Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2016

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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 and aims, according to the Statute, that "to deepen the existing knowledge on the interdependence between physico-chemical state of the human body and the expression of the psyche: that is, on the causes and treatment of mental insanity."

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 center for Neuroscience and chose Dr. Carlos Dotti, a foreign researcher, as scientific director. For some years, the research group of the Foundation was hosted at the San Luigi Hospital, Orbassano (Torino) while the construction of the building began in 2001 and was completed in 2009. Meanwhile, Dr. Dotti moved abroad with his group.

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

Aims of NICO

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

2) Our researchers are engaged in many activities of scientific dissemination, dedicated to the public (Open days at the NICO, Stem Cell Day and Night of Researchers, public conferences) and to high school students (Neuroscience Olympics and Scientific Summer Academy). These and other initiatives are designed both to bring young people to science, by sharing the commitment and passion that drives scientific research, both 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 toward the prevention, diagnosis and treatment of neurological disorders and in line with this principle, the research is

focused on mechanisms that govern normal neural maturation and defects involved in mental retardation syndromes.

THE COLLABORATIVE VISION AT NICO

Since its foundation in 2011, the NICO adopted a new (relative to the Departments of origin) view of sharing all facilities, supplies and instruments by all groups. Excepted for the clinical-relevant activities, which have to be performed in dedicated and isolated rooms to maintain privacy related to human material, all instruments are located in common facilities which are shared by all the members of NICO. 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 and develop multidisciplinary projects, and also acts as a whole institutional body in front of the scientific community and to the public. Finally, it represents a fundamental breakthrough to save money and to exploit the use of expensive instruments.

POSITIONING OF NICO IN THE UNIVERSITY OF TURIN

NICO is part of the University Interdepartmental center 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 and Veterinary Medicine) of the University of Turin, and, as lecturers at the schools of Biology, Biotechnology, Pharmacy, Medicine, Psychology and Veterinary Medicine, are involved in the preparation of many theses for Graduate and Master degrees. NICO collaborates with several other research centers of the University of Turin, such as the Molecular Biotechnology center, the IRCCS Candiolo and the Brain Imaging Center.

The scientific director of NICO has participated to an Institutional visit of the University of Turin and the Polytechnic of Turin to the University of Haifa, as the University representative for Neuroscience.

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

Starting from 2016, microscopy facilities at the NICO will be part of the Open Access lab program of the University of Turin.

POSITIONING OF THE NICO IN ITALY AND IN THE WORLD

NICO researchers have several international collaborations in the world, 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 also 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 and 2015 editions were organized with the administrative help of the Ottolenghi Foundation at the Teaching center of the San Luigi Hospital. 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 (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, in 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). As a result of this collaboration, a grant agreement within the Horizon 2020 program has just been signed in which the director of NICO is the coordinator.

THE NICO SPINOFF

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

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

Organization of the NICO (Neuroscience Institute Cavalieri Ottolenghi)

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

Our activities are organized into **eight groups**:
Adult Neurogenesis (PIs Luca Bonfanti and Paolo Peretto)
Brain Development and Disease (PI Alessandro Vercelli)
Clinical Neurobiology (PI Antonio Bertolotto)
Nerve Regeneration (PI Stefano Geuna)
Neurobiology of Brain Plasticity (PI Annalisa Buffo)
Neuroendocrinology (PI Giancarlo Panzica)
Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)
Neuropsychopharmacology (PI Carola Eva)

From the end 2016, **another group joined NICO**:
Embryonic Neurogenesis (PI Ferdinando Di Cunto).

Staff

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

We have a contract with a **Press Agent**, dr. Barbara Magnani, who is helping us in all dissemination activities, acting as web-manager and social promoter.

According to the Convention with the University of Turin and with San Luigi Hospital of Orbassano, the NICO hosts:
- **University staff**: 4 full professors, 4 associate professors, 8 university research assistants, 1 technician, 12 post-docs and 14 doctoral students;
- **Hospital staff**: 1 Head physician, 1 manager biologist, 4 specialists in Clinical Biochemistry, 3 post-doc fellows, 3 laboratory technicians.

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

Labs and Equipment

Molecular and cellular neurobiology
Neuroanatomy
This laboratory is equipped with numerous, excellent quality research light microscopes. We have two confocal microscopes (Leica SP5 and Nikon). A two-

photon microscope Nikon (A1MP) has just been acquired. There is also an electron microscope 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 facility.
For neurohistological studies, sliding or rotational microtomes, 3 vibratomes and 4 cryostats are available.
Animal facility
The structures dedicated to the experimental animals include rooms dedicated to farming and livestock buildings, spaces dedicated to behavioral tests and, finally, rooms equipped for surgery on rodents. The laboratory for behavioral tests is equipped with mazes and infrared camera for the behavioral analysis of locomotor activity, anxiety, depression and memory. There is also a computerized video analysis (Ethovision XT video track system) to analyze scanned images of behavioral tests.

There are spaces equipped with P2 for the use of viruses.

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

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

Clinical Neurobiology Laboratory (CNL)
The CNL offers diagnostic services and consulting for the interior (San Luigi Hospital) and external (10 departments of neurology in the region) diagnosis of multiple sclerosis.
The diagnostic tests offered include cytochemical examination of cerebrospinal fluid, immunoisoelectrofocusing to search for oligoclonal bands and several essays for the detection of viral nucleic acids. In addition, the laboratory provides a diagnostic service for neuronal paraneoplastic antibodies.

Currently the CNL is one of the few laboratories in Italy capable of providing a diagnostic service for the detection of antibodies NMO-IgG and anti-AQP4.
Finally, the CNL offers various services for monitoring patients with multiple sclerosis treated with different drugs; in this regard the lab performs a service in Italy and Europe for the serological titration of antibodies against interferon-beta (using three different methods) and natalizumab (Tysabri) potentially produced by patients treated with these drugs.
The laboratory is also equipped with a service for the evaluation of the biological activity of interferon-beta through the measurement of gene expression of specific proteins induced by interferon (such as MxA).

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

OUTREACH ACTIVITIES

From the perspective of educational and scientific dissemination the aims of NICO are:
• to promote scientific culture, and in particular knowledge of neuroscience, in high schools, through multimedia tools that reduce the economic impact of training initiatives
• provide basic skills on the normal functioning of the brain and neurodegenerative processes
• explain the importance of basic research and the impact on society of tomorrow
• 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

The NICO is engaged in scientific **activities dedicated to students** of high schools - Scientific Summer Academy, Olympic Neuroscience and Unistem Day, national and international - and to public (Researchers' Night, Open Day and Brain Awareness Week).
These activities - thanks to the network of partnerships that, starting by the University of Turin in the years has expanded throughout the country at 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) - have allowed to establish direct contacts with teachers and students of high schools.
NICO is organizing the regional competition of the World Olympics in Neuroscience (local responsible dr. Boido): 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. In 2016, 600 students, from 21 schools, participated to the regional 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).

SCIENTIFIC SEMINARS AT NICO

Over the last years, an internal committee (Annalisa Buffo and Silvia De Marchis) has been charged of the promotion and organisation of the seminar activities at NICO. The committee established a procedure according to which speakers to be invited are first proposed by NICO researchers and then selected based on a poll by all the NICO community. The committee also took the responsibility to organize the 'DISFEB meets NICO' series, agreed by the director of NICO and prof Melcangi of the Department of Pharmacological and Biomolecular Sciences-Center of Excellence on Neurodegenerative Diseases, Milan.

For invited speakers, see the attached list.

ON SITE VISIT

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Clinical neurobiology

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

Antonio Bertolotto — Birthdate (12/02/1952)
Degree: MD — Gender: M
Nationality: Italian — Phone: 00 39 011 670 66 00
Email: antonio.bertolotto@gmail.com

- **Personnel**

1. Arianna Sala — Birthdate (22/05/1972)
   Degree; 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)
   Degree; MSc and Board Certification in Clinical and Analytical Biochemistry
   Gender: F
   Role: Pharmacist — Nationality: Italian
   Expertise: relevant experience in drug immunogenicity, cell cultures, gene expression analysis and cerebrospinal fluid analysis. She is in charge of an Italian Service for the detection of anti-Interferon and anti-Natalizumab antibodies in multiple sclerosis patients. Currently her studies is focused on personalization of therapy and identification of new biomarkers to establish the efficacy of treatment.

3. Fabiana Marnetto — Birthdate (14/12/1980)
   Degree; 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)
   Degree: MSc and Master in *Medical and Genomic Statistics* Gender: F
   Role: Medical Biotechnologist Nationality: Italian
   Expertise: Molecular biology, data management, gene expression and genotype data analysis, biostatistics

5. Francesca Montarolo Birthdate (14/05/1983)
   Degree: 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)
   Degree: MSc, PhD in Molecular and Experimental Pathology and Board Certification in Clinical and Analytical Biochemistry Gender: F
   Role: Medical Biotechnologist Nationality: Italian
   Expertise: Previous research activity concerned the investigation of the molecular mechanisms underlying the physiological pathological neuronal aging in "in vitro" primary neuronal and glial cultures and in vivo mice models and disease biomarkers research in biological fluids (cerebrospinal fluid and serum) through the application of proteomics and biochemical techniques. Her current research activity is relates to the molecular mechanisms involved in the pathogenesis of multiple sclerosis (MS). In particular this research is carried on performing gene and protein expression analysis in peripheral blood mononuclear cells or in sub-population isolated from whole blood obtained from patients and healthy controls; immunohistochemically and immunofluorescence analysis in post-mortem MS human brain tissues and in the EAE mouse models of MS.

7. Michela Spadaro Birthdate (10/03/1975)
   Degree: MSc and PhD in in Immunology and Cellular Biology
   Gender: F
   Role: Biologist Nationality: Italian
   Expertise: Technical skills to work "in vivo" with experimental murine model and human samples to explore the immune mechanisms underlying multiple sclerosis

pathology by flow cytometry and functional assays, molecular biology and data management.

8. Paola Valentino Birthdate (11/08/1981)
   Degree: 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)
   Degree: 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)
    Degree: 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)
    Degree: 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.

2. PRINCIPAL INVESTIGATOR

H index, 43; Citations 5421

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

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2014-2016	National	Comp	FISM	*"Ruolo della deubiquitinasi A20/TNFAIP3 nell'immuno-patologia della SM".*	FISM code 2014/R/14	100.000	5%#
2016	National	PI	Biogen	*SERVIZIO DOMPÈ per dosaggio anticorpi anti Tysabri*	-	27.000	10%
2015-2016	National	PI	Merck	*Regulatory cells: evaluation of the effect of IFN-beta treatment in MS patients"*	-	60.000	10%
2015-2017	National	PI	Ministero Salute	*«Improving therapeutic appropiateness of Multiple Sclerosis treatments using biomolecular approaches to personalize therapy and save pharmaceutical spending»*	-	381.880	10%*
2016	National	PI	FISM	*Una banca biologica ed un laboratorio dedicati alla raccolta ed alla distribuzione di campioni biologici di SMPP, la replicazione e la condivisione di dati e la validazione di metodi biologici*	-	74.800	5%#
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%

*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.

[#]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.

Honours, prizes or awards received

Member of the jury "Neurology Merck Prize 2016"

Outreach activities:

- International collaborative experiences.

University of Munchen (anti-KIR antibodies);
Muenster (European Bio-bank and LSelectin);
MAGE (European project for antibodies anti-biological drugs);
Tel Aviv (SNPS for prevention of PML in Natalizumab treated patients)
San Francisco – Prof. Oksenberg (Evaluation of responsiveness in Fingolimod treated patients)

- Invited talks

13 XII Milano "L'induzione nel nuovo scenario terapeutico" "Excemed, Nuove terapie, nuove strategie"
24 X Venezia "Strategie di induzione: L'esperienza del CRESM su Lemtrada"
14 X Torino "ECTRIMS Highlight: focus su fingolimod" in "Socrate"
7 X Torino "PDTA" in Riunione Congiunta SIN – SNO triregionale
4 X Milano "PRIMUS Expert Panel"
7 VII Napoli "la scelta terapeutica: rischi benefici. Relazione introduttiva"
9 VI Palermo "Il trattamento della SM tra presente e futuro: induction versus escalation therapy" in "Donne in neuroscienze"
7 VI Parma "Slerosi multipla e gravidanza" in "Sesualità, Fertilità e gravidanza"
26 V Roma "A bio-bank and a laboratory devoted to the collection and supply of biological samples of PPMS, the replication and sharing of data and the validation of biological methods"
21 V Baveno Controversies in MS "All CIS must be treated" Yes
19 V Catania "Influenza delle comorbidità nella gestione della terapia della SM", 56° Convegno SNO
18 V Catania "Terapia della SM: ieri, oggi e domani"
10 V Torino "Benefit/Risk profile – posizionamento delle risorse terapeutiche nel percorso di cura del paziente con SM2 in "Socrate"
6 V Milano "Best Evidence in MS" "Le comorbidità"
28 IV Milano "IMuse: innovation in Multiple Sclerosis Education"
8-9 IV Torino "MS therapy: Past, present and future" in "Giornate Neurologiche Torinesi"
10 III Gallarate "Gestione del paziente con SM e grave disabilità"
25 II Milano "La banca biologica" in "Progetto registro italiano SM"
14-15 I Milano "Monitoraggio della terapia" in "Must: Multiple Sclerosis Trends"

- 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

Organizational activities:

- Workshops, Schools or Conferences organized

21 XI 2016 Milano "Neurologo e infermiere a confronto: analisi ed evoluzione del team in SM parte II
11 XI 2016 Pollenzo (CN) MUST Multiple Sclerosis: a Touching Story
24 VI 2016 Milano "Neurologo e infermiere a confronto: analisi ed evoluzione del team in MS" parte I
17 VI 2016 Enna MUST Multiple Sclerosis: a Touching Story
29 IV 2016 Pollenzo (CN) "La gestione della persona con SMRR: focus su efficacia e sicurezza"
5 II 2016 CRESM "Cambio di prospettiva su placebo, gravidanza e Devic"

3. PI's PUBBLICATIONS:

Valentino P, Marnetto F, Granieri L, Capobianco M, Bertolotto A. (2016). Aquaporin-4 antibody titration in NMO patients treated with rituximab: A retrospective study. Neurol Neuroimmunol Neuroinflamm. 4(2):e317. IF= not available; R = not available

Malucchi S, Capobianco M, Lo Re M, Malentacchi M, di Sapio A, Matta M, Sperli F, Bertolotto A. (2016). High-Risk PML Patients Switching from Natalizumab to Alemtuzumab: an Observational Study. Neurol Ther. [Epub ahead of print]. IF= not available; R = 187/322

Chiavazza C, Cistaro A, Fania P, Bertolotto A, Cavalla P, Rudà R, Pinessi L, Soffietti R. (2016). Reversible disconnection syndrome in a case of acute tumefactive demyelinating lesion: a PET study. Neurol Sci. 37(12):2019-2023. IF= 1.749; R = 812/2156

Marola S, Ferrarese A, Gibin E, Capobianco M, Bertolotto A, Enrico S, Solej M, Martino V, Destefano I, Nano M. (2016). Anal sphincter dysfunction in multiple sclerosis: an observation manometric study. Open Med (Wars) 11(1):509-517. IF= 0.294; R = 403/2156

Montarolo F, Perga S, Martire S, Navone DN, Marchet A, Leotta D, Bertolotto A. (2016). Altered NR4A Subfamily Gene Expression Level in Peripheral Blood of Parkinson's and Alzheimer's Disease Patients. Neurotox Res. 30(3):338-44. IF= 2.942; R = 21/108

Waters P, Reindl M, Saiz A, Schanda K, Tuller F, Kral V, Nytrova P, Sobek O, Nielsen HH, Barington T, Lillevang ST, Illes Z, Rentzsch K, Berthele A, Berki T, Granieri L, Bertolotto A, Giometto B, Zuliani L, Hamann D, van Pelt ED, Hintzen R, Höftberger R, Costa C, Comabella M, Montalban X, Tintoré M, Siva A, Altintas A, Deniz G, Woodhall M, Palace J, Paul F, Hartung HP, Aktas O, Jarius S, Wildemann B, Vedeler C, Ruiz A, Leite MI, Trillenberg P, Probst M, Saschenbrecker S, Vincent A, Marignier R. (2016). Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. J Neurol Neurosurg Psychiatry 87(9):1005-15. IF= 7.349; R = 4/372

Schwab N, Schneider-Hohendorf T, Pignolet B, Spadaro M, Görlich D, Meinl I, Windhagen S, Tackenberg B, Breuer J, Cantó E, Kümpfel T, Hohlfeld R, Siffrin V, Luessi F, Posevitz-Fejfár A, Montalban X, Meuth SG, Zipp F, Gold R, Du Pasquier RA, Kleinschnitz C, Jacobi A, Comabella M, Bertolotto A, Brassat D, Wiendl H. (2016). PML risk stratification using anti-JCV antibody index and L-selectin. Mult Scler. 22(8):1048-60. IF= 4,840; R = 56/322

Borghi M, Carletto S, Ostacoli L, Scavelli F, Pia L, Pagani M, Bertolotto A, Malucchi S, Signori A, Cavallo M. (2016). Decline of Neuropsychological Abilities in a Large Sample of Patients with Multiple Sclerosis: A Two-Year Longitudinal Study. Front Hum Neurosci. 10:282. IF= 3.209; R = 7/66

Troni W, Melillo F, Bertolotto A, Malucchi S, Capobianco M, Sperli F, Di Sapio A. (2016). Normative Values for Intertrial Variability of Motor Responses to Nerve Root and Transcranial Stimulation: A Condition for Follow-Up Studies in Individual Subjects. PLoS One. 11(5):e0155268. IF= 3.54; R = 12/163

Rosato R, Testa S, Bertolotto A, Confalonieri P, Patti F, Lugaresi A, Grasso MG, Toscano A, Giordano A, Solari A. (2016). Development of a Short Version of MSQOL-54 Using Factor Analysis and Item Response Theory. PLoS One. 11(4):e0153466. IF= 3.54; R = 12/163

Martire S, Navone ND, Montarolo F, Perga S, Bertolotto A. (2016). A gene expression study denies the ability of 25 candidate biomarkers to predict the interferon-beta treatment response in multiple sclerosis patients. J Neuroimmunol. 292:34-9. IF= 2.720; R = 62/322

Bertolotto A, Malentacchi M, Capobianco M, di Sapio A, Malucchi S, Motuzova Y, Pulizzi A, Berchialla P, Sperli F. (2016). The use of the 25 Sprotte needle markedly reduces post-dural puncture headache in routine neurological practice. Cephalalgia. 36(2):131-8. IF= 3,609; R = 113/2156

Hegen H, Adrianto I, Lessard CJ, Millonig A, Bertolotto A, Comabella M, Giovannoni G, Guger M, Hoelzl M, Khalil M, Fazekas F, Killestein J, Lindberg RL, Malucchi S, Mehling M, Montalban X, Rudzki D, Schautzer F, Sellebjerg F, Sorensen PS, Deisenhammer F, Steinman L, Axtell RC. (2016). Cytokine profiles show heterogeneity of interferon-β response in multiple sclerosis patients. Neurol Neuroimmunol Neuroinflamm. 3(2):e202. IF= not available; R = not available

Carletto S, Borghi M, Francone D, Scavelli F, Bertino G, Cavallo M, Malucchi S, Bertolotto A, Oliva F, Ostacoli L. (2016). The efficacy of a Mindfulness Based Intervention for depressive symptoms in patients with Multiple Sclerosis and their caregivers: study protocol for a randomized controlled clinical trial. BMC Neurol. 16:7. IF= 2,006; R = 307/2156

4.GROUP's PUBBLICATIONS:

Most relevant publications of the other members of the group in 2016.

Pellegrino RM, Boda E, Montarolo F, Boero M, Mezzanotte M, Saglio G, Buffo A, Roetto A. (2016). Transferrin Receptor 2 Dependent Alterations of Brain Iron Metabolism Affect Anxiety Circuits in the Mouse. Sci Rep. 6:30725. IF= 4.259; R = 4/77

5. GROUP's additional information:

List of honours, prizes or awards received by other members of the group.

Dr. Martire:
- Travel grant, XXV National AINI Congress. Lecce, Italy (2016)

Outreach activities of other members of the group:

- Invited talks

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 (3-5/05/2016).

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 all over Piedmont centers. 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's important to provide an important research tool. In 2016, 288 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 (573 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 2016, 614 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 (490 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 (97 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

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. We demonstrated that this impairment was mainly due to monocytes. Thus, we first aimed to elucidate how an altered expression of these genes could influence MS pathogenesis both in myeloid 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.

The aims of our study were:

1. To dissect the role of TNFAIP3 and NR4A2 in monocytes, MO and DCs. This issue was firstly addressed taking advantage of primary monocytes isolated from healthy donors' buffy coats (BCs). In order to simulate the pathological condition, we started to set up small interfering RNA (siRNA) experiments against TNFAIP3 and NR4A2. Normal and silenced monocytes were stimulated in vitro to generate pro- and anti-inflammatory MO and mature DCs (mDC). Cellular behaviour was evaluated by monitoring a panel of functional and phenotypical markers.
2. To examine the localization of TNFAIP3 and NR4A2 in normal and MS human post-mortem brain. We aimed to compare TNFAIP3 and NR4A2 protein and gene expression in both active and chronic active lesions and normal appearing areas from white and grey matter 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.
3. To investigate the impact of reducing TNFAIP3 expression in a mouse model of MS. To this aim we used transgenic mice lacking TNFAIP3 in myeloid-derived cells including microglia or only in mDCs. These mice were immunized with the myelin oligodendrocyte glycoprotein (MOG_{35-55}) to induce the EAE, the best model of chronic MS. The role of NR4A2 in the MS murine model has been previously investigated by our group and results are already published.

e. Results (4000 characters)

1. We characterized the primary culture of immature DC (iDC), mDC, M1 and M2 macrophages obtained by CD14+ monocytes isolated from BCs.
   We performed time-course experiments to evaluate the protein level of TNFAIP3 and NR4A2 during the maturation of DC and MO. We observed that these molecules were low or not expressed in the immature DC and MO, but were rapidly induced after maturation in mDC and M1 cells. These results were confirmed by real-time PCR analysis. A preliminary investigation demonstrated the modulation of the NF-

kB, STAT1and JNK pathways in mDC and of STAT1 and p38 MAPK kinases in M1 macrophages. SiRNA experiments are still ongoing.

2. 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.
   We demonstrated that TNFAIP3 is present in control human brain tissues in both white matter, in highly ramified cells and in grey matter, in morphologically-like neuronal cells. In MS brain, we observed a massive TNFAIP3 expression in the lesions in both perivascular infiltrates and ramified cells. In particular, in active and pre-active lesions, TNFAIP3 is expressed in the active core, whereas in chronic active lesions, TNFAIP3 is mainly expressed on the active-margin. Preliminary double immunofluorescence staining unveiled that TNFAIP3 is expressed infiltrating macrophages and by resident astrocytes and a subpopulation of microglial cells. Studies on NR4A2 are ongoing.
3. We obtained the TNFAIP3lox/lox mice from the no-profit RIKEN BioResource Center (Japan). In parallel, we purchased from the Jackson laboratory (USA) the transgenic Cre recombinase mice to remove the target gene from myeloid cells including microglia (Cx3Cr1-cre) and dendritic cells (Cd11c-cre). By crossing these mice we generated TNFAIP3lox::Cx3cr1-Cre and TNFAIP3lox:cd11c-Cre mice, respectively.
   The TNFAIP3lox::Cx3Cr1 transgenic mouse has never been generated before. For this reason, we aimed to characterize in detail the phenotype of this mouse model. These mice are viable, but, although the heterozygous are fertile, the homozygous born with difficulties and have a mortality rate of 50% between the first and second month of life. Moreover, we observed that the reduction of TNFAIP3 in myeloid cells was related to weight reduction. Anatomic-pathological studies on this mouse model are ongoing.
   The TNFAIP3lox::CD11c conditional mouse model has been previously characterize and have just begun the EAE experiments on the heterozygous and homozygous mice.

f. Advancement in the field

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

2. BIOMARKERS FOR MULTIPLE SCLEROSIS

2.1. Anti-KIR4.1 Antibodies in MS patients

a. Summary

KIR4.1 antibodies were specifically found in a subset of MS patients. This could have a significant impact on disease management. The aim of this study was to verify this finding. The procedure for isolating pure KIR4.1 is complex: high variability in the assay was observed. Thus, stringent criteria were established in order to identify sessions in which the pure KIR4.1 was isolated: this allowed us to detect KIR4.1 antibodies in 28% of MS patients and 5% of HCs.

b-d. Background, rationale and objectives

The presence of KIR4.1 antibodies has been proposed to be a characteristic of MS. This could have a significant impact on disease management. However, the validation of the initial findings has failed till date. Conflicting results have been attributed to difficulties in isolating the lower-glycosylated (LG) KIR4.1 expressed in oligodendrocytes, the putative target antigen of autoantibodies.
The aim of this study is to verify the presence of KIR4.1 antibodies in MS patients, by independently replicating the originally-described procedure.

e. Results
Assay procedure consisted of KIR4.1 expression in HEK293 cells, 3-step elution to isolate LG-KIR4.1 in elution fraction 3, and ELISA. Sera of 48 MS patients and 46 HCs were studied.
In a preliminary analysis, we observed different KIR4.1 antibody levels between MS patients and Healthy Controls (HCs): however, a high variability across working sessions was observed and the sensitivity of the assay was very low. Thus, stringent criteria were established in order to identify working sessions in which the pure LG-KIR4.1 was isolated: applying these criteria, we detected LG-KIR4.1 antibodies in 28% of MS patients and 5% of HCs.

f. Advancement in the field
We propose further efforts towards a uniform method to establish the detection of KIR4.1 antibodies in MS patients. We are actually working at this objective.

2.2. Titration of Anti-AQP4 Antibodies in NMOSD patients

a. Summary
We studied the usefulness of AQP4 antibodies titration in the management of NMO patients treated with (RTX. 322 serum samples from 7 NMO patients were tested by a cell-based assay. AQP4-Ab titres correlated with the disease activity showing higher titres during and preceding relapses than during remission. A reduction of AQP4-Ab titers in short-term and long-term period was observed during RTX treatment. Reduction of AQP4-Ab titres was specifically observed in responder patients.

b-d. Background, rationale and objectives
The detection of anti- AQP4-Abs is a milestone in the diagnosis of Neuromyelitis Optica (NMO). RTX is widely used to prevent relapses. We undertook an observational retrospective study to investigate the usefulness of AQP4-Ab titration in the management of NMO patients treated RTX by studying 1) the correlation between AQP4-Ab titre and disease activity, 2) the influence of RTX on antibody levels, 3) the association between AQP4-Ab levels and responsiveness to RTX .

e. Results
A cell-based assay was used for AQP4-Ab titration in 322 serum samples from 7 NMO patients treated with RTX (median follow-up 65 months), according to a treatment-to target approach. Serum samples were collected every month following standardized procedures. Results:

1. In group analysis, AQP4-Ab titres correlated with the disease activity showing higher titres during and preceding relapses than during remission. However, in individual analysis, an increase in AQP4 Ab titres and CD19+ B cells did not always precede a relapse.
2. A reduction of AQP4-Ab titers in short-term and long-term period was observed during RTX treatment.

3. Reduction of AQP4-Ab titres was observed in responder patients both 3 months after RTX infusion, and in the long-term follow up. In one non-responder patient AQP4-Ab levels never decreased during the treatment period.

f. Advancement in the field

Titration of AQP4-Abs could be useful in the clinical management of NMO patients treated with RTX providing information about responsiveness to RTX.
As described by other studies, the decrement of AQP4-Ab levels may be due to the mechanism of action of RTX, that may lead over time to decreased production of immunoglobulins G and M (IgG and IgM). This may predispose the patient to hypogammaglobulinemia and associated infections. For this reason we decided to continue the study with the evaluation of total IgG and IgM. (see Future Projects).

3) CRESM BIOBANK

a. Summary

Biological research suffers from poor reproducibility of published data, because of the lack of rigor in the collection of biological samples, the insufficient validation of the methods and limited sharing of data. In a chronic disease such as Primary progressive (PP) MS there are two further obstacles: the small number of patients, and the need of careful collection of clinical, biological, MRI, neuro-cognitive and neurophysiological data. This project aimed to address these problems by joining the activity of the bio-bank (BB) and of the Clinical Neurobiology Lab of CRESM that overlooks 1800 patients with MS, of which 250 PPMS patients.

b. Objectives

1. Expansion of the BB by collecting different biological samples from PPMS patients, others types of MS and controls, with paired clinical and laboratory data.
2. Distribution of aliquots of the samples to projects funded by the IPMSA (or other institutions)
3. Researcher receiving samples from the BB will be committed to providing the bio-bank with detailed protocols of the methods used in the research, and sharing raw data of his experiments with the bio-bank so that could be available for others studies.
4. Technical support from the lab of CRESM i) co-validation of methods; ii) replication of data obtained by researchers who have used biological samples of the BB, iii) implementation of educational courses for technicians/biologists
5. Cooperation with other bio-banks.

c. Results

- Establishment of sampling time points during follow up of treated MS patients Improvement of the procedures for samples collection. A laboratory technician (supported by FISM 2014/PMS/1 funding), is in charge to handle the patients'samples.
- Improvement of the procedures for samples storage and data managing: a complete bio-banking system for samples storage and data collection was bought.
- Enlargement of the location in which the biobank is housed: two deep-freezer have been bought.
- Increase of the amount of stored samples: in 12 months, 885 blood samples have been collected and stored in CRESM bio-bank, joining the more than 1500 samples previously collected .
- Distribution of samples to 1 Italian and 3 European laboratories. In addition, 2 laboratories requested the samples and another group requested the availability of samples to submit a FISM project. 4 projects from the PI research group have been realized using BB samples.

- Cooperation with a European bio-bank network (Germany, England, Spain, Denmark, Sweden, Poland) collecting samples from MS patients treated with the new generation of monoclonal antibodies.
- Collection of RNA in different kind of tubes to evaluate the influence of pre-analytical and handling conditions.
- Improvement of extracted RNA quality using an automatic instrument.
- Validation of results obtained by other laboratories.
- Collaboration with a bioethicist to revise the existing Informed Consent.
- Establishment of a procedure to obtain biological material from CReSM BB.

d. Advancement in the field (1000 characters)

The collection and distribution of samples and is ongoing. FISM is actually evaluating the CReSM proposal to re-fund this project

7. Future Projects (Next 3 years)

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

BIOMARKERS FOR TREATMENT RESPONSE IN MS

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

a. Summary:

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

b. Background and Significance:

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

c. General aim and integration with mission of the Institute

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

d. Specific objectives and strategies:

1) To analyze gene expression level of NR4A2 in blood obtained from healthy controls (HC), treatment naïve and FTY720 treated MS patients by using Real Time PCR technique. . Interesting results will be corroborated by protein expression analysis using the western blot technique in a smaller cohort of subjects. The association between RNA levels and clinical and demographical parameters, will be analyzed.

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

e. Unique features of the project research:

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

2) «Improving therapeutic appropiateness of Multiple Sclerosis treatments using biomolecular approaches to personalize therapy and save pharmaceutical spending»

a) Summary

Non-responding patients may undergo irreversible disease progression and severe outcomes during ineffective treatments; the cost of failed treatment puts tremendous pressure on public funds. We propose a strategy to improve therapeutic appropriateness by using biological approaches:
1. early identification of biological non responders (NRs)
2. better timing of drug administration,
3. study of the relationship between T-cells adhesion-molecule expression and the risk to develop adverse events during treatment.

b) Background and Significance

Total expense for MS patients in Italy 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. A new IFNb formulation (IFNb PEGylated) is now available and its immunogenicity, biological activity and cross-reactivity with old IFNbs 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 RTX (and other anti-CD20 drugs). 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-d) General aim , specific objectives and startegies

Biological methods to evaluate biological activity of a drug and to analyse biomarkers identifying NR patients can improve allocation of a large amount of pharmaceutical spending.

1. Early detection of NRs. Detection and titration of Antibodies against NAT and RTX will be performed. The biological activity of PEG-IFNb will be tested by gene expression analysis. Biological data will be correlated with the responsiveness to the treatment measured by EDSS, MRI and clinical activity. An economic analysis will be performed.
2. Optimization of dose and time of infusion of NAT and RTX. 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.
3. To analyze CD62L expression in T-cells to detect the risk to PML. The risk of PML limits the use of the highly effective NAT and PML early detection and management are very expensive.

f) Methodology: <u>please fill-out this section only in the case of innovative technologies</u>

Droplet digital PCR (DDPCR) will be used for gene expression analysis. DDPCR allows absolute quantification of the number of copies of the specific RNA in the reaction with higher sensitivity than traditional Real Time PCR.

3) "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

With this study we propose to study the biological activity of Peg-IFN and IFNbeta non pegylated to compare the two drugs in terms of mechanism of action, cellular populations involved, evaluation and identification of biomarkers of response to the treatment.

b) Background and Significance

Several products containing IFNb were already approved for 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 one (Avonex) although it is administrated every 2 weeks instead of ones a week. The exact mechanism of action of IFNb in MS is not completely known. To better understand the mechanism of action of a drug it is necessary to study its biological activity. The measurement of IFNb biological activity can allow the identification of the subset of patients who are non-responsive to the drug. Till now, the biological activity of IFNb has been studied by measuring a number of Interferon Stimulated Genes (ISGs) at protein or mRNA level by real-time PCR

c) General aim , specific objectives and startegies

This 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 Next-Generation Sequencing (NGS) technology. Data obtained from this pilot study can help to better understand IFNb pharmacodynamic, which cellular subset is most influenced by treatment and the efficacy of treatment for every single patient.

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

f) Methodology: <u>please fill-out this section only in the case of innovative</u>

The use of NGS technology represent an innovation of the present project. In particular RNA seq (RNA sequencing) represent the most current and reliable method to evaluate ISGs levels revealing a snapshot of RNA presence and quantity from a genome at a given moment in time.

4) "Side effects of long-term rituximab treatment in NMOSD patients: hypogammaglobulinemia and impairment of specific humoral immunity."

a) Summary

RTX is used in the treatment Neuromyelitis Optica Spectrum Disorders (NMOSD). Major side effects including hypogammaglobulinemia (Hypo-Ig) have been reported after a prolonged treatment. We aim at evaluating the long-term effects of RTX in NMOSD patients on total IgG, IgA and IgM levels and on levels of different pathogen-specific serological antibodies.

b) Background and Significance

RTX is an effective therapy in many autimmune disease and in NMOSD as well. Patients of several hematological and rheumatological diseases demonstrate side effects including hypogammaglobulinemia after a prolonged treatment with RTX. However, there are no such studies with long and detailed follow up in patients of neurological disorders treated with RTX.

Treating physicians must consider that prolonged treatment could lead to hypogammaglobulinemia and infections.

c-d) General aim , specific objectives and strategies

1. To evaluate the long-term effects of RTX on total Ig, pathology-specific IgG (AQP4 IgG) and humoral immunity-related Ig (anti-tetanus IgG, anti-Varicella zoster IgG, anti-EBV IgG) in the serum of NMOSD patients
2. To evaluate the relationship between total IgG and AQP4-IgG during RTX treatment
3. To confirm our previous data regarding the usefulness of AQP4 titration in a larger cohort

Data obtained in this study could provide neurologists important indications for the daily management of NMOSD patients. In addition, data obtained in this project could give important information about treatment with anti-CD20 drugs in other autoimmune neurological disorders such as Multiple Sclerosis.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Adult neurogenesis

1. LABORATORY DESCRIPTION – PERSONNEL:

NOTE: Since the birth of NICO (in 2010), the group **Adult Neurogenesis** was created from two independent research groups (already working and collaborating in Turin since 1994), which joined their expertise on structural plasticity and neurogenesis. Since then, the group at NICO has been organized with two PI coordinating four main, distinct but complementary, research lines.

- **Principal Investigator 1**

Paolo Peretto — Birthdate: (18 september 1963)
Degree: Associate professor — Gender: M
Nationality: Italian — Phone: 011 6706605
Email: paolo.peretto@unito.it

- **Principal Investigator 2**

Luca Bonfanti — Birthdate: (19 may 1962)
Degree: Associate professor — Gender: M
Nationality: Italian — Phone: 011 6706606
Email: luca.bonfanti@unito.it

- **Personnel**

1. Silvia De Marchis — Birthdate (14/09/66)
   Degree: Associate professor — Gender: F
   Role: Senior researcher — Nationality: Italian
   Expertise: in vivo and in vitro molecular and cellular analyses

2. Federico Luzzati — Birthdate (20/10/1974)
   Degree: Assistant professor — Gender: M
   Role: Senior researcher — Nationality: Italian
   Expertise: in vivo morphological analyses and 3D reconstructions

3. Sara Trova — Birthdate (25/04/89)
   Degree: PhD student (third year) — Gender: F
   Role: researcher — Nationality: Italian
   Expertise: behavioural aspects of AN in the olfactory system

4. Sara Bonzano — Birthdate (22/03/87)
   Degree: Postdoc — Gender: F
   Role: researcher — Nationality: Italian
   Expertise: cellular and molecular analyses of AN in the hippocampus

5. Chiara La Rosa — Birthdate (01/07/88)
   Degree: PhD student — Gender: F
   Role: PhD student (second year) — Nationality: Italian
   Expertise: comparative analyses of AN in wild mammals

6. Isabella Crisci — Birthdate (17/12/89)
   Degree: PhD student (first year) — Gender: F
   Role: just starting PhD — Nationality: Italian
   Expertise: cellular and molecular analyses of AN in the hippocampus

2. PRINCIPAL INVESTIGATOR

2a. PRINCIPAL INVESTIGATOR 1 (Paolo Peretto)
2b. PRINCIPAL INVESTIGATOR 2 (Luca Bonfanti)

H index, 29; Citations, 3301
H index, 26; Citations: 2393

Relevant discoveries:
- Evidence that opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis
- Evidence that SVZ is not neurogenic in dolphins, and it contains differentiated neurons

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Availabl e to NICO
Jan 2017 - Jan 2019	National	PI Local Unit Coordi nator (LB)	PRIN - MIUR (bando 2015)	A new non-invasive approach to the investigation of cerebral activity in domestic animals using functional near-infrared spectroscopy	2015Y5 W9YP	€ 32.000	8%
2016-17	National Local research (UNITO)	Coordi nator (LB)	University of Turin	Popolazioni di neuroni immaturi nell'amigdala e capsula esterna della pecora adulta	BONL_ RILO_16 _01	€ 6.000	8%
2016-17	National Local research (UNITO)	Coordi nator (PP)	University of Turin	Regolazione della neurogenesi adulta nell'interazione con l'ambiente	PERP_R ILO_16_ 01	€ 4.665	8%
Pending	Internati onal	Coordi nator (LB)	National Geographi c Foundation	Identification of brain structural plasticity in mammals: is there a trend?	243181	$ 25.000	
Pending	National (Local)	Coordi nator (PP)	Fondazion e CRT (Cassa di Risparmio di Torino)	Corsa, benessere psico-fisico e corretti stili di vita	CorBios	€ 50.000	

Name of supervised PhDs.

Roberta Parolisi (degree in 2016, February)
Chiara La Rosa (first year) (LB)
Giulia Nato (degree in 2016, January)
Sara Trova (third year) (PP)

Outreach activities

- International collaborative experiences.

- 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*
- CNRS, UMR6175, F-37380 University of Tours, Nouzilly, F (Dr. Frederic Levy) (LB)
- Institute of Anatomy, University of Zurich, CH (Dr Irmgard Amrein) (LB) *Comparative aspects of brain structural plasticity in mammals*

- Invited talks

- Neurogenic niches: theme and variations (Stem cells, work in progress; Università di Pavia, Laurea in Neurobiologia e dottorato di ricerca) (LB)
- Neurogenesi adulta nei mammiferi: in cerca di una logica (Università di Milano, Dip. Scienze farmacologiche) (LB)

- Editorial duties

In the Editorial Board of international journals:

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

Guest Editor for Special Issues:

- Adult neurogenesis: beyond rats and mice (2016) *Front. Neurosci*. (with I. Amrein) (LB)

Organizational activities:

- Speakers invited
  Alice Powers (Stonybrook University, USA) may 2016 (LB)
  Paolo Forni (University of Albany, USA) december 2016 (PP)
- Workshops, Schools or Conferences organized (each year, since 2010: organization of *UNISTEM day* in Turin - National event on research, science and stem cells, involving university researchers and students of the secondary schools) (LB)
- In the scientific board of the Meeting "*More than neurons*", Turin, november 2016 (LB)

3. PI's PUBBLICATIONS:

Bonfanti L. (2016) Adult Neurogenesis 50 Years Later: Limits and Opportunities in Mammals. *Front Neurosci.* 10:44.
IF=3.4; R=88/256

Lipp H.P., **Bonfanti L**. (2016) Adult neurogenesis in mammals: Variations and confusions. *Brain Behav Evol*. 87(3):205-221.
IF=2.1; R=32/51

Schellino R, Trova S, Cimino I, Farinetti A, Jongbloets BC, Pasterkamp RJ, Panzica G, Giacobini P, De Marchis S, **Peretto P**. (2016). Opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis. *Sci Rep.* 26;6:36063.
IF= 5,228; R=7/63

Bonzano S, Bovetti S, Gendusa C, **Peretto P**, De Marchis S. (2016). Adult born olfactory bulb dopaminergic interneurons: Molecular determinants and experience-dependent plasticity. *Front Neurosci.* 6;10:189.
IF= 3,4; R=88/256

4.GROUP's PUBBLICATIONS:

Fornasari BE, El Soury M, De Marchis S, Perroteau I, Geuna S, Gambarotta G. (2016). Neuregulin1 alpha activates migration of neuronal progenitors expressing ErbB4. *Mol Cell Neurosci.* 77:87-94.
IF=3,597; R=78/256

Casoni F, Malone SA, Belle M, Luzzati F, Collier F, Allet C, Hrabovszky E, Rasika S, Prevot V, Chédotal A, Giacobini P. (2016) Development of the neurons controlling fertility in humans: new insights from 3D imaging and transparent fetal brains. *Development* 143(21):3969-3981.
IF= 6; R=4/41

5. GROUP's additional information:

Grants (current and pending) of the other members of the group

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Availabl e to NICO
2016-17	National Local reseach (UNITO)	Coordinator (De Marchis)	University of Turin	Ruolo di CUPF1 nello sviluppo cerebrale	DEMS_ RILO_16 _01	€ 6.166	8%
2016-17	National Local reseach (UNITO)	Coordinator (Luzzati)	University of Turin	Neuroge nesi indotta con attivazio ne di astrociti striatali	LUZF_R ILO_16_ 01	€ 2.848	8%

Silvia De Marchis

- International collaborative experiences.

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

- Invited talks

- November 2016 – Role of the transcription factor COUP-TFI in adult hippocampal neurogenesis - 1° Neuronline Conference on Current Advances in Experimental Neuroscience Jagiellonian University, Krakow, Poland.
- October 2016 "Exploring the role of the transcription factor COUP-TFI in neurogenic regions of the adult mouse brain" - Department of Physiology, Anatomy and Genetics, University of Oxford, UK.
- March 2016 "Multiple roles of COUP-TFI in adult neurogenesis" DiSFeB, Università degli Studi di Milano.

Federico Luzzati

- International collaborative experiences:

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.

*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 2016* (Torino, marzo 2016), Aula Magna del Rettorato Cavallerizza reale (with 400 students of the secondary school)

- Debate science: European student parliament. Lectio magistralis: "Come dialogano ricerca e diritto. L'etica nella ricerca, nella politica, nell'informazione" (with Francesco Pallante, lawyer) Grattacielo San Paolo, TO, aprile 2016
- Partecipation to "*Geni a bordo" the Biotech future tour 2016*; series of conferences-spectacle sponsored by FARMINDUSTRIA; Istituto tecnico industriale Amedeo Avogadro, Torino
- *La scienza fa bene*. Book presentation at: Liceo scientifico Biagio Pascal (Romentino, NO); Liceo classico statale Vittorio Alfieri (TO);
- *Nuovi neuroni: che farne?* Article on "Sapere" (Dedalo); n. 2, 2016, pp. 16-21
- *Il caso stamina: finta o vera controversia scientifica?* Article on Scienza & Società (Egea - Unibocconi, Milano); n. 25/26, 2016, pp. 99-111

PAOLO PERETTO
- *Pint of science* (corsa e cervello)
- *Hack UniTo* (corsa, benessere psico-fisico e invecchiamento)

FEDERICO LUZZATI
- *Il cervello è uno sportivo.* Practical conference at Palazzetto dello sport (for Giovedì-Scienza)

SILVIA DE MARCHIS
- Organization of the National Prize Aldo Fasolo 2016 (for the PhD School in Neuroscience – in collaboration with NICO) - http://dott-neuroscienze.campusnet.unito.it

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

6 .Past Research activity

In 2016 most of the effort has been focused on understanding how social stimuli can influence the reproductive behavior through an interplay between adult neurogenesis and gonadal hormones. We found that the level of testosterone is critical to regulate the survival of newborn neurons in the accessory olfactory bulb and in turn to activate several nuclei involved in control of sex behavior. In parallel, we closed a three-year project aimed at defining the occurrence/degree of neurogenesis in dolphins (mammals devoid of olfaction). The results of this study showed that adult neurogenesis is absent in these aquatic mammals although a vestigial SVZ-like region is still present, thus confirming that neurogenic plasticity is highly heterogeneous through phylogeny, with an evident decrease in large-brained, long-living mammals (e.g., humans).

7. Future Projects (Next 3 years)

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 will be articulated into four main research lines:

I. ***Molecular mechanisms regulating AN*** : we will focus on molecular factors which we have recently shown to be involved in the control of OB neurogenesis to get deeper insights on their role in AN, extending the analysis to the dentate gyrus 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***: we will establish the definition of common and divergent traits in the process of AN in mammals through extensive comparative analyses.

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 in brain homeostasis and disregulation 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).

In particular we are developing new projects aimed at highlighting translational/social aspects related to brain plasticity. Among them, a project on "running, brain and reproduction" is trying to fill the gap between basic research on animal models and humans. We will

investigate the effects of running, as an aerobic exercise performed at moderate intensity over extended periods (e.g., amateur runners), on both cognitive and reproductive functions. Running has become a social phenomenon; to unravel its effects on brain function and other related activities (i.e., reproduction) is key to develop preventive and non-invasive therapeutic approaches. To this aim, we will study in parallel humans and mice models. This dual approach enables to couple macro and systems-level changes in humans with a mechanistic understanding of the impact of running at molecular and cellular levels in animal models. The project will be developed within a synergistic net, which includes researchers from national and international institutions (e.g., UMR-S 1172**,** Lille University, FR), and will be sustained by sports associations (e.g. CONI, UISP), which will be the source for amateur runners. The project will also benefit of the patronage of the "Assessorato allo Sport" City of Turin and the collaboration of the association CentroScienza to promote dissemination of the scientific findings to the citizens.

In parallel, a comparative project has been started aimed at understanding the possible phylogenetic trend (or, at least distribution) of non-neurogenic structural plasticity in a wide range of mammalian species and orders. Our preliminary data suggest that "immature neurons (non-newly generated, DCX+ neurons) could be more abundant in some mammals with respect to laboratory rodents. In addition, they could be present not only in cortical areas (as described in literature) but also in subcortical, white and grey matter, regions. This project is established in collaboration with other laboratories and tissue banks, in order to obtain the brains from a wide range of species.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Neurobiology of brain plasticity

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: Senior PostDoc — Nationality: Italian

   Expertise: Oligodendroglial heterogeneity and physiopathology

   Lead responsible of research on oligodendroglial physiopathology

3. Valentina Cerrato — Birthdate (21/07/1988)

   Degree: MSc in Biotechnology — Gender: F

   Role: PhD Student — 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 (*from March 2016*)

5. Giulia Nato — Birthdate (08/05/1986)

   Degree: PhD — Gender: F

   Role: PostDoc — Nationality: Italian

   Expertise: Astrocyte reactivity and neurogenic activation, brain tumors (*from February 2016*)

2. PRINCIPAL INVESTIGATOR

H index, 20; Citations, 2037

Relevant discoveries:

- We discovered that the Transferrin Receptor 2 (Tfr2) is a key regulator of brain iron homeostasis and proposed a role for Tfr2 alpha in the regulation of anxiety circuits (Pellegrino et al Sci Reports 2016)
- We showed in a model of genetic cerebellar ataxia that preventive motor training increases the survival of cerebellar neurons with a moderate positive impact on the motor phenotype. This therapeutic approach was proved more effective than perinatal transplant (Elisa Fucà, Neurobiol Dis, manuscript submitted)
- We found that a combination of inhibitors of GLUT/SLC2A Enhance the Action of BCNU and Temozolomide against High-Grade Gliomas (Giulia Nato, coll with Prof Lorenzo Magrassi, Univ of Pavia, manuscript submitted)

Advanced studies not yet submitted for publication:

- We performed clonal analyses of cerebellar astrocyte lineages and discovered that distinct embryonic progenitors exist with different fate potentials. Our data show for the first time that astroglial heterogeneity does not emerge from stochastic developmental events but occurs according to a spatiotemporally defined development program with deterministic features. We also demonstrate the existence of a postnatal in situ astrogliogenesis in the cerebellar cortex, where intermediate progenitors are capable of producing all cortical phenotypes. (Valentina Cerrato, coll with prof L Lopez-Masquaraque, Madrid, manuscript in preparation)
- Deletion of Sox2 during cerebellar development determines defects of cerebellar morphogenesis and alterations of Bergmann glia development associated with an ataxic phenotype (Valentina Cerrato, Elisa Fucà, collaboration with Silvia Nicolis, Univ Bicocca, Milano, manuscript in preparation)
- We revealed a distinct response of ventral and dorsal telencephalic oligodendroglia to Citron K deletion based on different ROS production and activation of antioxidant mechanisms (Enrica Boda, coll with Ferdinando di Cunto, manuscript in preparation)
- We found that the expression of Sox2 is necessary for the neurogenic activation of striatal astrocytes in the mouse damaged striatum and that locally neogenerated neuroblasts are connected with the host neurons (Giulia Nato, coll with Federico Luzzati)

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	Team Component (Enrica	Fondazione Cariplo	Characterization of a novel microRNA involved in	ID: 2014-1207)	80000	8%

		Boda PI)		myelination: a new potential pathogenetic mechanisms in multiple sclerosis			
2014-2018	International	Team Component PI: A Vercelli	FP7 European Union	Neurostemcell repair		400000	8%
2014-2017	National (Local)	PI	University of Turin (ricercal locale)	Meccanismi molecolari per il potenziamento dell'astroglia reattiva		2601,54 (2014-2016) 2.246,95 (2015-2017)	8%
Pending	National	PI	Ministero della salute	From Preclinical Astrocyte-Based Neurorestorative Approaches toward Diagnosis and Therapy in Parkinson's Disease	RF-2016-02364091	90000 requested	

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

PhDs students supervised in 2016:

Elisa Fucà (PhD degree obtained in 2016)
Valentina Cerrato, PhD student in Neuroscience

Outreach activities

- Major international collaborations:

- Dr. Andreas Bosio, Director of the Research Division in Neuroscience, Miltenyi Biotec (Köln, Germany): antigenic phenotyping of glial populations – research stay in Torino of Christine Kantzer to assess the fate potential of progenitors with distinct immunophenotypic profiles by grafting into the mouse cerebellum. 'Prospective isolation of neurogenic cerebellar precursors by ACSA2 and GLAST', in preparation
- Prof. Laura Lopez-Masquaraque (Cajal Institute, Madrid, Spain): clonal approaches for astrogliogenesis and neurogenesis. Collaborative exchange to fate map cerebellar progenitors. Short stay of Maria Figueria Onates, PhD student in Madrid, in Torino.
- Prof M Parmar, University of Lund, rabies virus based tracing of human medium spiny neuron transplantation into the rat damaged striatum

- Invited talks

Science communication
Tra geni ed esperienza, Brain Awareness Week, March 2016, Torino
MALLEABILE COME LA PLASTILINA? COME L'AMBIENTE E L'ESPERIENZA MODIFICANO I CIRCUITI NERVOSI NELLA PRIMA INFANZIA E DURANTE LA VITA, Festival dell'Educazione, November 24 2016 Torino

- Editorial duties

Guest reviewer for the following journals:
The Journal of Neuroscience, Glia, Journal of Neurochemistry, Journal of Cerebral Blood Flow and Metabolism, Molecular and Cellular Neuroscience, PlosOne, Neuroimmunology, Cell death and disease, Frontiers in Neuroscience.
Agencies: French National Research Agency (ANR), ARSEP, DAAD, FISM (Federazione Italiana Sclerosi Multipla), University of Milano, Medical Research Council (GB).

Organizational activities:

Ferdinando Rossi Lecture in Neuroscience, March 16 2016
Alexandra Joyner Aula Magna della Cavallerizza Reale dell'Università di Torino
Via Verdi 9, Torino Constructing the cerebellum: cross-talk between cell types underlies scaling of circuits
Organization of Open days at NICO
Organization of The Researcher Night, September 2016

- Speakers invited:

Ida Biunno (CNR, Milano); Maria Figueres Onate (Cajal Institute, Madrid); Alexandra Joyner (Sloan-Kettering center, New York); Antonello Mallamaci (Sissa, Trieste). Together with Silvia De Marchis we have organized most part of the seminars and instituted a procedure according to which speakers to be invited are first proposed by NICO researchers and then selected based on a poll by all the NICO community. We also collaborated to organize the 'DISFEB meets NICO' series.

Technology transfer achievements
AB is Co-founder and CEO of the Start-up S&PBrain (http://www.spbrain.com/)

3. PI's PUBBLICATIONS:

De Luca A, Cerrato V, Fucà E, Parmigiani E, **Buffo A,** Leto K. (2016) Sonic hedgehog patterning during cerebellar development. *Cell Mol Life Sci*. 73:291-303.
IF = 5.808; R = 40/289

Pellegrino RM, Boda E, Montarolo F, Boero M, Mezzanotte M, Saglio G, **Buffo A***, Roetto A. (2016) Transferrin Receptor 2 Dependent Alterations of Brain Iron Metabolism Affect Anxiety Circuits in the Mouse. *Sci Rep*. 1;6:30725. *co-last author
IF = 5.228; R = 7/63

Leto K, Arancillo M, Becker EB, **Buffo A**, Chiang C, Ding B, Dobyns WB, Dusart I, Haldipur P, Hatten ME, Hoshino M, Joyner AL, Kano M, Kilpatrick DL, Koibuchi N, Marino S, Martinez S, Millen KJ, Millner TO, Miyata T, Parmigiani E, Schilling K, Sekerková G, Sillitoe RV, Sotelo C, Uesaka N, Wefers A, Wingate RJ, Hawkes R. (2016) Consensus Paper: Cerebellar Development. *Cerebellum* 15(6): 789–828.
IF = 2.86; R = 152/256

4.GROUP’s PUBBLICATIONS:

5. GROUP's additional information:

Grants (current and pending) of the other members of the group

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	Fondazione Cariplo	Characterization of a novel microRNA involved in myelination: a new potential pathogenetic mechanisms in multiple sclerosis	ID: 2014-1207)	80000	8%
Pending	National	Enrica Boda PI, Simona Perga, CRESM San Luigi Hospital coordinator	Ministero della Salute	Does the low A20 blood expression level identify a subgroup of multiple sclerosis patients with a worse prognosis?	NA	100000 requested	

Honours, prizes or awards received by other members of the group.

Enrica Boda
International collaborations:
- V Taylor, University of Basel, oligodendrocyte heterogeneity

National collaborations:
- D. Lecca, MP Abbracchio, University of Milan, oligodendrocyte maturation

Awards:
Best poster award at the Meeting of the French Glial Cell Club, 1-3 June 2016, Carry-le-Rouet, France

- Invited talks:

- Inherent heterogeneity in dorsal and ventral OPCs of the mouse CNS unveiled by Citron-kinase deletion. 23 November 2016, Convegno del Gruppo Italiano di Neuromorfologia (GISN), Verona, Italy
- Inherent heterogeneity in dorsal and ventral OPCs of the mouse CNS unveiled by Citron-kinase deletion. 3 December 2016, "More than Neurons Meeting: toward a less neuronocentric view of brain disorders", Convegno con il Patrocinio della Società Italiana di Farmacologia (SIF), Torino, Italy

- Editorial duties

Guest reviewer for the following journals:
Front Neurosci, Neurochemical Research, Plos One

Roberta Parolisi

- International collaborative experiences

-Merck-Serono Grant Consortium (Pierre Gressens, Claudia Verderio) to study microglia-derived extracellular vesicles
National collaborations:
Dr. Claudia Verderio (Institute of Neuroscience, CNR, Milan, Italy): study of microglia-derived extracellular vesicles effects on proliferation and differentiation of oligodendrocyte precursor cells.
- Dr. Alessandro Gozzi (Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto, Italy): ultrastructural analysis of corpus callosum in 16p1.2 +/- mice (model of autism).
- Dr. Andrea Marcantoni (Department of Drug Science and Technology, University of Turin, Italy): study of neuromodulatory functions of oligodendrocyte precursor cells

- Invited talks:

- 'Microglia-derived extracellular vesicles regulate proliferation and differentiation of oligodendrocyte precursor cells', congress 'More than neurons: toward a less neuronocentric view of brain disorders', December, 3, 2017, Turin, Italy
- 'Non-neurogenic SVZ-like niche in aquatic mammals devoid of olfaction', congress XXVI Convegno Nazionale Gruppo Italiano per lo studio della neuromorfologia, November,24, 2016, Verona, Italy

Giulia Nato

- International collaborative experiences.

- connectivity of striatal newborn neurons produced by activated astroglia after quinolinic acid lesion (collaboration with Federico Luzzati, and B Berninger, University of Mainz, Germany)

National collaborations:
- physiopathology of patient-derived induced cerebellar neurons grafted into the rodent cerebella (coll with Prof Lorenzo Magrassi, Univ of Pavia)
- novel therapy against glioblastoma and role pf PTEN signalling in cerebellar tumors (coll with Lorenzo Magrassi, Univ of Pavia)

Valentina Cerrato
Awards:
- 2016. One of the 45 students selected to attend the Route28 Summit in Neurobiology "Adult neurogenesis: form follows function and function follows form", DZNE (Deutsches Zentrum für Neurodegenerative Erkrankungen), Frauenchiemsee, Germany.
- September 2016. Poster prize at D-Day 2016, organized by the Doctoral School in Life and Health Sciences, University of Turin.
- November 2016. Prize awarded as one of the best young oral presentation at the XXVI Congress G.I.S.N. Verona, November 24-25, 2016.

- International collaborative experiences.

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

- Invited talks

- Seminar for the teaching course of Developmental Biology at the Master's degree in Molecular and Cellular Biology, entitled "Generation of astroglial diversity in the CNS", April 26th 2016.
- "Origin and development of astroglial heterogeneity in the cerebellum", oral presentation at the 46th SfN annual meeting, San Diego (USA), 12-16 November 2016.
- "Astroglial heterogeneity in the cerebellum results from distinct embryonic and postnatal progenitors with different proliferative behaviors", oral presentation at the XXVI Congress G.I.S.N. , Verona, November 24-25 2016.

- Editorial duties

Ad hoc reviewer for International Journal of Developmental Neuroscience

Organizational activities:

- Workshops, Schools or Conferences organized by members of the group *Science Communication*
  - May 2016. Organizing member of the "Beautiful mind" sessions for "Pint of Science 2016", Turin, May 23th-25th.
  - September 2016. organizing member of NICO stand at the European Researchers Night, Turin, September 30th.

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

- Alternanza Scuola-lavoro: 10-days- long stages (13-23/06/2016) for high school students (Liceo Pascal, Giaveno (TO) (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

Summary

Cell replacement and training protocols were applied to promote functional recovery in neurodegenerative models. We identified rehabilitative motor strategies effective in both promoting the survival of neurons and in ameliorating the motor phenotypes in Ataxia mutants. We showed hES-derived striatal progenitors transplanted in animal models differentiate and integrate. We showed that abrogation of the transferrin receptor Tfr2 caused an overactivation of neurons in the limbic circuit with the emergence of an anxious behaviour. We found that pharmacological inhibition of GLUT/SLC2A increases the effect of BCNU against high-grade gliomas.

Background

Building upon research developed over the last years, in 2016 we have focused on the role of glia and progenitor cells in brain plasticity, repair and pathology, and on the implementation of cell replacement therapies.
Issues of glial biology considered to be the most promising to unveil the role of astrocytes in pathology and brain repair include how their diversity is generated and how parenchymal astrocytes can be instructed to acquire stem cell properties in the injured CNS. Further, among the hot topics in the field of oligodendrocyte physiopathology, there is the inquiry of how to promote OPC differentiation and how to protect myelin in disease.
Astrocytes comprise extremely heterogeneous phenotypes, including stem cells (NSC) in the neurogenic niches and parenchymal subsets that spontaneously activate neurogenesis after damage. 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 much it impacts on CNS functions is unknown. Moreover, the intrinsic and extrinsic mechanisms that trigger the transition of some astrocytes to a NSC status are largely unknown. Further, much remains to be understood also on how glial cells form tumors and what treatments can actually halt the growth of brain tumors, for which there is no cure at the moment.
OPCs 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 are interested in identifying factors promoting myelination or damaging myelin, and in understanding the feature and impact in physiology and pathology of oligodendroglial heterogeneity.
Knowledge gained from developmental studies and from investigations on mechanisms governing circuit remodelling provides crucial information to design effective therapeutic strategies based on cell replacement and/or rehabilitative training in case of neuronal loss or establishment of dysfunctional circuits. Our studies addressed these issues to help the development of therapies for neurological diseases based on adaptive cell replacement and/or manipulation of circuit plasticity.

Rationale

The capability of the adult mammalian CNS to regenerate or repair after damage is very limited as a consequence of the postnatal decline of neurogenesis and gliogenesis, and of the upregulation of molecules inhibiting circuit remodelling. However, it retains significant levels of structural plasticity that, if fostered, might promote brain repair.
We therefore sought to understand the cellular and molecular mechanisms that underlie key components of CNS structural plasticity to exploit this knowledge for both understanding pathogenic mechanisms of neuro-developmental disorders, and defining strategies implementing functional and anatomic repair in developmental diseases and neurodegenerative pathologies.

To address these issues, we focussed on the rodent cerebellum as an experimental model to study physiological astrocyte differentiation and alteration of developmental processes leading to Ataxia-like motor dysfunctions. We further explored the functional contiguity between forebrain parenchymal astroglia and neural stem cells. Finally, we investigated mechanisms promoting oligodendrocyte differentiation and functioning.
Overall, evidence gained with these studies provided the background knowledge to address the efficacy of preventive or therapeutic cell replacement approaches vs rehabilitative training (vs the combination of the two) in rodent models of Ataxia and Hungtington's disease (HD).

Objectives

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

Specific aims:
a) disclosing developmental processes guiding astrogliogenesis and cerebellar morphogenesis;
b) understanding similarities and differences between parenchymal astroglia and stem cells and regulatory mechanisms of adult stem cells;
c) understanding the physiopathology of oligodendrocyte progenitor cells (OPCs);
d) exploiting the knowledge gained with these studies to:
- define effective strategies based on preventive/therapeutic cell replacement approaches in rodent models of Ataxia and HD;
- define molecular manipulations and rehabilitative training protocols helpful to promote neuroprotection and recovery of lost functions through the broadening of glial and neuronal plasticity;
- implement the reparative properties of non-germinal glia upon brain damage and discover mechanisms to limit tumor growth.

Results

Major results published or submitted for publication in 2016:
Preventive motor training strategies and cell replacement approaches in rodent models of Ataxia and HD
We showed in a model of genetic cerebellar ataxia that preventive motor training increases the survival of cerebellar neurons with a positive impact on the motor phenotype. Of note, this action occurred through an attenuation of dysregulated autophagy, which is a key feature of the mouse model examined. This therapeutic approach was proved to be more effective than perinatal transplant. These results therefore enforce the use of preventive motor exercise in inherited ataxia to promote a delay in the progression of the disease, and indicate that this approach could be a promising step toward the amelioration of patients' quality of life. These results were submitted to Neurobiology of Disease.

Along this line of research, in collaboration with Alessandro Vercelli's group and Elena Cattaneo's lab, Elisa Fucà showed that Gsx2 and Ebf1 combined overexpression in hES cells achieves high yields of medium spiny neurons expressing Darpp32 and Ctip2 in vivo after transplantation in the damaged rat striatum. Thus, hES-derived striatal progenitors can be transplanted in animal models and can differentiate and integrate into the host, extending fibers over a long distance. These data were submitted to PNAS.

Understanding the physiopathology of oligodendrocyte progenitor cells
Iron is one major regulator of oligodendrocyte physiology. With the expectation to observe alteration in myelin integrity we studied mutants lacking the Transferrin Receptor 2. However, no alterations in oligodendroglia features were observed. Nevertheless, we found

that Tfr2 abrogation caused an accumulation of iron in specific districts in the nervous tissue that was not accompanied by a brain Hepc response. Moreover, Tfr2-KO mice presented a selective overactivation of neurons in the limbic circuit and the emergence of an anxious-like behaviour. Furthermore, microglial cells showed a particular sensitivity to iron perturbation. Thus, Tfr2 is a key regulator of brain iron homeostasis and propose a role for Tfr2 alpha in the regulation of anxiety circuits. These data were published in Scientific Reports.

Discovering mechanisms to limit tumor growth
Glucose transport across glioblastoma membranes plays a crucial role in maintaining the enhanced glycolysis typical of high-grade gliomas and glioblastoma. We contributed to show that low doses of the inhibitors of the glucose transporters GLUT/SLC2A superfamily, ritonavir, and BCNU synergize in vivo at low doses to limit the growth of established GL261 tumors. This drug combination increased the overall survival of the grafted mice and allowed a five-fold reduction in the dose of BCNU. These data were submitted to Neoplasia.

Other major advancements:
Cerebellar astrogliogenesis, morphogenesis and function
Clonal analysis of cerebellar astrocyte lineages revealed that distinct embryonic progenitor exists with different fate potentials. Our data show for the first time that astroglial heterogeneity starts earlier than expected and does not emerge from stochastic events but occurs according to a spatiotemporally defined developmental program with deterministic features. In a parallel study, we investigated the effects of the deletion of Sox2, a master regulator of stem cell functioning, in the cerebellar primordium. This determines a defect of cerebellar morphogenesis and specific alterations of Bergmann glia development associated with an ataxic phenotype. We are currently examining mechanisms downstream of Sox2 and effects on cerebellar circuits.
Understanding the physiopathology of oligodendrocyte progenitor cells
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. These data represent one of the first examples of functional heterogeneity of oligodendrocytes derived from distinct embryonic sources.

Advancement in the field

- Effect of preventive motor exercise in inherited ataxia to promote a delay in the progression of the disease, indicating that this approach could be a promising step toward the amelioration of patients' quality of life;
- hES-derived striatal progenitors proceed toward differentiation into medium spiny neurons upon transplantation into the damaged rat striatum;
- Combinatorial action of low doses of ritonavir and BCNU in contrasting glioblastoma growth in vivo;
- Unprecedented role of iron and Tfr2 alpha in the regulation of anxiety circuits.

7. Future Projects (Next 3 years)

a. Summary :

Our research will focus on the role of glia and progenitor cells in brain plasticity and repair, and on the implementation of cell replacement approaches and/or training protocols to promote functional recovery in CNS diseases.

We believe that specific issues regarding glia and neural progenitors as well as their reciprocal relationships are particularly promising to unveil new keys to the understanding of physiology, disease and repair. As for astrocytes, very little is known on how distinct astroglial subtypes are specified and how much it impacts the shaping of the circuits. Further, mechanisms underlying the acquisition of stem cell properties in parenchymal astroglia remain undefined. We will address these issues by studying the specification of astroglial subtypes and their morphogenetic programs in the cerebellum, and the latent stem cell properties of adult striatal astroglia, respectively. Oligodendrocyte progenitors self-maintain but have limited capability to repair myelin. Understanding their biology may help fostering myelin regeneration and reveal unsuspected functions of these progenitors in integration of information in the CNS, as provided by communication with neurons.

Recent advancements in human stem cell technology and reprogramming prompt the need of developing strategies to obtain proper differentiation into specific neuronal identities and functional integration in the recipient brain. Moreover, based on the efficacy of external stimuli and training to promote circuit plasticity, rehabilitation protocols appear as promising tools to promote adaptive remodelling of defective circuits and to enhance the integration of transplanted cells into the recipient tissue, therefore boosting functional recovery. We will perform preclinical studies to define therapies for neurological diseases based on adaptive cell replacement and/or manipulation of circuit plasticity.

b. Background and Significance :

Issues of glia biology considered to most promising to unveil how glia contributes to pathology and may promote brain repair include how the various astrocyte phenotypes are generated and how parenchymal astrocytes can be instructed to acquire stem cell properties in the injured CNS. Further, among the hot topics in the field of oligodendrocyte physiopathology are how to push the oligodendrocyte progenitor cells (OPC) towards differentiation, and how OPC and neurons communicate. In our studies we will address all these aspects.

Astrocytes comprise extremely heterogeneous phenotypes, including stem cells (NSC) in the neurogenic niches and parenchymal subsets that spontaneously activate neurogenesis after damage. 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 much it impacts on CNS functions is unknown. Understanding these aspects may reveal unknown features in the aetiology and progression of neurologic and psychiatric disorders such as Ataxia and Autism Spectrum Disorders. Further, the intrinsic and extrinsic mechanisms that trigger the transition of some astrocytes to a NSC status are largely unknown. Understanding how to implement these features in all astrocytes may lead to exploit their reparative plasticity not only to elicit neurogenic attempts, but, more broadly, to evoke in situ the 'bystander' neurosupportive/immunomodulary actions well-known for grafted and endogenous NSC reacting to damage (Martino and Pluchino 2006; Butti 2012).

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 in progenies with distinct regenerative

potential in disease; ii) factors promoting remyelination; iii) if and how OPC communicate to neurons. These studies aim at disclosing novel aspects of oligodendrocyte biology in view of fostering their capability to regenerate myelin. Further, they aim at revealing an unprecedented level of integration of information in the CNS as provided by OPC-to-neuron communication.
Knowledge gained from developmental studies and from investigations on mechanisms governing circuit remodelling provides crucial information to design effective therapeutic strategies based on cell replacement and/or rehabilitative training after neuronal loss or establishment of dysfunctional circuits. Here, advancements in human stem cell technology and reprogramming prompt the need of preclinical studies to develop approaches to obtain endurable donor cell engraftment in the host, including acquisition of specific neuronal identities and functional integration in the recipient brain. Moreover, based on the efficacy of external stimuli and training to promote circuit plasticity and functional recovery, generalized and/or specific rehabilitation protocols appear as promising tools to promote adaptive remodelling of defective circuits and to enhance the integration of transplanted cells into the recipient tissue, therefore boosting functional repair. Our studies will address these issues to help the development of therapies for neurological diseases based on adaptive cell replacement and/or manipulation of circuit plasticity.

c. General aim and integration with mission of the Institute

Our work has two major aims:

- understanding glial/progenitor heterogeneity and physiology at the molecular, cellular and functional levels and clarify how such features impact on CNS pathophysiology in order to exploit adult glia and progenitors as therapeutic actors to treat disease;
- developing therapies for neurological diseases where loss/dysfunctional cells have to be replaced by either endogenous sources or through transplantation.

The contribution of our group will be to deliver innovative evidence and expand expertise on fundamental processes of brain plasticity implicated in developmental psychiatric disorders and neurodegenerative diseases, which may be fostered or manipulated to propose preclinical therapeutic approaches for CNS diseases.

d. Specific objectives and strategies :

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

To unravel mechanisms of astrocyte specification and plasticity we will:

-understand phenotypic specification of cerebellar astrocytes and their role in neuronal functions. We found that distinct embryonic progenitors produce the 4 types of cerebellar A (Cerrato, in preparation). Preliminary data suggest that Sox2 specifies Bergmann glia (Cerrato, in preparation), possibly through the action of the downstream target Coup1. Deletion of Sox2 leads to ataxic-like behavior. We will study the role of these targets, and their impact on the formation and function of the cerebellar circuits. Coll: S Nicolis, Univ Bicocca, Milan, F Tempia, NICO.

- identify mechanisms underlying the acquisition of NSC properties in reactive astrocyte Upon damage striatal A acquire NSC properties and activate neurogenesis (Nato et al.,2015). We found that expression of Sox2 in astrocytes is crucial for the early phases of this activation. Action of Sox2 will be dissected at the molecular level together with possible extrinsic cofactors (Wnt, Shh, inflammation). We will also address the clonal identity of these astrocytes with a latent neurogenic potential. In parallel, the connections and limited life span of the newly generated neurons will be examined as well as the effects of the ablation of this neurogenic response in terms of secondary neuronal death. Coll: F Luzzati, NICO, S. Nicolis, Univ Bicocca, Milan, B. Berninger, Mainz.

To understand oligodendroglia physiopathology, we will:

- investigate oligodendrocyte heterogeneity in Citron K KO mice

The study of the effect of Citron K deletion will be extended to adult ages to understand whether this impact OPC response to demyleination and the formation of myelin lamellae. Coll: F. di Cunto, NICO.

- identify strategies to promote OPC remyelination

a) miR-125a is upregulated during OPC differentiation and affects their maturation in vitro (Lecca, unpublished). We will examine its expression pattern in models of toxic demyelination. Functional manipulations will be applied and, those promoting repair, extended to MS models. Coll: D Lecca, MP Abbracchio, Univ of Milan.

b) Extracellular vesicles released by alternatively activated/mesenchymal stem cells exposed-microglia promote myelination in vitro (Verderio, unpublished). We will assess if they also prompt remyelination in vivo after toxic demyelination, and, if confirmed, in MS models. Underlying molecular mechanisms will be investigated. Coll: C Verderio, CNR, Milan, A Uccelli, Unv of Genoa.

- define unconventional roles of OPCs

Preliminary data (Boda, Marcantoni, unpublished) show that OPCs trigger the maturation of neuronal networks and enhance GABAergic transmission in mature circuits. We will assess how this impact on glutamatergic and GABAergic contacts and esamine the role of contact or diffusible factors. Coll: E Carbone, Turin, G Martino, HSR, Milan

To understand cerebellar development in physiology and pathology we will:

-extend clonal analysis of astrogliogenesis to **cerebellar neurons and oligodendroglia** to unveil their lineage relationships and morphogenetic programs. Given the emerging roles of the cerebellum in **Autism spectrum disorder** these studies will be performed in wild type and mutant model of Autism (Autism-associated 16p11.2 microdeletion, Coll A Gozzi, IIT Rovereto) to reveal the underlying developmental alterations.

-investigate the pathogenetic mechanisms of Ataxia-telangiectasia we will graft cerebellar neurons derived from patients' iPSC or healthy controls in mouse embryos to examine if and how how they mature and identify potential pathological mechanisms (Coll. Lorenzo Magrassi, Univ of Pavia)

As a side explorative project, we will also focus on the role of PTEN in rare cerebellar tumors. We will ask whether PTEN deletion in the mature cerebellum leads to the formation of tumors similar to that of Malattia di Lhermitte-Duclos, and assess the nature of human cells forming these tumors (Coll. Lorenzo Magrassi, Univ of Pavia; Associazione PTEN ITALIA)

To devise therapeutic approaches based on cell replacement and training:

Examine whether hES derived striatal neuron progenitors differentiate, form connections and promote functional repair when grafted into a rat model of Huntington disease. We will also assess whether training protocol facilitate cell survival, differentiation, integration and functional recovery (Coll. A. Vercelli, M. Boido, NICO; E. Cattaneo, Univ of Milan; M. Parmar, Univ of Lund).

e. Unique features of the project research :

Several of the addressed questions (eg understanding and awakening latent reparative/regenerative potentials in glial cell, disclosure and understanding of neuromodulatory roles of OPCs, biological substrate of Autism) are highly innovative and essentially unanswered in the field. Moreover, we will combine several innovative approaches including the use of extracellular vesicles released by alternatively activated microglia as therapeutic agents, cutting edge technology for clonal analyses in vivo (see Parmigiani et al., 2015 to get insight on this technology), gene expression analyses on selected glial cell populations isolated from the CNS, and specific rehabilitative training protocols. This will be further implemented by the development of clarification processes and two-photon microscopy, which we are currently developing at out Institute. Thanks to our collaborative network, we will also employ induced human neuron precursors for transplantations in rodents, being therefore directly exposed to the fast evolving technology of reprogramming, and use rabies virus to trace monosynaptic connections. On top of this, we have generated and will develop mutant mouse lines that will constitute unique experimental models.

f. Methodology : <u>please fill-out this section only in the case of innovative technologies</u>

In collaboration with Filippo Molinari (Politecnico di Torino) we have developed a first version for the automatized analysis and interpretation of confocal images suitable to produce unbiased data and perform quantitative analyses for clonal studies or dispersion of cells in the nervous tissue. Federico Luzzati (NICO) has contributed a tomography-based approach to facilitate 3D reconstructions. The system is currently under further development to: i) perform multiscale analysis, at low and high magnification, for the analysis of single cells or clusters or for the spatial analysis of the whole sample (ed mouse cerebellum, hemispheres etc); ii) couple automatized cellular detection and algorithms for numerical analysis and modelling, so to provide parameters related to cell dispersion in the tissue; iii) support morphofunctional modelling. We aim at developing a versatile tool to be applied for different mapping-related purposes and released as an open source tool.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

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, 22; Citations: 2190

Relevant discoveries:

I have developed my career doing both experimental and computational biology research.
For what concerns experimental biology, I have always been strongly attracted by the investigation of fundamental mechanisms controlling cell proliferation, as well as by their relationship with cell differentiation. This interest led me, as a post-doc, to discover a dual role of the cyclin-dependent kinase inhibitor p21/WAF1 in the terminal stages of primary keratinocyte differentiation (1). So far, my main scientific achievement was to discover that the absence of the Citron kinase protein (CIT-K) leads to a severe form of microcephaly in mammals. This discovery, originally obtained in mice in 2000 (2), has been instrumental to the identification of CITK as a human disease gene in 2016 (3). Indeed, inactivating mutations in CITK have been found in patients affected by a syndrome known as Primary Microcephaly 17 (MCPH17), characterized by severe microcephaly, intellectual disability, spastic paralysis and ataxia (3).My group recently discovered that, besides being involved in control of cytokinesis, CITK regulates spindle orientation in concert with the microcephaly protein ASPM (4), that it regulates microtubule dynamics (5) and that it is required for genomic stability of neural progenitors (6). The study of a second isoform of the Citron gene, named CITN, led my group to establish that it controls the organization of Golgi apparatus in young neurons (7), and the maintenance of dendritic spines in mature neurons (8). Finally, we discovered that CITK and CITN cooperate with the Down syndrome critical region protein TTC3 in regulating neuronal differentiation (9).

In the computational biology field, my main contribution has been to develop the concept of conserved coexpression analysis as a useful strategy for prediction the function of non-annotated genes and to imply new genes in the pathogenesis of human disorders (Ala et al., 2008), especially those characterized by intellectual disability (Piro et al., 2011). We have recently adapted this technique to the identification of possible new treatments for orphan genetic disorders, through drug-repositioning strategies (Molineris et al., 2013).

1. Di Cunto F, Topley G, Calautti E, Hsiao J, Ong L, Seth PK, Dotto GP. (1998). Inhibitory function of p21Cip1/WAF1 in differentiation of primary mouse keratinocytes independent of cell cycle control. *Science* 280:1069-1072.
2. Di Cunto F, Imarisio S, Hirsch E, Broccoli V, Bulfone A, Migheli A, Atzori C, Turco E, Triolo R, Dotto GP, Silengo L, Altruda F. (2000). Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. *Neuron* 28:115-127.
3. Harding BN, Moccia A, Drunat S, Soukarieh O, Tubeuf H, Chitty LS, Verloes A, Gressens P, El Ghouzzi V, Joriot S, Di Cunto F, Martins A, Passemard S, Bielas SL. (2016). Mutations in Citron Kinase Cause Recessive Microlissencephaly with Multinucleated Neurons. *Am J Hum Genet* 99:511-520.
4. Gai M, Bianchi FT, Vagnoni C, Verni F, Bonaccorsi S, Pasquero S, Berto GE, Sgro F, Chiotto AM, Annaratone L, Sapino A, Bergo A, Landsberger N, Bond J, Huttner WB, Di Cunto F. (2016). ASPM and CITK regulate spindle orientation by affecting the dynamics of astral microtubules. *EMBO Rep* 17:1396-1409.
5. Sgro F, Bianchi FT, Falcone M, Pallavicini G, Gai M, Chiotto AM, Berto GE, Turco E, Chang YJ, Huttner WB, Di Cunto F. (2016). Tissue-specific control of midbody microtubule stability by Citron kinase through modulation of TUBB3 phosphorylation. *Cell Death Differ* 23:801-813.

6. Bianchi FT, Tocco C, Pallavicini G, Liu Y, Verni F, Merigliano C, Bonaccorsi S, El-Assawy N, Priano L, Gai M, Berto GE, Chiotto AM, Sgro F, Caramello A, Tasca L, Ala U, Neri F, Oliviero S, Mauro A, Geley S, Gatti M, Di Cunto F. (2017). Citron Kinase Deficiency Leads to Chromosomal Instability and TP53-Sensitive Microcephaly. *Cell Rep* 18:1674-1686.
7. Camera P, da Silva JS, Griffiths G, Giuffrida MG, Ferrara L, Schubert V, Imarisio S, Silengo L, Dotti CG, Di Cunto F. (2003). Citron-N is a neuronal Rho-associated protein involved in Golgi organization through actin cytoskeleton regulation. *Nat Cell Biol* 5:1071-1078.
8. Camera P, Schubert V, Pellegrino M, Berto G, Vercelli A, Muzzi P, Hirsch E, Altruda F, Dotti CG, Di Cunto F. (2008). The RhoA-associated protein Citron-N controls dendritic spine maintenance by interacting with spine-associated Golgi compartments. *EMBO Rep* 9:384-392.
9. Berto G, Camera P, Fusco C, Imarisio S, Ambrogio C, Chiarle R, Silengo L, Di Cunto F. (2007). The Down syndrome critical region protein TTC3 inhibits neuronal differentiation via RhoA and Citron kinase. *J Cell Sci* 120:1859-1867.
10. Ala U, Piro RM, Grassi E, Damasco C, Silengo L, Oti M, Provero P, Di Cunto F. (2008). Prediction of human disease genes by human-mouse conserved coexpression analysis. *PLoS Comput Biol* 4:e1000043.
11. Piro RM, Ala U, Molineris I, Grassi E, Bracco C, Perego GP, Provero P, Di Cunto F. (2011). An atlas of tissue-specific conserved coexpression for functional annotation and disease gene prediction. *Eur J Hum Genet* 19:1173-1180.
12. Molineris I, Ala U, Provero P, Di Cunto F. (2013). Drug repositioning for orphan genetic diseases through Conserved Anticoexpressed Gene Clusters (CAGCs). *BMC Bioinformatics* 14:288.

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	8%
01/08/13 31/08/17	National	Component	Telethon Foundation	Relevance of the axonal SMN protein (a-SMN) for spinal muscular atrophy: novel cell models, transgenic mice and therapeutic approaches.	GGP13081	130000	8%
01/01/16 31/12/18	National	Component	CNR, Epigen flagship project.	Disruption of circadian rhythms and epigenetic modifications		53000	8%

				in D. melanogaster.			
01/08/16 31/07/17	International	Component	LouLou Foundation : CDKL5 pilot program	Exploiting computational biology for target identification and drug repositioning in CDKL5 disorder		30000	8%
01/04/17 31/03/18	National	PI	Fondo ricerca locale Ex-60%	Validazione di interattori molecolari di Citron Kinase implicati nella riparazione del danno al DNA.		5879	8%
PENDING three years project	National	PI	Telethon Foundation	Dissection of the Citron kinase pathway in relation to common mechanisms of primary microcephaly		226000	
PENDING two years project	International	PI	Fondation Jerome Lejeune	Identification and initial validation of new possible treatments for intellectual disability in Down syndrome through drug repositioning.		50000	

PhDs supervised in 2016:

- Alessandra Maria Adelaide Chiotto
- Gianmarco Pallavicini

Outreach activities

- 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

Nov. 2016, International mini-conference 'Chromosomes and Mitosis', Novosibirsk, Russia. Title of the talk: Mitotic alteration in human microcephaly: the crucial role of Citron kinase

- Editorial duties

- Associate Editor of PLoS ONE
- Associate Editor BMC Research Notes

3. PI's PUBBLICATIONS:

El Ghouzzi V, Bianchi FT, Molineris I, Mounce BC, Berto GE, Rak M, Lebon S, Aubry L, Tocco C, Gai M, Chiotto AM, Sgro F, Pallavicini G, Simon-Loriere E, Passemard S, Vignuzzi M, Gressens P, **Di Cunto F.** (2016). ZIKA virus elicits P53 activation and genotoxic stress in human neural progenitors similar to mutations involved in severe forms of genetic microcephaly. *Cell Death Dis* 7:e2440.
IF= 5.378; R = 38/187

Gai M, Bianchi FT, Vagnoni C, Verni F, Bonaccorsi S, Pasquero S, Berto GE, Sgro F, Chiotto AM, Annaratone L, Sapino A, Bergo A, Landsberger N, Bond J, Huttner WB, **Di Cunto F**. (2016). ASPM and CITK regulate spindle orientation by affecting the dynamics of astral microtubules. *EMBO Rep* 17:1396-1409.
IF= 7.739; R = 26/289

Harding BN, Moccia A, Drunat S, Soukarieh O, Tubeuf H, Chitty LS, Verloes A, Gressens P, El Ghouzzi V, Joriot S, **Di Cunto F**, Martins A, Passemard S, Bielas SL. (2016). Mutations in Citron Kinase Cause Recessive Microlissencephaly with Multinucleated Neurons. *Am J Hum Genet* 99:511-520.
IF= 10.794; R = 8/166

Sgro F, Bianchi FT, Falcone M, Pallavicini G, Gai M, Chiotto AM, Berto GE, Turco E, Chang YJ, Huttner WB, **Di Cunto F**. (2016). Tissue-specific control of midbody microtubule stability by Citron kinase through modulation of TUBB3 phosphorylation. *Cell Death Differ* 23:801-813.
IF= 8.218; R = 23/289

4.GROUP’s PUBBLICATIONS:

5. GROUP's additional information:

Outreach activities of other members of the group:

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

6 .Past Research activity

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.

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 Zyka 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.

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 wich 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.

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.

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.

Advancement in the field

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

7. Future Projects (Next 3 years)

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

Summary:

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

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

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

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.

General aim and integration with mission of the Institute

The general aim of our group is to significantly advance our knowledge on neurodevelopmental dysorders, in particular microcephaly and Down syndrome. In the next thre 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.

Specific objectives and strategies:

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

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

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

We found that CITK controls cytokinesis and spindle orientation by altering microtubule dynamics, a scenario supported by the results of our proteomics screen. These functions

involve at least in part the capability of CITK to modulate TUBB3 phosphorylation through CK2a recruitment. Moreover KIF14, whose loss leads to microcephaly in mice and humans, is a partner of CITK in regulation of midbody stability. Since kinesins play established roles in microtubule dynamics and CK2a has been involved in kinesins' regulation, we will set out to obtain more information about the interplay between all these molecules.

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

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

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

We aim at translating our mechanistic findings to experimental models directly relevant for the human disease. Since most MCPH17 patients carry kinase dead mutations, we have undertaken the production a new mouse model, characterized by a similar alteration. We would also like to explore the potential usefulness of neural progenitor cells derived from MCPH17 patients as a possible platform for drug screening and validation, by transferring to these cells our discovery that the effects of CITK loss can be alleviated by P53 inactivation.

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

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

6. Identification of new genes involved in NDD.

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

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.

Methodology: <u>please fill-out this section only in the case of innovative technologies</u>

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 2016

Laboratory name: Neuropsychopharmacology

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

Carola Eugenia Eva — Birthdate (21/07/1957)
Degree: PhD — Gender: F
Nationality: Italian — Phone:+390116706608
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- **Personnel**

1. Alessandra Oberto — Birthdate (24/10/1967)
   Degree: PhD in pharmacology — Gender: F
   Role: Research Associate — Nationality: Italian
   Expertise: Biotechnology, behavioral analysis, immunohistochemistry

2. Ilaria Bertocchi — Birthdate (13/04/1982)
   Degree: PhD in pharmacology — Gender: F
   Role: Senior Postdoctoral fellow — Nationality: ItalianExpertise: Biotechnology, behavioral analysis, immunohistochemistry

3. Angela Longo — Birthdate (19/03/1982)
   Degree: PhD in pharmacology — Gender: F
   Role: Postdoctoral fellow — Nationality: Italian
   ExpertiseBehavioral analysis, immunohistochemistry

4. Paolo Mele — Birthdate (22/06/1973)
   Degree: PhD in pharmacology — Gender: M
   Role: senior Postdoctoral fellow — Nationality: ItalianExpertise: Behavioral analysis, immunohistochemistry

5. Mattia Ghigo — Birthdate (12/08/1989)
   Degree: Master in Psychology — Gender: M
   Role: PhD Student — Nationality: Italian
   Experise: Learning behavioral analysis, immunohistochemistry

2. PRINCIPAL INVESTIGATOR

H index, 19; Citations 1141

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 menopausa. The results of Dr. Eva's studies are published in 69 publications, 54 listed in PUB Med.

PI's Grants (current and pending)

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

PhDs supervised in 2016:
Mattia Ghigo

Outreach activities

- international collaborative experiences.
- With 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 Ritcher (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).

Invited talks
- Festival dell'Educazione 2016
- Presentation of S&PBrain start-up (see below)

- Editorial duties

Reviewer of manuscripts for Endocrinology and Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity

Technology transfer achievements

Founding member and President of the spinoff S&P BRAIN (may 2016). S&P BRAIN has been selected for Mentoring program of the Camera di Commercio di Torino (Mentor: Dr. A.Pellacani, Scientific Director of Menarini in 2016 and 2017. CE has been invited to present S&P BRAIN at – the Molecular Biotechnology Center, University of Turin and at the Ciber group meeting in Barcellona.

3. PI's PUBBLICATIONS:

Mele P, Zammaretti F, Longo A, Panzica G, Oberto A**, Eva C**. (2016) Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor gene expression in leptin treated obese (ob/ob) or lean mice. *Brain Res*. 1649:102-9. IF= 2.56; R = 143/256

Bo E, Farinetti A, Marraudino M, Sterchele D, **Eva C,** Gotti S, Panzica G. (2016) Adult exposure to tributyltin affects hypothalamic neuropeptide Y, Y1 receptor distribution, and circulating leptin in mice. *Andrology*. 4(4):723-34. IF= 2.51; R = 2/5

4.GROUP's PUBBLICATIONS:

Obenhaus HA, Rozov A, Bertocchi I, Tang W, Kirsch J, Betz H, Sprengel R. (2016). Causal Interrogation of Neuronal Networks and Behavior through Virally transduced Ivermectin Receptors. *Front Mol Neurosci*, 9:75. IF= 5.15; R = 35/256

5. GROUP's additional information:

6 .Past Research activity

Summary

We used Npy1rrfb mice, with the conditional inactivation of *Npy1r* in forebrain principal neurons, to study the role of limbic Npy1r in: **1)** resilience to psychopathologies, **2)** susceptibility to Metabolic syndrome (MetS) after menopause, **3)** learning and structural plasticity and **4)** we used Npy1rY5R-/- mice, with the conditional inactivation of Npy1r in Y5R expressing neurons, to investigated the role of Npy1r on behavioral flexibility and OFC neural activity.

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 memory, but the underlying mechanisms are far from clear. During spatial memory training, hippocampal NPY gene expression is increased and NPY knock-out mice display impaired spatial memory.
4) Cognitive flexibility is defined as the ability to rapidly adapt established patterns of behaviour in the face of changing circumstance and depends critically on the orbitofrontal cortex (OFC). NPY regulates cognition and emotional behaviour via the activation of Y1 and Y5R receptors. In rodents, Npy1r and Npy5r have an overlapping role in regulating anxiety and colocalize in several forebrain regions belonging to circuits of cognitive and emotional functions.

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

2) The incidence of MetS increases significantly after menopause suggesting the potential involvement of ovarian steroids. In the arcuate nucleus, estrogen increases activity of anorexigenic POMC neurons and represses synthesis of orexigenic AgRP and NPY. Given

that Npy1rrfb male but not female mice show an increased vulnerability to metabolic challenges in adulthood, we investigated whether limbic Npy1r represents a key target gene through which estrogens in brain modulate energy metabolism in relation to reproductive functions.
3) PNNs play crucial role in learning and memory. In the amygdala PNNs protect fear memories from erasure, and disruption of the PNNs in the hippocampus or the mPFC impairs long-term fear memory. Moreover, degradation of PNNs in the cortex chondroitinase enzymatic digestion enhances object recognition memory. To elucidate whether Npy1r plays a in learning and memory and affects PNNs, we tested spatial memory of Npy1rrfb and we analyzed PNN expression and distribution in the dorsal hippocampus,
4) Npy1rY5R-/- mice show an anxious phenotype that might be related to inactivation of the Y1R in the BLA. Additionally, Npy1rY5R-/- mice display increased spatial reference memory, suggesting an inflexible-perseverative phenotype and habit learning (Longo et al, 2014; 2015).

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) "*Gender difference in vulnerability to metabolic challenges*": to determine the effect of a moderate to high fat, high-energy diet on Npy1r gene expression in the hypothalamus of male and female mice.
3) "*Neuropeptide Y pathways in learning and memory":* to examine whether selective ablation of Npyr in forebrain excitatory neurons may affect learning and memory and PNN expression.
4) "*Npy1r and Behavioural inflexibility*": to uncover whether the targeted disruption of Npy1r gene in Y5R containing neurons affects cognitive flexibility and neuronal activity in the OFC and whether treatment with the SSRI escitalopram normalizes OFC neuronal activity and restores behavioural flexibility in Npy1rY5R-/- mice.

Results

1) We analyzed the emotional and cognitive behavior analysis of control and Npy1rrfb mice adopted by mothers known to have a different maternal approach to their offspring, We have confimed the differences observed in maternal care between the FVB/J or C57BL/6J strain of mice used as foster mothers. We then performed an extensive battery of behavioral tests for anxiety and cognitive abilities. We analyzed anxiety by using the open field arena, the elevated plus maze and the light/dark box test. By now, the latter showed the best results in validating the expected anxious phenotype of the Npy1rrfb and LMC mice compared to control HMC control mice. In addition we tested cognitive abilities using the burrowing test, that detects deterioration in the ability to perform daily activities and the puzzle box test, a problem-solving test with increasing difficulty. We found that Npy1rrfb HMC and LMC-mice presented deficits in burrowing behavior, due to the presence of the knockout and to the maternal care received, respectively.
2) We demonstrated that, when fed with either standard or high fat diet, Npy1rrfb female mice display no differences in calories intake, body weight growth, perigonadic WAT and plasma glycaemia, compared with their control littermates. Conversely, ovariectomized (ovx) Npy1rrfb female mice (a condition mimicking menopause) fed with either standard or high fat diet show a significant increase of body weight growth, visceral and subcutaneous WAT weight, plasma leptin levels and with lower glucose tolerance compared to ovx control females. This phenotype is associated with a decrease in spontaneous locomotor activity, and, at the molecular level, with a decreased POMC and increased NPY immunoreactivity in the

paraventricular nucleus (PVN) of the hypothalamus. Interestingly, Npy1rrfb females, but no male, mice, show a significant decrease of Npy1r mRNA in the PVN.
3) Npy1rrfb mice show a delayed spatial learning in both the Morris water maze and the Barnes maze compared to control mice. Moreover, Npy1rrfb mice showed a significant increase of the number of strongly stained PNNs in the stratum pyramidale and the stratum oriens of the CA1 region. We found that in Npy1rrfb animals PNN coating is significantly more branched and more extended in length only in the stratum oriens when compared to control animals. 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.
4) Npy1rY5R-/- mice display reversal learning impairment in the early phase of both MWM and WTM reversal tasks, as shown by the longer latency (MWM) and the increased number of trials (WTM) required to learn the new platform location, compared to their controls littermates. Reversal learning impairment of Npy1rY5R-/- mice is associated with an anxiety-like behavior but it appears to be otherwise highly specific and not associated with changes in spatial learning memory, stereotypic-repetitive and compulsive behaviors, sociability and social memory. Furthermore, Npy1rY5R-/- male mice display increased c-Fos-IR in the layers II and III of the OFC, where ≈80% of Y1R/Y5R positive neurons colocalize with pyramidal neurons. Acute treatment of Npy1rY5R-/- mice with escitalopram reverses the inflexible phenotype and decreases OFC neural hyperactivity suggesting that a dysregulation of serotonin within the OFC might be responsible for the reversal impairment induced by the conditional inactivation of Npy1r gene in these mice. Our results suggest that, in the corticostriatal circuit, this phenotype is specifically associated to increased neuronal activity in the OFC since no significant changes in c-Fos-IR were observed in the dorsomedial striatum of saline or escitalopram treated mutant mice (Longo 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 anxiety. Moreover, maternal environment fails to affect cognitive abilities.
2) We have shown, for the first time, a sexual dimorphism of Npy1r expression in the PVN that might underline the interaction between the Npy1r signal and estrogens in the modulation of energy metabolism in relation to reproductive functions.
3) We demonstrated that local enzymatic digestion of PNNs reverses the learning deficits of Y1 knock-out mice, highlighting a previously unknown link between NPY-Y1 function and PNN regulation.
4) This study has demonstrated that targeted deletion of Npy1r gene in Y5R coexpressing neurons leads to increased anxiety, behavioral inflexibility, OFC baseline hyperactivity and it is sensitive to SSRI, suggesting that Npy1rY5R-/- mice may provide a new neurobehavioral mouse model of the cognitive inflexibility endophenotypic trait within OCD with a degree of predictive, as well as construct and face validity.

7. Future Projects (Next 3 years)

Summary:

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

Background and Significance:

1) "*Vulnerability to psychopathologies*"
We previously demonstrated that lower expression levels of Npy1r in the limbic system of adult mice, induced by low levels of maternal care or by postnatal inactivation of Npy1r in excitatory neurons, increases anxiety and HPA axis activity but fails to affect cognition. Preliminary results also suggest that conditional inactivation of limbic Npy1r or low levels of maternal care increases PNN thickness in the PFC.
Given the role that PNN development and myelin maturation during juvenile development retain in regulating synaptic activity and structural stability and given that impaired PFC function and plasticity is thought to be a core pathological feature of several neuropsychiatric disorders, our main goal is to evaluate whether: i) a rearrangement of PNNs (in terms of thickness, molecular composition, sulfation pattern, physical properties) and myelin development in limbic areas is involved in maternal care programming of anxiety-like phenotype; ii) limbic Npy1r mediated transmission has an important function in maternal environment modulation of PNN. Development of the present project will be based on the synergic collaboration among groups working at NICO (A. Buffo and D. Carulli) and international groups characterized by diverse, but strongly complementary, scientific expertise. By connecting molecular interactions and matrix properties to behavior, we expect that this collaborative research will offer mechanistic insight into the role of plasticity modulators in inducing anxiety in early-life stressed mice.
2) "*Gender difference in vulnerability to metabolic challenges*"
Prior studies demonstrated that estrogen, not progesterone, is responsible for the control of energy homeostasis. In mammals ovaries and liver are the main targets of estrogen action and the liver estrogen receptor (the isoform ERα) is essential for the regulation of lipid metabolism. Previous results obtained by A. Maggi's laboratory (University of Milano) suggest that the liver is a key element at organismal level for the co-ordination of the hormetic response. The aim of the research, that will take advantage from previuos collaboration among NICO, the University of Milano (A. Maggi, E. Nisoli) and of Parma (P. Palanza) is to demonstrate the mechanistic and biological consequences of liver ERα stimulation on organs other than liver and relevant for energy homeostasis (fat, muscle and brain) in mice fertile or with surgical menopause. The study will be done in the presence/absence of hormetic stimuli (e.g. overnutrition, exercise). In particular our laboratory will investigate physiological and neurochemical mechanisms underlying the cross talk between estrogen and brain Npy1r in the control of energy homeostasis and, most importantly, whether such interaction could involve bidirectional signals between liver and brain.
3) *Npy1r and Behavioural inflexibility*
To further demonstrate that reversal learning deficit of Npy1rY5R-/- is specific for Npy1r deletion in the OFC we will analyze the effect of OFC-specific Npy1r deletion on reversal learning task performance and OFC cfos expression (see methods). We will also analyze whether escitalopram treatment will normalize OFC neuronal activity and restore behavioural flexibility in OFC-specific Npy1r deletion.

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

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

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

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

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 behavior on anxiety and stress susceptibility may be mediated by a precocious development of plasticity brakes;

- alterations of myelin structure and myelin plasticity inhibitors.

- remodeling of GABAergic, glutamatergic and NPYergic innervation and modifications of dendritic branching and dendritic spines.

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

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

Moreover:

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

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

2) *"Gender difference in vulnerability to metabolic challenges"*

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

-body weight, food consumption, glucose and insulin tolerance tests, locomotor activity, blood pressure

- Npy1r, NPY and CRH mRNA expression in limbic system and hypothalamus

-histochemical analysis of αMSH and CART (anorexigenic peptides) and NPY and AgRP (orexigenic peptides) in the hypothalamus.

-in liver, fatty acids, FGF21, Sirt1, lipid metabolism, mitochondrial biogenesis, activity and respiration; ii) in serum, IGF-1, GH, leptin, tryglicerides, FFA, LDL, HDL, cholesterol, FGF21 Glucose, Insulin; iii) in WAT, BAT, muscle and brain, mitochondrial biogenesis, UCP1, mitochondrial analysis by electron and confocal microscopy, ATP and O2 consumption, glucose concentration, lipid metabolism, and insulin sensitivity, Sirt1; in WAT and BAT, white adipose fat and sympathetic parameters.
In addition:
- to investigate the physiological relevance of the liver-CNS axis for the control of energy metabolism, in collaboration with Dr. A. Maggi (University of Milano) we will analyze the effect of the different diets in combination with ovx on NPY signaling in limbic and hypothalamic areas of wild type and liver specific ERα ko mice.

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, and optogenetic manipulation of neuronal activity , to address the role of plasticity-regulatory molecules in early-life stress-induced anxiety from entirely novel angles, spanning different levels of complexity, from molecules to the living organism. We expect to gain further insight into the complex and fine-tuned mechanisms underlying developmental programming. Moreover, we expect to elucidate whether pathological behaviours can be reverted to normal by enriched experience and/or pharmacological treatment in adulthood. The knowledge that will originate from this application has a tremendous potential in view of finding novel therapeutic approaches and early intervention strategies for the cure and possibly prevention of mental disorders, such as anxiety and stress-related disorders that have a strong relationship with early life adversities.
"Gender difference in vulnerability to metabolic challenges"
This is an innovative study focused on novel pathogenetic mechanisms that may lead to the age-related disorders in females. It is based on the integration of the knowledge of multiple disciplines (molecular biology, cell biology, behavioral science and metabolic phenotyping), as well as on the synergic collaboration between groups characterized by diverse, but strongly complementary, scientific expertise. It is expected that this collaborative research will allow: i.) to demonstrate the extent by which changes of ovarian estrogens and liver ERs are responsible for the increased incidence of MetS during menopause; ii.) to identify the potential mechanisms involved in this phenomenon, including chronic social stress; iii.) to evaluate the effects of specific dietary regimens in females with impaired ovarian functions. In case we were able to demonstrate a major involvement of liver ER in the dysmetabolisms consequent to the cessation of ovarian functions, our study will open the way to the generation of an entire class of novel Estrogen Receptor Modulators to be used for the therapy of MetS, thus allowing a major step forward in the development of therapies for a disorder that so far cannot be satisfactorily treated.
Methodology : please fill-out this section only in the case of innovative technologies
To generate OFC-specific *Npy1r* gene knockout mice, we will apply combinatorial genetic approach using viruses for conditional Cre recombinase (*Cre*) gene expression in genetically engineered mice (*Npy1r*^{2lox}) 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_{tet}*bi-Cre/tdTOM*) is equipped with a bidirectional tet promoter (P_{tet}*bi*) to simultaneously express two different genes encoding for the Cre recombinase protein and a red fluorescent protein variant, the tdTomato (tdTOM), for

visualizing virus-targeted neurons. The *Cre* and the *tdTOM* genes are expressed when rtTA binds to $P_{tet}bi$ in the presence of Dox. To increase the spread of virus in the OFC by hyperosmolarity, D-mannitol/virus mixture will be injected in the brain and D-mannitol was also delivered into mice systemically by intraperitoneal injection. Viruses will be injected in the OCD of age-matched wild-type littermates and $Npy1r^{2lox}$ mice. tdTOM and Cre expression in the OFC will be analysed three weeks after virus injection. Virus (v)-delivered Cre expression in neurons enabled, by Cre-loxP-mediated gene recombination, the generation of highly specific *Npy1r* gene knockout mice, *Npy1r*-$v\Delta^{OFC}$, in which the *Npy1r* gene will be selectively deleted (Δ) in the OFC. The virus-injected, age-matched wild-type littermates will serve as controls (*Contr*-v^{OFC}).

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Nerve Regeneration

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

Stefano Geuna — Birthdate (25/09/1965)

Degree: MD — Gender: M

Nationality: Italian — Phone: +39 011/67054

Email: stefano.geuna@unito.it

- **Personnel**

1. Stefania Raimondo — Birthdate (25/02/1977)

   Degree: Biological Sciences — Gender: F

   Role: Assistant Professor — 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 — Gender: F

   Role: PostDoc fellow — 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 — Gender: F

   Role: Post-Doc fellow — Nationality: Italian

   Expertise: Optical microscopy and confocal analysis of histological and cytological specimens prepared by histological techniques, histochemical and immunofluorescence; Electron microscopy and ultrastructural analysis; Analysis of recovery of motor function in rats and mice using behavioral tests; Quantitative morphological analysis of the regeneration of nerve fibers by stereological methods; Extraction and culture of primary lines of ganglion sensory neurons; Analysis of protein expression (western blotting) and mrna (qualitative rt-pcr and real time quantitative rt-pcr); Cell culture, transient and stable transfection, proliferation assays, migration assays, time lapse; Recombinant techniques of molecular biology (cloning, production of fusion proteins with gfp or flag, preparation of constructs in plasmids, adenoviral and lentiviral vectors); Validation of microarray analysis.

4. Luisa Muratori — Birthdate (02/05/1984)

Degree: Master degree in Neurobiology Gender: F

Role: PhD Student Nationality: Italian

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

5. Benedetta Elena Fornasari Birthdate (11/07/1989)

Degree: Master degree in Molecular and Cellular Biology Gender: F

Role: PhD Student Nationality: Italian

Expertise: Biomolecular Techniques: DNA, RNA and protein extraction, quantitative Real-time PCR, primers study and design, Western blot; Cellular Biology: cell colture, primary colture of Schwann cells and dorsal root ganglia, cell transfection, cell migration assays, proliferation and viability assays, time lapse assays; Morphological Analysis: immunocitofluorescence, resin embedding procedures; Animal Care; Biomaterial Production: production of fibres through electrospinning technique

6. Michela Morano Birthdate (29/10/1988)

Degree: Master degree in Molecular and Cellular Biology Gender: F

Role: PhD Student Nationality: Italian

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

7. Loredana Grasso Birthdate (13/08/1982)

Degree: Master degree in biological science Gender: F

Role: Research fellow Nationality: Italian

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

2. PRINCIPAL INVESTIGATOR CURRICULUM VITAE

H index, 39; Citations, 4433

Relevant discoveries:
The results of our recent research, in the context of the European Project funded (Biohybrid), allowed the development of chitosan-based medical device for improving functional recovery after radical prostatectomy. The device has already achieved a clinical use for the peri-prostatic nerve plexus protection and a patent (Application reference: 102016000070911).

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

PhDs supervised in 2016

.

- Morano Michela
- Muratori Luisa

Outreach activities

- Invited talks

Scientific Meeting on Peripheral Nerve Regeneration, Lund University - Malmo, Sweden October 2016

- Editorial duties

Member of Editorial Board of *Microsurgery*

Technology transfer achievements

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

3. PI's PUBBLICATIONS:

1: Fornasari BE, El Soury M, De Marchis S, Perroteau I, **Geuna S**, Gambarotta G. Neuregulin1 alpha activates migration of neuronal progenitors expressing ErbB4. *Mol Cell Neurosci.* 2016 Dec;77:87-94. doi: 10.1016/j.mcn.2016.10.008. Epub 2016 Oct 27. PubMed PMID: 27989735. IF 3,597 Q2

2: Gnavi S, Fornasari BE, Tonda-Turo C, Laurano R, Zanetti M, Ciardelli G, **Geuna S**. In vitro evaluation of gelatin and chitosan electrospun fibers as artificial guide in peripheral nerve repair: a comparative study. *J Tissue Eng Regen Med.* 2016 Nov 12. doi: 10.1002/term.2351. [Epub ahead of print] PubMed PMID: 27860458. IF 4,710 Q1

3: Fregnan F, Ciglieri E, Tos P, Crosio A, Ciardelli G, Ruini F, Tonda-Turo C, **Geuna S,** Raimondo S. Chitosan crosslinked flat scaffolds for peripheral nerve regeneration. *Biomed Mater.* 2016 Aug 10;11(4):045010. doi: 10.1088/1748-6041/11/4/045010. PubMed PMID: 27508969. IF 3,362 Q2

4: Papalia I, Magaudda L, Righi M, Ronchi G, Viano N, **Geuna S**, Colonna MR. Epineurial Window Is More Efficient in Attracting Axons than Simple Coaptation in a Sutureless (Cyanoacrylate-Bound) Model of End-to-Side Nerve Repair in the Rat Upper Limb: Functional and Morphometric Evidences and Review of the Literature. *PLoS One.* 2016 Feb 12;11(2):e0148443. doi: 10.1371/journal.pone.0148443. eCollection 2016. PubMed PMID: 26872263; PubMed Central PMCID: PMC4752340. IF 3,057 Q1

5: Meyer C, Stenberg L, Gonzalez-Perez F, Wrobel S, Ronchi G, Udina E, Suganuma S, **Geuna S**, Navarro X, Dahlin LB, Grothe C, Haastert-Talini K. Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves. *Biomaterials.* 2016 Jan;76:33-51. doi: 10.1016/j.biomaterials.2015.10.040. Epub 2015 Oct 21. PubMed PMID: 26517563. IF 8,387 Q1

6: **Geuna S,** Raimondo S, Fregnan F, Haastert-Talini K, Grothe C. In vitro models for peripheral nerve regeneration. *Eur J Neurosci.* 2016 Feb;43(3):287-96. doi: 10.1111/ejn.13054. Epub 2015 Sep 28. Review. PubMed PMID: 26309051. IF 2,975 Q2

7: Tos P, Crosio A, Pellegatta I, Valdatta L, Pascal D, **Geuna S**, Cherubino M. Efficacy of anti-adhesion gel of carboxymethylcellulose with polyethylene oxide on peripheral nerve: Experimental results on a mouse model. *Muscle Nerve.* 2016 Feb;53(2):304-9. doi: 10.1002/mus.24739. Epub 2015 Aug 8. PubMed PMID: 26082205. IF 2,713 Q2

8: Ronchi G, Haastert-Talini K, Fornasari BE, Perroteau I, **Geuna S**, Gambarotta G. The Neuregulin1/ErbB system is selectively regulated during peripheral nerve degeneration and regeneration. *Eur J Neurosci.* 2016 Feb;43(3):351-64. doi: 10.1111/ejn.12974. Epub 2015 Jul 8. PubMed PMID: 26061116. IF 2,975 Q2

9: Shapira Y, Tolmasov M, Nissan M, Reider E, Koren A, Biron T, Bitan Y, Livnat, M, Ronchi G, **Geuna S**, Rochkind S. Comparison of results between chitosan hollow tube and autologous nerve graft in reconstruction of peripheral nerve defect: An experimental study. *Microsurgery.* 2016 Nov;36(8):664-671. doi: 10.1002/micr.22418. Epub 2015 Apr 22. PubMed PMID: 25899554. IF 2,054 Q2

10: Meyer C, Wrobel S, Raimondo S, Rochkind S, Heimann C, Shahar A, Ziv-Polat O, **Geuna S,** Grothe C, Haastert-Talini K. Peripheral Nerve Regeneration Through Hydrogel-Enriched Chitosan Conduits Containing Engineered Schwann Cells for Drug Delivery. *Cell Transplant.* 2016;25(1):159-82. doi: 10.3727/096368915X688010. Epub 2015 Apr 14. PubMed PMID: 25876520. IF 3,427 Q3

4. GROUP's PUBBLICATIONS:

5. GROUP's additional information:

Please list the grants of the other members of the group in the last year (2016) 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%
2015-2016	National (Local)	S. Raimondo PI	Università degli Studi di Torino	Studio della regolazione del sistema neuregulina/erbB da parte dei miRNA durante la rigenerazione e del sistema nervoso periferico	Fondo per la Ricerca Locale	€ 10.654	8%
2016- 2017	National (Local)	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 (Local)	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%

Outreach activities of other members of the group:

- Collaborative experiences.

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

- Editorial duties

- Dr. Stefania Raimondo: Reviewer for Scientific International Journal (Acta Biomaterialia, International Review of Neurobiology, BioMed Research International).

- Dr. Giulia Ronchi Reviewer for Life Science and BioMed Research International.

6. Past Research activity (2016)

Summary

The research activities of Geuna's group is focused on the study of peripheral nerve repair and regeneration. 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.

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 loss of substance.

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). Transected 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.

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 bring 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.

Objectives
The objectives of Geuna's group activities are 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) Development of chitosan-based medical device for improving functional recovery after radical prostatectomy. 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 conduct 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 analyses have 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.

Results
Research activities carried out during 2016 are summarized below:

<u>Role of Neuregulin1 alpha in migration of neural progenitors</u>
Deficits in neuronal migration during development in the central nervous system may contribute to psychiatric diseases. In vitro and in vivo analysis were performed in order to study the the ability of the four ErbB4 isoforms to confer a different migratory activity to neuronal progenitors. The ligand neuregulin1 (NRG1) and its receptor ErbB4 are genes conferring susceptibility to schizophrenia, playing a key role in the control of neuronal migration both during development and adulthood. Our hypothesis was strengthened by the observation that both NRG1α and NRG1β and the four ErbB4 isoforms are expressed in the medial and lateral ganglionic eminences and in the cortex during development in rat. In vitro analysis of the signal transduction pathways activated by the different ErbB4 isoforms following the treatment with soluble recombinant NRG1α or NRG1β and the ability to stimulate migration showed that two ErbB4 isoforms, strongly activate AKT phosphorylation, conferring high migratory activity to neuronal progenitors, demonstrating that both NRG1α and NRG1β can play a role in neuronal progenitors migration.

<u>Neuregulin1/erbb system: the implication in skeletal and cardiac muscle recovery after injury.</u>
Nrg1/ErbB system has been deeply studied in peripheral nerve where Nrg1 is a key factor for determining axon ensheathment, myelin thickness and the formation of neuromuscular junctions at the muscular level during development. Several studies demonstrated that endogenous Nrg1 is necessary for nerve repair in adulthood, suggesting that therapies base on recombinant Nrg1 could be effective for nerve regeneration and functional recovery after injury. In particular the soluble Nrg1 isoforms have been shown up-regulated after injury. They drive the dedifferentiation of Schwann cells and their migration in the site of injury to create the *Bands* of Büngner, a tubular structure that direct growing axons to their original targets. Moreover a transmembrane Nrg1 expressed by the axons guides the deposit of myelin layers by Schwann cells expressing ErbB2/ErbB3 receptors, regulating remyelination process. Different approaches have been already investigated for the delivery of Nrg1 in the site of

injury to accelerate and improve nerve repair, but, despite the encouraging results, we are far away to a complete functional recovery.
Therapies for nerve regeneration should monitor also nerve target behavior, avoiding muscle atrophy and promoting the correct reinnervation of muscle fibers to obtain total functional recovery. After nerve injury the target muscle undergoes to a molecular and morphological changes that result in muscle atrophy, and when denervation persists permanent changes occur, reducing the possibility to recover the complete functionality after reinnervation. Meanwhile the role of Nrg1 and ErbB receptors is well defined in nerve, little is known about Nrg1/ErbB system in muscle after nerve injury. It is known that Nrg1 has a role in muscle development and controls spindle maintenance, glucose uptake and neuromuscular junction formation. Moreover ErbB2/ErbB3 expression in satellite cells are able to induce pro-survival signalling in activated cells. How Nrg1/ErbB system is influenced by nerve acute injury, and if the system could be a good therapeutic target to maintain the muscle receptive for nerve reinnervation.
In another muscular tissue, the cardiac tissue, is becoming more and more clear that Nrg1/ErbB system is a potential target for therapy in heart failure. Nrg1/ErbB system is essential for a correct cardiac development, furthermore, it is now clear that also in adult heart this signalling plays a critical role in the normal function as well as in ischemia or other pathological conditions. For its pro-survival effect Nrg1 has been proposed as a potential drug for heart failure treatment.

Peripheral nerve repair with conduits: enrichement of chitosan tubes with skeletal muscle fibres to improve peripheral nerve regeneration

To repair nerve gaps following severe peripheral nerve injuries, chitosan tubes were proved to give good results in the context of "Biohybrid" project, comparable with nerve autografts, the gold standard technique.
To further improve peripheral nerve regeneration using chitosan tubes, a conduit enrichment strategy was developed using longitudinal skeletal muscle fibres, which have been previously shown to be good fillers in the "muscle in vein" experimental paradigm, where muscle played a trophic and a structural role.
To this aim, rat median nerve gaps were repaired using chitosan tubes filled with a longitudinal piece of *pectoralis major* muscle ("muscle in tube") and hollow chitosan tubes. Different biomolecular and morphological analysis were carried out at early time points post surgery. Furthermore functional assay and stereological analysis were performed on the distal part of regenerated nerve 3 months after nerve repair, showed no significant differences in the regeneration outcome between hollow chitosan tube and "muscle in tube" groups.

Development of chitosan-based medical device for improving functional recovery after radical prostatectomy.

The chitosan membranes tested in this project are made of the same material that has already been successfully tested for the repair of somatic nerves in the form of hollow conduits, in the context of Biohybrid project.
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 disfunction often results after radical prostatectomy due to damage of the peri-prostatic nerve bundles.
The aim of this in vitro study is to assess the regenerative and anti-cancer properties of a biomedical device consisting of chitosan (CS), which has already achieved a clinical use for the peri-prostatic nerve plexus protection and a patent (Application reference: 102016000070911).
CS is a derivative of chitin, a natural material 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.
The CS-anti-proliferative properties on cancer cells were tested on different human prostate cancer cell lines (PC-3, DU145, LNCap) seeded on CS films and evaluated in terms of cell proliferation and morphology. Results showed that, after 2-5-7 days from plating, the direct contact of the cell lines with the CS substrate determines not only a substantial change in morphology, but also a significant decrease in proliferation.
The regenerative potential of CS films was assessed through primary neuronal cultures derived from different ganglia: giugular and nodose ganglia originating from the vagus nerve, as model for the parasympathetic segment, stellate ganglion, as model for sympathetic ganglion and dorsal root ganglia allowed to study the regenerative process on sensory somatic neurons. Different parameters have been tested: cell survival, neurite outgrowth and cell morphology, proving that the material represents a permissive substrate for neurite regeneration.
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.

Study of the postponing nerve repair in peripheral nerve regeneration.
Peripheral nerve regeneration and functional recovery after nerve injury are affected by many factors including lesion site, injury severity, patient gender and age, neuronal plasticity and time interval between nerve injury and surgical repair. This study focused the attention on nerve regeneration after delayed nerve repair.
A rat surgical model of delayed nerve repair consisting of a cross suture between 3/6 months degenerated median nerve distal stump and freshly axotomized ulnar proximal stump was used. Before the suture, a segment of degenerated median nerve stump was harvested for analysis. Functional, morphological, morphometric and biomolecular analyses were carried out on regenerated nerves 6 months after repair.
After delayed nerve repair, functional recovery is compromised. Morphometric analysis shows a significant reduction of number and size of regenerated myelinated fibers. Biomolecular analysis shows that soluble NRG1 is still strongly downregulated and that Schwann cell marker expression is still impaired.
Despite a long delay, fibres are still able to regenerate, even if fewer and smaller. The poor outcome after delayed nerve repair might be explained by Schwann cell impairment and their ineffective support for nerve regeneration. Providing a source of soluble NRG1 might be a good strategy to improve the outcome.

Advancement in the field
Results of Geuna's group research in the context of the European Biohybrid project allowed to develop an innovative chitosan-based membranes for protecting nerve damage and erectile functional impairment after prostatic cancer resection. Translational and regulatory work for clinical application are ongoing activities.
In addition, enrichement of conduit with skeletal muscle fibres represents an innovative advancement in the regenerative context.

7. Future Projects

Summary:

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.

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.

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

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 both 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 disorgers.

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, physical stimulation of tissue repair and the use of decellularized allografth nerve.

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.

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 been 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 marked of the Reaxon© nerve guides the patent on iron-conjugated neurogulin-1.

Methodology: <u>please fill-out this section only in the case of innovative technologies</u>

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 2016

Laboratory name: Neuroendocrinology

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

GianCarlo Panzica — Birthdate (17/08/1949)
Degree: PhD — Gender: M
Nationality: Italian — Phone: 011 670 6607
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- Personnel

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

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

3. Alice Farinetti — Birthdate: (23/12/1981)
Degree: PhD — Gender: F
Role: Post-Doc — Nationality: Italian
Expertise: Neurogenesis, Gonadal hormones

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

2. PRINCIPAL INVESTIGATOR

H index, 37; Citations, 4686

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): the alteration of the brain circuits dedicated to the control of food intake and energy metabolism.

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2014-2016	National (Local)	PI	UNITO-ex 60% 2013	Effetti cerebrali degli obesogeni: azione della TBT sul circuito a NPY		2.127	8%
2013-2016	Internation al Foundation		Postfinas teride Foundati	Effects of finasteride on behavior and		14.656	10%

			on	neurosteroids			
2015-2017	National (Local)	PI	UNITO-ex 60% 2014	Distruttori endocrini e circuiti che regolano l'assunzione di cibo ed il metabolismo energetico		2.343	8%
2016-2019	National (Spain)	C	Ministerio de Ciencia e Innovación (Spain)	Involvement of estradiol on feeding neurohormonal circuit programming in the rat		70.000	0

PhDs supervised in 2016.

Marilena Marraudino

Outreach activities

- International collaborative experiences.

Dr. Panzica had several international cooperations, and many of them are still active. They are chronologically listed here:
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.
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.
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.
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 Ministery 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
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
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
http://www.endocrine.org/membership/email-newsletters/endocrine-insider/2014/april-17-2014/society-expands-eu-advocacy-experts-talk-edcs-to-policymakers-in-brussels
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

Interferenti endocrini e circuiti neuroendocrini che controllano l'assunzione del cibo e il metabolismo energetico (INN: Prospettive di neuroscienze, Verona, June 2016)

- Editorial duties.

Member of the Editorial board of:

Frontiers in Endocrinology (2015-today)

Guest editor of the following special issues:

- Gender influence on neurodegenerative and psychiatric disorders (by R.C. Melcangi and G.C. **Panzica**) *Special Issue* Neuroscience & Biobehavioral Reviews vol. 67, 2016, pp 1-146 ISSN: 0149-7634.
- Steroids & Nervous System (by R.C. Melcangi and G.C. **Panzica**) *Special Issue* The Journal of Steroid Biochemistry and Molecular Biology, vol. 160, pp 1-226 ISSN: 0960-0760.

3. PI's PUBBLICATIONS:

For each publication, please indicate:
* if you contributed equally to the fist-author, as stated in the published article

Schellino R, Trova S, Cimino I, Farinetti A, Jongbloets BC, Pasterkamp RJ, **Panzica G**, Giacobini P, De Marchis S, Peretto P. (2016). Opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis. *Sci Rep.* Oct 26;6:36063.
IF= 5.228; R = 7/63

Mele P, Zammaretti F, Longo A, **Panzica G**, Oberto A, Eva C. (2016) Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor gene expression in leptin treated obese (ob/ob) or lean mice. *Brain Res.* Oct 15;1649 (Pt A):102-109.
IF= 2.561; R = 143/256

Kortenkamp A, Bourguignon JP, Slama R, Bergman Å, Demeneix B, Ivell R, **Panzica G**, Trasande L, Zoeller RT. (2016). EU regulation of endocrine disruptors: a missed opportunity. *Lancet Diabetes Endocrinol.* Aug;4(8):649-50.
IF= 16.320; R = 2/133

Bourguignon JP, Slama R, Bergman Å, Demeneix B, Ivell R, Kortenkamp A, **Panzica G**, Trasande L, Zoeller RT. (2016). Science-based regulation of endocrine disrupting chemicals in Europe: which approach? *Lancet Diabetes Endocrinol.* Aug;4(8):643-6.
IF= 16.320; R = 2/133

Bo E, Farinetti A, Marraudino M, Sterchele D, Eva C, Gotti S, **Panzica G**. (2016). Adult exposure to tributyltin affects hypothalamic neuropeptide Y, Y1 receptor distribution, and circulating leptin in mice. *Andrology*. Jul;4(4):723-34.
IF= 2.515; R = 2/5

Melcangi RC, **Panzica GC.** (2016). Gender influence on neurodegenerative and psychiatric disorders. *Neurosci Biobehav Rev.* Aug;67:1.
IF= 8.580; R = 3/51

Panzica G, Melcangi RC. (2016). Structural and molecular brain sexual differences: A tool to understand sex differences in health and disease. *Neurosci Biobehav Rev.* 2016 Aug;67:2-8.
IF= 8.580; R = 3/51

Slama R, Bourguignon JP, Demeneix B, Ivell R, **Panzica G**, Kortenkamp A, Zoeller RT. (2016). Scientific Issues Relevant to Setting Regulatory Criteria to Identify Endocrine-Disrupting Substances in the European Union. *Environ Health Perspect*. Oct;124(10):1497-1503.
IF= 8.44; R = 5/225

Porcu P, Barron AM, Frye CA, Walf AA, Yang SY, He XY, Morrow AL, **Panzica GC**, Melcangi RC. (2016). Neurosteroidogenesis Today: Novel Targets for Neuroactive Steroid Synthesis and Action and Their Relevance for Translational Research. *J Neuroendocrinol.* Feb;28(2):12351.
IF= 3.172; R = 53/133

Giatti S, Foglio B, Romano S, Pesaresi M, **Panzica G**, Garcia-Segura LM, Caruso D, Melcangi RC. (2016). Effects of Subchronic Finasteride Treatment and Withdrawal on Neuroactive Steroid Levels and Their Receptors in the Male Rat Brain. *Neuroendocrinology*.;103(6):746-57.
IF= 2.583; R = 74/133

4.GROUP's PUBBLICATIONS:

5. GROUP's additional information:

Grants (current and pending) of the other members of the group

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
01/01/2015-31/12/2017	National (Local)	Gotti S; PI	UNITO-ex 60% 2014	The Kisspeptin neuronal system: study of the distribution and of the pubertal development		1.854	8%
01/10/2015-31/10/2018	National (Local)	Gotti S; PI	UNITO-ex 60% 2015	The Kisspeptin neuronal system: study of the distribution and of the pubertal development		1.669	8%
28/06/2016-30/06/2018	National (Local)	Gotti S; Co-PI	UNITO-ex 60% 2016-progetti traslazionali	Anorexia nervosa, physical activity, hormones and morphological alteration in hippocampus. A translational study		27206	8%
22/12/2015-05/01/2018	National (Local)	Gotti S; PI	CRT, richieste ordinarie	Ruolo dei neuropeptide ipofisari e degli ormoni gonadici nel disturb da deficit di attenzione/iperattività		28000	8%
20/02/2017-20/02/2019	National (Local)	Gotti S; PI	UNITO-ex 60% 2016-2017	Effetti della somministrazione prenatale di chlorpyrifos nei circuiti ipotalamici del topo		4755	8%
Pending	National	Gotti S; PI	ARISLA	ALS and environmental risk factors: new insight		60000	
Pending	National	Gotti S; PI	FISM	Environmental factors in		30000	

				multiple sclerosis: bisphenols exposure effects.			
1/1/2015 - 31/1/2016	National (Local)	Ponti G; PI	UNITO-ex 60% 2014	Analisi dell'effetto dei fitoestrogeni sullo sviluppo del sistema nervoso centrale nei mammiferi		10.000	8%
1/1/2016 - 31/01/2017	National (Local)	Ponti G, Co-PI	UNITO-ex 60% 2015	Pattern di espressione genica nel differenziamento mammario della specie bovina		11984	8%
1/01/2017- 31/01/2018	National	Ponti G, Co-PI	UNITO-ex 60% 2016	Valutazione innovativa del benessere animale e della resilienza attraverso biosensori e biomarcatori nei ruminanti		14.256	8%
1/01/2017- 31/01/2018	National (Local)	Ponti G, Co-PI	UNITO-ex 60% 2017	Valutazione multifattoriale del benessere animale in avicunicoltura		10120	8%

Honors, prizes or awards received by other members of the group

Ponti G.

- 2-2-2017 Has achieved the Academic Qualification as Lector in Life Sciences from the Agència per a la Qualitat del Sistema Universitari de Catalunya (AQU) Please list outreach activities of other members of the group:
- since 2016 partecipates to the University of Turin working group in the "HackUNITO for aging"

- Editorial duties

Ponti G

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

Gotti S
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

Organizational activities:

- Speakers invited by members of the group

Ponti G

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

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

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

6 .Past Research activity

Summary
The central focus of our researches has been the study of the interactions among steroids and nervous circuits. In particular, last year we demonstrated the importance of testosterone on the neurophysiology of olfaction and the role played by neurosteroids in many disease. Moreover, we studied the effect of endocrine disruptors in the derangement of the circuit involved in the control of energetic metabolism and we contributed in the realization of several critical papers related to endocrine disruptors and European legislation.

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 (ERα and ER β) 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 endocrine disrupting chemicals (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 this 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.

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.

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

Results
Role played by neurosteroids (Translational research). We have reviewed the role played by neurosteroids in many diseases, as evidenced in both animal models and patient surveys **(Porcu et al., 2016).** We have highlighted how a drug used to cure alopecia (finasteride) is able to induces rather important behavioral effects (depression) and interferes with the normal levels of neurosteroids in an animal model **(Giatti et al., 2016).**

Gender Differences in the Brain and Relationship with Nervous System Diseases. We critically discussed the latest findings in the field of gender differences at the cerebral level, the different causes that induce these differences and their relevance especially in relation to psychiatric and neurodegenerative diseases **(Panzica and Melcangi, 2016).** Moreover, we demonstrated the importance of testosterone on the neurophysiology of olfactory bulb in the adult, to explain the attraction between opposing sexes **(Schellino et al., 2016).**

European legislation and endocrine disruptors.
We contributed in the realization of three papers that describe the issues surrounding the characterization and definition of endocrine disrupters, the uncertainties of the European Commission in the promulgation of laws and regulations in this area, and the resistance of industry to the introduction of new rules **(Bourguignon et al, 2016; Kortenkamp et al, 2016; Slama et al, 2016).**

Food intake and metabolism. We described the involvement of the NPY system and its Y1 receptor in obese or normal mice and its relationships with the treatment of exogenous leptin **(Mele et al., 2016).** In addition, we showed that the exposure of adult animals to a molecule classified as obesogenous (tributyltin, TBT) results in a complete derangement of the axis leptin – NPYsystem - Y1 receptor, confirming that metabolic disrupters have among their

targets not only the adipose tissue, but also the neuroendocrine hypothalamic circuits that control metabolism and food intake **(Bo et al., 2016).**

Anorexia nervosa (a translational study).
The anorexia nervosa is a severe mental disorder with a complex pathogenesis and typically affects women. The neonatal period and the influence of attachment to the mother is a critical life phase. For this reason, we have analyzed male and female adolescent rats and tested the effect of emotional deprivation, induced by the maternal separation in a model of Anorexia nervosa. Our preliminary results indicate that the maternal separation induces in the females a greater hyperactive behavior than in males. This study has potential translational implications for better understanding the role of early environment in the pathogenesis of this disorder and we are preparing a manuscript with these data.

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 3 years)

Summary:
A large effort of our team is directed to the study of the kisspeptin (Kiss) system (see our participation to a COST action) as a tool to understand the action of endocrine disrupters over the reproduction and metabolism control. One of the major targets of the Kiss system, is the paraventricular nucleus, where are located two major peptidergic systems controlling energy metabolism: CRF- and TRF-producing neurons. We want to investigate relationships among Kiss positive fibers and various systems located in the pVN, in particular the CRF and TRF systems, that are located in the medial part of the PVN, where Kiss positive fibers show a higher density. One important point is to demonstrate the origin of these fibers and the involved receptor, in fact, in the PVN the classical Kiss1 receptor is missing.
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 EDCs (TBT, or genistein), in order to see how the exposure to these compounds will impact on these steroid hormone-dependent neuronal systems and behaviors.
Finally, we will expand our cooperation with our colleagues in Psychiatry to test the effects of maternal separation and oxytocin administration in our newly developed ABA model.

Background and Significance:
Steroid hormones, which are synthesised 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 nove synthese active steroids (*neurosteroids*) which may control the activity and survival of nerve cells. In the living animal, there are mechanisms (i.e. the alpha-fetoprotein in rodents or the sex hormone binding globulin in primates) that protect the brain from the circulating gonadal hormones, in order to prevent “mistakes” in the differentiation of gonadal hormone-dependent circuits during specific windows of activity (critical period) that occurs in the pre- or/and postnatal development.

Endocrine disrupting chemicals (EDCs) are compounds that are biologically active and mimic endogenous hormones (often estrogens or androgens), thereby altering hormone-modulated responses. They are not blocked by protective mechanisms as the alpha-fetoprotein, therefore they have been shown to disrupt embryonic development, sexual differentiation, reproduction, immune function, behavior, and responses mediated by hormones. The issue of EDCs has gained increasing attention as it has become clear that these environmental contaminants have endocrine activity in humans, as well as in wildlife and domestic animal species. Some of these chemicals, most notably the plant phytoestrogens largely present in the food, may play an important role in the reproductive cycles of small rodents as well as have positive (or negative) effects in other animals including humans.

A range of EDC effects has now been documented in a number of animal species, both in laboratory studies and in wild populations, demonstrating that xenoestrogenic or xenoandrogenic compounds may exert deleterious effects, even long time after exposure. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. Experimental data in galliforms and rodents showed that EDCs exposure, though nonlethal, left the individual impaired or even incapable of reproducing (Panzica et al., 2007). A recent acquisition is the concept of metabolic disruptors, i.e. substances that are able to induce profound alterations of the metabolism and inducing diseases like the diabetes (Heindel et al., 2015).

Many studies on EDCs have a toxicological approach and are performed in vitro. Among those done in vivo, only a few studies have considered the brain as a major target. In this field, our laboratory is very active and we will continue our researches covering several research lines dealing with different aspects of the interactions gonadal hormones-nervous system.

One research line is strictly related to the control of reproduction and the interaction with the metabolic state of the animals. It appears from our preliminary studies that the hypothalamic paraventricular nucleus could be a privileged site for these interactions. In fact, in addition to TRH and CRH system, we have observed a large supply of kisspeptin fibers ending in this region that could potentially interact with the other peptidergic and neurotransmitter systems. Kisspeptin system is particularly vulnerable to EDCs and part of the metabolic effects of these compounds could be explained through the action over the kisspeptin system.

Another research line involves the study of the effects of estrogens over the orexigenic and anorexigenic neuroendocrine circuits (NPY, Orexin, and POMC/MSH). The anorexigenic effect of estradiol in the adult life is well known. We want to investigate its effects during early development and those of EDCs administered both in adult and in early life.

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 ALS and Multiple Sclerosis with EDCs/environmental factors.

Specific objectives and strategies:

Researches in the field of neuroendocrinology always face on the problem of receptors, trying to match effects and exact localization of hormone receptors. Part of our research will be, therefore, devoted to the study of the distribution of classical estrogen (alpha and beta) and

androgen receptors and of the recently discovered estrogen membrane receptor (GPR-30 or GPER-1) in specific regions of interest for our studies (the subventricular zone, the hyppocampus, the paraventricular nucleus, the arcuate nucleus). To test what receptors are involved in the observed effects, we will use agonist and antagonist of different receptors (these are now on the market ensuring a good reliability of results). This technique has been previously used in our studies on the regulation of the expression of vasopressin *in vivo* (Grassi et al., 2010) and *in vitro* (Grassi et al., 2013a), as well as on the regulation of the NADPH-diaforase activity (Grassi et al., 2013b, 2017). In addition, we will investigate the effects of adult of precocious exposure to EDCs for two specific reasons: one is to investigate the perturbations of normal physiology when the experimental animals are exposed to these compounds, and the second is the use of these compounds as an additional tool to investigate the mechanisms of actions of steroid hormones.
The EDCs' administration will be during late pregnancy and/or during the first week of life, during the prepuberal period, or during adult life. This strategy is to understand if different EDCs may have organizational and permanent effects (pregnancy or early life exposure), or they may alter the postnatal events, like the onset of puberty (prepuberal exposure), or, finally, if they may alter also correctly established circuits (adult exposure).
We plan to use 3 EDCs: bisphenol A (BPA), tributyltin (TBT), and genistein (GEN). BPA and GEN are xenoestrogens that should act mainly through ERα (BPA) or ERβ (GEN), while TBT has an anti-androgenic activity. At the moment, it is not known if one or more of these compounds may act also to the GPR-30 (membrane estrogen receptor). BPA and TBT are largely diffused in the world, due to their presence in the plastic and the PVC and are under strong discussion by the regulatory authorities. GEN is a phytoestrogen, with little or no regulation at all, that is largely present in human and animal food. For example, it has been calculated that more than 4.5 millions of babies drink soy-milk each day only in USA. Our recent study (Rodriguez et al., 2014) demonstrated that prenatal administration of GEN to pregnant mice, induced behavior and neural alterations in the puppies when adults. We think therefore, that more efforts should be put in this research line to discover potential dangers linked to the exposure to GEN and other phytoestrogens.
We want therefore to study also hypothalamic postnatal effects of a GEN treatment. Finally all these compounds are suspected of an obesogenic action (i.e. induce fat tissue accumulation), but very few studies tried to investigate if this effect is due to only a peripheral action or to some imbalance of neuroendocrine circuits controlling food intake and energy metabolism. Our previous data indicate that TBT may act directly on the neurons of the arcuate nucleus (Bo et al., 2011), and with a complete derangement of the axis leptin – NPYsystem - receptor Y1 (Bo et al., 2016), confirming that metabolic disrupters have among their targets not only the adipose tissue, but also the neuroendocrine hypothalamic circuits that control metabolism and food intake**.** Our intention is to investigate if a chronic treatment with TBT in adults induces alterations also in the POMC system
Regarding the ABA project, our intention is to investigate the brains of the animals involved in this study in order to correlate better what we have behaviorally observed with possible modification of nervous circuits.

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 lobbing 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.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Neurophysiology of neurodegenerative diseases

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

Filippo Tempia — Birthdate (20/08/1960)
Degree: MD, PhD — Gender: M
Nationality: Italian — Phone: +39-011-670-6609
Email: filippo.tempia@unito.it

- **Personnel**

1. Eriola Hoxha — Birthdate (26/01/1981)
   Degree: PhD — Gender: F
   Role: Postdoc — Nationality: Albany
   Expertise: patch-clamp, behavior, immunohistochemistry, molecular biology

2. PRINCIPAL INVESTIGATOR

H Index, 22; Citations 1543

Relevant discoveries:

- synaptic integration and maturation of electrophysiological properties of synaptic currents of Purkinje cells from embryonic cerebellum grafts
- postsynaptic currents due to metabotropic glutamate receptors in Purkinje cells
- electrophysiological alterations of Purkinje cells in an animal model of the human genetic disease ataxia telangiectasia
- synaptic physiology of deep cerebellar nuclei
- cerebellar synaptic plasticity induced by fear conditioning
- expression and physiological roles of voltage-dependent potassium currents (I_A, Kv3, erg) and resurgent sodium current in Purkinje cells
- role of neuronal activity on the progression of the pathological lesions in murine models of Alzheimer's disease
- mechanisms of spreading of pathological lesions in Alzheimer's disease models, by means of transplantation
- structural and functional alterations in animal models of hereditary ataxia
- cognitive impairment of mice with genetic deletion of the gene *Fgf14*

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2015-2018	National (Local)	PI	Ricerca Locale	Role of neuronal excitability in the prefrontal cortex of mice model of psychiatric disorders	n.a.	€ 2.605	8%
2014-2017	National (Local)	PI	Ricerca Locale	Cerebellar symptoms and cellular mechanisms in a murine model of spino-cerebellar ataxia type 38 (SCA38)	n.a.	€ 2.400	8%
2014-2018	National	co-PI	Telethon -Italy	Translating molecular pathology into a therapeutic strategy in SCA38, a newly	GGP14225	€ 86.400	10%

				identified form of spinocerebellar ataxia			
2013-2016	National (Local)	PI	Ricerca Locale	Integration of healthy embryonic nervous tissue in the adult brain of mice models of Alzheimer's disease	n.a.	€ 2.414	€ 193
2012-2017	National	co-PI	Telethon -Italy	Spinocerebellar ataxia type 28: cellular and animal models to unravel the pathogenesis and to identify potential therapeutic targets	GGP12217	€ 156,800	10%
2012-2016	National	co-PI	MIUR-PRIN	Morphological and functional alterations in knock-in mice, model of spinocerebellar ataxia SCA28		€ 110.000	110.000

Outreach activities

- 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)
  -Collaboration with Prof. Giorgio Casari, Vita-Salute San Raffaele University, Milan (Italy)
  -Collaborations for the SCA38 project: Prof. Borroni, University of Brescia; Prof. Caruso and Prof. Mitro, University of Milano; Dr. Brusco, University of Turin.
- Invited talks: none.

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

Organizational activities:

- Speakers invited: none.
- Workshops, Schools or Conferences organized: none.

3. PI's PUBBLICATIONS:

For each publication, please indicate:
* if you contributed equally to the fist-author, as stated in the published article

Lippiello P., Hoxha E., Speranza L., Volpicelli F., Ferraro A., Leopoldo M., Lacivita E., Perrone-Capano C., **Tempia F**. and Miniaci M.C. (2016). The 5-HT7 Receptor Triggers Cerebellar Long-Term Synaptic Depression via PKC-MAPK. *Neuropharmacol* 101: 426-438.
IF: 4,936; R = 19/255

Cupolillo D, Hoxha E, Faralli A, De Luca A, **Tempia F**, Rossi F, Carulli D (2016) Autistic-like traits and cerebellar dysfunction in Purkinje cell PTEN knock-out mice. *Neuropsychopharmacol* 41:1457-1466.
IF: 6,399; 11/255

Alshammari TK, Alshammari MA, Nenov MN, Hoxha E, Cambiaghi M, Marcinno A, James TF, Singh P, Labate D, Li J, Meltzer HY, Sacchetti B, **Tempia F,** Laezza F. Genetic deletion of fibroblast growth factor 14 recapitulates phenotypic alterations underlying cognitive impairment associated with schizophrenia. *Transl Psychiatry* (2016) 6, e806.
IF: 5,538; 16/142

Borroni B., Di Gregorio E., Orsi L., Vaula G., Costanzi C., **Tempia F**., Mitro N., Caruso D., Manes M., Pinessi L., Padovani A., Brusco A., Boccone L. (2016) Clinical and neuroradiological features of spinocerebellar ataxia 38 (SCA38). *Parkinsonism & Related Disorders* 28: 80-86.
IF: 3,794; 39/193

Hoxha E, **Tempia F**, Lippiello P, Miniaci MC. (2016) Modulation, Plasticity and Pathophysiology of the Parallel Fiber-Purkinje Cell Synapse. *Front Synaptic Neurosci*. 2016 Nov 3;8:35.
IF: n.a.

4.GROUP’s PUBBLICATIONS:

5. GROUP's additional information:

Honours, prizes or awards received by other members of the group.

ERIOLA HOXHA: Best Poster Award at the international meeting "The cerebellum inside-out: cells, circuits and functions. 1-5 December 2016, Erice (Italy).

6 .Past Research activity

Summary
In the year 2016, a first project determined the role of the 5-HT_7 receptor in the control of the cerebellar synapse between parallel fibers and Purkinje cells. A second research addressed the question of the role of the cerebellum and Purkinje cells in autism, by exploiting a novel animal model, in which a gene was selectively knocked out in Purkinje cells. A third study allowed us to advance the knowledge about the pathogenic mechanisms of spino-cerebellar ataxia 38. A fourth study was devoted to assess hippocampal and cognitive deficits of the Fgf14 mouse, which is a model of ataxia and intellectual disability.

Background
Aim 1. The serotonergic (5-HT) axons are widely distributed throughout the brain and modulate key aspects of physiological functions including sensory processing, cognitive control, emotion and motor activity. The 5-HT system has been identified as a target of many pharmacological treatments for psychiatric and neurological conditions such as depression, anxiety, schizophrenia and drug abuse. Moreover, neurodevelopmental disorders such as autism and schizophrenia are associated with a change in 5-HT receptor expression in the cerebellum. However, the action of 5HT on the most important plastic site of the cerebellar cortex, the parallel fiber (PF)- Purkinje cell (PC) synapse, are not know.
Aim 2. Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders affecting around 1% of the population worldwide. ASDs are characterized by abnormal social interaction, deficits in verbal and nonverbal communication, repetitive behaviors and restricted interests. Mutation of *phosphatase and tensin homolog missing on chromosome 10* (*PTEN*) is a causative factor in 5-10% of autism patients. The cerebellum is also involved in higher order emotional, social and cognitive functions and PC loss is one of the most common anatomical abnormalities seen in autopsy studies of autistic patients. However, a mechanistic link between PC dysfunction and autism still needs to be established.
Aim 3. Recently, we contributed to the identification of spino-cerebellar ataxia (SCA) 38 (MIM 611805), caused by mutations within the *ELOVL5* gene, which encodes an enzyme involved in the synthesis of long-chain fatty acids with a high and specific expression in PCs. The consequences of *ELOVL5* mutations are unknown, but the main hypotheses are a toxic gain of function of the mutated protein or a loss of function of ELOVL5 activity.
Aim 4. SCA27 is due to loss-of-function mutations of *FGF14*. Symptoms include ataxia, dyskinesia and intellectual disability. While the mechanism of ataxia has been extensively studied, the role of *FGF14* in cognitive functions has not been addressed. Alterations of cognition are strongly involved in most psychiatric disorders including schizophrenia. Mice with deletion of *Fgf14* recapitulate ataxia and dyskinesia, but their cognitive functions have not been studied so far.

Rationale
Aim 1. The PF-PC synapse plays a pivotal role in cerebellar function and presents a variety of types of plasticity, related to learning and memory. Recent findings imply the cerebellum in psychiatric disorders like autism and schizophrenia, in which the 5-HT system plays a central role. Therefore it is important to know how serotonin controls the dynamic signal processing and how it regulates plasticity in the cerebellar cortex.
Aim 2. An incontrovertible demonstration of the causative role of PCs dysfunction in autism requires a genetic manipulation restricted to this cell type. To this end we generated mice with a conditional deletion of *Pten* in PCs. This was the basis to address the questions of what type of PC dysfunction can cause autism.
Aim 3. To test the loss-of-function hypothesis we started a study on *Elovl5* knockout mice. In agreement with this theory, *Elovl5* knockout mice showed motor deficits similar to ataxia displayed by patients with SCA38. Such validation of the loss of function hypothesis suggests exploiting these mice to investigate the pathogenic mechanisms of SCA38.

Aim 4. *Fgf14* knockout mice are a model of SCA27 loss-of-function mutations. Since patients display cognitive alterations, we hypothesized that *Fgf14* knockout mice might represent a novel model of psychiatric disorders characterized by cognitive disturbances. Since the hippocampus plays a pivotal role in cognitive control, we focused our study to this brain structure.

Objectives

Aim 1. Objective of the first study was to find the effects of 5-HT on signal transmission at the PF-PC synapse and to find whether and how it controls its synaptic plasticity.

Aim 2. Objective of the second study was to generate a mouse model of autism with a gene deletion selectively in PCs, and to determine the functional alterations of these cells, related to autistic symptoms.

Aim 3. Objective of the third study was to investigate, in *Elovl5* knockout mice, the cerebellar alterations responsible for ataxic symptoms.

Aim 4. Objective of the fourth study was to assess, in *Fgf14* knockout mice, cognitive function especially in relation to psychiatric disorders. Moreover, we aimed at finding structural or functional alterations in the hippocampus of these mice, in order to compare them to findings in patients with psychiatric disorders.

Results

Aim 1. A first project determined the role of the 5-HT_7 receptor in the control of the PF-PC synapse. The activation of this serotonin receptor by a selective agonist produced a long-lasting synaptic depression between PFs and PCs, indicating a role in the control of the gain of signal processing at this synapse. Such depression was postsynaptically expressed and required the activation of protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) signaling pathways. Moreover, bath application of a 5-HT_7 antagonist prevented long-term depression. On the other hand, a 5-HT agonist blocked long-term potentiation. These results suggest that the activation of 5-HT_7 receptors shifts cerebellar synaptic plasticity towards depression, via PKC and MAPK pathways. Surprisingly, 5-HT_7 receptor activation was not only necessary, but also sufficient to trigger cerebellar LTD. These results might provide a basis for the development of new therapeutic agents and strategies for the treatment of learning and memory disorders involving the cerebellum.

Aim 2. A selective deletion of *Pten* in cerebellar PCs was sufficient to cause autistic-like symptoms, providing a proof of principle that a pure cerebellar deficit can cause autism. In these mice we observed autistic features, including impaired sociability and repetitive behavior. We went on to investigate the functional and structural alterations responsible for these symptoms. Mutant PCs appeared hypertrophic and showed structural abnormalities in dendrites and axons. In particular, our laboratory conducted a detailed electrophysiological analysis of PC-*Pten* mutant mice, finding decreased excitability with a reduced generation of action potentials. Therefore, the sole output signal of the cerebellar cortex (PC action potentials) is impaired in these mice with autistic symptoms. Moreover, the PF and climbing fiber synapses were profoundly altered with increased efficacy of the former and decreased of the latter synapse, creating an unbalance between incoming input signals.

Aim 3. The analysis of behavioral tests in *Elovl5* knockout mice showed, already at 3 months of age, a marked deficit in the balance beam task, with a progressive worsening at 6 and 12 months. In accordance with the fact that SCA38 patients have hyposmia, related to *ELOVL5* expression in mitral cells of the olfactory bulb, *Elovl5* knockout mice also showed hyposmia. The cellular determinants of ataxia were investigated by histological and immunohistochemical analyses, showing a thinning of the molecular layer, more prominent in specific lobules. The dendritic trees of PCs, which are the main content of this layer, showed a reduced extension, pointing to a primary defect of PC dendrites. However, no PC loss was detected, suggesting that the lack of *Elovl5* causes an aberrant morphology of PC dendrites

together with functional deficits responsible for ataxic symptoms. A future study is required to investigate PC functional deficits in *Elovl5* knockout mice.

Aim 4. A fourth study was devoted to assess structural and cognitive deficits of the *Fgf14* knockout mouse. We found impairment of working memory and of gamma frequency oscillations, which are also clinical finding in patients with psychiatric disorders including schizophrenia. In order to find brain alterations responsible for such deficits we studied the hippocampus of *Fgf14* knockout mice. Parvalbumin-positive GABAergic interneurons, which are known to orchestrate the cortical network to enable cognitive functions, were reduced in number, together with a decreased expression of the key proteins of GABAergic neurons, GAD67 and VGAT. Analysis of postsynaptic currents due to GABAergic contacts onto hippocampal pyramidal cells showed a decreased frequency, in line with a deficit originating from GABAergic interneurons. Bioinformatic analysis of transcriptomics from patients with schizophrenia revealed clustering and correlatively decreased expression of *FGF14*, *PVALB*, *GAD67* and *VGAT*. These results point to *FGF14* as a novel candidate mechanism in schizophrenia.

Advancement in the field

In the last few years the cerebellum has been implied in a broader range of physiological functions, and in neurologic and psychiatric disorders in which it was classically considered not involved. Regarding the modulation of cerebellar function by serotonin, it is interesting to note that treatment with selective 5-HT_7 antagonists has been shown to have anxiolytic and antidepressant effects in mice and some antipsychotics appear to interact directly with the 5-HT_7 receptor. Our results provide a first basis to address the question of the mechanism of these antipsychotic effects of $5HT_7$ receptor activation. Our study on the animal model of SCA38 is completely new because of the recent discovery of this disease, for which we found the first neuronal basis responsible for ataxic symptoms. The paramount role of cerebellum in autism in incontrovertibly demonstrated by our study on mice with a selective knockout of *Pten* in PCs, with results that will bring remarkable benefits for studies aimed at finding a therapy for this subset of patients. Our study on *Fgf14* mice provided a novel model of psychiatric disorders, which is a field in which scientific progress has been hampered by the scarcity and inadequacy of animal studies.

7. Future Projects (Next 3 years)

Summary:

Starting from our results that the dendritic tree of PCs is altered in *Elovl5* knockout mice, we plan to identify the pathogenic mechanism linking the loss of this elongase enzyme to SCA38 ataxia. We expect functional deficits of PCs. Moreover, we aim at finding novel functions of Elovl5. Finally, we plan to try different rescue strategies to revert the ataxic phenotype of *Elovl5* knockout mice.

Mice with a selective *Pten* deletion in PCs will be used as a model to investigate the mechanism, by which a cerebellar dysfunction causes autistic-like symptoms. In addition, we plan to determine the critical period time window and the cerebellar area relevant for the development of autism.

FGF14 has been linked, by very recent studies, to psychiatric diseases like schizophrenia and mood disorders. In the next three years we plan to develop a new line of research investigating the role Fgf14 in cognitive functions and in the regulation of mood and motivation. The most relevant brain areas related to these functions are hippocampus, prefrontal cortex and ventral striatum. This is our only research line about structures other than the cerebellum.

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. In a large collaborative study, we recently identified a novel form of spinocerebellar ataxia (SCA38) due to missense mutations in the gene *ELOngase of Very Long chain fatty acids 5*, *ELOVL5*. The molecular pathogenesis of SCA38 has not been studied yet. We have recently demonstrated that the deletion of *Elovl5* in mice causes symptoms that recapitulate SCA38, suggesting that human mutations found in patients act by a loss-of-function mechanism. The most abundant brain long chain PUFAs are the omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the omega-6 arachidonic acid. These molecules are substrates for the production of a huge variety of active substances, including prostaglandins, protectins and recoverins, involved in induction and resolution of inflammation. Since the actions of these lipidic mediators in the brain is largely unknown, it is likely that important roles in physiology and in reaction to pathology are yet to be discovered. The *Elovl5* knock mouse is an excellent model to discover new molecular mechanisms, in addition to allowing studies about the pathogenic mechanism of SCA38.

Aim 2. Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders affecting around 1% of the population worldwide. ASDs are characterized by abnormal social interaction, deficits in verbal and nonverbal communication, repetitive behaviors and restricted interests. The cerebellum is frequently altered in ASD patients, but the mechanism liking this structure to autism is not known. We recently demonstrated that, in mice, a selective deletion of *Pten* in PCs is sufficient to cause autistic-like deficits. We showed that PC-*Pten* knockout mice have a reduced PC action potential firing and an unbalance between their two glutamatergic inputs, PFs and climbing fibers. These behavioral symptoms and the functional deficits of PCs constitute an exceptional occasion to investigate the mechanisms by which a cerebellar deficit causes autism.

Aim 3. Mood disorders, including depressive and bipolar disorders, are an important health problem of the modern society. The major depression disorder alone has a lifetime prevalence greater than 10% of the population, constituting a higher cause of disability than any other disorder. *FGF14* has recently been listed among the top candidate genes associated with psychiatric disorders and in a genome wide association study it was a top scoring gene for depression, suggesting a major role in mood disorders. In line with these findings, preliminary results of our laboratory show that *Fgf14* knockout mice have reduced levels of anxiety, accompanied by a decreased susceptibility to depression. This observation might open a new avenue in the study of the molecular determinants of depression, which are almost

completely unknown. In fact, current pharmacological treatments for depression are ineffective in about one third of patients. Moreover, responding subjects start to recover after at least one week of treatment, indicating that the currently used drugs do not act on the pathogenic mechanism. We hypothesize that the level of Fgf14 activity is directly correlated with the susceptibility to depression. A validation of this hypothesis would provide a novel molecular target for antidepressant drugs, acting for the first time on the pathogenic mechanism.

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 the pathogenic mechanism and to design a specific therapy. Regarding autism, we want to provide a mechanistic explanation of the role of the cerebellum in this disorder, so that therapeutic interventions can be envisaged. The unexpected chance to start a line of research on psychiatric diseases like mood disorders completes the range of investigations of brain pathologies that will be conducted by our research group. The mission of the Institute is exactly the same as ours, namely to advance scientific knowledge regarding brain disorders, including neurologic and psychiatric diseases.

Specific objectives and strategies:

Aim 1: **Mechanisms of SCA38 ataxia**

Starting from our demonstration of ataxic signs and of alterations in the dendritic tree of PCs in *Elovl5* knockout mice, we plan to investigate the functional alterations of PCs, due to the lack of this enzyme. PCs of *Elovl5* knockout mice will be patch-clamp recorded in acute cerebellar slices. Intrinsic excitability of PCs and the function of synapses impinging on them will be analyzed. We expect that functional PC deficits are correlated with the onset and progression of ataxic deficits. More specifically, we hypothesize that Elolv5 activity in PCs is required for the synthesis of lipid mediators that play a major role in signal processing in the cerebellar cortex. First, we plan to find which PC functions are altered in *Elovl5* knockout mice. Then such functions will be studied in detail with rescue experiments based on supplementation of different molecules downstream to Elovl5. Finally, in wild-type mice, after strong and prolonged activation of the Elovl5-dependent function, the slice perfusate will be analyzed in collaboration with a laboratory of the University of Milano, in order to find the lipid molecules whose production is triggered by the stimulation.

Aim 2: **Role of cerebellum in autism spectrum disorders**

Starting from the results obtained in mice with a selective *Pten* deletion in cerebellar PCs, we plan to investigate the mechanisms responsible for autistic-like symptoms. The transduction pathways downstream Pten will be assessed by specific inhibitors. The relevant cerebellar region will be localized by pharmacological rescue delivered via *in vivo* injection or by implantation of an osmotic pump in specific areas of the cerebellar cortex. The critical period in which the cerebellar deficit induces autistic-like symptoms will be identified by pharmacological rescue at different time points, from neonatal age to adulthood.

Aim 3: **role of *Fgf14* in psychiatric disorders**

With a large battery of behavioral tests of *Fgf14* knockout mice we found that they show reduced levels of motivation and anxiety and that they are resilient to depression. Therefore we hypothesize that Fgf14 is a key element to modulate mood, so that its lack results in protection against anxiety and depression. We plan to verify this hypothesis by pharmacological positive or negative modulation of Fgf14 in the brain regions involved in mood disorders, starting with medial prefrontal cortex and ventral striatum. The regions critical for mood modulation will be identified by local injection of an Fgf14 inhibitor or by *in vivo* knockdown of the gene. We plan to use quantitative RT-PCR in order to verify if there is an altered gene expression in these regions in *Fgf14* knockout mice, starting with pathways already implied in depression like the dopaminergic and the endocannabinoid systems. On the basis of the results obtained from these experiments, we will proceed with patch-clamp recordings in prefrontal cortex and ventral striatum.

Unique features of the project research:

Aim 1. From our knowledge, ours is the only laboratory, which is studying *Elovl5* knockout mice as a model of the loss of function hypothesis of SCA38. At present there is no indication in the literature of why a failure to elongate unsaturated fatty acids causes a cerebellar selective deficit responsible for ataxia. We plan to use multiple approaches, including the identification of the main functional mechanism of PCs disrupted by Elovl5 deletion, the rescue by downstream molecules and the attempt to identify a novel lipid mediator essential for PC function.

Aim 2. The link between cerebellar PC dysfunction and autism is still completely enigmatic. Our experiments will try to make a breakthrough in the understanding of important and very frequent mechanisms of autism, caused by aberrant PC signaling. Our project is aimed at filling several gaps in the pathogenic mechanisms of autism, including specific molecular pathways involved, the relevant cerebellar regions, the critical periods. All these aspects are highly innovative.

Aim 3. *FGF14* has been linked, in very recent studies, to psychiatric diseases like schizophrenia and mood disorders. At present no study has been published about Fgf14 in mood disorders. Our project on the role of *Fgf14* in psychiatric disorders has the potentiality to be a pioneering research opening a new pathway in the study of these diseases, for which only elusive results are currently available. The demonstration of a molecular mechanism, like Fgf14, directly related to anxiety and depression would be an absolute breakthrough in the field, because current theories are based on the effects of drugs, which fail to modulate mood in the first few weeks of administration, suggesting that they do not target the mechanism, but act indirectly.

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

The recent acquisition of a two photon microscope, which is now located at NICO, will allow us to complement patch-clamp studies with *in vitro* and *in vivo* imaging of calcium to measure neuronal activity in large networks of neurons like the cerebellar cortex, the prefrontal areas of cerebral cortex and the ventral striatum. In collaboration with Prof. Thomas Knöpfel of the Imperial College of London we plan to extend this technique to optical measures of voltage signals, exploiting novel, genetically encoded fluorescent probes.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2016

Laboratory name: Brain development and disease

1. LABORATORY DESCRIPTION – PERSONNEL:

- **Principal Investigator**

Alessandro Vercelli — Birthdate (09/07/1961)
Degree: MD PhD — Gender: M
Nationality: Italian — Phone: 011/6706617
Email: alessandro.vercelli@unito.it

- **Personnel**

1. Adriano Ceccarelli — Birthdate (28/10/1957)
   Degree: MD PhD — Gender: M
   Role: Associate Professor — Nationality: Italian
   Expertise: Molecular Biology

2. Elena Tamagno — Birthdate (14/07/1967)
   Degree: PhD — Gender: F
   Role Associate Professor — Nationality: Italian
   Expertise Alzheimer's disease

3. Marina Maria Boido — Birthdate (06/09/1980)
   Degree: PhD — Gender: F
   Role: Assistant Professor RTD — Nationality: Italian
   Expertise: Spinal cord injury, motoneuron diseases, Huntington disease, stem cells

4. Michela Guglielmotto — Birthdate (28/02/1977)
   Degree: PhD — Gender: F
   Role: Post-doc — Nationality: Italian
   Expertise: Alzheimer's disease

5. Debora Monteleone — Birthdate (06/02/1986)
   Degree: PhD — Gender: F
   Role: Post-doc — Nationality: Italian
   Expertise: Alzheimer's disease

6. Roberta Schellino — Birthdate (11/02/1985)
   Degree: PhD — Gender: F
   Role: Post-doc — Nationality: Italian

Expertise Neurogenesis, motoneuron diseases, Huntington disease, behavior

7. Giusi Manassero — Birthdate (01/11/1979)
   Degree: PhD — Gender: F
   Role: Post-doc — Nationality: Italian
   Expertise: Alzheimer's disease, neuropathic pain

8. Ivan Enrico Repetto — Birthdate (11/09/1986)
   Degree: Biotechnology degree — Gender: M
   Role: doctorate student — Nationality: Italian
   Expertise: neuronal death, models of stroke

9. Matilde Ghibaudi — Birthdate (19/09/1988)
   Degree: Biotechnology degree — Gender: F
   Role: doctorate student — Nationality: Italian
   Expertise: Spinal cord injury, miRNA, molecular biology

10. Marta Tropiano — Birthdate (09/06/1985)
    Degree: Biotechology degree — Gender: F
    Role: doctorate student — Nationality: Italian
    Expertise: confocal microscopy

11. Elena Signorino — Birthdate (06/10/1976)
    Degree: Biology degree — Gender: F
    Role: technician — Nationality: Italian
    Expertise: molecular biology

2. PRINCIPAL INVESTIGATOR

H index, 27; Citations, 3225

Relevant discoveries:
Characterization of pyramidal dendritic bundles, stem cell therapy for ALS, role of JNK in neuronal death

PI's Grants (current and pending)

Starting-end date	Origin	Role	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
2016-2019	International	coordinator	Horizon 2020	My-AHA		395000	8%
2014-2017	International	PI (with A.Buffo)	FP7	Neurostemcellrepair		400000	8%
2017-2020	National	PI	PRIN (MIUR)	Generation of functional striatal neurons for brain repair in Huntington Disease	2015AY9AYB_002	75330	8%
2016	Local	PI	CRT			30000	10%
2016	Patient association	PI	Smarathon			16000	10%
2016	Patient association	PI	Atrofia spinale Onlus			25000	10%

PhDs supervised in 2016

Matilde Ghibaudi
Ivan Enrico Repetto
Marta Tropiano

Outreach activities

- 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

29 January 2016 L'interazione uomo-macchina nell'assistenza dell'anziano. Convegno: "I Disturbi Neurocognitivi: stato dell'arte e nuove frontiere". Torino
26 May 2016 Meccanismi molecolari della morte neuronale: un tempo per vivere e uno per morire. Convegno Neuropatologia Roma
4 October 2016 Towards early detection of age-related health risks: understanding users' needs, unobtrusive sensing and data analysis, Brussels
21 October 2016 Animal models of neurodegenerative diseases and stem cell transplantation: a thing of the past or a requisite for clinical studies? GISM, Brescia
4 November 2016 Science Agora Tokyo
23 November 2016 Presentazione My-AHA. Hackunito for Ageing Torino
16 December 2016 Il cervello che invecchia. From Silver to Golden Age Torino
17 December 2016 Le sfide etiche della medicina moderna. Corso di Bioetica Avanzata, ciclo di Specializzazione in Teologia Morale con indirizzo sociale. Torino

Organizational activities:

- Speakers invited

Alessio Cardinale
Matteo Cerri
Jean Pierre Hornung
Pier Cesare Grimaldi

- Workshops, Schools or Conferences organized

February 2016 My-AHA Kick-off meeting, Turin
March 2016: Organization of the Brain Awareness Week (Siamo davvero liberi?), Circolo dei Lettori, Torino
November 2016 My-AHA meeting at the Italian Embassy, Tokyo
December 2016 My-AHA Consortium meeting, Valencia

3. PI's PUBBLICATIONS:

1: Atassi N, Beghi E, Blanquer M, Boulis NM, Cantello R, Caponnetto C, Chiò A, Dunnett SB, Feldman EL, Vescovi A, Mazzini L; attendees of the International Workshop on Progress in Stem Cells Research for ALS/MND. Collaborators: Bendotti C, Bersano E, Brajkovic S, Car P, De Marchi F, Fantozzi R, Follenzi A, Gelati M, Giorgi C, Grilli M, Guenzi P, La Bella V, Mancardi GL, Panzarasa G, Poloni M, Profico D, Silani V, Sorarù G, Spataro R, Stecco A, **Vercelli A**. Intraspinal stem cell transplantation for amyotrophic lateral sclerosis: Ready for efficacy clinical trials? *Cytotherapy*. 18(12):1471-75.
IF = 3.2; R = 26/70

2: Brancia, C., Noli, B., Boido, M., Boi, A., Puddu, R., Borghero, G., Marrosu, F., Bongioanni, P., Orrù, S., Manconi, B., D'Amato, F., Messana, I., Vincenzoni, F., **Vercelli, A**., Ferri, G.L., Cocco, C. (2016). VGF Protein and Its C-Terminal Derived Peptides in Amyotrophic Lateral Sclerosis: Human and Animal Model Studies. *PLoS One*., 11(10):e0164689.
IF = 3.06; R = 11/63

3: Repetto, I.E., Monti, R., Tropiano, M., Tomasi, S., Arbini, A., Andrade-Moraes, C.H., Lent, R., **Vercelli, A**. The Isotropic Fractionator as a Tool for Quantitative Analysis in Central Nervous System Diseases. *Front Cell Neurosci*., 5;10:190.
IF = 4.61; R = 2/256

4: Vercelli U, Diano M, Costa T, Nani A, Duca S, Geminiani G, **Vercelli A**, Cauda F. Node Detection Using High-Dimensional Fuzzy Parcellation Applied to the Insular Cortex. *Neural Plast.* 016:1938292. doi: 10.1155/2016/1938292.
IF = 3.054; R = 115/258

5: Boido, M., **Vercelli, A**. Neuromuscular Junctions as Key Contributors and Therapeutic Targets in Spinal Muscular Atrophy. *Front Neuroanat*., 3;10:6.
IF = 3.26; R = 2/21

6: Mazzini L, Vescovi A, Cantello R, Gelati M, **Vercelli A**. Stem cells therapy for ALS. *Expert Opin Biol Ther.* 16(2):187-99.
IF = 3.684; R = 35/158

4.GROUP's PUBBLICATIONS:

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

Filippi, M.*, Boido, M.*, Pasquino, C., Garello, F., Boffa, C., Terreno, E. (2016). Successful in vivo MRI tracking of MSCs labeled with Gadoteridol in a Spinal Cord Injury experimental model. *Exp Neurol*., 282:66-77.
IF = 4.66; R = 51/256

Pascente, R., Frigerio, F., Rizzi, M., Porcu, L., Boido, M., Davids, J., Zaben, M., Tolomeo, D., Filibian, M., Gray, W.P., Vezzani, A., Ravizza, T. (2016). Cognitive deficits and brain myo-Inositol are early biomarkers of epileptogenesis in a rat model of epilepsy. *Neurobiol Dis.*, 93:146-55.
IF = 4.86; R = 43/256

Manassero, G., Guglielmotto, M., Zamfir, R., Borghi, R., Colombo, L., Salmona, M., Perry, G., Odetti, P., Arancio, O., Tamagno, E., Tabaton, M. (2016). Beta-amyloid 1-42 monomers, but not oligomers, produce PHF-like conformation of Tau protein. *Aging Cell* 15(5):914-23.
IF = 5.76; R = 36/187

Guglielmotto, M., Reineri, S., Iannello, A., Ferrero, G., Vanzan, L., Miano, V., Ricci, L., Tamagno, E., De Bortoli, M., Cutrupi, S. (2016). E2 Regulates Epigenetic Signature on Neuroglobin Enhancer-Promoter in Neuronal Cells. *Front Cell Neurosci*., 1;10:147
IF = 4.61; R = 2/256

Klionsky, D.J., Abdelmohsen, K., Abe, A., et al. (2016). Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy*., 12(1):1-222.
IF = 9.11; R = 19/187

5. GROUP's additional information:

Grants (current and pending) of the other members of the group

Starting-end date	Origin	Name and Role of Group Member	Funding Source	Project Title	Project ID	Overall Amount Funded	Directly Available to NICO
17/11/14 - 18/09/16	National	M. Boido (Component)	CRT	Utilizzo di scaffold biomimetici e di cellule staminali per sostenere la rigenerazione del midollo spinale lesionato	41491	€ 30.000	8%
23/03/15 - 31/12/16	National	M. Boido (Component)	Ricerca Locale 2014	Il ruolo neuroprotettivo di Nurr1 nella SLA		2.488,46 €	8%
25/02/16 - 31/12/17	National	M. Boido (Component)	Ricerca Locale 2015	Nurr1: un nuovo possibile target terapeutico nella SLA		2.012,24 €	8%
23/03/15 - 31/12/16	National	E. Tamagno (Component)	Ricerca Locale 2014	Study of the different effects of Amyloid β oligomers and monomers on aggregation of tau protein. Role on the pathogenesis ofAlzheimer's disease.		10789,44 €	8%
25/02/16 - 31/12/17	National	E. Tamagno (Component)	Ricerca Locale 2015	The role of proteasome and autophagy impairment in the pathogenesis ofAlzheimer's disease.		2751,94€	8%
pending	International	M. Boido (Component)	9th Call for Research Proposal	Autophagy/ubiquitin-proteasome system: the	21244	requested 150.000 €	

			s (2016)	smoking gun in SMA?			

- Editorial duties

Boido M. – Webdoc production (video-lessons with scientific research experts) for the following books published by Mondadori: “Fisica! Pensare la natura” di Caforio (2016; ISBN 120900044662), “Fisica! Pensare l'universo - Edizione TECH” di Caforio (2016; ISBN 120900044999), “Fisica intorno a te” di Stroppa (2017; ISBN 120900052931).

Technology transfer achievements, if applicable

Boido M./Tamagno E.: Founders of the academic spin-off S&P BRAIN SRL

6 .Past Research activity

Summary

We study the development of the CNS from the embryo to the aged, and the common neurobiological mechanisms and molecular pathways which lead to normal development and to neurodegeneration. We are interested in the molecular pathways leading to neuronal cell death, which we study in development and in experimental models of transient/permanent cerebral ischemia, acute/chronic glaucoma, epilepsy, SMA (spinal muscular atrophy) and AD (Alzheimer disease). Finally, we are studying cell therapy in ALS (amyotrophic lateral sclerosis), SCI (spinal cord injury) and HD (Huntington disease).

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 an holistic approach, from molecules to brain areas, from development to disease and from a multidisciplinary point of view can provide new insights and concept on brain function, disease and repair.

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 amount of data with IoT (Internet of things) and big data pose new challenges to Neuroscience and we would like to participate to this new era.

Objectives

We aim to the structural and 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 and the organization of their dendritic bundles and axonal projections. At a larger extent, 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, in order to prevent them. In particular, 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, motoneuron diseases 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, ALS and SMA and how to prevent it to delay the onset and the development of disease.
Stem cells are a growing field of research related to normal development, disease and cancer. We study the integration of neural stem cells and neural progenitors grafted into the striatum (in HD) or spinal cord (in SCI). 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.

Results

Development of cerebral cortex

We are studying the development of corticofugal axons originating from cortical pyramidal neurons. In collaboration with P. Arlotta (Harvard Medical School) we have previously shown that the myelin sheath of cortical axons is layer dependent and may be interrupted in the gray matter. Also, in collaboration with G.M. Innocenti (now at EPFL, Lausanne) we have shown that the diameter of cortical axons depends on their target. In collaboration with A. Buffo (another PI at NICO) we are currently investigating the axon/oligodendrocyte relationship, and the role of OL activity. In particular, we have identified a MAP-kinase, JNK, as a key molecule in oligodendrocyte development and axon myelination, and we are studying its role both in vitro and in vivo (also in ko mice for the three different isoforms of JNK).
Also, we are studying the development of corticostriatal axons, and their specific relationship with neurons of the direct and indirect pathways in the striatum.

Mechanisms of neuronal death and neuroinflammation in motor neuron diseases

We are investigating role/interaction of two of the major types of programmed cell death, i.e. autophagy and apoptosis. The injection of 3-MA (an autophagy inhibitor) can significantly improve motor performance, extend the lifespan and delay motor neuron death in SMA spinal cord. Moreover autophagy inhibition also suppresses apoptotic activation, underlining that these two pathways are intricately intertwined (*submitted*). We have also demonstrated that the JNK-signaling pathway is involved in the SMA pathogenesis: the administration of a synthetic JNK-inhibitor peptide (DJNKI) exerted positive effects on motor neuron survival and muscular atrophy (*in preparation*).
As concerns ALS, we evaluated the role of Nurr1, a nuclear receptor implicated both in neuroprotection and immunomodulation in PD and MS: in our mouse model (SOD1 G93A), Nurr1 activation seems to modulate neuroinflammation and to protect motor neurons, at least at the onset of disease.
As concerns AD, we investigated the effect of Aβ1-42 monomers and oligomers on Tau, using mice expressing WT human Tau that do not spontaneously develop Tau pathology, thus the mechanistic relationship between Aβ1-4 and the alteration of Tau protein are debated in the pathogenesis of AD. After ICV injection of Aβ1-42, mice were sacrificed after 3h or 4 days. The short lasting treatment with Aβ monomers, but not oligomers, showed a

conformational PHF-like change of Tau, together with hyperphosphorylation. The same treatment increased the concentration of GSK3 and MAP kinases. The inhibition of the kinases rescued the Tau changes. It is known that Aβ1-42 monomers foster synaptic activity. Our results suggest that Aβ monomers physiologically favor Tau activity and dendritic sprouting, whereas their excess causes Tau pathology. Moreover, our study indicates that anti-Aβ therapies should be targeted to Aβ1-42 monomers too.

Stem cell therapy in Huntington's disease

With E. Cattaneo and A. Buffo, we are exploring the potential of human embryonic stem (hES) cells in an experimental model of HD, with good results in terms of cell replacement and establishment of new connections. Indeed we showed that hES-derived striatal progenitors can be transplanted in HD animal models and can differentiate and integrate into the host, extending fibers over a long distance (Faedo et al., PNAS, in press).

Spinal cord injury

Our group has previously demonstrated the therapeutic effects of stem cells (NPs and MSCs) in case of SCI. Recently, in collaboration with the group of Prof. Terreno (MBC, Torino), we have developed a reliable method to track in vivo the grafted cells and to monitor their fate after transplantation: using the clinically approved MRI agent Gadoteridol through a procedure based on the hypo-osmotic shock, we have been able to perform in vivo imaging on injured mice after transplantation of 300,000 labeled MSCs, enabling to circumscribe their spinal distribution and to follow their migratory dynamics for 10 days (Filippi et al., 2016).
Moreover, in order to improve the graft success (in terms of cell survival, uncontrolled proliferation…), in collaboration with Dr. Tonda-Turo (Polito), we are developing and testing biomimetic injectable hydrogels (chitosan) in which stem cells can be encapsulated. The obtained in vitro results are encouraging and preliminary in vivo approaches are ongoing.
Finally, in collaboration with Prof. Dalmay and Prof. De Pietri Tonelli, we have performed a profiling of miRNA expression in a mouse model of SCI: we have identified miR-7b-3p as a key-miRNA probably involved in the regulation of axon growth. In vitro experiments are still running to confirm such hypothesis, in order to find a possible new therapeutic target for SCI. A manuscript on the role of miRNAs in CNS and PNS disease is 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 with pre-frailty symptoms. It will empower older citizens to better manage their own health, providing new ways of health monitoring and disease prevention through individualized profiling and personalized recommendations, feedback and support. An ICT-based platform will detect defined risks in the frailty domains early and accurately via non-stigmatising embedded sensors and data readily available in the daily living environment of older adults. When risk is detected (pre-frail), My-AHA will provide targeted ICT-based interventions. These interventions will follow an integrated approach to motivate users to participate in physical exercise, cognitively stimulating games and social networking to achieve long-term behavioural change, sustained by continued end user engagement with My-AHA. A randomized controlled study (RCT), involving 300 subjects receiving intervention, and 300 controls from many EU and non EU countries, to evaluate intercultural aspects, is ongoing in order to evaluate efficacy of the my-AHA platform.

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.

7. Future Projects (Next 3 years)

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

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). 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 and optogenetics for in vivo analysis, Multisem microscopy for ultrastructural 3D reconstructions. 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). Other applications on the same line of research will be presented in 2017 to EEC, to Regione Piemonte (poli innovazione), to the Ministry of Education (PON).

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): 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.

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 is fully integrated with the mission of the Neuroscience Institute.

Specific objectives and strategies :

Axonal growth. A fundamental issue in the building of neural connections and in their conduction 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 oligodendrocytes and their precursors, and some molecules which may affect this interaction in the normal brain and in experimental models of disease, such as schizophrenia. Moreover we will study the activity-dependence of this process in the normal brain and in disease, for example, in collaboration with G. Fisone in L-Dopa induced dyskinesia in PD.
Moreover, 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.

miRNA networks. A novel class of regulatory molecules called microRNAs (miRNAs) seem to be implicated in several neurological diseases such as neurodegenerative/psychiatric disorders and traumatic CNS injury. It has been demonstrated that miRNAs control the activity of at least 20-30% of human protein-coding genes and a large number of these regulators has been found in mammalian nervous system, including brain and spinal cord, where they exert a key role in neurodevelopment and plasticity. 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.
Moreover we will study the involvement of miRNA-206 (a skeletal muscle specific miRNA) in SMA. MiRNA-206 was proposed to act as a compensatory mechanism to promote nerve re-innervation: we will modulate miRNA-206 expression in muscles of SMA mice, in order to identify a novel potential therapeutic target.

Stem cell therapy. We will continue previous work on stem cell transplantation in experimental models of neurodegenerative diseases and SCI. On one hand, we will dissect the neuroprotective and immunomodulatory properties of stem cells, in particular of mesenchymal stem cells, as reservoirs to deliver molecules to the diseased brain. On the other hand, we will aim at cell replacement, using iPS and reprogrammed MSCs to replace lost neurons, using HD, PD and ALS as paradigms. To this aim we are involved, together with A. Buffo, in Italian and European Consortia to obtain a translation to clinics within two years. As a secondary aspect, we aim to model disease in vitro by iPS technology after obtaining tissue samples from patients of neurological and psychiatric diseases, and growing it in cell culture or into organoids. This is an essential step to drug testing and personalized medicine.

Molecular mechanisms of cell death. We will continue to investigate the role of specific genes and molecules in neuronal cell death in neurodegenerative diseases. With A. Chiò, we want to study the interrelationship between genes involved in two motoneuron diseases, ALS and SMA. In fact, TDP-43 and FUS/TLS (genes involved in ALS) and SMN (involved in SMA) are involved in RNA metabolism, colocalize in nuclear Gems and play a role in maintaining the spliceosome. This alteration in the integrity of the spliceosome could be the basis for the selective vulnerability of motoneurons.

Moreover, in ALS, we have identified in Nurr1 a powerful nuclear receptor able to positively modulate neuroinflammation: we intend now to pharmacologically stimulate the Nurr1 activation in ALS mice, in order to early counteract inflammation. We also intend to evaluate gender-related Nurr1 expression in ALS, in order to clarify its mechanisms of neuroprotection in this pathology.
Starting from the obtained 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.
As concerns AD, we will further explore the role of Uch-L1 an enzyme found decreased in AD brains in the pathogenesis of the disease. We will investigate this aim in P301L mice harboring a mutation in a sequence of Tau within exon 10 and develop numerous intracytoplasmic Tau deposits ICV injected with different preparations, monomeric as well as oligomeric, of Aβ1-42. Therefore, drugs regulating Uch-L1 functions could have a potential to offer therapeutic strategies against AD.
Moreover we will study gender differences on Tau pathology. Thus the main objective of the project will be investigate if the different sex-related effect of Aβ42 monomers on pathological Tau conformational change depends on estrogens decline as well as oxidative stress.

Unique features of the project research:

Some of our research topics, the methodologies employed and the external collaborations with top institutes and scientists, 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.

Methodology: <u>please fill-out this section only in the case of innovative technologies</u>

We are collaborating with IIT (Italian Institute of Technology, D. De Pietri Tonelli) in the study of miRNA networks, and with T. Dalmay (University of East Anglia) to develop new techniques to exploit miRNA analysis from small samples of cells.
In collaboration with groups of the Polytechnic of Turin, we intend to develop three-dimensional cellularized constructs by bioprinting technique for SCI treatment: with this innovative approach, we will encapsulate stem cells in new 3D materials “printed” to recreate the longitudinal course of the nerve fibers of the spinal cord, and improve their ability to fill the lesion gap.

22/1

Gli anticorpi intracellulari come potenziali strumenti terapeutici per le malattie neurodegenerative

Alessio Cardinale, Laboratorio di Neurobiologia Molecolare e Cellulare - Istituto San Raffaele, Roma

19/2

Emx2 in cortico-cerebral astrogenesis and glioblastoma A novel, non-CRISPR RNA-programmable artificial transactivator

Antonello Mallamaci, Head, Lab of Cerebral Cortex Development - Neuroscience Area
SISSA, Trieste - Italy

4/3

Understanding Hibernation: the Quest for Future Medical Applications

Matteo Cerri, MD PhD, Department of Biomedical and NeuroMotor Sciences, University of Bologna

15/4

Tracing lineages from single neural progenitors: UbC-StarTrack

Maria Figueres Oñate, Developmental Neurobiology, Cajal Institute, Spanish National Research Council Madrid, Spain

16/6

Repurposing FDA-and EMA-approved drugs for their remyelination properties: the hurdles and the good news for regenerative medicine in multiple sclerosis

Antonella Ragnini-Wilson, Department of Biology, University of Rome "Tor Vergata", Rome and Department of Translational Pharmacology, Fondazione Mario Negri Sud, Santa Maria Imbaro (CH), Italy.

17/6

SEL1L: a molecular biological clock in neuronal differentiation

Ida Biunno, Senior researcher at CNR - Italian National Research Council Univesity of Chieti (Italy) - Biotechnology Center of Temple University Philadelphia-USA

1/7

Effects of Experience on Adult Neurogenesis in Turtles

Alice S. Powers, Ph.D., Research Professor of Psychology, Stony Brook University - NY

23/9

5-HT1a receptors orchestrate hippocampus development: impact on morphology, activity and behavior.

Prof. Jean-Pierre Hornung, Département des Neurosciences fondamentales, Directeur de la Plateforme de Morphologie - Faculté de Biologie et de Médecine, University of Lausanne - Suisse

27/9

Dai neuroni enterici al movimenti dell'intestino; una lezione anche per il cervello

Prof. Marcello Costa, Matthew Flinders Distinguished Professor - Flinders Medical Centre - Adelaide, Australia

18/10

NGF and neuro-behavioural plasticity: coping with stress in mice, rats and parachutists

Prof. Enrico Alleva, Direttore del Dipartimento di Biologia cellulare e Neuroscienze - Istituto Superiore di Sanità, Roma – Professore Associato di Etologia all'Università La Sapienza di Roma

22/12

Unraveling the machanisms recognition and decision in the macaque

Prof. Piercesare Grimaldi, University of California, Los Angeles - Department of Psychiatry and Biobehavioral Sciences

23/12

Terminal differentiation of vomeronasal sensory neurons and GnRH-1 neuronal migration, from new models to new stories

Paolo E Forni, Department of Biological Sciences - University at Albany, NY, USA

Ferdinando Rossi
Lectures on Neuroscience
Neuroscience Institute Cavalieri Ottolenghi

14/3 - Brain Awareness Week 2016

Constructing the cerebellum: cross-talk between cell types underlies scaling of circuits

Alexandra Joyner, Sloan-Kettering Center di New York

DiSFeBmeetsNICO
Neuroscience builds a bridge from Milan to Turin

20/5

New mechanisms regulating oligodendroglial differentiation: focus on the GPR17 receptor and a related microRNA.

Davide Lecca, Laboratorio di Farmacologia Molecolare e Cellulare della Trasmissione Purinergica - Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB) - Università degli Studi di Milano