265. <https://doi.org/10.1016/j.bbr.2018.08.034>. IF : 3.173; R = 11/68 (16%)

Cecilia Mancini\*, Eriola Hoxha\*, Luisa Iommarini, Alessandro Brussino, Uwe Richter, Francesca Montarolo, Claudia Cagnoli, Roberta Parolisi, Diana Iulia Gondor Morosini, Valentina Nicolò, Francesca Maltecca, Luisa Muratori, Giulia Ronchi, Stefano Geuna, Francesca Arnaboldi, Elena Donetti, Elisa Giorgio, Simona Cavalieri, Eleonora Di Gregorio, Elisa Pozzi, Marta Ferrero, Evelise Riberi, Giorgio Casari, Fiorella Altruda, Emilia Turco, Giuseppe Gasparre, Brendan J. Battersby, Anna Maria Porcelli, Alfredo Brusco\*, Filippo Tempia\* (2018) Mice harbouring a SCA28 patient mutation in AFG3L2 develop late-onset ataxia associated with enhanced mitochondrial proteotoxicity. *Neurobiol Dis.* 2018 Oct 30. pii: S0969-9961(18)30320-6. doi: 10.1016/j.nbd.2018.10.018.

\*contributed equally.

IF: 5.227; R= 8/142 (5%)

Eriola Hoxha, Pellegrino Lippiello, Fabio Zurlo, Iliaria Balbo, Rita Santamaria, Filippo Tempia\*, Maria Concetta Miniaci The emerging role of altered cerebellar synaptic processing in Alzheimer's Disease. *Front. Aging Neurosci.* (in press).

\* corresponding author.

IF: 3.582; R = 11/90 (12%)

## 6 Past Research activity

### f. Summary

The main project for the year 2018 was the study of the Elov15 knock-out mouse as an animal model to understand the pathogenesis of spino-cerebellar ataxia type 38 (SCA38). We performed a comprehensive study of the generation of action potentials in Purkinje cells and of synaptic function. We found an impairment of the endocannabinoid-mediated suppression of excitation, a form of short-term synaptic plasticity. On the other hand, the conduction velocity of peripheral and central axons was significantly reduced, suggesting of role of Elov15 products in the function of myelin. We also concluded a study on the behavioral alterations of Fgf14 mice and in collaboration with other research groups we demonstrated the important role of the transcription factor Sox2 in cerebellar physiology.

### g. Background

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.

### h. Rationale

Mice with a targeted deletion of Elov15, kindly provided by Dr. Horton and Dr. Moon, University of Texas Southwestern Medical Center, Dallas (TX, USA), display similar symptoms and pathology as patients with SCA38. Therefore they are a valid model of this disease. Since motor symptoms can be attributed to cerebellar dysfunction, it can be hypothesized that some of the main mechanisms of neuronal signalling in the cerebellum is altered. A second hypothesis is based on the fact of Elov15 is necessary for the synthesis of long-chain fatty acids, which are present in myelin: if myelin sheaths are altered we expect an impairment in the conduction of action potentials along myelinated fibers.

### i. Objectives

The aim of the study was to find functional alterations of cerebellar function, which might explain the motor deficits. A first subaim was to probe the main electrophysiological features of the cerebellar cortex. A second subaim was to test the conduction of action potentials.

### j. Results

In cerebellar Purkinje cells of Elov15 knock-out mice, by cell-attached recording the spontaneous action potential firing was not altered. Evoked firing recorded in whole-cell configuration was also normal. Synaptic transmission was intact in the synapses formed by parallel fibers and climbing fibers. Since the mGlu1 receptor expressed by Purkinje cells requires phospholipids, which are altered due to the lack of Elov15, we also tested the postsynaptic current mediated by such receptor. Also this signaling mechanism was normal in Elov15 knock-out mice. Since endocannabinoids require unsaturated fatty acids for their synthesis, we studied the Synaptically evoked Suppression of Excitation (SSE), which is a form of short-term synaptic plasticity (duration 20-30 seconds) mediated by the action of an endocannabinoid on CB1 receptors. SSE was less pronounced in Elov15 knock-out mice, suggesting a role of Elov15 in endocannabinoid signaling.

The velocity of action potential conduction was measured in a peripheral nerve, finding a consistent and significant reduction. This suggests that Elov15 is required for proper myelin function. In order to assess action potential conduction in a cerebellar axon, we evoked antidromic spikes by stimulation of the Purkinje cell axon. Also in this case the velocity was significantly slower. These results indicate a role of Elov15 and of its enzymatic products in functions in which unsaturated fatty acids are involved, including endocannabinoid signaling and in the formation and maintenance of myelin sheaths.

In Fgf14 knock-out mice we found a complex pattern of behavioral alterations, including reduced aggressivity, reduced sexual behavior, reduced behaviors directed toward objects. These results have important implications regarding the functions controlled by Fgf14.

Sox2 deletion is associated with motor symptoms and with alterations of cerebellar astrocytes. We found a subtle alteration of synaptic transmission at the parallel fiber-Purkinje cell synapse, in line with an aberrant function of nearby astrocytes.

In a mice model of the Phelan McDermid syndrome we found that excitatory synaptic transmission was intact. However, preliminary data suggest an alteration of GABAergic signaling.

**k. Advancement in the field**

The pathogenesis of spino-cerebellar ataxias is very heterogeneous, and at present no therapy is available for any type of SCA. Our results pointing to a role of Elov5 in endocannabinoid signaling and in the maintenance of myelin provide the first functional explanation of the pathogenic mechanism linking Elov5 loss of function to the motor impairment present in knock-out mice and in patients with SCA38.

## 7. Future Projects (2018)

### a. Summary:

We found that in the *Elovl5* knock-out cerebellum synaptic transmission is intact but a form of short-term synaptic plasticity (SSE) is impaired. In the next year we plan to extend this study to long-term synaptic plasticity at the parallel fiber-Purkinje cell synapse, which can undergo either potentiation or depression depending on the induction protocol, with different transduction pathways. Our findings in action potential conduction in *Elovl5* knock-out mice suggest an alteration of myelin. We plan to investigate at the ultrastructural and molecular level the myelin of this animal model. Thanks to the data provided by a collaboration with the laboratory of lipid biochemistry of the University of Milan, we plan to design a diet with specific molecules downstream *Elovl5*, to attempt a rescue of behavioral and physiological deficits.

Preliminary data suggest that *Fgf14* knock-out mice have an altered sensitivity to depression. We plan to study the mechanisms at the level of brain regions involved and of neuronal mechanisms.

In the animal model of the Phelan McDermid syndrome we plan to study in detail the alterations in GABAergic signaling and their consequences on cerebellar function.

### a. Background and Significance:

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. 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. In addition, although some redundancy is present among *Elovl* enzymes, the lack of *Elovl5* causes a complex disruption of the lipidic pattern, as shown by preliminary lipidomics data of the laboratory of Milan. This fact suggests that functions dependent on long chain lipids might be affected. Several types of synaptic plasticity rely on lipid signals like endocannabinoids and phosphatidylinositols. Since impairments in synaptic plasticity are usually associated with ataxia, it is highly relevant to test the main forms of long-term synaptic potentiation and depression in the cerebellum. Moreover, proper function of myelin sheaths in allowing high action potential conduction requires a correct lipid composition. Cerebellar function is based on precise timing of neuronal signals, so that a delay due to a myelin defect might disrupt the cerebellar contribution to motor control and cause ataxia. For this reason it is highly relevant to investigate the consequences of *Elovl5* loss on myelin.

Aim 2. Preliminary results suggest that *Fgf14* knock-out mice have a reduced susceptibility to depression. If confirmed, this suggests that *Fgf14* might be a molecule involved in the modulation of mood, so that a loss of function causes resilience to depression while hyperactive might be responsible for depression. Currently the molecular mechanisms of depression are not understood and antidepressant drugs have a low rate of efficacy. If *Fgf14* can be confirmed as a central player in the control of susceptibility to depression, this finding would open a new avenue to the study of the molecular basis of this disease, which is the leading cause of lifelong disability due to its high prevalence in the population.

Aim 3. Loss of function of SHANK3 is the cause of the Phelan McDermid syndrome, which is characterized by intellectual disability, hypotonia, epilepsy and autism like features. SHANK3 is a scaffold protein located in the postsynaptic density, which is required for proper synapse development and plasticity. Mice with a deletion of exon 11 of *Shank3*, as found in patients with the Phelan McDermid syndrome, recapitulate the main symptoms of the disease and have a deficit in mGlu5 receptor mediated signaling in the hippocampus. Since the most brain region most frequently involved in autism is the cerebellum, it is mandatory to study the consequences of the *Shank3* exon 11 deletion on synaptic transmission in the cerebellar cortex. The results might have important implications on the mechanisms of autism.

**a. General aim and integration with mission of the Institute**

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 its pathogenic mechanism and to design a specific therapy. Regarding Fgf14, a possible link with depression would open a new field of research on the molecular and neuronal mechanisms of this psychiatric disease, which is a main mission of our Institute. Regarding autism, we want to provide mechanistic explanations 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 diseases like spino-cerebellar ataxias, and psychiatric diseases such as depression and autism.

**a. Specific objectives and strategies:**

**Aim 1: Mechanisms of SCA38 ataxia**

Starting from our validation of *Elovl5* knockout mice as a model of SCA38 and from the finding that basic synaptic transmission is intact in the cerebellum of these mice, in the next year we firstly aim at concluding this study by testing long term potentiation and long term depression in the cerebellar cortex. We have recently conducted a study on the role the 5HT<sub>7</sub> serotonin receptor in these forms of synaptic plasticity, and we plan to apply the experimental protocols for this research on *Elovl5* knockout mice. A second aim of our study of this animal model of SCA38 concerns the role of Elovl5 in providing the lipid molecules for myelin formation. Our preliminary results show that action potential conduction is significantly affected by the loss of Elovl5, in both peripheral and central axons. We expect to find fine ultrastructural defects of myelin and alterations in the expression pattern of the main proteins of myelin. Therefore, we plan to perform a detailed analysis of myelin by electron microscopy, protein levels assessment by western blot and protein localization analysis by immunohistochemistry. The results will be discussed with the collaborators of the lipidomics laboratory in Milan, and a rescue strategy will be designed based on the administration of the lipids, which are deficient in the myelin of *Elovl5* knockout mice.

**Aim 2: Role of FGF14 in the modulation of depression**

Firstly we want to confirm that the deletion of Fgf14 in mice confers resilience to depression. Fgf14 is a cytoplasmic protein that binds to the C-terminal domain of Nav voltage-dependent sodium channels and modulates their biophysical properties. Fgf14 has a wide expression in most brain regions. Our aim is to find the brain areas nuclei involved in the modulation of depression by Fgf14. Our strategy is to induce a depressive-like behaviors in Fgf14 knock-out mice and to use a reported of neuronal activity to mark the neuronal activation. Comparison with wild-type mice will provide information about the brain regions where Fgf14 exerts its effect of protection from depression. In a second phase, that will probably extend beyond next year, we plan to record the neuronal activity in such brain areas to find the mechanisms related to depression. Part of this research will be performed in collaboration with laboratories like the one of Prof. D'Ascenzo (Catholic University School of Medicine in Rome) and Prof. Laezza (University of Texas Medical Branch at Galveston).

**Aim 3: Role of cerebellum in autism spectrum disorders**

Our latest results showed that Shank3 mutant mice have intact excitatory synaptic transmission in the cerebellar cortex, including the mGlu1 receptor mediated postsynaptic current. Preliminary results show that GABAergic signaling is altered in these mice. In the next year we plan to search for the alterations of GABAergic synaptic transmission in the cerebellum of Shank3 mutant mice and to relate them to the symptoms.

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

We plan to perform some of the measures of neuronal activity by *in vivo* two photon imaging, to confirm results derived from experiments in tissue slices. We plan to acquire mice with a genetically encoded calcium sensitive fluorescent protein or with a voltage-sensitive one (in collaboration with Dr. Knopfel of the Imperial College of London). In the next year we don't plan to perform stimulation experiments, but, following identification of the brain areas or nuclei where Fgf14 modulates depression, we'll use *in vivo* optogenetic stimulation to assess the effects of activation or inhibition of specific neuronal populations in the relevant structures. This will allow us to identify the neurons and the pathways involved in the control of depression.



*Fondazione Cavalieri Ottolenghi*

*Neuroscience Institute Cavalieri Ottolenghi*

**Internal Annual Report 2018**

Laboratory name: Brain development and disease

## 2. 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 B Nationality Italian  
Expertise Spinal cord injury, motor neuron diseases, Huntington disease, stem cells
  
4. Michela Guglielmotto 28/02/1977  
PhD (from December Role Assistant Professor RTD B) Female  
Role Post-doc Nationality Italian  
Expertise Alzheimer's disease
  
5. Roberta Schellino 11/02/1985  
PhD Female  
Role Post-doc Nationality Italian  
Expertise Neurogenesis, motor neuron diseases, Huntington disease, behavior
  
6. Matilde Ghibaudi<sup>^</sup> 19/09/1988  
Biotechnology degree Female  
Role doctorate student Nationality Italian



Expertise Spinal cord injury, miRNA, molecular biology

- |   |                     |
|---|---------------------|
| 7. Giulia Pasini  | 14/02/1986          |
| Pharmacy degree   | Female              |
| Role Fellowship recipient                               | Nationality Italian |
| Expertise molecular biology, mitochondria analysis      |                     |
| 8. Elena Signorino                                      | 06/10/1976          |
| Biology degree  | Female              |
| Role technician   | Nationality Italian |
| Expertise molecular biology                             |                     |
| 9. Serena Stanga*                                       | 03/06/1983          |
| Biology degree  | Female              |
| Role postdoc  | Nationality Italian |
| Expertise molecular biology, neurodegenerative diseases |                     |
| 10. Angela Coppola*                                     | 16/10/1985          |
| MD  | Female              |
| Doctorate student                                       | Nationality Italian |
| Expertise neurosurgeon                                  |                     |

^until February 2018

\*From October 2018

### 3. PRINCIPAL INVESTIGATOR

**H index 19, citations 3830 (Scopus)**

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%
2017-20	National	PI	PRIN (MIUR)	Generation of functional striatal neurons for brain repair in Huntington Disease	2015AY9A YB_002	75530	8%
2016-18	Local	PI	CRT			30000	10%
2018	Local	PI	Smarathon	SMA		14000	10%
2018	Local	PI	Atrofia spinale onlus	SMA		25000	10%
2018	Local	PI	FORB	SCI		20000	10%
Pending	National	Coordinator	PRIN (MIUR)	Targeting neuroinflammation to enhance cell therapy for Huntington Disease (NICEHUE)		Pending	8%
2019	National	Collaboration	Telethon	Identification of new druggable targets and potential therapeutic compounds for Spinal Muscular Atrophy, using a C. elegans model of neurodegeneration		28000	

Please list the name of PhDs you have supervised.

Angela Coppola  
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

22 February 2018: "Insula e neuroni di von Economo", SOPSI, Rome  
23 February 2018: My-AHA PhD meeting, Italian Society for Neuroscience, Naples.  
12 March 2018 Development, morphology and connectivity of puramidal neurons. Palermo  
12 March 2018 My-AHA Palermo

17 March 2018 Il robot: utile, empatico, amico. Brain Awareness Week, Circolo dei Lettori, Torino  
19 March 2018: Il robot. Rotary Polaris, Torino.  
12 May 2018: Digitcult presentation. Bookfair, Torino  
30 May 2018 Esperienze precoci e periodi critici nello sviluppo della corteccia cerebrale. Alba.  
16 April 2018: “*My-AHA project*” University of KAUST, Saudi Arabia  
August 28, 2018 Neuronal diversity and circuitry in cerebral cortex: pyramidal neurons. Politecnico di Torino.  
August 30, 2018: Neuronal diversity and circuitry in cerebral cortex: cortical interneurons. Politecnico di Torino.  
October 10, 2018 Il cervello che invecchia. Festival dell’innovazione. Settimo Torinese  
October 22, 2018: EU-FUNDED RESEARCH IN TECHNOLOGIES FOR A HEALTHY AND ACTIVE AGING: AN ITALIAN SUCCESS STORY A satellite event of the 2018 ISSNAF Annual Event. Italian Embassy in Washington.  
October 26, 2018: JNK: a double-edged sword in NS. Department of Neurology, UTMB Health, Galveston, USA  
November 8, 2018 Il diritto all’informazione e alla qualità della vita con lesione midollare. Coordinamento Para/Tetraplegici Torino.  
November 16, 2018 Da Superman a Schauble, le lesioni del midollo spinale. UNITRE Rivoli

- Editorial duties

Member of the Board of Editors of the Journal Digitcult  
Associate Editor of Frontiers in Ageing Neuroscience

Please list your organizational activities:

- Speakers invited

Elia Di Schiavi  
Giorgio M. Innocenti  
Luca Guglielmo Pradotto  
Serena Stanga

- Workshops, Schools or Conferences organized

Brain Awareness Week 2018, Torino  
Meetings of the My-AHA Consortium (Siegen, June 2018 and Catania, December 2018).

#### 4. PI's PUBLICATIONS:

Schellino R, Boido M, Borsello T, Vercelli A. Pharmacological c-Jun NH(2)-Terminal Kinase (JNK) Pathway Inhibition Reduces Severity of Spinal Muscular Atrophy Disease in Mice. *Front Mol Neurosci*. 2018 11:308. IF = 3.902; R = 74/261

Zamboni V, Armentano M, Berto G, Ciralo E, Ghigo A, Garzotto D, Umbach A, DiCunto F, Parmigiani E, Boido M, Vercelli A, El-Assawy N, Mauro A, Priano L, Ponzoni L, Murru L, Passafaro M, Hirsch E, Merlo GR. Hyperactivity of Rac1-GTPase pathway impairs neurogenesis of cortical neurons by altering actin dynamics. *Sci Rep*. 2018 8:7254. IF = 4.122; R = 12/64

Brancia C, Noli B, Boido M, Pilleri R, Boi A, Puddu R, Marrosu F, Vercelli A, Bongioanni P, Ferri GL, Cocco C. TLQP Peptides in Amyotrophic Lateral Sclerosis: Possible Blood Biomarkers with a Neuroprotective Role. *Neuroscience*. 2018 pii: S0306-4522(18)30214-8. IF = 3.382; R = 105/261

Boido M, De Amicis E, Valsecchi V, Trevisan M, Ala U, Ruegg MA, Hettwer S, Vercelli A. Increasing Agrin Function Antagonizes Muscle Atrophy and Motor Impairment in Spinal Muscular Atrophy. *Front Cell Neurosci*. 2018 12:17. IF = 4.300; R = 62/261

Burla R, La Torre M, Zanetti G, Bastianelli A, Merigliano C, Del Giudice S, Vercelli A, Di Cunto F, Boido M, Verni F, Saggio I. p53-sensitive epileptic behavior and inflammation in Ft1 hypomorphic mice. *Frontiers in Genetics*. ACCEPTED IF = 4.151; R = 36/171

Summers MJ, Rainero I, Vercelli AE, Aumayr G, de Rosario H, Mönter M, Kawashima R; My-AHA Consortium. The My Active and Healthy Aging (My-AHA) ICT platform to detect and prevent frailty in older adults: Randomized control trial design and protocol. *Alzheimers Dement (N Y)*. 2018 4:252-262. IF = 12.764; R = 4/197

## 5. GROUP'S PUBLICATIONS:

Piras A, Boido M. Autophagy inhibition: a new therapeutic target in spinal muscular atrophy. *Neural Regen Res.* 2018 May;13(5):813-814.

IF = 2.234 R = 183/261

Tamagno E, Guglielmotto M, Monteleone D, Manassero G, Vasciaveo V, Tabaton M. The Unexpected Role of A $\beta$ 1-42 Monomers in the Pathogenesis of Alzheimer's Disease. *J Alzheimers Dis.* 2018;62(3):1241-1245.

IF = 3.476; R = 96/261

Testa G, Staurengi E, Giannelli S, Gargiulo S, Guglielmotto M, Tabaton M, Tamagno E, Gamba P, Leonarduzzi G. A silver lining for 24-hydroxycholesterol in Alzheimer's disease: The involvement of the neuroprotective enzyme sirtuin 1. *Redox Biol.* 2018 17:423-431.

IF=6.86; R = 1/119

Crociara P, Chieppa MN, Vallino Costassa E, Berrone E, Gallo M, Lo Faro M, Pintore MD, Iulini B, D'Angelo A, Perona G, Botter A, Formicola D, Rainoldi A, Paulis M, Vezzoni P, Meli F, Peverali FA, Bendotti C, Trolese MC, Pasetto L, Bonetto V, Lazzari G, Duchi R, Perota A, Lagutina I, Quadalti C, Gennero MS, Dezzutto D, Desiato R, Boido M, Ghibaudi M, Valentini MC, Caramelli M, Galli C, Corona C, Casalone C. Motor neuron degeneration, severe myopathy and TDP-43 increase in a transgenic pig model of SOD1-linked familiar ALS. *Neurobiology of Disease.* ACCEPTED

IF = 5.227; R = 40/261

## 6. 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
Febbraio 2017- Febbraio 2019	Local	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	Local	M. Boido (Component)	Ricerca Locale 2016-2017	Nurr1, un promettente target terapeutico per la SLA		7150,16 €	8%
2016-2022	Local	M. Boido (Component)	Ricerca autofinanziata			2292,77€	8%
2017-2020	Local	M. Boido (Component)	CRT	I mitocondri nell' Atrofia Muscolare Spinale: disfunzioni e mitofagia	2017.2052	28.000 €	8%
pending	National	M. Boido (Component)	PRIN	CombinatoriALS: a polytherapeutic approach for the cure of ALS	201778XF HZ	Requested: Total 582.980€ (180.000€ for Dr Boido)	8%
pending	International	M. Boido (Component)	AFM Telethon	Development of combinatorial therapies for SMA	22346	Requested: Total 200.000€ (70.000€ for Dr Boido)	8%
pending	International	M. Boido (Proponent)	IRP Research Grant 2018	Cells meet biomedical engineering: the new frontier to boost spinal cord regeneration		Requested: 149.728 CHF	10%
pending	National	E. Tamagno (Component)	PRIN (MIUR)	Molecular heterogeneity of Alzheimer's disease		requested 306.637€	
pending	National	E. Tamagno (Component)	Ricerca Finalizzata	Identification and Evaluation of new biomarkers for the early diagnosis of Alzheimer's disease (IDEAL)		requested 78.317€	

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

- Boido M. - Best study on paediatric neurology awarded by Eu-Brain (BraYn - Brainstorming Research Assembly for YOUNG Scientists, 29-30 June 2018, Genoa)
- Schellino R. - Fellowship as selected participant at the ISN-JNC Flagship School. International Society of Neurochemistry. Alpbach, Austria. 9-16/09/2018

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), Spain (Rosa Soler, University of Lleida-IRBLLEIDA; Artero Ruben, University of Valencia)
- Invited talks
  - Boido M. Mesenchymal stem cells in neurodegenerative diseases: immunomodulation and neuroprotection. 2018 GISM Annual Meeting. Assisi, 12-13/04/2018.
  - Boido M. Nanotecnologie per le Neuroscienze. Brain Awareness Week, Turin, 13/03/2018.
  - Boido M. Lesioni spinali: le sfide della ricerca e della forza di volontà. Festival dell'Innovazione, Settimo T.se (TO), 17/10/2018.
  - Boido M. Le speranze e le certezze nel campo della rigenerazione del midollo: cosa può riferire una ricercatrice. Seminario sul diritto all'informazione e sul diritto alla qualità della vita delle persone con lesione midollare, Torino, 08/11/2018.
- Editorial duties

Please list your organizational activities:

- Speakers invited by members of the group
- Workshops, Schools or Conferences organized by members of the group
  - Boido M. Olympics in Neuroscience, regional stage, 17/03/2018 (Turin)

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

- Alternanza Scuola-lavoro: 10-days- long stages (11-21/06/2018) 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

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

Boido M., Schellino R. – Patent: International publication number: WO2018/100483A1. International publication date: 7 June 2018

## **7. Past Research activity**

### **a. Summary**

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**

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 a 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**

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 amounts 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**

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**

### *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 the pathogenesis of SMA both at spinal and muscular level. On one side, we clarified that the JNK-signaling pathway (apoptotic cascade) is involved in the SMA pathogenesis: the administration of a JNK-inhibitor peptide (DJNKI) exerted positive effects on motor neuron survival and muscular atrophy (Schellino et al., 2018). On the other side, we demonstrated that the therapeutic agrin biological NT-1654 can significantly antagonize muscle atrophy, NMJ denervation and motor impairment in SMA (Boido et al., 2018). Re ALS, we have evaluated 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 (*in preparation*).

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 (animals were housed in standard cages or enriched environment conditions, to further boost the cell integration), observing good results in terms of cell replacement, establishment of new connections and behavioral performance.

### *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 developed and tested biomimetic injectable hydrogels (chitosan) in which stem cells can be encapsulated. The in vitro and in vivo results revealed the capability of chitosan to support survival and paracrine activity of mesenchymal stem cells (paper submitted to Biomaterials Science journal).

Finally, with Prof. Dalmay, we performed a profiling of miRNA expression in a mouse model of SCI, in order to identify key-miRNAs involved in the regulation of axon growth (*in preparation*).

### *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**

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.

## 8. Future Projects (2018)

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

### a. Summary:

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 in the frail elderly subject, a group of which is undergoing physical, cognitive, psychological and social intervention to prevent conversion to mild cognitive impairment.

### b. Background and Significance:

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), , 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

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:**

*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: in collaboration with Prof. Terreno (MBC Turin) we are currently developing similar nanocarriers to label lymphocytes (B and T) and evaluate their trafficking in MS (FISM pilot grant, Dr. Boido co-applicant).

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. 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 and three-dimensional cellularized bioprinted constructs, in collaboration with Dr Tonda-Turo, Politecnico of Turin). Moreover, we are testing the ICV injection of MSC-derived exosomes in SMA mice (in collaboration with Prof. Mariotti, Univ. Verona), to counteract neuroinflammation and apoptosis, and to delay the disease progression.

Finally, 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): preliminary experiments are ongoing. 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 are now pharmacologically stimulating the Nurr1 activation in ALS mice, in order to early counteract inflammation.

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: by proteomic approach (2D electrophoresis), we are identifying specific mitochondrial proteins affected by SMA. We have also collected spinal cord samples that will be analyzed by multiSEM (3D electron microscope, in collaboration with Dr. Cali). 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, we discovered a role ubiquitin C-terminal hydrolase L1 (Uch-L1), a key neuronal enzyme involved in the elimination of misfolded proteins, in transgenic AD mouse model subjected to Bengal Rose, a light-sensitive dye that induces a cortical infarction through photo-activation. Thus, 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. Now we will further investigate this issue in cerebral stroke and ischemic injury and in particular we will confirm a connection between the decrease the Uch-L1 and neuroinflammation by studying triggering receptor expressed on myeloid cells (TREM) -2 pathway.

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

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: 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. We have started a collaboration with dr. Elia Di Schiavi (CNR, Naples), stemming from the use of *C.Elegans* as a clinical model to study new therapeutic strategies for SMA.

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.

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.



23/11

**Amerigo Pagoto**, PostDoc, Department of Molecular Biotechnology and Health Sciences, University of Turin

**NEURO-PORTRAITS - Imaging applications in CNS diseases**

20/11

**Elia Di Schiavi**, PhD - Institute of Biosciences and BioResources, IBBR  
Department of Biology, Agriculture and Food Science – CNR, Naples

**Identification of neuroprotective molecules using a C. elegans model of Spinal Muscular Atrophy**

28/9

**Jenny Sassone**, Vita-Salute University and San Raffaele Scientific Institute

**Synapse transmission as potential target in Juvenile Parkinsonism**

19/7

**Enrique Lanuza**, Universitat de València, Facultat de Ciències Biològiques - Depto. de Biología Celular

**Evolution of the amygdala (and the neural circuits for the important things)**

25/6

**Luca Guglielmo Pradotto**, MD, PhD, Division of Neurology and Neurorehabilitation  
IRCCS Istituto Auxologico Italiano, Piancavallo (VB)

**Pathogenic Mechanisms and New Molecular Therapies in Transthyretin-related Amyloidosis**

29/5

**Jeroen Pasterkamp**, Professor of Translational Neuroscience, University Medical Center Utrecht (The Netherlands) - Director MIND Facility | Chair Brain (Center Rudolf Magnus)

**Molecular mechanisms of motor neuron disease**

18/5

**Serena Stanga**, PhD - Alzheimer Research group/CEMO, Institute of Neuroscience (IoNS)  
Université catholique de Louvain (UCL) - Brussels (Belgium)

**APP and Presenilins: biological function and role in neurodegenerative and neuromuscular diseases**

10/4

**Sathya Puthanveetil**, PhD - Department of Neuroscience - The Scripps Research Institute  
Scripps Florida, USA

**From sea slugs to developing novel neurotherapeutics**

27/3

**Prof. Giorgio M. Innocenti**, Karolinska Institute, Stockholm, Sweden - EPFL, Lausanne, Switzerland

**Cortical connectivity: axonal tracing, diffusion MRI and dynamics**

23/3

**Simona Lodato**, PhD - Dep. of Biomedical Sciences, Humanitas University- Humanitas Clinical and Research Center, IRCCS – Milan

**Decoding neuronal diversity of the mammalian cerebral cortex in development, evolution and disease**

22/3

**Jeroen Pasterkamp**, Professor of Translational Neuroscience, University Medical Center Utrecht (The Netherlands) - Director MIND Facility | Chair Brain (Center Rudolf Magnus)

**Molecular mechanisms of neural circuit development**

9/3

**Alessandra Raspanti**, PhD - Lab of Cellular and Molecular Determinants of Intellectual Disabilities - Department of Neuroscience, University of Turin - Netherland Institute for Neuroscience, Amsterdam

**Development of multisensory inputs to higher order visual cortex**

22/2

Ferdinando Fiumara, MD, PhD - Department of Neuroscience, University of Turin

**Physiological and pathological roles of homopolymeric amino acid repeats**

15/2

**Serena Quarta**, Medical University Innsbruck, Austria

**The role of the inflammation mediators gp130 receptor and sphingosine-1-phosphate in peripheral neuronal regeneration**

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06 - 07 novembre 2018

**ZEISS Academy Workshop**

**Microscopia Correlativa 3D - Le ultime novità nella Microscopia Correlativa Multi-modale**

26 settembre 2018

**Vision4D - Analisi quantitativa delle immagini in 3D**

workshop applicativo con: Maurizio Abbate, ARIVIS - Prof. Ferdinando Di Cunto, NICO - UNITO

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**gennaio - novembre 2018**

## **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 **770 iscritti** (con un aumento di circa 100 un anno, grazie anche agli eventi che danno visibilità all'Istituto e creano interesse verso le sue attività).

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](#).

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### **PAGINA FACEBOOK**

[www.facebook.com/NeuroscienceInstituteCavalieriOttolenghi](http://www.facebook.com/NeuroscienceInstituteCavalieriOttolenghi)

La pagina NICO **ha raggiunto i 2667 'amici'** (crescendo di oltre 500 in un anno).

Si sottolinea che non sono state effettuate campagne a pagamento per incrementare il numero di follower della pagina che - sebbene non altissimo rispetto agli standard di Facebook (con pagine seguite da decine di migliaia di persone) - rappresenta un target selezionato e di qualità.

I post hanno una media di 1.500 – 2.000 visualizzazioni, con picchi che superano le 4-5000.

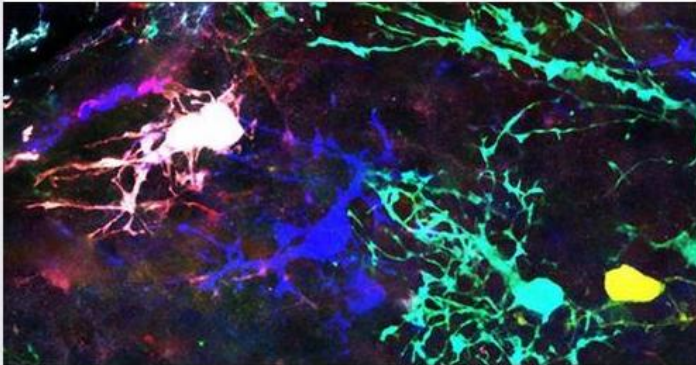
La pagina è una vetrina degli eventi e soprattutto delle news di ricerca dell'Istituto (con rimando alle pagine dedicate del sito).

Qui di seguito alcuni esempi di post pubblicati sulla pagina NICO durante l'anno, con il numero di interazioni e il totale delle persone raggiunte (visualizzazioni).



☆☆ Come nasce una stella: origine e sviluppo degli ASTROCITI, le vere "star" del cervello ☆☆☆

Lo hanno svelato per la prima volta Valentina Cerrato ed Elena Parmigiani (ora all'Università di Basilea) - le nostre ricercatrici del gruppo guidato da Annalisa Buffo - nell'articolo pubblicato sulla prestigiosa rivista PLOS Biology con Federico Luzzati Università degli Studi di Torino IRCCS Ospedale San Raffaele Università Vita-Salute San Raffaele

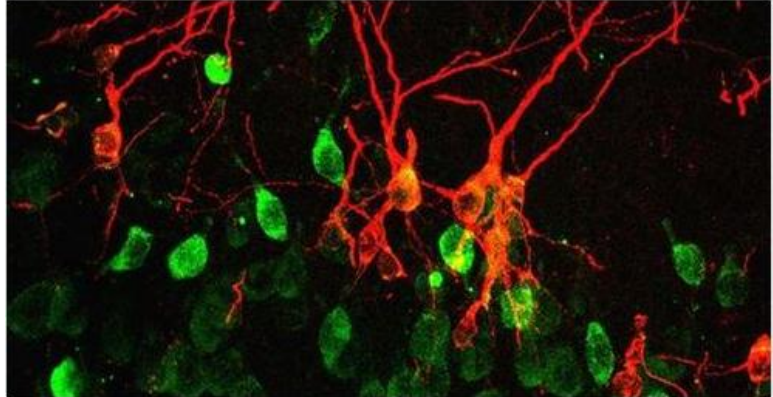


NICO.OTTOLENGHI.UNITO.IT  
**Come nasce una stella**  
Neuroscience Institute Cavalieri Ottolenghi

**4946** Persone raggiunte **499** Interazioni

Alessio Coscia, Paola Camoletto e altri 75 Condivisori: 21

Il gruppo di ricerca di Luca Bonfanti ha individuato una riserva di neuroni "immaturi" in zone inedite del cervello. Lo studio, pubblicato sul Journal of Neuroscience, apre nuovi scenari per compensare la scarsa capacità del cervello di rigenerarsi  
#NICOmetticilaFIRMA #neuroscienze #invecchiamento



NICO.OTTOLENGHI.UNITO.IT  
**Neuroni "immaturi": la riserva che può prevenire l'invecchiamento**

**2835** Persone raggiunte **240** Interazioni

Stefano Gualtierio, Piera Aimar e altri 50 Condivisori: 19

Steroidi, stress, asse intestino-cervello e #salute: da questa intersezione nasce un'entusiasmante area di studio che la #ricerca dovrà affrontare in futuro. Senza dimenticare che gli alimenti sono fonti naturali di sostanze, come i neurotrasmettitori, che possono esercitare effetti cruciali sul nostro sistema nervoso. Ne parla in due pubblicazioni il prof. GianCarlo Panzica, direttore del Dipartimento di Neuroscienze dell'Università degli Studi di Torino, qui al NICO responsabile del gruppo di ricerca di Neuroendocrinologia #NICOmetticilaFirma



NICO.OTTOLENGHI.UNITO.IT  
**Cibo, intestino e cervello**  
Una nuova frontiera per capire le malattie del sistema nervoso?

**5772** Persone raggiunte **320** Interazioni

Antonella Losa, Elena Signorino e altri 39 Condivisori: 19

**CAPIRE IL CERVELLO PER CURARLO**  
La strada per trovare una terapia per le malattie del sistema nervoso parte dalla ricerca di base: vieni a scoprirla, i nostri ricercatori ti aspettano a Orbassano (compressorio dell'Ospedale San Luigi Gonzaga) con Le Settimane della Scienza.  
L'invito è aperto a tutti, ma i posti sono limitati! Prenota subito la tua visita sul nostro sito.  
Università degli Studi di Torino #SettimanedellaScienza #ricerca #Alzheimer #SclerosiMultipla #SindromediDown #SMA #lesionispinali #invecchiamento



NICO.OTTOLENGHI.UNITO.IT  
**PorteAperte@NICO sabato 12 maggio**  
Settimane della Scienza 2018

**3764** Persone raggiunte **353** Interazioni

Antonella Losa, Elena Signorino e altri 39 Condivisori: 19

## UFFICIO STAMPA

[www.nico.ottolenghi.unito.it/ita/Press/Comunicati-stampa/\(year\)/2018](http://www.nico.ottolenghi.unito.it/ita/Press/Comunicati-stampa/(year)/2018)

I comunicati stampa (12 in totale nel 2018) sono tutti disponibili nella sezione dedicata del sito (PRESS).

I comunicati dedicati alle pubblicazioni scientifiche sono stati ripresi diverse volte con lanci di **ANSA Scienza**, e sono pubblicati spesso da **LeScienze.it** - **nella sezione DAL MONDO DELLA RICERCA** presente in home page:

**10/1/2010** - NICO, Università di Torino - Neuroni 'immaturi': ecco la riserva che può prevenire l'invecchiamento cerebrale [ [link](#) ]

**9/07/2018** - NICO/Università degli studi di Torino - Tumore al cervelletto dei bambini: silenziare la proteina citk può bloccarne la progressione [ [link](#) ]

**16/7/2018** - Università di Torino: Scoperto il gene che guida il destino delle staminali nel cervello [ [link](#) ]

**7/11/2018** - NICO, Università di Torino - Identificato un nuovo bersaglio per contrastare la SMA [ [link](#) ]

# Le Scienze

EDIZIONE ITALIANA DI SCIENTIFIC AMERICAN

Le Scienze **MIND** [microbiologia](#) [neuroscienze](#) [computer quantistici](#)

07 novembre 2018

## NICO-UNITO: Identificato un nuovo bersaglio per contrastare la SMA

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[Consiglia 106](#) [Tweet](#)

[G+](#)

[neuroscienze](#) [medicina](#) [genetica](#)

Torino, 7 novembre 2018 - L'inibizione della proteina JNK rallenta la progressione della malattia che colpisce i motoneuroni ed è la prima causa genetica di morte nell'infanzia. Lo studio, realizzato dal gruppo di ricerca guidato da Alessandro Vercelli del NICO - Università di Torino in collaborazione con Tiziana Borsello dell'Università di Milano, chiarisce uno dei meccanismi molecolari alla base della SMA e apre la strada allo sviluppo di nuove terapie.



## COMUNICATI STAMPA - 2018

9 novembre 2018

### **CAPIRE IL CERVELLO PER CURARLO - Invito a scoprire la ricerca di base in Neuroscienze**

PorteAperte@NICO sabato 24 novembre. I ricercatori del NICO - Università di Torino invitano a visitare i laboratori di Orbassano – Ospedale San Luigi Gonzaga

7 novembre 2018

### **IDENTIFICATO UN NUOVO BERSAGLIO PER CONTRASTARE LA SMA**

Su Frontiers in Molecular Neuroscience di settembre - L'inibizione della proteina JNK rallenta la progressione della malattia che colpisce i motoneuroni ed è la prima causa genetica di morte nell'infanzia. Lo studio, realizzato dal gruppo di ricerca guidato da Alessandro Vercelli del NICO - Università di Torino in collaborazione con Tiziana Borsello dell'Università di Milano, chiarisce uno dei meccanismi molecolari alla base della SMA e apre la strada allo sviluppo di nuove terapie.

12 ottobre 2018

### **COME NASCE UNA STELLA: ORIGINE E SVILUPPO DEGLI ASTROCITI, LE VERE "STAR" DEL CERVELLO**

Su Plos Biology di settembre. Svelato per la prima volta il processo che genera diversi tipi di astrociti nel cervello. Lo studio del gruppo di ricerca guidato da Annalisa Buffo del NICO - Università di Torino conferma il ruolo cruciale degli astrociti nel corretto sviluppo e mantenimento della funzionalità neuronale, e apre nuove strade per comprendere i disturbi del neurosviluppo.

16 luglio 2018

### **SCOPERTO IL GENE CHE GUIDA IL DESTINO DELLE STAMINALI NEL CERVELLO**

Il gene COUP-TFI controlla che le staminali producano la giusta quantità di nuovi neuroni o astrociti. Un eccesso dei secondi è tipico di processi infiammatori associati a patologie tra cui l'Alzheimer. La scoperta di un team guidato dalla prof.ssa Silvia De Marchis del NICO e DBIOS dell'Università di Torino

9 luglio 2018

### **TUMORE AL CERVELLETTO DEI BAMBINI: SILENZIARE LA PROTEINA CITK PUÒ BLOCCARNE LA PROGRESSIONE**

Inattivare la proteina Citron Kinase determina la morte delle cellule tumorali. Su Cancer Research i risultati del team di ricerca guidato dal prof. Ferdinando Di Cunto del NICO - Università di Torino. Lo studio, sostenuto dall'AIRC, apre nuove prospettive per la terapia del medulloblastoma.

25 giugno 2018

### **NeuroPsicoTOFest**

Dal 27 al 29 giugno Le Giornate delle Neuroscienze @UNITO: un'occasione per capire l'impatto della ricerca sulla vita di tutti i giorni.

3 maggio 2018

### **CAPIRE IL CERVELLO PER CURARLO - PorteAperte@NICO**

In occasione delle Settimane della Scienza, sabato 12 maggio i ricercatori del NICO - Università di Torino invitano a visitare i laboratori di Orbassano – Ospedale San Luigi Gonzaga

7 marzo 2018

### **SETTIMANA DEL CERVELLO 2018**

Un viaggio in 5 tappe dal 12 al 17 marzo al Circolo dei Lettori di Torino per indagare l'integrazione tra Tecnologie e Neuroscienze che sta aumentando capacità e abilità umane, rivoluzionando il campo del lavoro, della ricerca e della medicina.

23 gennaio 2018

### **NEUROCHIRURGIA E RICERCA DI BASE INSIEME PER RIPARARE LE LESIONI DEL MIDOLLO SPINALE**

L'alpinista Hervé Barmasse ospite del NICO per il battesimo del nuovo gruppo di ricerca sulle lesioni spinali

19 gennaio 2018

### **OLIMPIADI DELLE NEUROSCIENZE 2018: in Piemonte sul podio 3 studenti di Novara e Mondovì**

Preparatissimi e appassionati i 120 finalisti di 24 scuole in gara al Dipartimento di Neuroscienze dell'Università di Torino per la finale regionale.

15 gennaio 2018

### **NELLA VITA, IN MONTAGNA... E NELLA RICERCA: L'IMPOSSIBILE NON ESISTE**

#### **NEUROCHIRURGIA E RICERCA DI BASE INSIEME PER RIPARARE LE LESIONI DEL MIDOLLO SPINALE**

Il 23 gennaio la presentazione del nuovo gruppo di ricerca, con l'intervento di Hervé Barmasse l'alpinista "figlio del Cervino"

10 gennaio 2018

### **NEURONI "IMMATURI": ECCO LA RISERVA CHE PUÒ PREVENIRE L'INVECCHIAMENTO CEREBRALE**

Sul Journal of Neuroscience - Il gruppo di ricerca del prof. Luca Bonfanti del NICO - Università di Torino ha individuato una riserva di neuroni "immaturi" in zone inedite del cervello: si aprono nuovi scenari per compensare la scarsa capacità del cervello di rigenerarsi.

## **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 **2018** conta **oltre 90 articoli** (su testate nazionali e locali) dedicati a ricerche ed eventi del NICO, tra cui **1 servizio del TG scientifico Leonardo di RAI 3** e **diversi articoli su testate nazionali.**

### **Estratto della rassegna stampa/web/TV**

8 novembre 2018 - Galileo Giornale di scienza

#### **Sma, un nuovo bersaglio per contrastarla**

L'inibizione della proteina JNK rallenta la progressione della Sma, malattia che colpisce i motoneuroni ed è la prima causa genetica di morte nell'infanzia. A dimostrarlo è un studio italiano pubblicato su Frontiers in Molecular Neuroscience

The screenshot shows the Galileo magazine website interface. At the top, the logo 'GALILEO' is displayed next to the text 'Giornale di scienza - 9 novembre 2018 - Redazione | Cont'. Below the logo are navigation tabs for 'SOCIETÀ', 'SALUTE', 'AMBIENTE', and 'SPAZIO'. A 'Hot Topics' section highlights 'SPAZIO', 'INFLUENZA', 'CAMBIAMENTI CLIMATICI', and 'INTEST'. The main featured article has a blue-toned image of neural networks and the headline 'Intestino, il "secondo cervello" si rinnova ogni due settimane'. Below this are two smaller article thumbnails. The first thumbnail shows a microscopic view of cells with the headline 'Sma, un nuovo bersaglio per contrastarla' and a sub-headline '8 NOVEMBRE 2018 REDAZIONE GALILEO'. The second thumbnail shows a glowing geometric structure with the headline 'Led super efficienti in arrivo dalla perovskite' and a sub-headline '8 NOVEMBRE 2018 CLAUDIA DE LUCA'.



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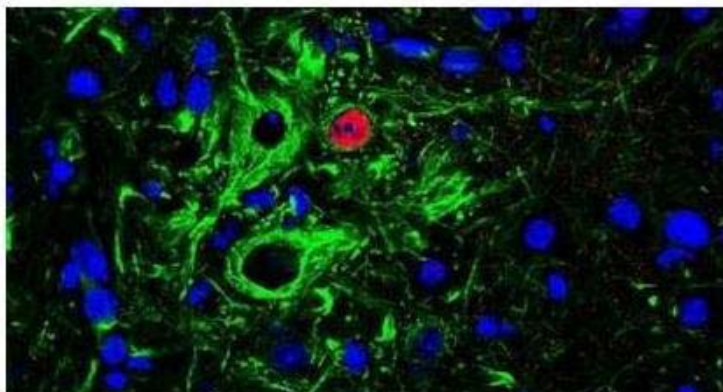
Redazione Galileo

Gli interventi a cura della Redazione di Galileo.

## Sma, un nuovo bersaglio per contrastarla

8 NOVEMBRE 2018 - REDAZIONE GALILEO - STAMPA

L'inibizione della proteina JNK rallenta la progressione della Sma, malattia che colpisce i motoneuroni ed è la prima causa genetica di morte nell'infanzia. A dimostrarlo è un studio italiano pubblicato su *Frontiers in Molecular Neuroscience*



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APRI



È considerata la più frequente causa genetica di morte nell'infanzia. L'atrofia muscolare spinale (Sma), grave patologia neurodegenerativa, caratterizzata dalla perdita dei motoneuroni del midollo spinale (le

cellule che innervano e fanno contrarre i muscoli scheletrici), causando progressiva debolezza, atrofia muscolare e complica respiratorie. A oggi, accanto alla disponibilità del medicinale nusinersen, sono in fase di sperimentazione clinica alcuni farmaci benché presentino ancora alcuni limiti, si tratta di un primo tentativo per contrastare la progressione della malattia aumentando la produzione di Smn, la proteina carente nella Sma.

### La proteina Jnk e il ruolo nella Sma

Tuttavia i meccanismi molecolari alla base di questa complessa patologia non sono ancora stati del tutto chiariti in particolare, comprendere quale tipologia di morte cellulare colpisca i motoneuroni potrebbe permettere lo sviluppo di nuove terapie. Recenti studi hanno dimostrato che la via di segnalazione mediata dalla proteina Jnk modula differenti processi neurodegenerativi (questa proteina si attiva in condizioni di stress neuronale, innescando la cosiddetta morte per apoptosi, una sorta di "suicidio programmato" della cellula) e sembra essere coinvolta nella patogenesi della Sma.



## Lo studio

Nell'articolo pubblicato sulla rivista **Frontiers in Molecular Neuroscience**, **Marina Boido** e **Roberta Schellino**, ricercatrici del gruppo guidato da **Alessandro Vercelli** del NICO – Neuroscience Institute Cavalieri Ottolenghi e Dipartimento di Neuroscienze dell'Università di Torino (affidenti all'INN – Istituto Nazionale di Neuroscienze), hanno approfondito il ruolo di **Jnk** nella malattia. In collaborazione con **Tiziana Borsello** dell'Università di Milano, Dipartimento di Scienze Farmacologiche e Biomediche e dell'IRCCS-Istituto Mario Negri di Milano, hanno somministrato un **inibitore sintetico** dell'attività di Jnk (chiamato D-JNKI1) in un modello sperimentale di **Sma**, valutandone gli effetti.

## I risultati

"L'inibizione di Jnk ha **rallentato** efficacemente la progressione della patologia: rispetto ai topi malati di controllo", spiegano Schellino e Boido, "abbiamo infatti riscontrato un significativo aumento della sopravvivenza dei **motoneuroni** e un miglioramento dell'innervazione dei **muscoli**, risultati di conseguenza più trofici. Inoltre – continuano – a riconferma di queste positive osservazioni molecolari e istologiche, le performance motorie degli animali trattati con D-JNKI1 sono risultate migliori rispetto agli animali di controllo. Infine – aggiungono le autrici dello studio – abbiamo osservato anche una parziale, ma significativa, estensione della **sopravvivenza** dei topi Sma, in seguito alla somministrazione del peptide".

## Un bersaglio per rallentare la Sma

"Questo studio, realizzato grazie al contributo della Fondazione CRT e delle associazioni Girotondo Onlus e SMARathon Onlus (che da anni sostengono il lavoro di ricerca del NICO), suggerisce quindi come l'inibizione della via di segnale di Jnk possa rappresentare una futura e valida **strategia terapeutica** per il rallentamento della **neurodegenerazione** e dell'atrofia muscolare nella Sma. La proteina Jnk e le altre molecole ad essa connesse – conclude **Alessandro Vercelli** – risultano quindi un valido bersaglio per lo sviluppo di terapie farmacologiche mirate, eventualmente in combinazione con quelle attualmente in trial".

Riferimenti: [Frontiers in Molecular Neuroscience](#); Nico, Università di Torino

15 ottobre 2018 - ANSA Scienza&Tecnica

## Così nascono le stelle del cervello

Sono gli astrociti, cruciali per la salute dei neuroni

**Sciienza&Tecnica**

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**Così nascono le stelle del cervello**  
Sono gli astrociti, cruciali per la salute dei neuroni

**L'ultimo studio di Hawking sui buchi neri**  
Tenta di capire cosa accade agli oggetti che vi cadono dentro

**Antartide, si sta staccando un altro iceberg gigante**

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BIOTECH **La prima retina umana coltivata in laboratorio**

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IN COLLABORAZIONE ASI - Agenzia

Giornale di scienza - 15 ottobre 2018 - Redazione | Contatti | Pubblicità

**GALILEO** SOCIETÀ SALUTE AMBIENTE SPAZIO

**Hot Topics** CANNABIS EBOLA TALASSEMIA TUMORE AL SENO INFERTILITÀ

RICERCA D'ITALIA

**Un orto su Marte: ecco il prototipo italiano**

11 OTTOBRE 2018  
Creare un orto su Marte: è il progetto di Luca Nardi, biotecnologo

**Astrociti vere "star" del cervello: ecco come nascono**

12 ottobre 2018 - Il Fatto Quotidiano - Scienza

## Così nascono le "stelle" del cervello: gli astrociti aiuteranno a comprendere i disturbi del sistema nervoso

Lo studio, condotto in collaborazione con l'Irccs San Raffaele di Milano, l'Istituto Cajal di Madrid e il Brain Research Institute dell'Università di Zurigo, ha svelato per la prima volta i meccanismi attraverso i quali sono generati i tre tipi di astrociti del cervelletto, centro di controllo del movimento

8 agosto 2018 - LaStampa.it - SALUTE

## Svolta contro uno dei tumori cerebrali più aggressivi tipico dell'età pediatrica

Ricercatori torinesi mettono a punto nuova tecnica per affrontare il medulloblastoma.



## Cervello, scoperto il gene-regista delle staminali

Copertina del canale Ansa.it Scienza&Tecnica e del canale Biotech

**Ai Scienza&Tecnica**  [Fai la Ricerca](#) [Vai a ANSA.it](#)

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**Cervello, scoperto il gene-regista delle staminali**  
Perché producano la giusta quantità di cellule nervose



**L'Antartide non è isolata, lo dimostra un'alga**



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17 luglio, 00:18 / [Biotech](#)

## Cervello, scoperto il gene-regista delle staminali

Perché producano la giusta quantità di cellule nervose





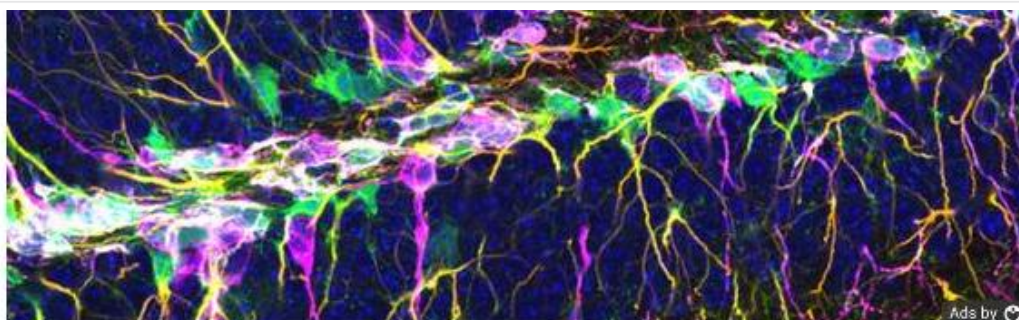
# Cervello, scoperto il gene-regista delle staminali

Perché producano la giusta quantità di cellule nervose



Redazione ANSA 17 luglio 2018 13:27

Scrivi alla redazione Stampa



Scoperto il gene che regola le cellule staminali del cervello (fonte: S. Bonzano et al., Cell Reports) © ANSA/Ansa

CLICCA PER INGRANDIRE +

È merito di un gene se le cellule del cervello vengono prodotte nella giusta quantità, senza carenze e soprattutto senza eccessi pericolosi che potrebbero portare a infiammazioni associate a gravi malattie, con l'Alzheimer. Lo ha scoperto la ricerca italiana pubblicata sulla rivista Cell Reports e coordinata dal gruppo dell'Università di Torino guidato da Silvia De Marchis, del Neuroscience Institute Cavalieri Ottolenghi (Nico) e del Dipartimento di Scienze della Vita e Biologia dei Sistemi (Dbios), in collaborazione con il laboratorio dell'Università di Nizza diretto da Michèle Studer.

Il risultato, ha osservato De Marchis, apre "interessanti prospettive per il trattamento di disfunzioni cognitive associate alle malattie neurologiche". L'esistenza di cellule staminali in alcune regioni del cervello dei mammiferi adulti è nota, ma resta molto da scoprire sui fattori che regolano la formazione di nuovi neuroni. Per questo scoprire che esiste un regista addetto a questa funzione costituisce un passo in avanti importante.

Il gene si chiama Coup-Tf1 e finora era noto per le numerose funzioni che ha nello sviluppo del cervello. Adesso la ricerca italiana ha scoperto che il suo compito consiste nel fare in modo che le cellule staminali producano la giusta quantità delle cellule nervose a forma di stella chiamate astrociti e che costituiscono la glia, ossia la struttura portante del cervello. L'attenzione dei ricercatori si è concentrata sulle cellule staminali della regione del cervello chiamata ippocampo, che ha un ruolo chiave in processi cognitivi come memoria e apprendimento.

In questa area le cellule staminali formano sia nuovi neuroni sia nuove cellule della glia e questo equilibrio assicura il corretto funzionamento dell'ippocampo. Lo studio ha inoltre messo in evidenza come l'attività del gene Coup-Tf1 risultasse ridotta "nelle staminali adulte in presenza di neuroinfiammazione", ha rilevato la prima autrice della ricerca, Sara Bonzano, e che la situazione è tornata normale ripristinandone la funzione.

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Scrivi alla redazione Stampa

12 marzo 2018 - TG Leonardo - RAI 3 [ minuto 7:30 ]

## **Hervé Barmasse e la ricerca sulle lesioni spinali**

Il famoso alpinista ha tenuto a battesimo il nuovo gruppo di ricerca sulle lesioni spinali del NICO - Università di Torino. Le interviste al neurochirurgo Diego Garbossa e alle ricercatrici del NICO Marina Boido e Annalisa Buffo.

22 gennaio 2018 - torino.repubblica.it

## **Torino, l'alpinista Barmasse sale in cattedra (di neurochirurgia)**

Torino

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### Torino, l'alpinista Barmasse sale in cattedra (di neurochirurgia)



*L'alpinista Hervé Barmasse in azione*

*Il "figlio del Cervino" in passato ha subito un intervento chirurgico che ha salvato la sua grande carriera. La sua esperienza al centro del confronto con i ricercatori dell'Istituto Cavalieri Ottolenghi che studiano come riparare le lesioni spinali*

di STEFANO PAROLA

21 gennaio 2018 - Corriere della sera - Torino

## **«La mia seconda vita sulle montagne. Oltre i limiti, grazie alla ricerca»**

Intervista a Hervé Barmasse. Una lesione ha rischiato di fermare l'alpinista, ma i medici lo hanno riportato in vetta.

10 gennaio 2018 - LaStampa.it Torino

## **Una riserva di neuroni per prevenire l'invecchiamento cerebrale**

La scoperta del gruppo di ricerca del NICO-Università di Torino apre nuovi scenari per compensare la scarsa capacità del cervello di rigenerarsi.

10 gennaio 2018 - Torino.Repubblica.it

## **Torino, il Centro di Neuroscienze dell'ateneo scopre i neuroni "antiinvecchiamento"**

Da una ricerca dell'équipe universitaria di Luca Bonfanti una speranza per la prevenzione della senescenza cerebrale

10 gennaio 2018 - ANSA Salute

## **Neuroni immaturi, la riserva che può prevenire invecchiamento. Studio apre nuovi scenari su capacità rigenerazione del cervello**





6



## Ricerca: neuroni immaturi per "prevenire" l'invecchiamento cerebrale



*Il gruppo di ricercatori dell'università di Torino ha individuato una riserva in zone inedite del cervello: si aprono così nuovi scenari per compensare la scarsa capacità di quest'organo di rigenerarsi*

ABBONATI A

Rep:



Lo leggo dopo

10 gennaio 2018

6



ESISTE una "riserva" che può prevenire l'invecchiamento cerebrale: si tratta di un insieme di neuroni "immaturi" in zone inedite del cervello. Ad individuarla il gruppo di ricerca di **Luca Bonfanti** del Nico-Università di Torino, con uno studio pubblicato sul *Journal of Neuroscience*. La riserva è stata evidenziata in modelli animali e studi ora in corso anche sull'uomo: "La scoperta - afferma Bonfanti - apre nuovi scenari per compensare la scarsa capacità del cervello di rigenerarsi".

Le ricerche sulla plasticità cerebrale hanno infatti puntato molto sulla scoperta che il cervello può generare nuovi neuroni (neurogenesi adulta). Tuttavia, oggi è noto come il fenomeno, pur presente nella specie umana, sia molto ridotto se paragonato a quanto avviene nel topo. Ci si è però accorti che esistono due tipi di neuroni "giovani": quelli generati ex novo nella neurogenesi adulta e altri che vengono prodotti prima della nascita ma rimangono in uno stato di immaturità per tempi indefiniti, in attesa di essere utilizzati. Questi neuroni immaturi sono stati osservati per la prima volta negli anni '90 da Bonfanti. Studi successivi hanno mostrato che gli stessi neuroni sono presenti anche in altre specie di mammiferi oltre ai roditori. Oggi il gruppo di Bonfanti può confermare che tutti i neuroni della corteccia cerebrale sono effettivamente immaturi e, con grande sorpresa, i ricercatori hanno scoperto che lo stesso tipo di neuroni è abbondante anche in altre regioni "inedite" del cervello, tra cui alcune importanti nella gestione delle emozioni e degli stati coscienti.

Lo studio si è avvalso di una tecnica che marca i neuroni in divisione già dalla vita fetale, usando la pecora come modello animale con aspettativa di vita estesa (15-20 anni) e cervello relativamente grande, situandosi a metà tra il topo e l'uomo. "Questi risultati - spiega Bonfanti - aprono la strada a studi nei diversi mammiferi, uomo incluso, e suggeriscono che questo tipo di plasticità potrebbe essere stato 'scelto' nel corso dell'evoluzione da specie con ridotte capacità di neurogenesi come la nostra. La possibilità di una riserva di neuroni giovani nel cervello è oggi un'allettante ipotesi per il futuro, con possibili ruoli nella prevenzione dell'invecchiamento cerebrale".

# CONFERENZE ED EVENTI DIVULGATIVI 2018

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](#) (l'edizione 2018 non si è svolta a Torino) e [News](#).



**23 gennaio 2018 - ore 11:00**  
Aula Magna dell'Istituto di Anatomia | Corso Massimo D'Azeglio 52 - Torino

**NELLA VITA, IN MONTAGNA... E NELLA RICERCA  
L'IMPOSSIBILE NON ESISTE**  
Neurochirurgia e ricerca di base insieme  
per ripare le lesioni del midollo spinale

con l'intervento dell'alpinista "figlio del Cervino"  
**HERVÉ BARMASSE**

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UNIVERSITÀ DEGLI STUDI DI TORINO  
NICO Neuroscience Institute Cavalieri Ottolenghi  
FONDAZIONE CAVALIERI OTTOLENGHI  
DIPARTIMENTO DI NEUROSCIENZE RITA LEVI MONTALCINI

Unire le forze per affrontare una delle più grandi sfide della ricerca: riuscire a riparare le lesioni del midollo spinale, che causano gravi disabilità e hanno un drammatico impatto sulla sanità pubblica. Con questo obiettivo è nato il nuovo gruppo che unisce Neurochirurgia e ricerca di base, presentato con un ospite d'eccezione, il celebre alpinista **Hervé Barmasse**.

Hanno tenuto a battesimo il nuovo gruppo di ricerca il Rettore dell'Università di Torino e Presidente della Fondazione Cavalieri Ottolenghi **Gianmaria Ajani**, il Vice Presidente della Fondazione **Paolo Bertolino** e il prof. **Giancarlo Panzica**, Direttore del Dipartimento di Neuroscienze "Rita Levi Montalcini".

Il **neurochirurgo Diego Garbossa** (Direttore della Scuola di Specializzazione in Neurochirurgia dell'Università di Torino - Città della Salute e della Scienza di Torino) e **le ricercatrici NICO Marina Boido e Annalisa Buffo** (afferenti al Dipartimento di Neuroscienze "Rita Levi Montalcini") hanno spiegato al pubblico il passaggio dall'approccio clinico alla ricerca di base sui traumi midollari e la rigenerazione nel midollo spinale.

**Hervé Barmasse, in occasione della sua visita a Torino, ha visitato l'Istituto e girato un video a sostegno alla ricerca di base svolta al NICO. Il video - disponibile sul sito e sulla pagina Facebook - è stato utilizzato per la campagna 5xmille del NICO.**



## GiovedìScienza | vendita biglietti lotteria Girotondo Onlus

In occasione di alcune conferenze del ciclo GiovedìScienza 2018 (20/1 e 15/2) 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).



## Settimana del Cervello 2018 | 12 > 17 marzo | TECNOLOGIE E NEUROSCIENZE

Organizzato da CentroScienza Onlus in collaborazione anche per questa edizione con il prof. Vercelli per la definizione del programma.

Questi gli appuntamenti che hanno visto protagonisti i ricercatori del NICO:

### 13 marzo | NANOTECNOLOGIE PER LE NEUROSCIENZE

Enzo Terreno, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino.  
Marina Boido, Dip. di Neuroscienze "Rita Levi Montalcini", NICO - Università di Torino.

### 17 marzo | IL ROBOT: UTILE, EMPATICO, AMICO

Alessandro Vercelli, Direttore del NICO e del NIT, Presidente del Consorzio INN

## giovedì 10 maggio | Salone Internazionale del Libro

### Aspettando il Festival della Scienza e dell'Innovazione di Settimo Torinese

Giovanna Ponti e Stefano Gotti (del Gruppo di ricerca NICO di *Neuroendocrinologia*) sono stati ospiti della Biblioteca Archimede con la conferenza ***Siamo ciò che mangiamo***



## sabato 12 maggio | PorteAperte@NICO con Le Settimane della Scienza 2018

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.

***Sabato 24 novembre è in programma il secondo e ultimo PorteAperte dell'anno.***

**27>29giugno2018**

## Giornate delle Neuroscienze

Dipartimento di Neuroscienze "Rita Levi Montalcini"  
Palazzo degli Istituti Anatomici | corso M. d'Azeglio 52, Torino

27/6 **Le Neuroscienze a Torino**

28/6 **Tra Neuroscienze e Psicologia: nuove prospettive**

29/6 **La neurodegenerazione:  
dove si incontrano ricerca biologica e ricerca clinica**

[neuroscienzeto.wixsite.com/neuropsico-tofest](http://neuroscienzeto.wixsite.com/neuropsico-tofest)



DIPARTIMENTO  
DI NEUROSCIENZE  
RITA LEVI MONTALCINI  
Dipartimento di eccellenza

Con il patrocinio di:



UNIVERSITÀ  
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DI TORINO



Istituto Nazionale di Neuroscienze  
Consorzio Interuniversitario di Neuroscienze



NICO  
Neuroscience Institute Cavalieri Ottolenghi

NEUROPSICO TOFEST



## 27>29 giugno | Giornate delle Neuroscienze

Considerato l'ampio coinvolgimento e la partecipazione dei ricercatori afferenti al NICO, l'Istituto ha dato sostegno alle attività di comunicazione dell'evento, promuovendolo attraverso i suoi canali.



## **14>21 ottobre | Festival dell'Innovazione e della Scienza**

L'Istituto ha partecipato attivamente all'organizzazione del programma del Festival, proponendo numerose tematiche di ricerca legate alla salute. Dalle proposte selezionate sono nati una serie di appuntamenti inseriti nel calendario del Festival:

### **martedì 16 ottobre - ore 18:00**

SETTIMO T.SE | BIBLIOTECA ARCHIMEDE | piazza Campidoglio, 50

#### **Dalle parole perse alle parole ritrovate**

prof. Alessandro Vercelli, Direttore scientifico del NICO - Università di Torino

prof. Claudio Marazzini, Presidente dell'Accademia della Crusca

### **mercoledì 17 ottobre - ore 18:00**

SETTIMO T.SE | BIBLIOTECA ARCHIMEDE | piazza Campidoglio, 50

#### **Lesioni spinali: le sfide della ricerca e della forza di volontà**

dott. Diego Garbossa, direttore della Scuola di Specializzazione in Neurochirurgia dell'Università di Torino - Città della Salute e della Scienza di Torino

dr.ssa Marina Boido, ricercatrice del NICO e Dipartimento di Neuroscienze dell'Università di Torino

dott. Marco Dolfín, Medico ortopedico e traumatologo dell'Ospedale San Giovanni Bosco di Torino

### **venerdì 19 ottobre - ore 21:00**

GRUGLIASCO | AULA MAGNA DELL'UNIVERSITÀ DI AGRARIA E VETERINARIA

a cura di *Seralmente Cultura Net* e Comune di Grugliasco

#### **Tra Geni ed Esperienza**

Prof.ssa Annalisa Buffo, Dipartimento di Neuroscienze Rita Levi Montalcini e NICO - Università di Torino

### **martedì 16 ottobre - ore 10:00**

MONCALIERI | LICEO SCIENTIFICO E. MAJORANA

#### **La plasticità cerebrale: una roba per giovani?**

Prof. Luca Bonfanti, Dipartimento di Scienze veterinarie e NICO - Università di Torino

## 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 2018 VIII edizione | Piemonte**

Organizzazione delle selezioni regionali, coordinate dalla prof.ssa Marina Boido.

Con **oltre 500 studenti di 24 scuole** da tutte le province in gara, il Piemonte si conferma una delle regioni con la maggior adesione all'iniziativa e con i migliori piazzamenti: una studentessa di Novara si è infatti aggiudicata il terzo posto alla finale nazionale ospitata dal CNR di Catania.

### **16 marzo | UniStemDay 2018 | 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).