FONDAZIONE
CAVALIERI OTTOLENGHI

NICO
Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2019

INDEX

Dissemination events are listed in the pages of the single groups.

NICO 2019 by the numbers

9
Research Groups
59 Scientists
85 Graduating Students

60
Peer-Reviewed
Publications

62
Collaborative Initiatives
with
International
Research Groups

53
On-going/Granted
Research Projects

5
Scientific
Conferences/workshops
organized
by NICO members

13
Invited speakers

1
Spin-off Company

1
Biobank

84
Outreach Activities
42 Invited Talks
42 Science Dissemination Initiatives

9
trained
PhD students

3100
Facebook Followers

BRIEF HISTORICAL NOTES

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

After decades in which the Foundation has funded scientific research projects and purchase of scientific equipment, in the 90s an international scientific committee of eminent personalities in Neuroscience proposed to build a 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).

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

Aims of NICO

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

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

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

THE COLLABORATIVE VISION AT NICO

Since its foundation in 2011, the NICO adopted a new (relative to the Departments of origin) view of sharing all facilities, supplies and instruments by all groups. Excepted for the clinically relevant activities, which have to be performed in dedicated and isolated rooms to maintain privacy related to human material, all instruments are located in common facilities which are shared by all NICO

members. This has initially created an organizational burden, but it has also obliged people to meet, share decisions, collaborate and interact, also in the formation of new researchers. Internal courses on the use of instruments and facilities have been organised to improve their correct usage. Starting from the practical needs of everyday research life, this attitude has boosted collaboration and exchange of ideas among the individual researchers and ameliorated the scientific production of single researchers. To sum up, it has created a scientific environment which, respecting the peculiarities of single researchers, interacts and operates as a real community to apply for grants, develop multidisciplinary projects, act as a whole institutional body in front of the scientific community and to the public. Finally, it represents a fundamental breakthrough to save money and to exploit the use of expensive instruments.

POSITIONING OF NICO IN THE UNIVERSITY OF TURIN

NICO is part of the University Interdepartmental 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, Molecular Biotechnology and Veterinary Medicine) of the University of Turin and hosts 9 PhD students. Moreover, as lecturers at the schools of Biology, Biotechnology, Pharmacy, Medicine, Psychology and Veterinary Medicine, they are involved in the preparation of many theses for Bachelor and Master degrees. Currently, NICO laboratories hosts 21 students who are developing their Bachelor or Master thesis projects and 23 stage students. NICO collaborates with several other research centers of the University of Turin, such as the Molecular Biotechnology center, the IRCCS Candiolo and the Brain Imaging Center. NICO members belong to the Departments of Neuroscience, Biotechnology, Veterinary Morphophysiology and Systems Biology. NICO members belonging to the Department of Neuroscience of UNITO participate to the project which was recently selected by the MIUR for the Departments of Excellence. The Department of Veterinary Medicine was selected as well. Therefore, NICO will be involved in these five-year projects, with a significant return in terms of personnel, upgrade of instrumentation and translational science collaborations.

Several groups of the NICO have projects and funding in collaboration with the Polytechnic of Turin. Starting from 2017, microscopy facilities at the NICO are part of the Open Access lab program of the University of Turin.

POSITIONING OF THE NICO IN ITALY AND IN THE WORLD

NICO researchers have several national and international collaborations, as shown by their publication record. They have also a strong rate of exchange of visits and seminars, as it can be argued from their reports in attachment. Also, they participate to exchange programs of bachelor, graduate and doctorate degrees, and NICO is often visited and attended by foreign students. They participated in the international Young Investigators Training Program established in Italy in occasion of the 2011 World International Brain Research Organisation Meeting and of the 2014 European Neuroscience Meeting. Every second year, an international meeting (Steroids and Nervous System) is organized by Prof. Panzica's group (with the cooperation of prof. R.C. Melcangi, University of Milan): the meeting has an average 150 people attendance and more than 40 invited speakers from all over the world. The 2013, 2015, 2017 andm2019 editions were organized with the administrative help of the Ottolenghi Foundation. The 2021 edition will be organized by the Ottolenghi Foundation as well. Since 2018, NICO gives its patronage to BraYn (Brainstorming Research Assembly for Young Neuroscientists), an international meeting devoted to under-40 scientists that is held every year (Dr. Enrica Boda is member of the organizing committee). The first edition of the meeting (2018) had 330 participants and more than 500 presented abstracts, the second (2019) had 452 participants and 231 abstracts. The Clinical Neurobiology group is organising local and national meetings on multiple sclerosis in the San Luigi Hospital.

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

Researchers of NICO are involved in several collaborative grants at a local (Cassa di Risparmio di Torino, Compagnia di San Paolo), national (PRIN) and international (7-FP and Horizon 2020) level, as detailed in the following reports.
NICO has been recently (July 2015) included by the MIUR (Italian Ministry of University and Research) in the list of Italian Research Institutes which are allowed to hire directly foreign researchers. Moreover, NICO has successfully applied to the MIUR to receive public funds to support private research institutes.
In 2014 NICO has signed an official agreement of collaboration with Italian Institute of Technology for the exchange of researchers and facilities, and in this frame researchers of the two institutions are already collaborating and preparing joint grant applications. Also, within the frame of NIT, an agreement has been signed with the Istituto Superiore Mario Boella (a research and innovation centre of the Compagnia di San Paolo, operating in the Information and Communication Technologies (ICT) domain, now Links) and with the Tohoku University in Japan. As a result of this collaboration, a grant agreement within the Horizon 2020 program has been signed in which the director of NICO is the coordinator.

THE NICO SPINOFF

In 2014 and 2015 some NICO researchers (prof. Eva, Panzica, Buffo, Boido and Tamagno) collaborated in preparing the application for an academic spinoff (S&P Brain) of the University of Torino, to provide services to researchers, institution and companies related to behavioral neurosciences. This will allow to provide an income to the NICO, and also to apply for cooperative grants as a company. The spinoff has been approved by the technology transfer committee of the University of Torino and approved by the Academic Senate and Council of Advisors of the University and constituted in 2016. S&P Brain allows to provide an income to the NICO, and also to participate in cooperative grant applications as a company.

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

Organization of the NICO (Neuroscience Institute Cavalieri Ottolenghi)

Scientific Director is prof. Alessandro Vercelli (confirmed in June 2018, up to March 2021). In addition to the scientific direction he performs also the function of Administrative Director. From November 2018, prof. Annalisa Buffo was appointed vice-Director for the activities at the NICO.

Our activities are organized into **nine groups**:
Adult Neurogenesis (PIs Luca Bonfanti and Paolo Peretto)
Brain Development and Disease (PI Alessandro Vercelli)
Clinical Neurobiology (PI Antonio Bertolotto)
Embryonic Neurogenesis (PI Ferdinando Di Cunto).
Nerve Regeneration (PI Stefania Raimondo – formerly S. Geuna)
Physiopathology of Stem Cells (PI Annalisa Buffo)
Neuroendocrinology (PI Giancarlo Panzica)
Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)
Neuropsychopharmacology (PI Carola Eva)

Staff

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

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

According to the Convention with the University of Turin and with San Luigi Hospital of Orbassano, the NICO hosts:
- **University staff**: 7 full professors, 8 associate professors, 11 university research assistants, 1 technician, 13 post-docs and 9 PhD students;
- **Hospital staff**: 1 Head physician, 1 manager biologist, 2 specialists in Clinical Biochemistry, 3 post-doc fellows, 1 laboratory technician.

About 85 graduating students of Biology, Biotechnology, Medicine and Psychology perform experiments for their thesis at the NICO.

Labs and Equipment

Molecular and cellular neurobiology, Neuroanatomy

The laboratory is equipped with several excellent quality research light microscopes, in particular, two confocal microscopes (Leica SP5 and Nikon) and a Nikon ViCo system. Moreover, a two-photon microscope Nikon (A1MP) has recently been acquired in the context of the Open Access laboratories project of the University. An electron microscope is available in the Department of Cell Biology, San Luigi Hospital, adjacent NICO.
There are also various imaging systems with computerized microscopes and photo / digital video cameras that allow morphometric investigations, studies densitometry quantitative autoradiography, image processing and statistical analysis. Two Neurolucida systems are in the microscopy facility.

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

Animal facility

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

Cellular and molecular biology

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

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

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

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.

Updates in 2019

New equipment

In 2019 the Neuroscience Institute Cavalieri Ottolenghi, which participated with its members of the Department of Neuroscience Rita Levi-Montalcini to the successful Department of Excellence project, could significantly implement its instrumentation.

First of all, the already available Leica confocal laser scanning microscope has been implemented, with an updated software, a new 20x objective (NA 0.75) and a hybrid detector (more sensitive than the common PMTs).

Moreover the installation of the Two-Photon confocal microscope was finally completed, and the recruitment of a researcher in the field (Dr. S. Bovetti, who joined the group of P. Peretto) allows to bring a specific competence in the Institute, of which all members will take profit. Dr. Bovetti will organize a "hands on" meeting/course for PhD students in 2020 together with external experts in the field.

A Zeiss Axioscan high throughput image acquisition station was bought and installed in 2019 and is currently used by researchers of the Institute.

Recently, an official tendering procedure was set for a light sheet microscope. The LaVision microscope was chosen and it will be installed in early 2020.

The CNL has recently bought and installed a SR-X Ultra-Sensitive Biomarker Detection System (Quanterix), an instrument based on the innovative Simoa (Single-molecule array) technology that allows the detection and quantification of proteins with a very high sensitivity, 1000 times greater than traditional ELISA, allowing the analysis of biomarkers (as Neurofilaments) previously difficult or impossible to measure, in numerous biological matrices, including serum, plasma, liquor, cell extracts.

Minor instruments (a new Leica cryostat, Noldus instruments for the behavior, a new high sensitivity Hamamatsu videocamera, and a single photon counting system for electrophysiology) were bought and installed in 2019. Neurolucida and Stereoinvestigator systems were updated.

Personnel

New personnel were recruited by the University Departments collaborating at NICO: in particular, Dr. Bertocchi joined the group of C. Eva, Dr. Ronchi that of S. Geuna, Dr. Hoxha that of F. Tempia, Dr. Guglielmotto and Dr. Stanga that of A. Vercelli. In the meanwhile, Dr. Boido and Dr. Gotti were promoted associate professors. One of the PIs (prof. S. Geuna) was recently elected Rector of the University, and therefore as such became President of Foundation Cavalieri Ottolenghi: his group will be temporarily led by Prof. S. Raimondo.

Dr. E Signorino was recently recruited as a lab technician by the Department of Neuroscience and assigned to the NICO.

A contract with Charles River was signed by the University of Torino for the animal house. One of the two technicians of NICO working in the facility was allocated to other duties as lab technician.

Upcoming projects on instrumentation, personnel and facilities

In order to further promote the implementation of instruments, the Scientific Director is organising a joint project for a distributed core facility for optic and electron microscopy of the Piedmont, together with the Politecnico of Torino.

Recently, Dr. Calì (from Magistretti's group in KAUST) won the competition for a researcher position at the Neuroscience Department, and from February 2020 will join A. Vercelli's group. He will bring new expertise in 3D EM and virtual reality.

In collaboration with the Doctorate School in Neuroscience and the Interdepartmental center for Neuroscience of the University of Torino, NICO members are organizing a series of "hands on" and thematic courses for PhDs and postdocs, open to the attendance of external people:

1) Boda, Cerrato and Buffo "Glial cells-neurons crosstalk in CNS health and disease"
2) Boido and Stanga "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies"
3) Bovetti and Bonzano "Imaging of cell and neural circuits: technical principles and applications"

Dr. Bertolotto is organizing a biobank for the Multiple Sclerosis. In the Department of Neuroscience, in the frame of the Department of Excellence project, a biobank for human samples for neurodegenerative diseases is being organized. A member of the NICO, prof. Tamagno, is involved in the organizing committee and the whole NICO will have access to samples.

Some considerations regarding research funding

The gross amount of active funds in which NICO PIs have direct responsibility (approximately 3,170,000 €) has remained stable in 2019, compared to 2018. The number of funded projects was 53 in 2019, as compared to 60 in 2018. In contrast, the requests for new funding have significantly increased. In 2018 NICO set 24 applications for 3,237,000 €, in 2019 16 applications for 8,820,000 €. These data indicate that NICO PIs increased their activity in fund raising in big competitive grants. The new competences and instruments acquired by the Institute could at least in part explain this improvement.

OUTREACH ACTIVITIES

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

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

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

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

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

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

of the region, the Association Girotondo Onlus for SMA patients in Biella, etc.). Together with the Coordination Committee for Tetraplegic and Paraplegic patients of Piedmont and the Neurosurgery Clinic of the Department of Neuroscience we have activated a help desk for the public for scientific research on Spinal Cord Injury. A famous mountain climber (Mr. Hervé Barmasse) is acting as a testimonial.

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). L. Bonfanti, together with other members of the NICO, organised a series of happenings called "10 piccoli neuroni per 10 grandi libri" for the public.

Organization and scientific supervision of **UNISTEM DAY** (yearly, national event; NICO organizes each year the Turin edition), Aula Magna del Rettorato Cavallerizza Reale (with 400 students of the secondary school). NICO also collaborated with IIT and INFN in the exhibition "Uomo Virtuale: corpo, mente, cyborg" (4 May-13 October 2019) at the Mastio della Cittadella in Turin.
Also, NICO is participating to the Festival dell'Innovazione and to the activities of the UNITRE (University of the Third Age) of Rivoli.

Next year NICO will be involved in the organisation of the Festival della Scienza di Settimo, fully devoted to neuroscience.

SCIENTIFIC SEMINARS AT NICO

Over the last six years, an internal committee (Annalisa Buffo and Silvia De Marchis) has been in charge for the promotion and organisation of the seminar activities at NICO. The committee established a procedure by which speakers to be invited are first proposed by NICO researchers and then selected, based on a poll by the NICO community. A Ferdinando Rossi's Lecture was organised in January.

For invited speakers, see the attached list.

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Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: Clinical Neurobiology

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Antonio Bertolotto

Degree: MD

Birthdate: 12/02/1952

Nationality: Italian

Gender:

Phone: 011 670 66 00

Email: antonio.bertolotto@gmail.com

Personnel

1) Serena Martire

Degree: MSc

Birthdate: 01/08/1987

Nationality: Italian

Gender: F

Phone: 011 670 6600

Email: serena.martire@gmail.com

Position: Biostatistician

Role & expertise: Design and conduct of epidemiological and experimental studies, data analysis.

2) Francesca Montarolo

Degree: MSc and PhD

Birthdate: 14/05/1983

Nationality: Italian

Gender: F

Phone: 011 670 6632

Email: francesca.montarolo@unito.it

Position: post-doc

Role & expertise: Experimental and behavioral murine model studies, histological and molecular analyses

3) Simona Perga

Degree: MSc, PhD and Board Certification

Birthdate: 29/03/1977

Nationality: Italian

Gender: F

Phone: 011 670 6600

Email: simona.perga@unito.it

Position: post-doc

Role & expertise: Proteomic and biochemical studies, histological and molecular analyses

4) **Fabiana Marnetto**

Degree: MSc, Board Certification

Birthdate: 14/12/1980

Nationality: Italian

Gender: F

Phone: 011 670 6635

Email: fabiana.marnetto@gmail.com

Position: biologist

Role & expertise: Quality management of CRESM Biobank, study of biomarkers for MS

5) Paola Valentino

Degree: MSc, and Board Certification | Birthdate: 11/08/1981

Nationality: Italian | Gender: F

Phone: 011 670 6635

Email: paolaval81@hotmail.com

Position: Medical biotechnologist

Role & expertise: diagnostic and prognostic tests for the detection of biomarkers for MS and NMO, evaluation of drug immunogenicity therapies; gene expression analysis.

6) **Federica Brescia**

Degree: 3-years level degree in Laboratory Techniques | Birthdate: 26/03/1984

Nationality: Italian | Gender: F

Phone: 011 670 6635

Email: fedeb2684@hotmail.it

Position: Technician

Role & expertise: CRESM Biobank technician.

7) Arianna Sala

Degree: MSc, and Board Certification | Birthdate: 22/05/1972

Nationality: Italian | Gender: F

Phone: 011 670 6601

Email: sala.arianna72@gmail.com

Position: resident biologist

Role & expertise: CSF analysis, diagnostic and prognostic tests for the detection of biomarkers for MS and NMO, evaluation of drug immunogenicity therapies.

2. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/ Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2018-19	Effects of siponimod on Nuclear Receptor subfamily (NR4As) in human blood and brain derived cells	Dr. Bertolotto and Dr. Montarolo	Novartis	PI of research unit	60.000	10%
2019-20	Comunicazione fra paziente e centro Sclerosi Multipla: implementazione di strumenti e sistemi informatici	Dr. Bertolotto	Roche	PI of research unit	50000	0%
September 2016-November 2020	"Improving therapeutic appropriateness of Multiple Sclerosis treatments using biologicalapproaches to personalize therapy and save pharmaceutical spending" RF-2013-02357497	Dr.Bertolotto	Ministero della Salute, Ricerca Finalizzata 2013	PI of research unit	381880	0%
Pending	Exploring the molecular mechanisms of A20 gene expression deregulation in multiple sclerosis patients: a multi-omics approach	Dr Perga	Fondazione Italiana Sclerosi Multipla (FISM)	PI of research unit	191.600	0%
Pending	Epigenetic mechanisms in A20 deregulation associated to Multiple Sclerosis: a possible target for	Dr. Montarolo	Ministero della Salute, Ricerca Finalizzata Giovani Ricercatori 2019	PI of research unit	449.900	0%

	repositioned drugs					
Pending	Early detection of disease progression in Relapsing Remitting multiple Sclerosis	Dr. Bertolotto	Ministero della Salute, Ricerca Finalizzata 2019	PI of research unit	441.200	0%

3. SCIENTIFIC ACTIVITIES IN 2019

Antonio Bertolotto (PI)
Supervised PhD students:NA
Honors, prizes, awards:

- ROCHE per la SM Awards
- MERCK AWARD

Outreach activities

- International collaborations: NA
- Invited talks: NA
- Science communication:NA
- Editorial duties:NA
- Others NA

Organizational activities and responsabilities at NICO: NA

Speakers invited:

- 16 Jan 2019, Milan Advisory Board "La sclerosi multipla secondaria progressiva: la definizione di progressione, i criteri della diagnosi nella pratica clinica e il nuovo approccio terapeutico alla luce dei risultati dello studio EXPAND" – Round table
- 12-13 Feb 2019, Rome "Sclerosi Multipla: quando l'innovazione incontra la tradizione. La gestione del paziente con attività di malattia lieve-moderata"
- 15 Feb 2019, Turin "La sclerosi multipla: gestione clinica e farmaco-economica"
- 26 Mar 2019, Milan "Ocrevus now: insieme per cambiare il futuro della SM"
- 29 Mar 2019, Gallarate "XXI approfondimenti monotematici nella sclerosi multipla"
- 18 May 2019, Imperia "Convegno Liguria SM – second edition". Talk: Terapie di seconda linea e gravidanza
- 20-21 Jun 2019, Milan "Brain experience" Aggiornamento multidisciplinare in SM Talk: A 10 anni dalla prima terapia orale: quanto è cambiato il trattamento della SM?
- 19 Sep 2019, Reggio Emilia "Encefaliti autoimmuni e malattie demielinizzanti atipiche: eziologia, approccio diagnostico e terapeutico.
- 25 Sep 2019, Moncrivello "SM2019: terapia e riabilitazione" Talk: Nuove terapie: decorso primario progressivo.
- 8 Nov 2019, Bologna "L'attuale scenario nel trattamento della SM" Talk: Algoritmo terapeutico nel trattamento del paziente con SM.
- 26 Nov 2019, Moncrivello "DMD in riabilitazione, una nuova strategia" Talk: Ruolo degli anticorpi monoclonali anti cellule B.

Other organizational activities:NA

Workshops, Schools or Conferences organized:

- 3-5 Jun 2019, Orbassano (Turin) "La gestione quotidiana del paziente con SM – XXII corso"

Technology transfer achievements (patents, etc.):NA

Serena Martire, Biostatistician
Supervised PhD students: NA
Honors, prizes, awards: Travel Grant for XXVII AINI Congress (Camogli, Italy) and 35th ECTRIMS (Stockholm, Sweden)
Outreach activities

- International collaborations: NA
- Invited talks: NA
- Science communication: NA
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: NA
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA

Francesca Montarolo, post-doc
Supervised PhD students: NA
Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: 2019 FISM Congress, "The de-ubiquitinase A20/TNFAIP3 in immunopathology of Multiple Sclerosis", Rome (Italy)
- Science communication: 2019 NICO-Conoscere, "Studio del fenotipo ADHD nel modello animale deficitario per il recettore nucleare NURR1".
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: NA
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA

Simona Perga, post-doc
Supervised PhD students: NA
Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: NA
- Science communication: NA
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: NA
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA

Fabiana Marnetto, Biologist
Supervised PhD students: NA

Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: 2019, Milan, Droplet Digital PCR Scientific Conference, Bio-Rad. “ddPCR approach for molecular monitoring of Rituximab therapy in patients with Neuromyelitis Optica Spectrum Disorders”
- Science communication: XXVII AINI Congress (Camogli, Italy): Development of a high sensitive molecular assay to improve individualized monitoring of Rituximab treatment in patients with NMOSD.
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: Member of the 2018-2019 ELSI (ethical, legal, and societal issues) Working group of the Italian node of BBMRI (Biobanking and BioMolecular Resources Research Infrastructure).
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA

Paola Valentino, Medical Biotechnologist
Supervised PhD students: NA
Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: NA
- Science communication: NA
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA

Federica Brescia, Technician
Supervised PhD students: NA
Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: NA
- Science communication: NA
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: NA
Workshops, Schools or Conferences organized: NA

Arianna Sala, Biologist
Supervised PhD students: NA
Honors, prizes, awards: NA
Outreach activities

- International collaborations: NA
- Invited talks: 27-28 May 2019, Catania- Ospedale Garibaldi, 17° Mediterranean Neuroscience Congress, 22° Etnean Epilepsy Workshop. “Patologie da anticorpi anti glicoproteina mielinica oligodendrocitaria e anti Aquaporina 4: il ruolo del laboratorio”

- Science communication: NA
- Editorial duties: NA
- Others: NA

Organizational activities and responsabilities at NICO: NA
Speakers invited: NA
Other organizational activities: NA.
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA

ALL LAB MEMBERS
Activities:
Dr. Bertolotto, Dr. Serena Martire, Francesca Montarolo, Simona Perga, Fabiana Marnetto, Paola Valentino, Arianna Sala: Official speaker for MS patients' care courses at CRESM "La gestione quotidiana del paziente con Sclerosi Multipla" organized by Biogen Idec.

Dr. Francesca Montarolo and Arianna Sala: “Alternanza Scuola-Lavoro”.

4. Research activity in 2019

a. Summary (500 characters)

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with an unpredictable course. As a part of the SCDO Neurologia- Centro di Riferimento Regionale Sclerosi Multipla (CRESM), the Clinical Neurobiology Laboratory is focused on both routine diagnostic and research activities, aimed to obtain a better understanding of the mechanisms involved in MS pathogenesis, identify diagnostic and prognostic biomarkers and define targets for novel treatment approaches.

b. Background and rationale (3000 characters)

MS is a chronic inflammatory demyelinating disease with no cure. It affects about 2.5 million people in the world and it represents the leading cause of non-traumatic disability in young adults. MS has an unpredictable course of a wide range of severity, but starting treatment early generally provides the best chance at slowing the progression of MS. In the last years new therapeutic agents have emerged, characterized by increased efficacy as well as higher costs and drug-related risks. Non-responding patients (NRs) are exposed to unnecessary risk and may undergo irreversible disease progression and potentially severe outcomes during ineffective treatments. Thus, there is a growing need for reliable markers to predict prognosis and therapeutic risk/benefit balance for each patient. In this context, it has been demonstrated that neurofilaments light chain (NfL) are a good biomarker for neuroaxonal damage, while the development of specific and persistent Anti Drug Antibodies (ADA) is a well-known biological mechanism that causes lack of response. Our MS Clinic is the Italian referral center for ADA against IFNb and Natalizumab (NAT), and last year we purchased the SR-X instrument (Quanterix) for the quantification of serum Neurofilaments (sNFL) and circulating drug levels.
The cause of MS is unknown, but it has a presumed autoimmune etiology. Accordingly, pregnancy acts as modulator of disease activity, since it strictly regulates maternal immune system to a state of transient tolerance in order to avoid rejection of the semi-foreign fetus. Unveiling the mechanism of the pregnancy induced immunomodulation would lead to a better understanding of the MS pathogenesis and to the identification of novel potential therapeutic targets. In the past years we identified a group of genes which are deregulated in unpregnant MS women but normalized during pregnancy. Among them we focused on two potent NF-KB inhibitors (TNFAIP3 and NR4A2) which are down-regulated in blood cells from MS patients and whose alteration might contribute to autoimmune processes. These genes now represent the target of studies aimed to unveil their role in the MS pathology.
However, the vast majority of biological research suffers from poor reproducibility of published data, even in prestigious journals, because of the lack of rigor in the collection of biological samples, the insufficient validation of the methods according to the instructions of FDA and limited sharing of data. This issue could be addressed by the creation of a structured Biobank able to collect, store and distribute data and samples obtained from MS patients to other researchers, following rigorous ethical and technical guidelines.

c. Objectives (1000 characters)

I) To early identify NR patients through the quantification of drugs level, ADA, biological activity and biomarkers such as sNfl.
II) To investigate the pregnancy-related mechanisms of immunomodulation though the evaluation of the immunomodulatory effects of placenta-derived extracellular vesicles (EV) on monocytes and regulatory T cells (Treg).
III) To examine the role of the TNFAIP3 gene in the MS pathology through the evaluation of the effect of the specific TNFAIP3 deficiency in myeloid cells including monocytes, monocyte-derived cells (M-MDC) and microglia in murine models.
IV) To create a structured Biobank able to collect, store and distribute data and samples from MS patients.

d. Results (4000 characters)

Diagnostic activity of the Clinical Neurobiology Laboratory includes cerebrospinal fluid (CSF) analysis from CRESM patients and other Piedmont hospitals and detection of anti-AQP4 and anti-MOG serum antibodies for differential diagnosis with neuromyelitis optica.
I) We collected serum and RNA samples from IFN beta treated patients to evaluate respectively ADA and biological activity; we enrolled NAT-treated patients to collect serum samples every month for 12 months to evaluate ADA. We tested samples from healthy subjects and MS patients for sNFL, in order to define a positivity cut off and to evaluate the analytical performance of the method. Analysis of data are still ongoing.
II) Thanks to the collaboration with Dr Luca Marozio, Head of the High Risk Pregnancy Unit and of the Research Laboratory of the Department of Surgical Sciences, Obstetrics and Gynaecology, University of Torino (Italy), we collected placental tissues from both MS and healthy women. We characterized for the first time the surface markers profile of placenta-derived EV, and we tested their ability to influence
the monocytes and CD4 T cells co-coltured with Treg. As a result, we observed that EV released by placental tissues of both HC and MS patients have a modest effect on monocytes activated with LPS in reducing the gene expression level of pro-inflammatory cytokines such as IL1-beta and TNF-alfa. On the other side, we observed an increased regulatory activity of Treg. In fact, the decrease of the proliferation level of activated CD4+ lymphocytes was greater after co-culture with Treg conditioned with EV compared to unconditioned Treg.
III) We found that the deletion of TNFAIP3 in myeloid cells of mice induces a reduced body weight, a decrease in the percentage number of M-MDC and of common monocyte and granulocyte precursor cells. We also reported that the deletion of TNFAIP3 in myeloid cells reports an increased microglial cell density in brain. The results suggest that the presence of TNFAIP3 in myeloid cells critically controls the development of M-MDC in lymphoid organ and of microglia in brain.
IV) We focused on the formal commitment of the BB-CRESM within the AOU San Luigi Gonzaga Hospital, in order to establish the previous Biobank project into a formal and structured part of the Institution. Also, we performed the collection of diagnostics remnant samples and the quality control of RNA samples stored in the Biobank.

e. Advancement in the field (1000 characters)

Results obtained may lead to the setup of biological assays that can improve the efficacy of treatment, selecting the best drug for each patient, and save, or better allocate, enormous amounts of NHS funds.
On the other side, we have contributed to elucidate the role of circulating human placental EV in the suppression of the immune system occurring in MS pregnant women and the role of the altered breaking molecules of inflammation in the disease pathogenesis.
Finally, our spontaneous collection of samples from patients with MS and healthy subjects is becoming an increasingly structured bio-research bank (informed consent, standardization of procedures for collecting and storing biological material and associated data) and is funded by Fondazione Italiana Sclerosi Multipla (FISM) since 2015.

f. Publications

Spadaro Michela, Montarolo Francesca, Martire Serena, Frezet Federica, Bruno Stefania, Grometto Alice, Perga Simona, Brescia Federica, Valentino Paola, Marnetto Fabiana, Botta Giovanni, Marozio Luca, Bertolotto Antonio. “Pregnancy: a powerful transient immunosuppressive phenomenon in Multiple Sclerosis women”, Poster presentation at the 2019 FISM Congress, Rome (Italy).

Montarolo Francesca, Martire Serena, Perga Simona and Bertolotto 1 Antonio. NURR1 Impairment in Multiple Sclerosis. Int. J. Mol. Sci. 2019, 20, 4858; doi:10.3390/ijms20194858.

Montarolo Francesca, Perga Simona, Tessarolo Carlotta, Spadaro Michela, Martire Serena, Bertolotto Antonio. "TNFAIP3 in circulating and parenchymal myeloid lineage critically controls monocytes, monocytes-derived cells and microglia", Journal of Neuroinflammation (submitted).

Assessing measurement invariance of MSQOL-54 across Italian and English versions.
Giordano A, Testa S, Bassi M, Cilia S, Bertolotto A, Quartuccio ME, Pietrolongo E, Falautano M, Grobberio M, Niccolai C, Allegri B, Viterbo RG, Confalonieri P, Giovannetti AM, Cocco E, Grasso MG, Lugaresi A, Ferriani E, Nocentini U, Zaffaroni M, De Livera A, Jelinek G, Solari A, Rosato R.
Qual Life Res. 2019 Nov 9. doi: 10.1007/s11136-019-02352-0. [Epub ahead of print]

Acknowledgement to Authors, Referees and Readers 2019.
Sabbagh M, Bertolotto A.
Neurol Ther. 2019 Dec;8(2):513-515. doi: 10.1007/s40120-019-00163-6. No abstract available.

Immunomodulatory Effect of Pregnancy on Leukocyte Populations in Patients With Multiple Sclerosis: A Comparison of Peripheral Blood and Decidual Placental Tissue.
Spadaro M, Martire S, Marozio L, Mastromauro D, Montanari E, Perga S, Montarolo F, Brescia F, Balbo A, Botta G, Benedetto C, Bertolotto A.
Front Immunol. 2019 Aug 16;10:1935. doi: 10.3389/fimmu.2019.01935. eCollection 2019.

NURR1 deficiency is associated to ADHD-like phenotypes in mice.
Montarolo F, Martire S, Perga S, Spadaro M, Brescia I, Allegra S, De Francia S, Bertolotto A.
Transl Psychiatry. 2019 Aug 27;9(1):207. doi: 10.1038/s41398-019-0544-0.

Drug Holiday of Interferon Beta 1b in Multiple Sclerosis: A Pilot, Randomized, Single Blind Study of Non-inferiority.
Romano S, Ferraldeschi M, Bagnato F, Mechelli R, Morena E, Caldano M, Buscarinu MC, Fornasiero A, Frontoni M, Nociti V, Mirabella M, Mayer F, Bertolotto A, Pozzilli C, Vanacore N, Salvetti M, Ristori G.
Front Neurol. 2019 Jul 16;10:695. doi: 10.3389/fneur.2019.00695. eCollection 2019.

Best Practices for Long-Term Monitoring and Follow-Up of Alemtuzumab-Treated MS Patients in Real-World Clinical Settings.
Barclay K, Carruthers R, Traboulsee A, Bass AD, LaGanke C, Bertolotto A, Boster A, Celius EG, de Seze J, Cruz DD, Habek M, Lee JM, Limmroth V, Meuth SG, Oreja-Guevara C, Pagnotta P, Vos C, Ziemssen T, Baker DP, Wijmeersch BV.
Front Neurol. 2019 Mar 22;10:253. doi: 10.3389/fneur.2019.00253. eCollection 2019. Review.

Neurology and Therapy: Looking Back on 2018 and Forward to 2019.
Sabbagh M, Bertolotto A.
Neurol Ther. 2019 Jun;8(1):1-3. doi: 10.1007/s40120-019-0126-3. Epub 2019 Jan 30. No abstract available.

7. Future directions and objectives for next years

Please describe the following information relevant to the research that you are planning to do – Character limit is mandatory. Please highlight the added value of collaborations within the NICO where applicable.

a. Summary (up to 2000 characters):

Based on the results obtained on the last 20 years of clinical and research activity, the aim of the Clinical Neurobiology Laboratory is still to be the investigation of the mechanisms involved in MS pathogenesis, the identification of diagnostic and prognostic biomarkers and the definition of targets for novel treatment approaches.

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

Aiming at addressing the need for reliable markers to predict MS prognosis, we tested samples from healthy subjects and MS patients for sNFL, which represent a promising candidate biomarker for neuroaxonal damage, in order to define a positivity cut off and to evaluate the analytical performance of the method. Samples collection and analysis of data are still ongoing and will be carry out for the next few years.

Aiming at identifying biomarkers for the identification of NR patients, we collected samples from IFN beta and NAT-treated patients evaluate ADA and IFN-biological activity. Another strategy to improve therapeutic appropriateness is to tailor time and dose of drug infusions each patient. This approach can be applied to NAT and Rituximab (RTX) and other anti-CD20 drugs that are infused at fixed schedule.

In the context of the study of the pregnancy-induced immunomodulatory mechanisms in MS disease, we focused on the deregulation of the anti-inflammatory gene TNFAIP3. We recently demonstrated its crucial role in myeloid cells and in the the development of M-MDC in lymphoid organ and of microglia in brain, and now we plan to investigate the cause underlying its deregulation in MS patients.

Finally, conscious that biobanks represent vital resources for the entire scientific community and beyond, we plan to continue the research in this area.

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

MS is a progressive disabling disease of CNS, which requires an early diagnosis and treatment to decrease the risk of progression of neurological dysfunction and also the burden on the health care system. Our efforts aim to provide an early diagnosis for the patients, a personalized therapy and monitoring of therapeutic response, and to identify novel therapeutic targets.

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

In the next few years, we plan to:

I) improve timing of drug administration, based on serum drug levels or specific drug biomarkers. We will optimize dose and time of infusion of NAT and RTX. NAT and RTX responders are defined as patients without clinical activity and without new MRI lesions. NAT and RTX serum concentration in samples stored in our bio-bank will be correlated with responsiveness. The frequency of RTX infusion will be decided according to the number of circulating CD19+ and CD27+ B cells detected by FACS and by a new molecular (RT-PCR and Droplet digital PCR) approach set up in our center. This latter method could be used also for the new anti-CD20 drugs. The cost of traditional infusions will be compared with the optimized procedures.

II) investigate epigenetic mechanisms of TNFAIP3 expression regulation focusing on DNA methylation and miRNA expression analysis. We will perform mRNA sequencing, genome-wide genotyping and characterization of genome-wide DNA methylation and small ncRNA expression. Next, we will compare TNFAIP3 gene expression and epigenetic profiles between MS patients and healthy controls, and we will evaluate the association of the methylation status of CpGs in the TNFAIP3 locus, the genetic variants in the A20 locus and the expression profiles of miRNA targeting the TNFAIP3 locus with the TNFAIP3 gene expression level. Also, we will apply a differential expression analysis of mRNA sequencing data to identify quantitative changes in expression levels between patients and controls, we will perform an Epigenome-Wide Association Study (EWAS) to identify MS-related interaction networks and we will analyse small ncRNA sequencing data to identify expression profiles of miRNA correlated to disease status. Furthermore, we plan to identify agency-approved drugs increasing TNFAIP3 expression, applying a drug repositioning approach. All these goals will be achieved by multidisciplinary approaches, involving the collaboration between counterparts providing expertise in MS management, genome-wide molecular analysis (Prof. Giuseppe

Matullo's lab, affiliated to the Medical Science Dept, Univ. of Turin, and the Italian Institute for Genomic Medicine) and drug screening analysis (GEMFORLAB and MEDSynth).
III) expand the biobank which is already in operation at the CRESM, through the collection of biological samples (serum, plasma, cerebrospinal fluid (CSF), urine, cells from blood and CSF for DNA and RNA,) of different types of MS and various controls, according to strict criteria and recorded in a database. Moreover, the biobank project aims to distribute samples to projects funded by FISM or other institutions. We will offer technical support for co-validation of methods and will perform quality controls on biological materials stored in the Biobank (i.e. evaluation of the influence of pre-analytical variables, time and temperature, on blood samples used in gene expression studies). Also, we will cooperate with other biobanks in the future network of biobanks dedicated to MS research.

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

Results obtained from the optimization of drug administration will allow to save, or better allocate, enormous amounts of NHS funds.
Our studies on molecular mechanism of TNFAIP3 deregulation will provide, for the first time to our knowledge, a biomarker fingerprint of the MS disease based on the integrated analysis of molecular data deriving from multi-omics approaches. This finding will offer promising prospects for the development of diagnostic (and maybe prognostic) tools.
The Biobank of the Clinical Neurobiology Laboratory will improve the reproducibility of data obtained by researchers who will use biological samples of the bio-bank.

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

FONDAZIONE
CAVALIERI OTTOLENGHI

NICO
Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: **Adult neurogenesis**

1. LABORATORY DESCRIPTION – PERSONNEL:

Since the birth of NICO (in 2010), the group **Adult Neurogenesis** was created from two independent research groups, which joined their expertise on structural plasticity and neurogenesis. The group at NICO has been organized with two PIs coordinating independent but complementary research lines. The group includes 4 senior researchers with a permanent position, a RTD-B, and 7 young researchers. The keyword is cooperation on complementary research topics on structural plasticity and neurogenic potential of the adult brain.

Principal Investigator 1

Paolo Peretto — Birthdate: (18/09/1963)

Full professor — Gender: M

Nationality: Italian — Phone: 011 6706605

Email: paolo.peretto@unito.it

Principal Investigator 2

Luca Bonfanti — Birthdate : (19/05/1962)

Associate professor — Gender: M

Nationality: Italian — Phone: 011 6706606

Email: luca.bonfanti@unito.it

Researchers (permanent position or RTD at the University of Turin)

1. First name: Silvia De Marchis — Birthdate (14/09/66)

Associate professor — Gender: F

Role: Senior researcher — Nationality: Italian

Expertise: in vivo and in vitro molecular and cellular analyses

2. First name: Federico Luzzati — Birthdate (20/10/1974)

Assistant professor — Gender: M

Role: Senior researcher — Nationality: Italian

Expertise: morphological analyses and 3D reconstructions

3. First name: Serena Bovetti — Birthdate (13/09/1977)

Assistant Professor (RTD-B) — Gender: F

Expertise: in vivo two-photon microscopy (functional and morphological analyses)

Researchers (Postdoc and PhD students)

4. First name: Chiara La Rosa Birthdate (01/07/88)
Postdoc (scholarship NICO – Neuroni alternativi) Gender: F
Role: young researcher (third year) Nationality: Italian
Expertise: comparative analyses of immature neurons in domestic and wild mammals

5. First name: Isabella Crisci Birthdate (17/12/89)
PhD student (fourth year) Gender: F
Role: young researcher Nationality: Italian
Expertise: cellular and molecular analyses of AN in the hippocampus

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

7. First name: Giulia Nato Birthdate (08/05/1986)
Postdoc (Fondazione San Paolo Fellowship) Gender: F
Role: young researcher Nationality: Italian
Expertise: cellular and molecular analyses of lesion-induced neurogenesis

8. First name: Marco Fogli Birthdate (23/09/1993)
PhD student (First year; since november 4th) Gender: M
Role: young researcher Nationality: Italian
Expertise: cellular and molecular analyses of lesion-induced neurogenesis

9. First name: Marco Ghibaudi Birthdate (29/05/1992)
PhD student (first year; since november 4th) Gender: M
Role: young researcher Nationality: Italian
Expertise: cellular and molecular analyses of immature neurons in mammals

10. First name: Yifei Liu Birthdate (22/01/1995)
PhD student from CSC (first year) Gender: F
Role: young researcher Nationality: Chinese
Expertise: cellular and molecular analyses of immature neurons in mammals

2. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2017-20	A new non-invasive approach to the investigation of cerebral activity in domestic animals using functional near-infrared Spectroscopy 2015Y5W9YP	Bonfanti	PRIN bando 2015	PI (Coordinator)	10.000 (out of 30.000)	8%
Annual	-	Bonfanti	Local Research UniTO	PI	3.100	8%
Always open for donations	Neuroni alternativi	Bonfanti	Progetto Neuroni Alternativi (NICO)	PI	7.000	10%
Annual	-	Peretto	Local Research UniTO	PI	2.701	8%
2019-20	-	Luzzati	Fondo Finanziamento Attività di base 2017 – Compagnia di San Paolo	PI	27.500 (out of 55.000)	8%
Annual	-	Luzzati	Local Research UniTO	PI	3.432	8%
Annual	-	De Marchis	Local Research UniTO	PI	2.647	8%
Annual	-	Bovetti	Local Research UniTO (+ starting grant)	PI	8.024	8%
2020-2023	Sounds and pheromones: neural networks merging olfactory and acustic cues in sexual impriniting	Bovetti/Peretto	Human Frontiers 2020-23	PI	350.000 Pending	8%
2020-21	Potenziare le cellule staminali neurali per combattere l'invecchiamento e il declino cognitivo	Bovetti/De Marchis	Fondazione CRT	PI	30000	

2. SCIENTIFIC ACTIVITIES IN 2019

Paolo Peretto (PI 1)
Supervised PhD students: Marco Fogli (co-tutored, starting from November 4th)
Honors, prizes, awards: na
Outreach activities

- International collaborations: Prof. Dustin Penn (Konrad Lorenz Institute of Ethology, Veterinary Medicine University, Vienna); Prof Sylvain Gigan (Laboratoire Kastler-Brossel Sorbonne Université, Paris); Dr. Paolo Giacobini (Inserm, UDSL, School of Medicine, Lille, France).
- Invited talks: na
- Science communication: DBIOS Conferences in Evolutionary Zoology "Running and Cerebral evolution" Turin, Italy, June 2019.
- Editorial duties: Associate Editor Frontiers in Neuroscience
- Others: Referee for Scientific Journals; Involvement in the "Scuola–lavoro" training June 14 2019.

Organizational activities and responsabilities at NICO: Representative of the personnel for safety
Speakers invited: na
Other organizational activities :na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Luca Bonfanti (PI 2)
Supervised PhD students: Chiara La Rosa (postdoc in the second part of the year); Marco Ghibaudi, Yifei Liu (starting from November 4th)
Honors, prizes, awards: na
Outreach activities

- International collaborations: Chet Sherwood (USA); Irmgard Amrein (Zurich); Frederic Lèvy and Elodie Chaillou (France); Juan Nacher (Spain).
- Invited talks: "Large-brained, gyrencephalic mammals as a model for studying brain plasticity" in the meeting: New animal models to understand the brain, Tours, France. "Immature neurons: A novel target to modulate neurogenesis?" Società Italiana di Farmacologia, Firenze.
- Science communication: 4 conferences in secondary schools; organization of UNISTEM DAY in Turin (a national/international day dedicated to research and stem cells for students of the secondary school), Aula Magna Cavallerizza reale; Lyons and Rotary dinner with conferences; Conference at "L'Uomo virtuale" (exposition); six lessons at the "Università delle 3 età" Asti. Conference at "Pint of Science". Organization and realization of a cycle of 10 conferences in a downtown book shop: "10 piccoli neuroni per 10 grandi libri" (also involving other members of NICO).
- Editorial duties: Editor in Chief of Frontiers in Neurogenesis.
- Others: Scientific supervisor of 4 undergraduate thesis.

Organizational activities and responsabilities at NICO: coordinating the NICO Open Day 2019.
Speakers invited: Francesco Bifari, University of Milan.
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Silvia De Marchis, Associate Professor
Supervised PhD students: Isabella Crisci
Honors, prizes, awards: na
Outreach activities

- International collaborations: Prof. Chichung Lie and Dr. Ruth Beckervordersandforth-Bonk, Institute of Biochemistry, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany; Dr. Michèle Studer, INSERM U636, Nice Sophia Antipolis; Dr. Saadia Ba-M'hamed, University of Marrakech, Marocco; Dr. Paolo Giacobini, Inserm, UDSL, School of Medicine, Lille, France; Wojciech Krezel INSERM, IGBMC, Strasbourg, France.
- Invited talks: na
- Science communication: na
- Editorial duties: na

- Others: Referee as Scientific expert for ANR Research Proposal Generic Call 2019 Referee for Scientific Journals (e.g. Cell Reports, Brain Structure and Function, Stem Cells…); Scientific supervisor of Sara Bonzano (post doc) and Hanaa Malloul (visiting post doc – three months); Scientific supervisor of 4 undergraduate thesis.

Organizational activities and responsabilities at NICO: Reference for Seminar organization
Speakers invited: Dr. Samuele Chiaramello, PhD, Milan.
Other organizational activities:na
Workshops, Schools or Conferences organized: Prize for Communication in Neuroscience "Aldo Fasolo" 2019 PhD Program in Neuroscience – University of Turin
Technology transfer achievements (patents, etc.): na

Federico Luzzati, Role (Researcher)
Supervised PhD students: Marco Fogli (co-tutored, starting from November 4th)
Honors, prizes, awards: na
Outreach activities

- International collaborations: Benedikt Berninger, University of Mainz, Germany; Philip
- Gerulich, University of Southampton, UK; Hongjun Song, University of Pennsylvania, USA.
- Invited talks: na
- Science communication: Public conference entitled "C'era una volta un neurone" (once upon a time there was a neuron) in the context of Mirafiori Scienza (Turin).
- Editorial duties: na
- Others: Referee for Scientific Journals

Organizational activities and responsabilities at NICO: Person in charge for Imaris/Neurolucida work station
Speakers invited:na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Serena Bovetti, Assistant Professor (RTD-B since June 2018)
Supervised PhD students: na
Honors, prizes, awards: na
Outreach activities

- International collaborations: Prof. Dustin Penn (Konrad Lorenz Institute of Ethology, Veterinary Medicine University, Vienna); Prof Sylvain Gigan (Laboratoire Kastler-Brossel Sorbonne Université, Paris); Dr. Paolo Giacobini, (Inserm, UDSL, School of Medicine, Lille, France).
- Invited talks: "High-speed two-photon fluorescence functional imaging of cortical and subcorti regions" 65° Congresso GEI, Ancona, June 2019.
- Science communication: na
- Editorial duties: na
- Others: Referee for Scientific Journals (e.g. Scientific Reports; Molecular Neurobiology); Scientific supervisor of 1 undergraduate thesis; Technical Report Nico "Two Photo Microscopy and probes for in vivo multicolor imaging of brain structure and function", December 2019.

Organizational activities and responsabilities at NICO: Person in charge of the BSL2 surgical room and of two-photon microscope. Involvment in the "Scuola –lavoro" training June 14 2019.
Speakers invited: Prof. Stefano Vicini; Georgetown University, Washington DC, USA; Dr Gabriele Losi, Istituto di Neuroscienze-CNR Padova.
Other organizational activities:
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Chiara La Rosa (Postdoc)
Supervised PhD students: na
Honors, prizes, awards: na
Outreach activities

- International collaborations: na
- Invited talks: "Cortical layer II immature neurons are more abundant in mammals with a large, gyrencephalic brain" at the Meeting "New Perspective in Neuroscience: Research Results of Young Italian Neuroscientists" March 1st 2019 -Napoli, Italy
- Science communication: Member of the organizing committee of the "Beautiful mind" sessions for The International Festival 'Pint of Science', Turin May 2019
- Editorial duties: na
- Others: Participation as poster presenter at the 4th Eurogenesis Meeting, June 2019, Bordeaux, France and at the Brayn 2nd Brainstorming Research Assembly for Young Neuroscientists, Nov 2019, Milan, Italy

Organizational activities and responsabilities at NICO: Person in charge for Nikon Eclipse 80i + VICO image acquisition system and of organization office space for PhD students and postdocs
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Giulia Nato (Postdoc, starting from February 2019)
Supervised PhD students: na
Honors, prizes, awards: "Assegno di ricerca''one year long, Compagnia San Paolo Call for the internationalization of research - Year 2019-2020
Outreach activities

- International collaborations: na
- Invited talks: na
- Science communication: dissemination activity at the "Liceo scientifico E Bérard" January 2019
- Editorial duties: na
- Others: Participation as poster presenter at the 4th Eurogenesis Meeting, June 2019, Bordeaux, France; the Brayn 2nd Brainstorming Research Assembly for Young Neuroscientists, Nov 2019, Milan, Italy; XIV European Meeting on Glial Cells in Health and Disease, Porto, Portugal, July , 2019; Talk: "Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion" Nico Progress Report, INN Open Neuroscience Forum, June 2019

Organizational activities and responsabilities at NICO: Person in charge for Nikon Eclipse 80i + VICO image acquisition system
Speakers invited: Caramello Alessia, Francis Crick Institute, London
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Sara Bonzano (Postdoc)
Supervised PhD students: na
Honors, prizes, awards: one year post-doctoral fellowship, Fondazione Umberto Veronesi (Grant 2019) and one year "Assegno di Ricerca" for 2020 (Research Internationalization Grant – University of Turin)

Outreach activities

- International collaborations: Prof. Chichung Lie and Dr. Ruth Beckervordersandforth-Bonk, Institute of Biochemistry, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany
- Invited talks: na
- Science communication: "COUP-TFI, mitocondri e staminali: alleati per la plasticità cerebrale" at "Fondazione Ferrero", Alba, Italy, April 2019. Secondary school conference "Ricercatori in classe" organized in collaboration with the Umberto Veronesi Foundation at the ITIS Pininfarina, Moncalieri (TO), Italy. May, 17, 2019.
- Editorial duties: Referee for Scientific Journals (Journal of Experimental Neuroscience; Metabolic Brain Disease)
- Others: Participation as poster presenter at the 4th Eurogenesis Meeting, June 2019, Bordeaux, France; the Brayn 2nd Brainstorming Research Assembly for Young Neuroscientists, Nov 2019, Milan, Italy

Organizational activities and responsabilities at NICO: In charge of calendar organization for the Leica SP5 confocal microscope
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Isabella Crisci (PhD student)
Supervised PhD students: na
Honors, prizes, awards: na

Outreach activities

- International collaborations: Wojciech Krezel INSERM, IGBMC, Strasbourg, France.
- Invited talks: na
- Science communication: na
- Editorial duties: na
- Others: Participation as poster presenter at the Brayn 2nd Brainstorming Research Assembly for Young Neuroscientists, Nov 2019, Milan, Italy; Talk: "Fate mapping of adult hippocampal neural stem/progenitor cells in a model of neuroinflammation" Nico Progress Report, INN Open Neuroscience Forum, February 2019

Organizational activities and responsabilities at NICO: In charge of calendar organization for the Leica SP5 confocal microscope
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Marco Fogli (PhD student, starting from November 4th 2019)
Supervised PhD students: na
Honors, prizes, awards: 4 years PhD Fellowship - PhD program in Neuroscience, Univ of Turin

Outreach activities

- International collaborations: na
- Invited talks: "Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion" at the 4th Eurogenesis Meeting, June 2019, Bordeaux, France

- Science communication: na
- Editorial duties: na
- Others: Participation as poster presenter at the Brayn 2nd Brainstorming Research Assembly for Young Neuroscientists, Nov 2019, Milan, Italy; XIV European Meeting on Glial Cells in Health and Disease, Porto, Portugal, July , 2019; Talk: "Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion" Nico Progress Report, INN Open Neuroscience Forum, September 2019

Organizational activities and responsabilities at NICO: na
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Marco Ghibaudi (PhD student, starting from November 4th 2019)
Supervised PhD students: na
Honors, prizes, awards: three-year PhD Fellowship - Research Doctorate in Veterinary Sciences for Animal Health and Food Safety.
Outreach activities

- International collaborations: na
- Invited talks: na
- Science communication: na
- Editorial duties: na
- Others: na

Organizational activities and responsabilities at NICO: na
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

Yifei Liu (PhD student from the China Scholarship Council, starting from November 4th 2019)
Supervised PhD students: na
Honors, prizes, awards: three-year PhD Fellowship after selection of the China Scholarship Council (May, 2019), to be performed at the University of Turin, Research Doctorate in Veterinary Sciences for Animal Health and Food Safety.

Outreach activities

- International collaborations: na
- Invited talks: na
- Science communication: na
- Editorial duties: na
- Others: na

Organizational activities and responsabilities at NICO: na
Speakers invited: na
Other organizational activities: na
Workshops, Schools or Conferences organized: na
Technology transfer achievements (patents, etc.): na

ALL LAB MEMBERS

Activities: Open days at NICO

4. Research activity in 2019

a. Summary (500 characters)

Different aspects of adult brain structural plasticity were addressed.
i) *progenitor fate:* cell-intrinsic control of adult neural stem cells in the hippocampus;
ii) *reproductive*: understanding GnRH system role in adult neurogenesis/plasticity;
iii) *comparative*: analysis of "immature" neurons in mammals, including small-brained and large-brained species;
iv) *repair*: mechanisms and dynamics of lesion-induced acquisition of a neurogenic competence in striatal astrocytes.

b. Background and rationale (3000 characters)

The discovery of adult neurogenesis (AN) in the mammalian brain produced a fully renewed vision of brain plasticity, involving stem/progenitor cells capable of generating new neurons throughout life, raising new hopes for regenerative therapeutic approaches. Yet, a lot of unanswered questions still remain, concerning both the rate and functional role of AN in different mammalian species/brain regions and the regulatory mechanisms of plasticity involved. Such lack of knowledge is confirmed by recent studies revealing conflicting results and interpretations on the existence of AN in the human brain, and unveiling new/alternative types of structural plasticity (i.e. immature neurons) depending on the species. Another set of new data reveal unexpected roles of astrocytes in lesion-induced paradigms (e.g., neurogenic activation of striatal astrocytes). Finally, a further level of complexity consists of the emerging tight interaction between the neuro-endocrine system and AN to sustain reproductive behavior in rodents.

In this new complex picture, some pivotal questions are:

A. how is regulated the fate of adult neural stem cells (NSCs) in physiological or pathological conditions?
B. how different types of plasticity (AN versus "immature" neurons) are phylogenetically distributed among mammals?
C. how the external and internal cues integrate with AN to sustain behaviors essential for survival, such as reproduction?

The rationale of the research carried out in 2019 can be summarized as follows:

i) The age-related decrease in hippocampal neurogenesis might contribute to brain pathologies, such as Alzheimer's disease and dementia. We recently showed that COUP-TFI/Nr2f1 is necessary and sufficient to favor neurogenesis over astrogliogenesis from young adult NSCs/progenitors in mice (Bonzano et al., 2018 Cell Rep) but the mechanistic insights underlying such function and its alteration during aging and in pathological condition are still largely unknown.
ii) Upon excitotoxic lesion, striatal astrocytes acquire a neurogenic competence in a SOX2 dependent manner and subsequently express it *in vivo* by clonally expanding through intermediate progenitors. The neuronal progeny are not committed to classic striatal cell types but still integrate into pre-existing circuits.
iii) On the basis of a previous work showing that "immature" neurons are more widely distributed in the sheep brain with respect to rodents (Piumatti et al., 2018, J Neurosci) we established a method to quantify in a comparable manner the amount of cortical immature neurons in 10 mammals, including small-brained and large-brained species.
iv) Previously, we showed modulation of AN during puberty in female mice (Oboti et al., 2016, Front Neuroanatomy). To establish whether sex-hormones set AN during this critical stage of life, we investigated the role of GnRH secretion, which orchestrates the hypothalamic-pituitary-gonadal axis, and that of gonadal hormones alone.

c. Objectives (1000 characters)

i) Determining the role of COUP-TF1/Nr2f1 function on adult NSC activity (i.e. proliferation and fate) in young adults and healthy aging.
ii) Establishing the activation mechanisms of the striatal astrocytes neurogenic potential and analyzing the identity and integration capacity of their neuronal progeny.
iii) Studying the influence of sex-hormones on peri-pubertal AN modulation. To this aim we exploited the GnRH::Cre:DicerloxP/loxP mice, a model in which GnRH secretion is inactivated in the infantile period, as well as females ovariectomized just before puberty, determining loss of sex-hormones around the onset of puberty.
iv) Establishing whether layer II cortical "immature" neurons are heterogeneous in mammals, and possibly more extended in large-brained species we systematically analyzed their occurrence, anatomical distribution, phenotype and amount in 12 different species, belonging to 8 orders.

d. Results (4000 characters)

Progenitor fate in the hippocampus. Our results show that COUP-TFI conditional loss in adult NSCs decreases cell proliferation, a typical sign of senescent NSCs, and that COUP-TFI is downregulated in the aged hippocampal neurogenic niche. Moreover, exploiting a model of conditional COUP-TFI overexpression in NSCs we obtained new data on its implication in migration, maturation and survival of adult born neurons. Interestingly, evidences obtained by ChIP-Seq analysis on adult mouse brain show that COUP-TFI could directly control expression of nuclear-encoded mitochondrial genes and preliminary data obtained by retroviral mediated labelling of mitochondria *in vivo* show an altered mitochondria morphology upon COUP-TFI manipulation.

Neurogenesis and reproduction. In GnRH::Cre:DicerloxP/loxP mice we examined proliferation and cell fate specification (neuronal vs glial) of the primary progenitors, and survival/differentiation of newborn neurons in the two main adult neurogenic regions (i.e. olfactory bulb and hippocampus) and sexes. The data obtained show a gender-specific alteration of these processes in both regions. Proliferation and neuronal progenitor specification was found affected in males, whereas survival of newborn neurons was reduced in the olfactory bulb of females. These results actually indicate that impaired GnRH secretion during the onset of puberty affects AN. In addition, data from pre-pubertal gonadectomized females support that such alterations are mostly mediated by gonadal hormones. Interestingly, we have also identified and characterized a new population of GnRH neurons located around the olfactory bulb, whose role is still unknown.

Lesion induced striatal neurogenesis: Through genetic lineage tracing and conditional mutagenesis, in collaboration with Annalisa Buffo we showed that the TF SOX2 is essential for the activation of a neurogenic competence in striatal astrocytes early after excitotoxic lesion. This competence is subsequently expressed in a SOX2 independent manner by the third week after lesion with the clonal expansion of multiple scattered astrocytes. The neuronal progeny disperses in the striatum and integrate into pre-existing circuits as assessed by rabies virus retrograde tracing and electrophysiological recordings. Interestingly, single cell RNAseq indicates that these cells are not committed to classic striatal cell types and may rather represent a novel neuronal subtype involved in the re-organization of the striatal circuits.

Immature neurons. In the adult mammalian brain, mainly composed of mature neurons, a limited amount of stem cell-driven neurogenesis can persist in postnatal life but is reduced in large-brained species. A population of immature neurons in the cortical layer II retains developmentally undifferentiated states in adulthood. In the 12 diverse mammalian species studied here (spanning from small, lissencephalic to large, gyrencephalic brains), in spite of well-preserved morphological and molecular features, the distribution of cortical immature

neurons was highly heterogeneous, particularly in the neocortex. While virtually absent in rodents, they are present in the entire neocortex of many other species and their linear density covaried with brain size. These findings suggest an evolutionary developmental mechanism for plasticity in large brains, granting a reservoir of young cells for the cerebral cortex.

e. Advancement in the field (1000 characters)

As concerns the regulation and fate of adult NSCs, we found that in the adult hippocampus COUP-TFI is a crucial pleiotropic factor to maintain neurogenesis. Moreover, we showed that the peculiar (astrocytic) origin of the lesion-induced striatal neurogenesis can represent a novel form of compensatory plasticity, potentially useful to drive these cells toward a striatal neuronal fate. As to reciprocal interaction/integration between AN and hormones, we showed that, at the onset of puberty, gonadal hormone secretions organize the process of AN in a sex-dependent way, thus supporting this setting is critical in sustaining the reproductive behaviour. Finally, regarding the different types of structural plasticity, the study of "non-newly generated, immature" neurons is revealing that these cells might represent a reservoir of "young" neurons for the (non-neurogenic) cerebral cortex of large-brained mammals.

f. Publications

La Rosa, C., Ghibaudi, M., Bonfanti, L. (2019). Newly generated and non-newly generated "immature" neurons in the mammalian brain: A possible reservoir of young cells to prevent brain ageing and disease? *J. Clin. Med.* 8(5), pii: E685. doi: 10.3390/jcm8050685.
IF: 5,7 Q1

Tomagra G, Picollo F, Battiato A, Picconi B, **De Marchis S**, Pasquarelli A, Olivero P, Marcantoni A, Calabresi P, Carbone E, Carabelli V. *Front. Neurosci.* 2019. https://doi.org/10.3389/fnins.2019.00288
IF: 3,6 Q2

Bovetti S, Moretti C, Fellin T (2019) Patterned two-photon illumination for high-speed functional imaging of brain networks *in vivo*. In "Advanced Optical Methods for Brain Imaging" (book chapter) Springer

Submitted:

La Rosa C, Cavallo F, Pecora A, Chincarini M, Ala U, Nacher J, Cozzi B, Sherwood CC, Amrein I, **Bonfanti L.** Greater occurrence of cortical layer II "immature" neurons in large-brained mammals (Original research article)

Bovetti S., Gribaudo S., **Nato G.,** Saraulli D., **Bonzano S.**, Gambarotta G ., **Luzzati F**, **Peretto P**., **De Marchis S.** Neurogranin regulates structural plasticity in adult-born olfactory neurons and odor-reward associative memory (Original research article)

Trova S., **Bovetti S.**, Giacobini P., **Peretto P.** Sexually-dimorphic peripubertal regulation of adult nurogeneis in mice (Original research article)

La Rosa C, Parolisi R, **Bonfanti L.** The never-ending story of brain structural plasticity: From adult neurogenesis to immature neurons (Mini-review)

Cozzi B, **Bonfanti L**, Canali E, Minero M. Brain waste: Animal brains that we neglect (Perspective)

Boda E, Lorenzati M, Parolisi R, Harding B, Pallavicini GM, **Bonfanti L**, Di Cunto F, Buffo A. Citron-kinase deletion unveils inherent molecular and functional heterogeneity in dorsal and ventral oligodendrocyte precursor cells of the mouse forebrain.
(In collaboration with NICO group "Pathophysiology of neural stem cells")

7. Future directions and objectives for next years

Please describe the following information relevant to the research that you are planning to do – Character limit is mandatory. Please highlight the added value of collaborations within the NICO where applicable.

a. Summary (up to 2000 characters):

In the previous years, and relative publications, our lab has contributed to set several new and alternative angles of the AN process: the molecular control of neuronal-glial switch in neurogenic sites, the activation of quiescent (neurogenic) astroglial progenitors in the lesioned striatum, the contribute of AN in some aspects of reproductive behavior and the phylogenetic approach to non-newly generated "immature" neuronal populations in mammals. In the next years, we will focus on an in depth analysis of the molecular/cellular mechanisms regulating adult NSC function in both physiological and pathological conditions, the identification of brain circuits in which AN integrates with different internal and external sensory cues to accomplish behavior, and a further characterization of the immature neuron "reservoirs" in widely different mammals, including humans.

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

After 30 years of research in AN, the interest of the scientific community on "classic" AN is progressively decreasing, also in relation to data coming from human brain tissue. On the other hand, different aspects are emerging related to "new nuances" or "theme variations" of AN (e.g., the neuronal-glial switch at the progenitor level or its integration with various brain functions and systems at the behavioral level) as well as to the discovery of other forms of plasticity (e.g. immature neurons), raising new problems, opportunities and questions, such as:

A) how is regulated the fate of adult neural stem/progenitor cells in physiological or pathological conditions?
It is of paramount importance to get insight into the mechanisms regulating the neuronal vs. glial switch in different conditions and brain regions (physiological and pathological). This is particularly promising also if/when/where a few (quiescent) progenitors are available (e.g., the adult human brain).

B) how different types of plasticity (AN versus "immature" neurons) are phylogenetically distributed among mammals?
It is now clear that different forms of brain plasticity, including AN and immature neurons, are differently present/distributed/active in different mammals. To get a picture of such heterogeneity in a high number of mammalian species and orders, including humans (and identify possible phylogenetic trends) is mandatory for correct translation of results and to identify new targets for therapeutic/preventive approaches.

C) how the external and internal cues integrate with AN to sustain behaviors essential for survival (reproduction)?
The study of adult neurogenic niches still offers the opportunity to understand how the brain integrates different sensory modalities and hormonal cues to optimize behaviors.

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

Only by knowing the multifaceted roles of AN and other forms of plasticity in brain homeostasis and dysregulation we could expect to use this biological process for translational purposes (novel therapeutic approaches for neurodegenerative diseases and preventive approaches for optimal brain function/plasticity in healthy adults and in aging; both goals ultimately in line with the NICO Mission). To understand how the brain adapt to different environmental stimulations during life (from young to old individuals) is fundamental to figure out preventive strategies. In particular, to find and modulate new sources of undifferentiated neurons or

new ways to drive quiescent (neuronal and glial) progenitors might be pivotal in translating results in large-brained species (e.g., humans) with reduced amount and/or different types of plasticity.

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

- *Mitochondrial alterations in hippocampal progenitors and their progeny*. Based on evidences suggesting that i) COUP-TFI controls adult NSC activity, ii) adult NSC behaviour relies on mitochondria, and iii) COUP-TFI regulates mitochondria functions, we aim to prove the link between COUP-TFI and mitochondrial function/dynamics in the adult hippocampal neurogenic niche. In order to do so, we will take advantage of multiple COUP-TFI mutant mouse models to perform thorough analyses of changes in adult NSC activity/lineage and mitochondria. In particular, alteration in mitochondria morphology and function will be evaluated by high resolution confocal microscopy, advanced imaging techniques *in vivo* (i.e. a new implementation of two-photon functional live imaging for studying mitochondrial dynamics within the adult DG neurogenic niche) and *in vitro* (mitochondria functional assays).

- *Role of GnRH cells located in the olfactory bulb in modulating the structural and functional plasticity of circuits involved in mating behavior*. To address this aim we will use in vivo two-photon imaging combined with functional ablation of GnRH-cells selectively in the OB by using Cre-dependent adeno-associated viral expression of inhibitory DREADDs or Caspase3, in the GnRH-Cre mouse line. In the context of this study a new multidisciplinary research line involving optical engineers and ethologists has been created in the last year. Major aims of the new research line are: i) determine the brain areas integrating salient olfactory and acoustic cues important for mating behavior; ii) develop a new advanced optical technology for in vivo imaging of neural circuits in freely-moving animals (living in the wild) during mating behavior. The project in now being evaluated in the second step of the Human Frontier Research Grant.

- *Mechanisms and role of astrocyte neurogenic activation.* Our data suggests that specific molecular players differentially regulate the acquisition of astrocytes neurogenic competence and its expression. In order to investigate the nature of these factors, in collaboration with Annalisa Buffo we will collect whole striatum and single cell astrocytes RNAseq data in the presence or absence of SOX2 during the early acquisition of neurogenic competence and its subsequent expression. In addition, a ChipSeq of SOX2 binding sites will be used to more specifically define SOX2 regulated pathways and genetic programs. In parallel, to establish the role of lesion induced neurogenesis we will define the identity of newborn neurons through cell RNAseq and we will analyze the anatomical and behavioral effects of neurogenesis ablation either by the SOX2 conditional deletion or in Nestin-TK mice. Particular attention will be devoted to the reorganization of the connections of striatal, cortico-striatal, cortico-thalamic, thalamo-striatal and thalamo-cortical neurons.

- *Characterization and quantification of immature neurons in different mammals*. By using the same method employed for cortical immature neurons in 12 mammalian species, molecular, cellular, quantitative analyses will be performed in the amygdala, claustrum and external capsule, namely the subcortical regions in which these cells are expected to be present, especially in gyrencephalic mammals. *Modulation of cortical immature neurons in the sheep neocortex*: 15 brains from young sheep kept in different environmental conditions for 7 weeks (enriched environment, stress (isolation), and control group) will be analyzed for DCX+ neuron quantification, expression of markers of maturity/immaturity, and Sholl analysis. *Search for cortical immature neurons in humans*: analysis of adult human tissue (NIH, USA) and fetal brains at different gestational stages (hospital S. Anna, Turin). The overall objective is to complete the study on the possible heterogeneity of "immature" neurons across mammals and start to explore their modulation in neocortex of large-brained species.

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

In our research group, different aspects or brain structural plasticity, spanning from classic AN to "immature" neurons, and involving progenitor specification, hormone-linked behavior, lesion-induced repair and "young" neuron reservoir, are addressed. A combination of basic and innovative technical approaches will be employed to study at the molecular, cellular and functional levels different types of plasticity occurring in different brain regions of different mammalian species. We think that such approach from molecule to behavior in a comparative vision could widen our view of brain plasticity, with the aim to figure out a correct translation of research data in animal models to humans. The identification of mechanisms underlying the neuronal-glial switch in both neurogenic and non-neurogenic sites remains a crucial point to be addressed with the aim of modulating endogenous progenitors. The study of "immature" neurons, as well as the use of imaging technology in wild-living mice, are novel topics directly addressed, at present, by a few laboratories in the world. In addition, we are searching for a promising neuronal population abundantly present in large-brained mammals characterized by reduced rates of AN (with particular reference to humans).
We think that such kind of approach, other than opening new insights in basic neurobiology, will help to overcome the current bottleneck of "classic" AN (intended as a constitutive, continuous genesis of new neurons in rodents) vision, by exploring the alternative (less-travelled) roads mentioned above.

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

Different innovative technologies are being developed to tackle the aims of our projects:
- A customized approach is being developed to standardize and automate the production of serial section reconstructions through hierarchical imaging at the confocal microscope in order to obtain 3D high-resolution reconstructions of large volumes. A block face imaging of the specimen during sectioning is used as reference for non-linear registration of the confocally acquired volumes to their original position in the intact brain. A preliminary version of this method has been used to reconstruct the distribution of GnRH+ cells in the entire brain, the composition of neurogenic niches in the lesioned striatum, the morphology of the newly generated neurons and the distribution of their afferents. This technique might be useful in the study of immature neuron populations and their possible modulation.
- To address the functional role of adult-generated olfactory neurons in reproductive behavior and understand whether exposure to specific pheromones recruit the activation of specific olfactory circuits we combine in vivo two-photon microscopy with fluorescent reporters of cell activity in head restrained anesthetized and awake mice. Two-photon imaging is also used to in vivo study mitochondrial dynamics in neurogenic regions.
- In case of positive outcome of Human Frontier Research grant, and in collaboration with Dr. S. Gigan (Laboratoire Kastler-Brossel Sorbonne Université, Paris) and Dr. D. Penn (Konrad Lorenz Institute of Ethology, Vienna), we will develop a high-throughput imaging technology based on multimodal optical fibers (integrated in a Wire-Free head-mounted device) to record the functional activity from multiple brain regions, with single unit resolution, low invasiveness and in freely-moving animals. This technique will be used to image simultaneously the regions involved in mating behavior primarily focusing on olfactory-related areas.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: **Physiopathology of stem cells**

3. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Annalisa Buffo

Degree: PhD | Birthdate: 25-12-1967

Nationality: Italian | Gender: F

Phone: 00 39 011 6706614

Email: annalisa.buffo@unito.it

Position: Associate Professor of Physiology

Personnel

1 Daniela Carulli (on leave of absence since 2015)

Degree: PhD | Birthdate: 17/04/1973

Nationality: Italian | Gender: F

Phone: 00 39 011 6706614

Email: daniela.carulli@unito.it

Position: Assistant Professor

Role & expertise: Extracellular matrix, perineuronal nets, on leave of absence from August 2015

2 Enrica Boda

Degree: PhD | Birthdate: 08/05/1981

Nationality: Italian | Gender: F

Phone: 00 39 011 6706615

Email: enrica.boda@unito.it

Position: Assistant Professor in tenure track

(RTD-B)

Role & expertise: Lead responsible of research on oligodendroglial physiopathology, expert in oligodendrocyte neurobiology

3 Roberta Parolisi

Degree: PhD | Birthdate: 23/01/1985

Nationality: Italian | Gender: F

Phone: 00 39 011 6706632

Email: roberta.parolisi@unito.it

Position: PostDoc

Role & expertise: Responsible of electron microscopy investigations and surgical procedures, expert in microglia-oligodendrocyte crosstalk and myelin ultrastructure

4 Giulia Nato

Degree: PhD Birthdate: 08/05/1986

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: giulia.nato@unito.it

Position: PostDoc

Role & expertise: Responsible of research on astrocyte neurogenic activation; expert in striatal lesion and astrocyte reactivity

5 Valentina Cerrato

Degree: PhD Birthdate: 21/07/1988

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: valentina.cerrato@unito.it

Position: PostDoc

Role & expertise: Responsible of research on astrocyte heterogeneity and cerebellar development; expert in clonal analyses

6 Martina Lorenzati

Degree: Master Program in Medical Biotechnology Birthdate: 30/10/1992

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: martina.lorenzati@unito.it

Position: PhD student co-supervised with AV

Role & expertise: expert in oligodendroglia biology, in vitro assays and protein analyses

4. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2019-2021	D11G19000270007 Mirage - Oligodendrocyte Precursor Cells for Myelin Repair and Gliomagenesis	Annalisa Buffo	Bando Ex-Post 2018 of the University of Turin and Compagnia di San Paolo, Turin	PI	56,840 €	4547 €
2020-2024	NSC-Reconstruct Novel Strategies for Cell-based Neural	Annalisa Buffo	H2020-SC1-BHC-2018-2020	PI of research unit, WP coordinator	680,000 €	54400 €

	Reconstructio n #874758					
2020-2022	Allele-specific siRNAs as therapeutic option for ADLD: in vitro pre-clinical validation on unique human experimental models	Annalisa Buffo	ELA Foundation	Coordinator	200,000 €	16000 €
2019	Attivazione neurogenica degli astrociti	Annalisa Buffo	Local Funds of the Department of Neuroscience (University of Turin)	PI	2,900 €	232 €
2019	Scientific Meeting Grant EA184	Annalisa Buffo	Company of Biologists	Proponent	2,000 £	--
2019	Unveiling oligodendrocyte precursor heterogeneity in CNS physiology and pathology	Enrica Boda	Local Funds of the Department of Neuroscience (University of Turin)	PI	2.471,48 €	197.72 €
2019	Scientific Meeting Grant EA107	Enrica Boda	Company of Biologists	Proponent	2,000 £	--
2019	European Society of Neurochemistry (ESN) Initiative Funding	Enrica Boda	European Society of Neurochemistry (ESN)	Proponent	1,000 €	--
2019	Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in neuroinflammation and demyelination. 2019/PR-Multi/003	Enrica Boda	Fondazione Italiana Sclerosi Multipla (FISM)	PI	30,000 € (20,000 € to our unit)	1,000 €
2020	Uncovering the unfolding of mouse and human astrocyte lineages through high	Valentina Cerrato	IBRO-PERC InEurope Short Stay Grant	Proponent	3,000 €	--

	throughput RNA sequencing in the cerebellum					
2019	Meeting Grant EA132	Valentina Cerrato	Company of Biologists	Proponent	1,500 £	--
2019	EBBS Sponsored International Lecturer Grant	Valentina Cerrato	EBBS	Proponent	700 €	--
2019	IBRO-PERC Worksh ops, Conferences and Meetings Grant	Valentina Cerrato	IBRO-PERC	Proponent	4,000 €	--
2020-2021	Inquinamento da particolato: ruolo nella sindrome da morte in culla	Roberta Parolisi	Banca d'Italia 24268E	Proponent	24,268 € PENDING	--

5. SCIENTIFIC ACTIVITIES IN 2019

Annalisa Buffo, PI

Supervised PhD students: Martina Lorenzati, co-supervised with Alessandro Vercelli

Honors, prizes, awards: na

Outreach activities

- International collaborations: Neural stem cell (NSC) reconstruct network (E Cattaneo, University of Milano, M Parmar, University of Lund, M Gotz, University of Muenchen); Laura López-Mascaraque (Inst Cajal, Madrid); Steve Goldman (University of Rochester); see also below for lab members
- Invited talks: - *Experimental results in cerebellar reserve - from autophagy to motor training*, SRCA Symposium, 16-17 May 2019, Sheffield, UK;
  - *Stem cell derived human striatal progenitors innervate striatal targets and alleviate sensorimotor deficit in a rat model of Huntington Disease*, NECTAR 2019, 28-29 November 2019 Cardiff, UK
- Science communication: - *Il neurone immortale*, Unistemday, March 15, 2019, Torino
  - Unistemtour pink edition, TIM Inclusion Week, October 23, 2019
  - *Tra Geni ed Esperienza*, Pint of Science, May 21, 2019, Torino
  - Participating in *Il neurone immortale*, Bardotto, October 29, 2019, Torino
  - Collaborating in setting up the exhibition UOMO VIRTUALE, May-October 2019, Torino
- Editorial duties: - Topic Editor: Pharmacology of neurogenesis, Current Opinion in Pharmacology;
  - Topic Editor: Engineering Adult Neurogenesis and Gliogenesis, Frontiers in Neuroscience;
  - Editorial board member of Scientific reports;
  - *Ad hoc* reviewer for the following journals: The Journal of Neuroscience, Glia, Frontiers in Neuroscience, Elife, The Cerebellum, Journal of Cell Science, PlosBiology, Trends in Molecular Medicine, Cancer letters, Molecular and Cellular Neuroscience.

• Others	- Member of the task force for Ataxia (Society for Research on the Cerebellum and Ataxias); member of the scientific committee of the Interdepartmental Center for Clinical and Experimental Pharmacology (University of Turin). - Member of the Evaluation Jury of the National Research Infrastructure, ANR, France, 25-29 June 2019, Paris. - Reviewer for the following Agencies/Charities: French National Research Agency (ANR), FISM (Federazione Italiana Sclerosi Multipla), Action for A-T (UK).
Organizational activities and responsibilities at NICO:	- Deputy Director of NICO - CEO of S&P Brain - Organization of Scuola lavoro at NICO - Responsible of BLS2 labs at NICO - Coordinator for NICO of the Student Exchange Agreement with the Paris Descartes University (established in 2019)
Speakers invited:	- prof. Mehrnaz JAFARIAN-TEHRANI, Université Paris Descartes, France - dr. Alan Perotti, ISI Foundation, Turin, Italy
Other organizational activities:	UNISTEM TOUR Torino; UNISTEM Day Torino, together with L. Bonfanti (since this year) (http://users2.unimi.it/unistem/)
Workshops, Schools or Conferences organized:	INTERNATIONAL WORKSHOP ON: GLIAL CELLS-NEURONS CROSS TALK IN HEALTH AND DISEASE (with V. Cerrato and E. Boda, see below), February 2020, Torino
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Enrica Boda, lead responsible of research on oligodendroglial physiopathology

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Prof. Brian Harding (Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, USA), Prof. Stephanie Bielas and Dr. Amanda Moccia (Dept. of Human Genetics, University of Michigan, Ann Arbor, MI, USA)
• Invited talks:	- *Strategies to support neural progenitor survival and maturation during CNS development: a lesson from the microcephalic Cit-K KO mouse model.* November 22 2019, 39th Meeting of the Italian Society of Pharmacology (SIF), Florence, Italy; - *Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model.* September 1st 2019, 23rd ESN (European Society of Neurochemistry) Meeting 2019, Milan, Italy; - *Heterogeneity of the response to DNA damage in oligodendroglia populations: a functional study in vivo and in vitro.* May 24 2019, Workshop "Advanced microscopy techniques for biomedical applications", Dept. of Clinical and Biological Sciences, Orbassano (Turin), Italy (host: Prof. Saverio Retta)
• Science communication:	- *Il neurone scoperto*, Bardotto, November 14 2019, Torino - Collaboration in setting up the exhibition UOMO

VIRTUALE https://home.infn.it/it/comunicazione/mostre-e-installazioni/mostre/3478-uomo-virtuale-corpo-mente-cyborg, May-October 2019, Torino
- Participation in NICO Open Days and Alternanza Scuola-Lavoro

- Editorial duties:
- Guest Editor for the Special Issue "Glial Cells in CNS Pathology and Repair", Journal of Clinical Medicine, MDPI (https://www.mdpi.com/journal/jcm/special_issues/Glial_Cell_CNS).
- Review Editor for Frontiers in Cellular Neuroscience – Section Non-neuronal cells.
- Ad-hoc reviewer for Front Neurosci, Mechanisms of Ageing and Development, Plos One, Biochemical Pharmacology, Neurochemical Research, Purinergic Signalling, International Journal of Molecular Sciences, BMC Molecular Biology, Theriogenology (Animal Reproduction), Cells, Experimental and Molecular Pathology.

- Others:

Organizational activities and responsabilities at NICO:
- Responsible for the Histology Lab at NICO;
- Responsible of the Neurolucida system II;
- Organization of the Progress Report seminar series at NICO.

Speakers invited:
- Dr. Giovanni Ferrara, Dipartimento di Neuroscienze, riabilitazione, oftalmologia, genetica e scienze materno-infantili (DINOGMI), Università di Genova;
- Prof. Stephanie Bielas, Dept. of Human Genetics, University of Michigan, Ann Arbor, MI, USA

Other organizational activities: na

Workshops, Schools or Conferences organized:
- Member of the Organizing and Scientific Committee of the BraYn (*Brainstorming Research Assembly for Young Neuroscientists)* Conference, November 14-16 2019, Milan, Italy. https://www.braynconference.com/
- Workshop GLIAL CELLS-NEURON CROSSTALK IN CNS HEALTH AND DISEASE (with V. Cerrato and A. Buffo), February 27-29 2020, Torino. http://www.nico.ottolenghi.unito.it/Agenda/GLIAL-CELLS-NEURON-CROSSTALK-IN-CNS-HEALTH-AND-DISEASE

Technology transfer achievements (patents, etc.): na

Roberta Parolisi, PostDoc, expert in microglia-oligodendrocyte crosstalk and myelin ultrastructure

Supervised PhD students: na

Honors, prizes, awards: na

Outreach activities

- International collaborations:
- Claudia Verderio, CNR Milan and Pierre Gressens, Inserm, France
- Prof. Marie-Ève Tremblay, Université Laval Axe Neurosciences, CRCHU de Québec, Canada.
- Invited talks: na
- Science communication: Participation in NICO Open Days and Alternanza Scuola-Lavoro
- Editorial duties: Ad hoc reviewer for Neurochemical Research, Journal of the Neurological Sciences and Frontiers Aging Neuroscience.

- Others:

Organizational activities and responsabilities at NICO:	- in charge of the maintenance of one of the confocal microscopes (Nikon CS1) hosted at NICO microscopy facility and of the Nikon Eclipse 80i + VICO image acquisition system - responsible of the 'electron microscopy lab' at NICO
Speakers invited:	na
Other organizational activities:	Organizing member of the "Beautiful mind" sessions for the national festival 'Pint of Science' (science communication). Turin. (11-13 May 2019)
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Giulia Nato, PostDoc responsible of research on astrocyte neurogenic activation under the joint supervision of AB and FL

Supervised PhD students:	na
Honors, prizes, awards:	"Assegno di ricerca''one year long, Bando Ex-Post 2018 of the University of Turin and Compagnia di San Paolo for the internationalization of research - Year 2020

Outreach activities

• International collaborations:	Benedikt Berninger, King's College, London, UK; Philip Greulich, Mathematical Sciences, University of Southampton, UK.
• Invited talks:	na
• Science communication:	- Dissemination activity at the "Liceo scientifico E Bérard", Aosta, January 2019 - Participation in NICO Open Days and Alternanza Scuola-Lavoro
• Editorial duties:	na
• Others:	

Organizational activities and responsibilities at NICO:	Assistance for the confocal microscopy Leica SP5 use and maintenance
Speakers invited:	Dr. Alessia Caramello, Francis Crick Institute, London, UK.
Other organizational activities:	
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Valentina Cerrato, PostDoc responsible of research on astrocyte heterogeneity and cerebellar development

Supervised PhD students:	na
Honors, prizes, awards:	- Fellowship granted by Fondazione Umberto Veronesi, Milan, Italy for the project *Uncovering the unfolding of mouse and human astrocyte lineages through high throughput RNA sequencing in the cerebellum* (for 2020). - SINS Travel Grant Award to attend the XVIII Congress of the Italian Society for Neuroscience (SINS), granted by the SINS society.

	- "Elena Benaduce" price awarded as best research project dedicated to the quality of life, in the frame of the 8th edition of the national science communication price "GiovedìScienza". - IBRO stipend to attend the XIV European Meeting on Glial Cells in Health and Disease, Porto, July 10-13, 2019.
Outreach activities	
• International collaborations:	Prof. Laura Lopez Mascaraque (Cajal Institute, Madrid, Spain); Prof. Ludovic Telley (University of Lausanne, Switzerland); Prof. Magdalena Götz (Ludwig-Maximilians-Universität München, Germany)
• Invited talks:	- *The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning*. 2nd Brayn Congress, Milan 14-16, 2019. - *The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning*. XVIII National Congress of the Italian Society for Neuroscience, Perugia 26-29, 2019. - *In vivo clonal analyses to study the ontogenesis of cerebellar astrocytes: from confocal microscopy, to automatic segmentation and 3D reconstruction tools*, May 24 2019, Workshop "Advanced microscopy techniques for biomedical applications", Dept. of Clinical and Biological Sciences, Orbassano (Turin), Italy (host: Prof. Saverio Retta)
• Science communication:	Participation in NICO Open Days and Alternanza Scuola-Lavoro
• Editorial duties:	Ad hoc reviewer for International Journal of Developmental Neuroscience and Neurochemical Research.
• Others	
Organizational activities and responsibilities at NICO:	- Responsible of the ZEISS Axio Scan.Z1 use at NICO - Responsible of the Neurolucida system II
Speakers invited:	Prof Ludovic Telley, Dept. Of Fundamental Neuroscience, University of Lausanne, Switzerland
Other organizational activities:	na
Workshops, Schools or Conferences organized:	- Symposium on *Astrocyte heterogeneity: from development to functional implications*. XVIII National Congress of the Italian Society for Neuroscience, Perugia 26-29, 2019 (https://www.sinsmeeting.com/program/) - Workshop GLIAL CELLS-NEURON CROSSTALK IN CNS HEALTH AND DISEASE (with E. Boda and A. Buffo), February 27-29 2020, Torino. http://www.nico.ottolenghi.unito.it/Agenda/GLIAL-CELLS-NEURON-CROSSTALK-IN-CNS-HEALTH-AND-DISEASE
Technology transfer achievements (patents, etc.):	na

Martina Lorenzati, PhD student under the joint supervision of AB and AV

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	

- International collaborations: na
- Invited talks: *Axo-glial interplay in oligodendrocyte specification and myelination: role of JNK1*. 2nd Brayn Congress, Milan 14-16, 2019.
- Science communication: - Sguardi sulla bellezza – Saluzzo (with the contribution of Fondazione CRC), October 29-30th 2019; invited speaker with the talk *Aletheia tra scienza e arte*
  - Participation in NICO Open Days and Alternanza Scuola-Lavoro
- Editorial duties: na
- Others:

Organizational activities and responsibilities at NICO: Co-responsible of Neurolucida system I at NICO.

Speakers invited: na

Other organizational activities: Organizing member of the "Beautiful mind" sessions for the national festival 'Pint of Science' (science communication). Turin. (11-13 May 2019)

Workshops, Schools or Conferences organized: na

Technology transfer achievements (patents, etc.): na

4. Research activity in 2019

a. Summary (500 characters)

In addition to established research lines on astrocyte (AS) and oligodendrocyte (OL) heterogeneity in development and disease, and on cell replacement, our group provided crucial functional evidence in two collaborative studies, published in prestigious journals. In the first, we disclosed the role of microglial-derived extracellular vesicles (EVs) in remyelination. In the second, we showed that allele-specific silencing reverted OL pathological readouts typical of ADLD leukodystrophy.

b. Background and rationale (3000 characters)

In 2019 we have put our main focus on the investigation of the contribution of glia to CNS physiopathology. In several pathologies such as Multiple Sclerosis (MS), oligodendrocyte progenitor cells (OPCs) are not able to support efficient myelin regeneration. Moreover, OL are themselves the main target of genetic diseases such as ADLD (autosomal dominant adult-onset demyelinating leukodystrophy), where duplication of one of the two LMNB1 alleles leads to myelin degeneration. Thus, there is need to identify new ways to foster the capability of OPCs to progress toward maturation and regenerate myelin in the diseased CNS, as well as to define strategies to rescue OL dysfunctions. To meet these needs, we worked to disclose both intrinsic and extrinsic factors affecting OPC maturation and myelin deposition, and participated in the development of an allele-specific silencing strategy to treat diseased OL.

In regard to AS lineages, mechanisms of astrocyte specification and plasticity are poorly understood. On the one hand, the disclosure that an ontogenetic program, tightly regulated in space and time, determines AS heterogeneity in the cerebellum (Cerrato et al., PLoS Biol 2018), prompted new questions on how mechanistically such diversity is achieved, and about lineage relationships between distinct AS types and cerebellar neurons. It further led to ask whether the same mechanisms and lineages occur in rodent and human samples, and stimulated efforts to develop new analytic tools and adopt cutting-edge technologies such as single cell RNA sequencing (scRNAseq), the most appropriate method to address all these issues in one shot. On the other hand, the spontaneous neurogenic activation of adult mouse striatal AS upon excitotoxic (quinolinc acid mediated, QA) lesion (Nato et al., Development 2015) offered a unique model to explore

molecular pathways driving quiescent AS toward a neural stem cell state, with potential proreparative and neuroregenerative actions. Understanding such transition is important to develop strategies to modulate AS reactivity and exploit glial cells for the treatment of CNS pathologies.

In parallel, we have continued the work to develop therapies for neurodegenerative diseases based on adaptive cell replacement and promotion of circuit plasticity. No disease modifying therapies are currently available for pathologies such as Huntington's disease (HD) and such approaches will not solve the problem of dead or severely dysfunctional neurons. Strategies aimed at replacing lost neurons with human embryonic stem cell (hESC) derivatives may represent promising options. However, replacement of lost neurons may be challenged by the low receptiveness for the full integration of grafted cells of the degenerating nervous tissue. We investigated the therapeutic effects of transplantation in a rat model of early HD stages where QA induces the degeneration of striatal neurons.

c. Objectives (1000 characters)

We have investigated the neurobiology of glial cells and devised strategies for cell replacement with the ultimate goal to identify cellular and molecular targets to promote repair in acute and chronic neurodegenerative pathologies. During this last year we specifically aimed to:
(a) identify intrinsic and extrinsic factors promoting OPC maturation, remyelination and, more in general, affecting OPC response to damage;
(b) define strategies to rescue diseased oligodendroglial phenotypes;
(c) further disclose how AS heterogeneity is achieved developmentally;
(d) understand mechanisms leading to the neurogenic activation of striatal parenchymal AS;
(e) explore cell replacement strategies based on hESC derivatives and promotion of circuit plasticity.

d. Results (4000 characters)

(a) We found that in lysolecithin induced demyelinated lesions in the mouse corpus callosum, EVs produced by proinflammatory microglia blocked remyelination, whereas EVs produced by microglia cocultured with immunosuppressive mesenchymal stem cells promoted OPC recruitment and myelin repair. In vitro assays implicated AS in mediating EV-induced remyelination failure. (Coll. with Prof C Verderio, CNR, Milano; Proff M Fumagalli and MP Abbracchio, Univ of Milano; Prof. A Uccelli, Univ of Genova). doi: 10.1007/s00401-019-02049-1

We also discovered that high levels of miR-125a-3p impaired OPC maturation in demyelinated lesions, whereas its silencing accelerated remyelination. Transcriptome analysis suggested that these effects are mediated by direct interaction of miR-125a-3p with Slc8a3, a Na^{+}/Ca^{++} membrane transporter expressed in OL, and Gas7, a neuronal protein so-far involved in axonal growth. (Marangon D, **Boda E, Parolisi R**, .., *Montarolo F, Perga S, Bertolotto A*, **Buffo A**, Abbracchio MP, Lecca D., under revision, Glia)

Finally, we showed that the developmental origin of OPCs impacts on their response to damage. Ablation of Citron-kinase (Cit-K), leading to accumulation of DNA damage, disrupts OPC fate resulting in cell death or senescence in dorsal and ventral cell subsets, respectively. This divergence correlates with differential anti-oxidant responses to DNA lesions in dorsal and ventral OPCs. Depending on their developmental origin, wild-type OPC subsets also show a diverse vulnerability to DNA damage. (**Boda E, Lorenzati M, Parolisi R**, ., *Pallavicini GM, Bonfanti L,.., Di Cunto F*, **Buffo A**., under rev, Nat Communication)

(b) We validated allele-specific silencing by RNA interference as an effective therapeutic approach for ADLD. This strategy reduced LMNB1 levels and reverted ADLD-specific phenotypes in relevant cellular models (murine OL overexpressing human LMNB1 and neurons directly reprogrammed from patients' fibroblasts). (Coll. with Prof. A Brusco, Univ of Torino; Prof. E Cattaneo, Univ of Milano, Prof. L Conti, Univ of Trento). doi: 10.1093/brain/awz139

(c) Toward a better understanding of the emergence of cerebellar AS heterogeneity, we re-analysed available single-cell data from the murine cerebellum with Prof S Benso (Politecnico, Torino). We successfully approached the analytic pipeline but data were limited in the number of sampled AS. Thus, we set out to obtain our own data and started a collaboration with Prof. L Telley (Univ of Lausanne). Further, we extended the analyses to human fetal cerebellar samples (Coll with Prof L Marozio, F Borella, Sant'Anna Hosp, Univ of Torino).

Prompted by AS clonal studies, with Prof. F Molinari (Politecnico, Torino) we implemented an automated method for the segmentation of fluorophore-labeled cells. doi: 10.1016/j.jneumeth.2019.108348.

(d) In regard to the interrelation between AS and neural stem cells and regulation of AS reactivity, results indicate that the stem cell-related transcription factor SOX2 is necessary for the acquisition of the neurogenic competence in striatal AS, but is dispensable for the subsequent competence expression. SOX2 also affects the quality of AS reactivity. Further work addressed the AS neurogenic lineage progression and the connectivity/phenotype of the generated neuroblasts (Adult Neurogenesis g.'s report). (Coll with Prof. F Luzzati, NICO; Prof S Nicolis, Univ of Milano).

(e) In replacement studies we found that hESC-derived striatal progenitors grafted into the striatum of a rat model of HD (QA lesion) mature into medium spiny neurons (MSN) and interneurons, extend neurites to striatal targets, receive intrastriatal inputs and improve sensorymotor reflex responses and, though to a lesser extent, more complex motor tasks (Besusso D*, *Schellino R**, *Boido M*., **Parolisi R**.., *Vercelli A*, **Buffo A**# and Cattaneo E#. Stem cell .., under rev, Stem Cell Report).
Italic, NICO collaborators; * cofirst,# colast authors

e. Advancement in the field (1000 characters)

Among published papers, the following had the major impact:
(i) Evidence that microglial EVs act in vivo as multimodal and multitarget signaling mediators affecting myelin repair and inhibiting myelin regeneration through the regulation of AS reactivity.
(ii) The first proof of principle that an ASP-RNAi strategy can treat diseases caused by gene duplications, thus opening new therapeutic opportunities for several pathological conditions linked to gene copy number gains.
(iii) FAST (Fluorescent cell Analysis Segmentation Tool) is the first fully automated method for the segmentation of stained cells and tissues labeled by multicolor combinations of fluorophores.

f. Publications

Lombardi M, **Parolisi R**, Scaroni F, Bonfanti E, Gualerzi A, Gabrielli M, Kerlero de Rosbo N, Uccelli A, Giussani P, Viani P, Garlanda C, Abbracchio MP, Chaabane L, **Buffo A**, Fumagalli M, Verderio C. (2019) Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure Acta Neuropathol. doi: 10.1007/s00401-019-02049-1.

Giorgio E, **Lorenzati M**, Rivetti di Val Cervo P, Brussino A, Cernigoj M, Della Sala E, Bartoletti Stella A, Ferrero M, Caiazzo M, Capellari S, Cortelli P, Conti L, Cattaneo E, **Buffo A**, Brusco A (2019) Allele-specific silencing as treatment for gene duplication disorders: proof-of-principle in autosomal dominant leukodystrophy. Brain. 2019 Jul 1;142(7):1905-1920. doi: 10.1093/brain/awz139.

Salvi M, **Cerrato V**, **Buffo A**, Molinari F (2019) Automated segmentation of brain cells for clonal analyses in fluorescence microscopy images J Neurosci Methods. 325:108348. doi: 10.1016/j.jneumeth.2019.108348.

Becerra-González M, Varman Durairaj R, Ostos Valverde A, Gualda EJ, Loza-Alvarez P, Portillo Martínez W, Berenice Gómez-González G, **Buffo A**, Martínez-Torres A (2019) Response to Hypoxic Preconditioning of Glial Cells from the Roof of the Fourth Ventricle. Neuroscience. pii: S0306-4522(19)30660-8. doi: 10.1016/j.neuroscience.2019.09.015.

Mancini C, Hoxha E, Iommarini L, Brussino A, Richter U, Montarolo F, Cagnoli C, **Parolisi R**, Gondor Morosini DI, Nicolò V, Maltecca F, Muratori L, Ronchi G, Geuna S, Arnaboldi F, Donetti E, Giorgio E, Cavalieri S, Di Gregorio E, Pozzi E, Ferrero M, Riberi E, Casari G, Altruda F, Turco E, Gasparre G, Battersby

BJ, Porcelli AM, Ferrero E, Brusco A, Tempia F. (2019) Mice harbouring a SCA28 patient mutation in AFG3L2 develop late-onset ataxia associated with enhanced mitochondrial proteotoxicity. Neurobiol Dis. 2019 Apr;124:14-28. doi: 10.1016/j.nbd.2018.10.018.

Cendelin J, **Buffo A**, Hirai H, Magrassi L, Mitoma H, Sherrard R, Vozeh F, Manto M (2019) Task Force Paper On Cerebellar Transplantation: Are We Ready to Treat Cerebellar Disorders with Cell Therapy? Cerebellum. 2019 Jun;18(3):575-592. doi: 10.1007/s12311-018-0999-1.

Boda E (2019) Myelin and oligodendrocyte lineage cell dysfunctions: New players in the etiology and treatment of depression and stress-related disorders. Eur J Neurosci. 2019 Nov 17. doi: 10.1111/ejn.14621.

Cerrato V and **Buffo A**, Gliogenesis. In: Gruol D, Koibuchi N, Manto M, Schmahmann JD, Sillitoe RV, editors. Handbook of the Cerebellum and Cerebellar Disorders. Springer, Cham. 2019 Feb 18 doi: 10.1007/978-3-319-97911-3_108-1.

Boda E, Rigamonti AR, Bollati V. Understanding the effects of air pollution on neurogenesis and gliogenesis in the growing and adult brain. Current Opinion in Pharmacology, *accepted*.

Mitoma H, **Buffo A**, Gelfo F, Guell X, Fucà E, Kakei S, Lee J, Manto M, Petrosini L, Shaikh AG, Schmahmann JD. Consensus paper. Cerebellar reserve: from cerebellar physiology to cerebellar disorders. Cerebellum, *accepted.*

7. Future directions and objectives for next years

a. Summary (up to 2000 characters):

Our research will remain focused on the role of glia in CNS physiopathology and cell replacement strategies.

In OL studies, we will build on results showing a distinct vulnerability of dorsal and ventral cells in response to DNA damage, extend the investigation on the underlying molecular mechanisms in both Cit-K KO and WT OPCs, and link such mechanism to conditions of OPC maturation failure such as the aged nervous tissue and chronic demyelinated lesions. With this, we aim to identify intrinsic and extrinsic factors limiting the OPC maturation potential and possibly targetable for preclinical intervention.

The investigation of AS heterogeneity will proceed through single-cell/-nuclei RNA sequencing of cerebellar cells, so to describe the unfolding of AS lineages in both mouse and human cerebella, disclose the mechanistic milestones driving distinct trajectories, and highlight features of each AS type specific to its interplay with defined neuronal subsets. Based on this knowledge we aim at uncovering new AS functions in cerebellar circuit formation and activity, and prospectively obtain cerebellar neurons from reprogrammed astrocytes and human pluripotent stem cells (hPSC).

In regard to cell replacement studies, we will work to develop strategies to replace striatal neurons by transplantation and in situ cell reprogramming, and to foster functional circuit reconstruction in rodent models of HD. We will first address (i) the promotion of human striatal progenitor graft survival, maturation and integration through the stimulation of circuit plasticity via behavioral training and (ii) employ chemogenetics to provide formal evidence of the implication of transplants in behavioral amelioration upon grafting. In parallel, the study of the spontaneous neurogenic activation and reactivity of adult mouse striatal AS will define core candidate factors to facilitate AS reprogramming into neurons or states supportive for repair and circuit remodeling.

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

Fundamental issues on glia biology are poorly understood: (i) mechanisms mediating OPC vulnerability to insults and regulating full differentiation into myelinating OLs; (ii) specification of AS types, their relationship with neural stem cells and crosstalk with neurons. Yet, these are most promising matters to unveil how glia contributes to CNS physiopathology and may promote brain repair.
In the more translational context of neurodegenerative pathologies, while disease modifying therapies are emerging, these will not solve the problem of dead or severely dysfunctional neurons. Thus, innovative therapies based on cell replacement, reprogramming and circuit reconstruction have the potential to transform how we treat a wide range of neurological diseases.
We will address these issues as follows:

- In CNS aging and MS, OL suffer from DNA damage and undergo apoptosis and cell senescence, which in turn contribute to diminished remyelination. We recently found that, depending on their developmental origin, dorsal and ventral OPCs respond to DNA damage with apoptosis or cell senescence, respectively (Boda et al., under review). In Cit-K KO mouse mutants, this is related to a differential ability to counteract oxidative stress depending on distinct levels of NRF2, a master regulator of cell detoxifying functions. Whether this or other mechanisms explain also WT dorsal/ventral OPC divergent responses remains to be clarified. Further, mechanistic aspects underlying OPC senescence and contributing to their functional decline in aging or pathology are still obscure. Both cell intrinsic and environmental factors may be implicated. Among these latter, the senescence-associated secretory profile (SASP) can "spread" aging-related dysfunctions in a paracrine manner by targeting crucial signal cascades in surrounding progenitors. However, active components in OPC SASP remain to be dissected.
- AS comprise extremely heterogeneous types. We have unveiled fundamental cellular mechanisms implicated in the generation of the diversity of cerebellar AS. However, much remains to be understood on the molecular actors of AS types specification. For instance, knowledge on implicated transcription factors is very limited. Conversely, it appears that environmental signals may be crucial factors for the induction of defined AS types. However, how such extrinsic cues are translated within the cells in a permanent phenotype remains to be understood. Additionally, the molecular identity of distinct AS progenitor and the genetic determinants of fate decisions or of the specification of each AS type are still unknown. Moreover, while common lineages are known for AS and interneurons, whether they occur for other cerebellar neurons needs to be elucidated. To tackle these issues, we will employ scRNAseq on mouse and human cerebella to characterize cellular heterogeneity and state transitions.
- Technological advancements offer the opportunity to devise regenerative treatments based on hPSC and reprogramming that will have the potential to reach out to many patients. In this context we will continue working on cell replacement approaches in a rat model of early HD based on QA lesions, where striatal MSN and striatal circuits are mostly affected. Work conducted so far shows a good maturation of hESC-derived striatal progenitors, local connections and behavioral improvements especially in spontaneous responses (Besusso,Schellino, under review). However, there is need to improve graft composition and circuit reconstruction with consequent functional impact.

An alternative strategy to support cell replacement is in situ reprogramming of AS. Approaches to reprogram AS into MSN currently await development. Yet, striatal adult mouse AS can spontaneously undergo a neurogenic activation upon QA lesion and produce neurons with so far not-well-defined phenotype. Understanding such activation is important to design successful strategies for AS reprogramming and, possibly, control AS reactivity.

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

In 2019 we will work toward these main general aims:

- understanding glial/glial progenitor heterogeneity and physiology at the molecular, cellular and functional levels and clarifying how such features impact on CNS pathophysiology in order to exploit adult glia and their progenitors as therapeutic actors to treat disease;
- developing cell replacement approaches focused on the substitution of functional striatal circuits.

The contribution of our group will be to: (i) deliver innovative evidence and expand knowledge on fundamental processes of neural progenitor/glial cell physiopathology. Knowledge on these processes may lead to identify mechanisms to be fostered or manipulated in view of proposing preclinical therapeutic approaches for CNS

diseases; (ii) contribute to pave the way for future CNS cell replacement therapies using functionally enhanced cells.

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

We will pursue the following aims:

- Characterization of the molecular mechanisms underlying OPC heterogeneity and maturation blockade in pathology Based on evidence of distinct vulnerability to injury of dorsal and ventral OPCs, we will first ask whether, as for Cit-K KO OPCs, a differential cell-intrinsic ability to buffer oxidative stress is at the basis of WT OPC divergent responses to DNA damage (cisplatin-induced). Through in vitro assays and biomolecular/biochemical analyses we will therefore monitor levels of intracellular/mitochondrial ROS, expression and activity of the NRF2 pathway, glycolytic vs. respiratory capacity, mitochondrial integrity and functionality. Should WT OPC heterogeneity not rely on these mechanisms, alternative factors will be explored, such as differential activation/modulation of p53 or GSK3b activity induced by DNA damage. We will also expand our investigation to the study of the mechanistic aspects of OPC senescence, with a special focus on (possibly druggable) SASP components. We will test in vitro if paracrine factors secreted by senescent OPCs promote senescence in healthy OPCs and block their maturation. If confirmed, we will investigate the secretome of senescent OPCs (by proteomic and lipidomic analyses) and compare its content with factors expressed in the diseased tissue, such as MS murine models (EAE) and human MS plaques. By this, we will identify candidates factors whose targeting in vivo/in vitro with pharmacological treatments may abrogate senescence and help stimulating OPC function and remyelination. Finally, we will assess whether the postnatal and/or prenatal administration of N-acetylcysteine, an FDA-approved glutathione precursor, is able to correct Cit-K KO mouse neuroanatomical and behavioral phenotypes, and extend their life span. Collab. with Prof. F Di Cunto and Dr. A Bertolotto (NICO)

- Identification of the molecular determinants over the generation and differentiation of distinct astrocyte lineages in mouse and human cerebella. ScRNAseq will be applied on embryonic and postnatal AS isolated from hGFAPGFP mice (Lausanne) and human fetal cerebellar samples (nuclei or cells, under validation, Torino). Analyses based on R software package (Seurat) and machine learning algorithm will integrate our, already published data, and data on neuronal cells from L Telley'lab. First aims of this analysis will be to i) describe the unfolding of AS lineages and understand their contiguity with defined cerebellar neuronal lineages; ii) disclose the transcriptional programs driving the progression of the various lineages and the transcription factors governing key cell fate switches or transitional stages; iii) identify AS machineries potentially implicated in cerebellar circuit physiology. Relevant targets will be confirmed histologically and the role of key candidate fate/transition determinants validated through pharmacological or genetic GoF/LoF approaches. Moreover, the identification of molecular milestones in the unfolding of AS lineages and in the gliogenic switch of putative multipotent progenitors will help to drive cerebellar AS towards neuronal fates.
(Coll: Prof L Telley, Univ Lausanne; Prof L Marozio, Dr F Borella, Univ of Turin; Prof S Lodato, Humanitas, Milano).

- Development of strategies to replace lost striatal neurons. Firstly, we will seek to enhance graft (G) survival, maturation and function through long-term (up to 6 months) housing in enriched environment (EE), known to increase trophic factor production and circuit plasticity. Besides high resolution histological analyses, behavioral tests, and virus-based afferent tracing, we will apply RNAseq on both the G and host tissue to disclose the full impact of EE. We will further test the functional role of the G through the transplantation of engineered cells amenable to chemogenetic regulation. Then, to enhance G functions we will test composite G with optimized cell composition (representing the human striatum), apply ad hoc physical training or stimulate graft activity chemogenetically. Over time, we will also test reprogramming of striatal AS toward striatal neuronal fates. This aim will benefit of studies on the mechanisms driving the neurogenic activation of striatal AS. By applying genome-wide surveys of gene regulation (RNAseq of bulk QA lesioned striatal tissue, AS scRNAseq, SOX2 ChipSeq) in the presence/absence of SOX2, we will define core transcription factor networks and predict candidate factors suitable to foster AS reprogramming into striatal neurons or cell states supportive for repair and circuit remodeling.

(Coll: Prof F Luzzati, NICO; Proff A Vercelli, M Boido, NICO; Prof. E Cattaneo, Univ of Milano; Prof. S Nicolis, Univ M Bicocca; Prof S Lodato, Humanitas, Milano; Prof M Parmar and T Bjorklund, Univ Lund).

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

Several of the addressed questions (eg identifying molecular substrates of OL diversity in health and disease, understanding the emergence of AS heterogeneity, its functional impact and mechanisms regulating AS neurogenic competence and reactivity) are fundamental questions essentially unanswered. Our studies will therefore provide unique insight to these evolving fields.
The generation of human neuronal types with clinically relevant functionality through either the transplantation of composite grafts or in situ spontaneous/induced reprogramming confers unique originality to this translational experimental activity.
Our approaches (eg bioinformatic approaches, gene expression analyses on select cell populations and on human cells, viral-mediated gene expression analyses, development of training protocols to human cell graft to favor function restoration) represent cutting edge techniques whose integration confers methodological originality to our studies.
Developed mutant mouse lines constitute unique experimental models, and focus on the cerebellum provides a specific advantage in the field of astrocyte diversity (which, at difference with other mouse CNS areas, is well established for this territory), reprogramming and hPSC-derivatives (very poorly explored so far).

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

We will adopt state of the art technologies and analytic tools but as for the next year we do not envisage to develop groundbreaking innovative technologies.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name:**Embryonic neurogenesis**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Ferdinando Di Cunto

Degree: MD, PhD — Birthdate: 20/12/1969

Nationality: Italian — Gender: M

Phone: 011 6706616

Email: ferdinando.dicunto@unito.it

Personnel

1 Gianmarco Pallavicini

Degree: MoS in Molecular Biotechnology — Birthdate: 10/10/1991

Nationality: Italian — Gender: M

Phone: 011 6706616

Email: gianmarco.pallavicin@edu.unito.it

Position: Graduate Student

Role & expertise: Molecular and cellular biology, analysis of genetically modified mouse models.

2 Giorgia Iegiani

Degree: Bacelor degree in Biotechnology — Birthdate: 17/04/1996

Nationality: Italian — Gender: F

Phone: 011 6706616

Email: giorgia.iegiani@edu.unito.it

Position: Master student

Role & expertise: Molecular and cellular biology, analysis of genetically modified mouse models.

3 Giada Onorato

Degree: MoS in Biology — Birthdate: 29/01/1993

Nationality: Italian — Gender: F

Phone: 011 6706616

Email: giadaonorato93@gmail.com

Position: Graduate Student

Role & expertise: Use of the genetically tractable model C. elegans

2. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
01/01/2020 31/12/2024	National	PI	AIRC	Validation of Citron kinase as a therapeutic target for	855000	(8%)

				medulloblastoma.		
01/01/2020 31/12/2022	National	PI	Fondazione CRT	Implementazione di modelli cellulari e genetici per la validazione di varianti genomiche associate a patologie neurodegenerative.	35000	(8%)
01/06/2017 31/12/2019	International	PI	Fondation Jerome Lejeune	Identification and initial validation of new possible treatments for intellectual disability in Down syndrome through drug repositioning.	40000	10%

3. SCIENTIFIC ACTIVITIES IN 2019

Name, Role (PI)
Supervised PhD students: Gianmarco Pallavicini
Honors, prizes, awards: NA
Outreach activities

- International collaborations:
  - 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

- Editorial duties:

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

- Organizational activities and responsibilities at NICO:

Speakers invited: Giovanni Petri (ISI) Brain functional shapes: how topology characterizes healthy and altered brain function (14/06/2019)
Other organizational activities: Data management.
Workshops, Schools or Conferences organized: NA
Technology transfer achievements (patents, etc.): NA
ALL LAB MEMBERS

Activities: Gianmarco Pallavicini, selected speaker, ABCD 2019 National Congress.

4. Research activity in 2019

a. Summary (500 characters)

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.

b. Background and rationale (3000 characters)

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. 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.

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.

c. Objectives (1000 characters)

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. CITK as a possible target for cancer therapy.

d. Results (4000 characters)

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. We have addressed the possibility that the function of CITK may be essential for proliferation in medulloblastomas, devastating brain tumors of the infancy that urgently require the development of new therapies. To do so, we have produced a conditional model for deleting CITK in medulloblastomas arising in mutant mice. We have also addressed whether the discoveries which we have published for Medulloblastoma may apply to more prevalent brain tumors, such as glioblastomas, and may increase the radiosensitivity of both tumor types.

e. Advancement in the field (1000 characters)

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

f. Publications

1: Chiotto AMA, Migliorero M, Pallavicini G, Bianchi FT, Gai M, Di Cunto F, Berto GE. Neuronal Cell-Intrinsic Defects in Mouse Models of Down Syndrome. Front Neurosci. 2019 Oct 10;13:1081. doi: 10.3389/fnins.2019.01081. eCollection 2019. PubMed PMID: 31649502; PubMed Central PMCID: PMC6795679.

2: Grasso S, Cangelosi D, Chapelle J, Alzona M, Centonze G, Lamolinara A, Salemme V, Angelini C, Morellato A, Saglietto A, Bianchi FT, Cabodi S, Salaroglio IC, Fusella F, Ognibene M, Iezzi M, Pezzolo A, Poli V, Di Cunto F, Eva A, Riganti C, Varesio L, Turco E, Defilippi P. The SRCIN1/p140Cap adaptor protein negatively regulates the aggressiveness of neuroblastoma. Cell Death Differ. 2019 Jul 8. doi: 10.1038/s41418-019-0386-6. [Epub ahead of print] Erratum in: Cell Death Differ. 2019 Sep 5;:. PubMed PMID: 31285546.

3: Pallavicini G, Berto GE, Di Cunto F. Precision Revisited: Targeting Microcephaly Kinases in Brain Tumors. Int J Mol Sci. 2019 Apr 28;20(9). pii: E2098. doi: 10.3390/ijms20092098. Review. PubMed PMID: 31035417; PubMed Central PMCID: PMC6539168.

4: Tran THY, Yang DW, Kim M, Lee DH, Gai M, Di Cunto F, Choi KW, Lim DS. Citron kinase interacts with LATS2 and inhibits its activity by occluding its hydrophobic phosphorylation motif. J Mol Cell Biol. 2019 Mar 13. pii: mjz013. doi: 10.1093/jmcb/mjz013. [Epub ahead of print] PubMed PMID: 30865227.

7. Future directions and objectives for next years

Please describe the following information relevant to the research that you are planning to do – Character limit is mandatory. Please highlight the added value of collaborations within the NICO where applicable.

a. Summary (up to 2000 characters):

During the next three years, we plan 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 address the role of CITK in brain tumors and in their radiosensitivity. In particular, if the AIRC grant proposal will be funded, we plan to concentrate on the development of specific CITK inhibitors.
3. 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.
4. We will strengthen our efforts to increase the collaborations between NICO and clinical researchers of the Department of Neuroscience. Specifically, we are working on the implementation at NICO of the genetically tractable model C. elegans, which will be of invaluable help in addressing the biological significance of mutations identified in a clinical setting, in patients affected by neurodevelopmental and neurodegenerative disorders.

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

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 druggable targets, which may potentially affect many different forms of ID. Even more importantly, the same methods may lead to the indication that some drugs, already in clinical usage for other disorders, have the potential of being useful for ID treatment. The latter approach, commonly referred to as 'drug repositioning', is especially interesting

because it does not require the huge financial resources necessary to perform phase-one and phase-two clinical trials on new molecules and would therefore allow to move directly from pre-clinical models to patients. We have previously developed a novel SB-inspired method, based on the identification of Anticoexpressed Gene Clusters (CAGCs), to obtain strong drug repositioning hypotheses for rare genetic diseases.

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

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

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

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

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

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

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

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

FONDAZIONE
CAVALIERI OTTOLENGHI

NICO
Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: Neuropsychopharmacology

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Carola Eugenia Eva

Degree: PhD Birthdate: 21/07/1957

Nationality: Italian Gender: Female

Phone: :+390116706608

Email: carola.eva@unito.it

Personnel

1 Alessandra Oberto

Degree: PhD Birthdate: 24/10/1967

Nationality: Italian Gender: Female

Phone: :+390116706611

Email: alessandra.oberto@unito.it

Position: Research associate

Role & expertise: Biotechnology, behavioral analysis, immunohistochemistry

2 Ilaria Bertocchi

Degree: PhD Birthdate: 13/04/1982

Nationality: Italian Gender: Female

Phone: +390116706632

Email: ilaria.bertocchi@unito.it

Position: Research contract

Role & expertise: Behavioral analysis, immunohistochemistry, biotechnology

2. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2020-2022	Nuove prospettive terapeutiche nel trattamento della sindrome dell'X-fragile	Carola Eva	Fondazione CRT	PI	**50000**	8%

3. SCIENTIFIC ACTIVITIES IN 2019

Carola Eva, PI

Supervised PhD students: Mattia Ghigo

Honors, prizes, awards: na

Outreach activities

- International collaborations:

-Martyn Goulding, Professor and Chair, Molecular Neurobiology Lab of The Salk Institute La Jolla, CA 92037, US. Role of spinal NPY1R neurons in mechanical itch.

- Mark Shlomchik, MD, PhD, UPMC Endowed and Distinguished Professor of Immunology Chair, Department of Immunology, University of Pittsburgh School of Medicine. 'Role of Npy1r gene expression in germinal center (GC) B cells'

- Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University 'Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

-William Wisden, Imperial College London Dept Life Sciences Lab of Molecular Neuroscience 'Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

- Invited talks:

-'Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor' 45th workshop on 'Sex differences, dimorphisms, divergences: impact on brain and behavior in health and disease'. Invited lecture, Erice, Sicily, May 20-25, 2019.

- Science communication:

Acton D, Ren X, Di Costanzo S, Dalet A, Bourane S, Bertocchi I, Eva C, Goulding M. *Spinal NPY1R+ Neurons form an Essential Excitatory Pathway for Mechanical Itch.* Cell Reports, Volume 28, Issue 3, 16 July 2019, Pages 625-639.e6

- Editorial duties:

-Nature Communications manuscript NCOMMS-18-34111A

-European J Neurosciences

Organizational activities and responsibilities at NICO: In charge for hygiene anti-smoke rules

Speakers invited: Dr. Prabahan Chakraborty (30/05/2019)

Other organizational activities: Founding member and President of the spinoff S&P BRAIN

Workshops, Schools or Conferences organized:

Informative meeting of scientific disclosure entitled 'Fragile X syndrome: research and therapy' (02/12/2019, Clinical surgery Morino room, Molinette Hospital) in collaboration with 'Associazione del Piemonte Sindrome X Fragile' and seminars hold by Prof. Chiurazzi (Medical genetic Institute of Catholic University 'Sacro Cuore' and geneticist at Gemelli Policlinico, Rome), Prof. Vitiello (Professor and Director of Child and Adolescent Neuropsychiatry, University of Turin, Regina Margherita Hospital) and Prof.ssa Carola Eva and Dott.ssa Ilaria Bertocchi, NICO)

Technology transfer achievements (patents, etc.):

S&P BRAIN 2019 activities

- Execution of the assisted IMP³rove Assessment compliant with the European standardisation documents (CEN TS 16555-1 and the CEN Workshop Agreement CWA 15899).

- ISO 9001:2015 second audit January 2019

- BioFIT participation, Marsiglia December 10-11, founded by F.E.S.R. 2014/2020, Thematic objective III.3 - Promoting the competitiveness of SMEs).

- Enterprise Europe Network registration

- Bioindustry Park association
- Business partnership with the CRO Accelera (Nerviano, MI)

Alessandra Oberto, Research Associate

Supervised PhD students: Mattia Ghigo

Honors, prizes, awards: na

Outreach activities

- International collaborations:

-William Wisden, Imperial College London Dept Life Sciences Laboratory of Molecular Neuroscience 'Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

- Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University 'Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

-Mazahir T. Hasan, PhD Ikerbasque Professor Laboratory of Memory Circuits Achucarro Basque Center for Neuroscience 'NMDA receptors and fear memory engrams'

- Mark Shlomchik, MD, PhD, UPMC Endowed and Distinguished Professor of Immunology Chair, Department of Immunology, University of Pittsburgh School of Medicine. 'Role of Npy1r gene expression in germinal center (GC) B cells'

- Invited talks: na
- Science communication:na
- Editorial duties: na
- others

Organizational activities and responsibilities at NICO: In charge for behavioral labs I and II (animal facility)

Speakers invited:na

Other organizational activities: na

Workshops, Schools or Conferences organized: na

Technology transfer achievements (patents, etc.): na

Ilaria Bertocchi, Research contract

Supervised PhD students: Mattia Ghigo

Honors, prizes, awards: na

Outreach activities

- International collaborations:

- Mazahir T. Hasan, PhD Ikerbasque Professor Laboratory of Memory Circuits Achucarro Basque Center for Neuroscience 'NMDA receptors and fear memory engrams'

-José María Delgado García Division de Neurociencias Universidad Pablo de Olavide Sevilla-41013, España (Spain) 'NMDA receptors and fear memory engrams'

-Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University ''Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

- Valery Grinevich, Head, Neuropeptide Research in Psychiatry Zentralinstitut für Seelische Gesundheit (ZI) J 5 68159 Mannheim, Germany 'NMDA receptors and fear memory engrams'

-William Wisden, Imperial College London Dept Life Sciences Laboratory of Molecular Neuroscience 'Treating GRIN2A-related epileptic encephalopathies: a preclinical study'

-Martyn Goulding, Professor and Chair, Molecular Neurobiology Lab of The Salk Institute La Jolla, CA 92037, US. Role of spinal NPY1R neurons in mechanical itch.

- Mark Shlomchik, MD, PhD, UPMC Endowed and Distinguished Professor of Immunology Chair, Department of Immunology, University of Pittsburgh School of Medicine. 'Role of Npy1r gene expression in germinal center (GC) B cells'

- Invited talks:

-'Limbic inactivation of NPY-Y1 receptors increases vulnerability to stress disorders and diet-induced obesity in male mice' EATING DISORDERS AND SUBSTANCE ABUSE, The impact of stressful lifestyles on brain functioning. Organized by Delegates of the PhD Students in Neuroscience. Invited lecture. Torino, 1st of March 2019

-‘Influenza dell'ambiente sul segnale NPY-Y1R e plasticità neuronale’. Brainstorm EMSA (European medical student’s association). Invited lecture. Torino, 7 Marzo 2019

-‘Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor’ 45th workshop on ‘Sex differences, dimorphisms, divergences: impact on brain and behavior in health and disease’. Invited lecture, Erice, Sicily, May 20-25, 2019

-‘Extracellular matrix and cellular interactions in neuroplasticity and memory’ 7th Mediterranenan Neuroscience Conference (MNS), Marrakech, Morocco, June 23-27 2019

- Science communication:

- Hasan MT, Althammer F, Silva da Gouveia M, Goyon S, Eliava M, Lefevre A, Kerspern D, Schimmer J, Raftogianni A, Wahis J, Knobloch-Bollmann HS, Tang Y, Liu X, Jain A, Chavant V, Goumon Y, Weislogel JM, Hurlemann R, Herpertz SC, Pitzer C, Darbon P, Dogbevia GK, Bertocchi I, Larkum ME, Sprengel R, Bading H, Charlet A, Grinevich V. Fear Memory Engram and its Plasticity in the Hypothalamic Oxytocin System. Neuron, DOI: 10.1016/j.neuron. 2019.04.029 (2019)
- Acton D, Ren X, Di Costanzo S, Dalet A, Bourane S, Bertocchi I, Eva C, Goulding M. Spinal NPY1R+ Neurons form an Essential Excitatory Pathway for Mechanical Itch. Cell Reports, Volume 28, Issue 3, 16 July 2019, Pages 625-639.e6

- Editorial duties: na
- others
- Organizational activities and responsibilities at NICO: In charge for behavioral labs I and II (animal facility) and BSL2 Cell Culture and Surgery Lab
- Speakers invited:na
- Other organizational activities: ‘The Science Bridge’ advisory board member (https://thesciencebridge.org/)
- Workshops, Schools or Conferences organized: Informative meeting of scientific disclosure entitled ‘Fragile X syndrome: research and therapy’ (02/12/2019, Clinical surgery Morino room, Molinette Hospital) in collaboration with ‘Associazione del Piemonte Sindrome X Fragile’ and seminars hold by Prof. Chiurazzi (Medical genetic Institute of Catholic University ‘Sacro Cuore’ and geneticist at Gemelli Policlinico, Rome), Prof. Vitiello (Professor and Director of Child and Adolescent Neuropsychiatry, University of Turin, Regina Margherita Hospital) and Prof.ssa Carola Eva and Dott.ssa Ilaria Bertocchi, NICO)
- Technology transfer achievements (patents, etc.): na

4. Research activity in 2019

a. Summary(500)

We used conditional mutant mice to investigate the role of NPY-Y1R in: 1) sex-related differences in brain control of energy homeostasis; 2) susceptibility to Metabolic Syndrome; 3) changes in plasticity and cognitive functions. 4) We elaborated data to understand the effects of the loss of the activity-controlled NMDA receptor Ca2+ signaling in Grin2A (N596S) mice. 5) We analyzed data related to a project aimed in better elucidating the role of NMDAR and circuit dynamics in fear memory engrams.

b. Background and rationale (3000 characters)

Neuropeptide Y (NPY) is one of the most abundant neuropeptide within the CNS that regulates several functions among which feeding behaviour and metabolism, cognition, anxiety and stress responses. We have previously generated a conditional knockout mouse model ($Npy1r^{rfb}$ mice), in which the Npy1r gene was specifically inactivated in forebrain principal neurons starting from juvenile age (Bertocchi et al., 2011).

1) Although sex-related differences play a central role in animal physiology and behavior, the vast majority of rodent researchers continue to use exclusively males in their studies, and information on sex differences in these regards is sparse. We conducted a sex-specific analysis of the above-cited model to explore how sex may differentially influence the physiology, behavior and the limbic expression of important neuropeptides in conditional knockout mice.
2) We investigated whether limbic Npy1r represents a key target gene through which estrogens in the brain modulate energy metabolism in relation to reproductive functions.

3) NPY is expressed in GABAergic neurons and acts as a homeostatic regulator of cortical and hippocampal excitatory neurotransmission in particular via Y1R, we asked therefore whether NPY-Y1R signaling affects cognitive functions through modulation of perineuronal nets (PNNs), extracellular matrix structures that form around some types of neurons at the end of critical periods, limiting neuronal plasticity.
4) To dissect the mechanisms underlying NMDA receptor coincidence detection, we analyzed the effects of the point mutation Grin2a(N615S) in gene-targeted mice, analogous to a *de novo* mutation found in a patient affected by early onset epileptic encephalopathy. We are actually working on a manuscript to publish the mouse model and we to use it in next studies because GluN2A(N615S) mice may represent the first valuable murine model for GRIN-associated early onset epileptic encephalopathies and one of the best existing model to elucidate the mechanisms underlying SUDEP, the most common epilepsy-related death (see future directions section).
5) Building fear memories is hypothesized to operate by involving different brain regions in a process called systems consolidation. However, the underlying mechanism of systems consolidation that facilitates recruitment of fear circuits for memory formation and retrieval is not known. It is also not known in what order and which circuit pathway(s) are recruited for the sequential printing of fear memory engrams across the brain regions for their dynamic retrieval. With advanced genetic tools, we interrogated basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) by combinatorially blocking synaptic plasticity and synaptic output and optogenetically activating cell assemblies tagged during cued fear learning.

c. Objectives

To uncover sex-related differences in control and Npy1r^{rfb} mice we evaluated differences in: i) hypotalamic Npy1r gene inactivation pattern ii) body weight, WAT weight and hormone serum levels in male and female Npy1r^{rfb} mice iii) hypothalamic NPY, CRH, POMC and AgRP ir iv) behavior. To unravel the role of NPY-Y1R signalling in metabolic challenges and in metabolic syndrome we analyzed physiological parameters and behavior in males and sham and ovariectomized female control and Npy1r^{rfb} mice, fed either with high fat diet or standard diet. To examine whether selective ablation of Npyr in forebrain excitatory neurons affects cognitive functions and PNN expression we analyzed in *Npy1r^{rfb}* and control mice i) spatial memory performance and PNN distribution and appearance in the hippocampus ii) working memory and executive performance and PNN distribution and appearance in the prefrontal cortex.

d. Results (4000 characters)

1) We demonstrated that *Npy1r^{rfb}* mice show a sex dimorphic phenotype, revealing the existence of NPY-Y1R neuronal subpopulations involved in sex-related differences of metabolic and behavioural functions (Eva, Bertocchi, Oberto, Longo, Sex-dependent regulation of hypothalamic neuropeptide Y-Y1 receptor, Special Issue of Neuroscience & Biobehavioral Reviews, due January 31st 2020; Bertocchi et al., submitted).
2) We demonstrated the existence of an estrogen dependent relay necessary to ensure the maintenance of the homeostasis also in case of Y1R malfunctioning in female, but not in male brains (Oberto et al., in preparation).
3) We demonstrated that the lower expression levels of Npy1r in the limbic system in adult mice, induced by low levels of maternal care or by postnatal inactivation of Npy1r in excitatory neurons in Npy1r^{rfb} mice, is coupled with a deficit of prefrontal cortex driven cognitive abilities in the puzzle box test, a problem-solving test with increasing difficulty, that is associated with a different distribution of PNN around PV+ neurons in the PFC (Bertocchi et al., in preparation). Moreover, we demonstrate that the targeted inactivation of Npy1r gene in forebrain principal neurons impairs spatial learning and significantly increases the number of pyramidal cells expressing c-Fos, an established marker for neural activity, and the thickness of PNNs coating around CA1 neurons of the dorsal hippocampus. Enzymatic ChABC-mediated disruption of the PNNs in the CA1, achieved by stereotaxic local delivery, reestablishes neuronal activity homeostasis in Npy1r^{rfb} mice, thereby restoring learning performance (Bertocchi and Mele et al., under revision J.Neurosci).
4) In collaboration with Prof. Sprengel (University of Heidelberg, Germany) we are currently working on the preparation of a manuscript where we describe the effects observed in gene targeted mice with Mg2+ block attenuated GluN2A-type NMDARs. These point mutated mice exhibit a complex phenotype characterized by mild muscular weakness, strong sensitivity to audiogenic seizures and cognitive deficits, likely as a result of attentional impairments. This phenotype reflects an overexcitation in the midbrain/brainstem circuitry and, surprisingly, an inhibition of the hippocampal formation (Bertocchi et al, in preparation).

5) We found that both presynaptic mPFC and postsynaptic LA/BLA N-methyl-D-aspartate receptors (NMDARs) are needed for fear memory formation. Our results show that NMDAR-dependent synaptic plasticity involving both presynaptic and postsynaptic NMDARs of interacting brain circuits, especially those located on the mPFC presynaptic compartments and LA/BLA postsynaptic compartments, respectively, facilitates sequential printing of cued-fear memory engrams from LA/BLA to mPFC and, subsequently, to the other brain regions. (SfN 2019. Chicago, IL, 19-23 October 2019. Bertocchi I, Hasan MT et al (2019). Poster session). We are currently analyzing the expression of different rAAVs in brain slices of new groups of animals needed to confirm our previous results and to assess new hypotheses (Bertocchi et al., in preparation for Neuron).

e. Advancement in the field (1000 characters)

Our work, together with others, suggest that in addition to feeding, Y1R mediates other metabolic effects of NPY related to energy homeostasis, including the control of energy expenditure and lipid metabolism. In particular, besides interacting with corticosteroids and other hypothalamic peptides, NPY is also highly connected and regulated by estrogens, and this interaction may explain the differences existing in this signalling system and in related physiological regulation between sexes. Our research regarding sex-dimorphic pathophysiological mechanisms in stress and anxiety and comorbid homeostasis-related disease could contribute to more personalized therapies in the future and more awareness in terms of sex-specific risk factors. In addition, our data reveal a previously unknown interplay between limbic NPY-Y1R signaling and PNNs, two key regulators of neuronal plasticity. Our findings may have major relevance for all those neuropathologies associated with altered E/I balance.

f. Publications

- Hasan MT, Althammer F, Silva da Gouveia M, Goyon S, Eliava M, Lefevre A, Kerspern D, Schimmer J, Raftogianni A, Wahis J, Knobloch-Bollmann HS, Tang Y, Liu X, Jain A, Chavant V, Goumon Y, Weislogel JM, Hurlemann R, Herpertz SC, Pitzer C, Darbon P, Dogbevia GK, Bertocchi I, Larkum ME, Sprengel R, Bading H, Charlet A, Grinevich V. Fear Memory Engram and its Plasticity in the Hypothalamic Oxytocin System. Neuron, DOI: 10.1016/j.neuron. 2019.04.029 (2019).

- Acton D, Ren X, Di Costanzo S, Dalet A, Bourane S, Bertocchi I, Eva C, Goulding M. Spinal NPY1R+ Neurons form an Essential Excitatory Pathway for Mechanical Itch. Cell Reports, Volume 28, Issue 3, 16 July 2019, Pages 625-639.e6

7. Future directions and objectives for next years

a. Summary (up to 2000 characters):

Neurodevelopmental disorders are characterized by multi-level differences (from genes to phenotype and response to pharmacological treatments) among individuals. Alterations in the fine equilibrium between triggers and brakes of plasticity during critical periods may cause mistimed trajectories of brain development that may contribute to neuropathology. A better understanding of the molecular mechanisms necessary in maintaining the balance between excitation and inhibition (E/I) required for normal neural circuit function can lead to the individuation of a common and innovative therapeutic target.

At this purpose, the main objects of our research in the next years will be: 1) to investigate a possible molecular target for the development of innovative therapies for fragile X syndrome (FXS), a rare genetic disorder of the autistic spectrum associated with intellectual disability (*New therapeutic perspectives in the treatment of fragile X syndrome)*; 2) to use a new genetic murine model which allows to dissect the different molecular and environmental factors contributing to epilepsy and cognitive dysfunctions (*Treating GRIN2A-related epileptic encephalopathies: a preclinical study)* 3) to examine the expression of perineuronal nets in an environmentally induced rat model of autism spectrum disorders (ASD) (*Role of neuronal plasticity inhibitors in a environmentally induced rat model of ASD*).

There is a great need to undergo functional assessment in model systems to understand how genes interact with genetic and epigenetic factors in an individual to result either in a severe form of impairment or in milder syndromes, or even in lack of symptoms. Our models allows to selectively modify background genetic and environmental factors to interrogate their interaction and to evaluate different treatments at different developmental stages.

a. Background and Significance (up to 4000 characters):

1) *New therapeutic perspectives in the treatment of fragile X syndrome.*
FXS is the main monogenic cause of hereditary mental retardation and the second cause of intellectual disability on a genetic basis, caused by the silencing of the FMR1 gene or, more rarely, by its point mutations/deletions. To date, there is no approved therapy for FXS and only drugs that partially mitigate the symptoms are used. In FXS, anomalies of the E/I equilibrium could underlie cortical response deficits, such as reduced ability to adapt, amplified cortical responses to sensory stimuli, EEG abnormalities and defects in cortical network synchronization and communication. It was recently shown that, in the murine FXS model, maladaptation of the response to acoustic stimuli is associated to elevated levels of type 9 matrix metalloproteinases (MMP-9) and a consequent reduction in PNN formation around the parvalbumine (PV+) interneurons of the auditory cortex and that the deletion of MMP-9 reverts the phenotypes FXS-associated in these mice. Given that PNNs play a key role in the early stages of brain development, they could constitute a promising target, totally innovative and potentially effective, for new FXS therapies more accessible, less invasive, and with less undesirable effects.
2) *Treating GRIN2A-related epileptic encephalopathies: a preclinical study.*
Early onset epileptic encephalopathies (EOEE) comprise a large, heterogeneous group of devastating epileptic disorders mainly characterized by pharmaco-resistant polymorphous epilepsy, severe EEG abnormalities, and developmental regression. Recently, several mutations have been identified in GRIN2A, encoding the GluN2A subunit of the NMDA receptor, in children suffering from EOEE with different mental and neurological disorders. This proposal aims to use gene-targeted mice expressing the GluN2A(N615S) mutation, analogous to a *de novo* mutation found in a patient affected by EOEE, to achieve a genetic and pharmaceutical rescue of the severe epileptic phenotype and perhaps some of the cognitive disabilities associated with the disease. GluN2A(N615S) mutants are strongly prone to audiogenic generalized convulsive seizures leading to respiratory arrest and exhibit cognitive deficits and behavioral endophenotypes associated with ADHD, representing the first valuable mouse model of GRIN-related EOEE. The validation of the model with further physiological and molecular analysis might be a precious tool to detect specific brain areas and new molecular targets for network-based drug discovery, and also to get increase knowledge about the mechanisms underlying epilepsy and the most common related death: sudden unexpected death in epilepsy (SUDEP).
3) *Role of neuronal plasticity inhibitors in a environmentally induced rat model of ASD*
Although the genetic bases of ASD are well documented, the recent increase in clinical cases of idiopathic ASD indicates that several environmental risk factors could play a role in ASD etiology. Among these, maternal exposure to psychosocial stressors during pregnancy has also been hypothesized to affect the risk of ASD in offspring. In addition, it has been demonstrated that parents with psychiatric disorders and women exposed to childhood/adolescent abuse experiences were more likely to have sons with ASD. The offspring of socially isolated (SI-O) rats displayed a behavior with many analogies to core diagnostic symptoms of ASD in children and in genetic mouse models of autism (Pisu et al., 2019). Common pathophysiological features of different neurodiseases, including ASD, are consistent with a removal of "brakes" on plasticity, such as altered E/I balance, myelin deficits or PNNs loss. The formation process of plasticity brakes during development may be altered in SI-O rats and may therefore be associated with the ASD-like phenotype and may also constitute an important difference between sexes.

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

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

Our projects well integrate with the mission since their intentions are all aimed in gaining more knowledge about genetic and environmental-induced disorders affecting young children with severe neurological impairments and potential risk of premature mortality, including sudden death. Thus, there is a great need to search for new treatments. However, this process faces considerable challenges.

c. Specific objectives and strategies (up to 4000 characters)

1) *New therapeutic perspectives in the treatment of fragile X syndrome*

The main objectives are:

- To study the development and functioning of brain PNNs in a mouse model of FXS, to expand the knowledge about the correlation between phenotype-genotype associated with the mutation
- Use the model to identify potentially useful drugs to prevent and correct functional deficits in children and adults with FXS, autism or other neurodevelopmental disorders
- Identify critical periods in brain circuit development when drug therapy can be effective

In particular, we will analyze the correlation between phenotype-genotype in Fmr1ko mice through the implementation of behavioral studies and the process of PNN formation at different times of postnatal development in brain nuclei important for cognitive and emotional functions. Based on these results, we will then analyze the effectiveness of different therapeutic approaches in various postnatal development periods on the same behavioral and molecular parameters, using drugs currently in use or undergoing clinical trials. For the choice of drugs and the interpretation of the results, we will collaborate with Prof. B. Vitiello, Child and Adolescent Neuropsychiatry, University of Turin. Furthermore, i) M Cambiaghi (University of Verona) will study the effect of the same treatments on *in vivo* EEG in Fmr1ko mice and ii) D. Carulli (Institute of Neurosciences, Amsterdam) will study the role of a different biochemical composition of networks on the FXS phenotype.

Treating GRIN2A-related epileptic encephalopathies: a preclinical study.

The main objectives are:

- validation of the animal model of GRIN2A-associated EOEE with a better analysis of phenotype-genotype correlation
- individuation of specific brain areas and new molecular targets
- assessment of different therapeutic approaches

These objectives will be develop thanks to the use of behavioral, electrophysiological, molecular, and epigenetic tools. Given that a hallmark of GRIN2A-related disorders is severe impairment in auditory language comprehension and speech (epilepsy–aphasia spectrum disorders), we will detect ultrasonic vocalizations (USVs) and assess different social behaviors, together with an analysis of myelin and perineuronal nets formation in the forebrain regions involved in the above cited functions. It is of relevance to analyze the cardiac functionality of these mutants, to understand if the expressed mutation in this system could be the main responsible of sudden death. The analysis of cardiac and sympathetic parameters will be done in collaboration with Prof. Palanza Lab. To study how nutritional regimen can affect gene expression, in particular, ketogenic diet, that demonstrated utility in EOEE and other epileptic syndromes, we will collaborate with Prof. Monti, University of Bologna, expert in epigenetic analysis. We will perform *in vivo* EEG recording as a tool to characterize epilepsy and behavior (in collaboration with Dott. Cambiaghi, University of Verona). Finally we aimed to rescue at least the epileptic phenotype by AAV-based NPY gene therapy.

2) *Role of neuronal plasticity inhibitors in a environmentally induced rat model of ASD*

To examine the maturation of neuronal plasticity inhibitors in male and female SI-O rats, we will carry out a detailed analysis of thickness, intensity and number of PNNs (including specific types of PNNs+ GABAergic neurons and parvalbumin (PV) expression and PV+ interneurons cell density), and of changes in myelin structure and myelin plasticity inhibitors in different brain areas where neuronal circuits were reported to be altered in a variety of neurodevelopmental disorders, including ASD. Finally, to address a putative impairment in neuroplasticity, we will evaluate the occurrence and distribution of selected molecules, chosen as possible biomarkers of the behavioral phenotype observed in SI-O rats, in areas of the limbic system involved in the regulation of behavioral flexibility, spatial learning and memory.

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

The proposed studies are expected to have implications of clinical research:

1) New therapeutic perspectives in the treatment of fragile X syndrome. This line of research has great clinical relevance for the development of therapies for children, adolescents and adults with neurodevelopmental disorders in which there is an imbalance between excitatory and inhibitory neuronal transmission. These disorders include not only FXS, but also other forms of autism or neurodevelopmental disorders, the genetic basis of which has not yet been completely clarified, but which are often associated with neuronal hyperexcitability. In fact, autism is often associated with sensory hypersensitivity and epilepsy. Neurodevelopmental disorders are associated with multiple symptoms and severity, resulting in different degrees of mental, emotional, physical, and economic consequences for individuals, and in turn families, social groups, and society.

2) Treating GRIN2A-related epileptic encephalopathies: a preclinical study

If validated, the novel animal model allows relevant molecules to be evaluated for their impact on behavioral and cognitive deficits associated with this mutation. In particular, agents that act on the glutamatergic transmission will be examined for their potential of preventing, reversing, or attenuating the behavioral manifestations. The administration of compounds can be conducted at different times of development, thus testing the relevance of the CNS developmental stage on treatment response. In case of drugs already used in clinical care for other purposes the impact on patient care of positive results from these studies is expected to be fast through repurposing. For molecules not yet approved as medications, the next step will be the process of safety assessment and phase I pharmacological development studies. The AAV-based gene therapy approach proposed has been demonstrated to be viable in humans.

3) Role of neuronal plasticity inhibitors in a environmentally induced rat model of ASD

This latter study may lead to the identification of biomarkers of risk for ASD in the offspring that could be sex-specific and that could be applicable to other environmentally related ASD models. Understanding the biological consequences of preconceptional stress exposure may aid to develop care guidelines for the general population and therapeutic interventions for the affected offspring.

e. Methodology (up to 2000 characters): <u>please fill-out this section only in the case of innovative technologies</u>

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: Peripheral Nerve Regeneration Unit

4. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Stefano Geuna Birthdate: 25/09/1965
Degree: Graduated in Medicine and Surgery, UNITO Gender: Male
Nationality: Italian
Phone: +39 011/6705433
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Personnel

1 Stefania Raimondo

Degree: Biological Sciences, University of Turin Birthdate: 25/02/1977

Nationality: Italian Gender: Female

Phone: +39 011/6705433

Email: stefania.raimondo@unito.it

Position: Associate Professor

Role & expertise: since October 1st 2019, supervisor of the research group.

2 Giulia Ronchi

Degree: Master degree in Neurobiology, University of Turin Birthdate: 27/11/1982

Gender: Female

Nationality: Italian

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Position: RTDB

Role & expertise: In vivo models for peripheral nerve regeneration study

3 Federica Fregnan

Degree: biological sciences, University of Turin Birthdate: 02/07/1976

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Position: Post-doctoral fellowship recipient

Role & Expertise: In vitro model for peripheral nerve regeneration study

4 Luisa Muratori

Degree: Master degree in Neurobiology, University of Turin Birthdate: 02/05/1984

Gender: Female

Nationality: Italian

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Position: Post-doctoral fellowship recipient

Role & Expertise: Study of autonomic system regeneration

5 Benedetta Elena Fornasari

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

Birthdate: 11/07/1989

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Position: Post-doctoral fellowship recipient

Role & expertise: Biomolecular analysis of peripheral nerve regeneration

6 Marwa El Soury

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

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Nationality: Egyptian

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Email: marwa.elsory@unito.it

Position: PhD Student, PhD Programme in Neuroscience

Role & expertise: Biomolecular analysis of peripheral nerve regeneration

7 Giacomo Carta

Degree: Master degree in rehabilitation science

Birthdate: 24/06/1986

Nationality: Italian

Gender: Male

Phone: +39 011/6705433

Email: giacomo.carta@unito.it

Position: PhD student, PhD Programme in Experimental Medicine and Therapy

Role & expertise: Functional treatment and analysis of peripheral nerve regeneration

5. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2018-2019	Fondo per la Ricerca Locale	Prof. Stefano Geuna	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2.859,70	228,77
2019-2021	Ricerca Finalizzata 2018	Prof. Stefano Geuna	Regione Piemonte	PI of Research Unit	50.000	4.000
2018-2019	Fondo per la Ricerca Locale	Prof.ssa Stefania Raimondo	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2.874,7	229,97
2018-2019	Fondo per la Ricerca Locale	Dr.ssa Giulia Ronchi	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2.721,39	217,71

6. SCIENTIFIC ACTIVITIES IN 2019

Name, Role: Stefano Geuna (PI)

Supervised PhD students: Giacomo Carta (PhD)
Monica Maurina (Md PhD)

Honors, prizes, awards: NA

Outreach activities

- International collaborations: Co- founder of the European Society for Peripheral Nerve Repair and Regeneration, Bruxelles.
- Invited talks: NA
- Science communication: NA
- Editorial duties: Member of Editorial Board of *Microsurgery*
- Others: NA

Organizational activities and responsabilities at NICO: NA

Speakers invited: NA

Other organizational activities:

Workshops, Schools or Conferences organized: Organization of the 5th International Symposium on Peripheral Nerve Regeneration (ISPNR) (Porto 8-9 July 2019)

Technology transfer achievements:

- Patent 102019000007115 "Dispositivo medico comprendente una struttura di supporto a base di chitosano"

- Patent extension n. 102015000071499
  "Coniugato della neuregulina 1 per il trattamento delle lesioni dei nervi periferici"

Name, Role: Stefania Raimondo (Associate Professor)

Supervised PhD students:	Alessandro Crosio (PhD)
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	- Board members of the European Society for the Study of Peripheral Nerve Repair and Regeneration (ESPNR) - Scientific advisory board member of the NANBIOSIS Research Infrastructure Advisory board (SAB) - Member of the academic staff of the master in tissue engineering and advance therapies of the University of Granada
• Invited talks:	NA
• Science communication:	NA
• Editorial duties:	- Member of Editorial Board of *Frontiers in Neuroanatomy* - Guest associate editor of *Frontiers in Cellular Neuroscience*
• others	
Organizational activities and responsabilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:	
Workshops, Schools or Conferences organized:	NA
Technology transfer achievements (patents, etc.):	NA

ALL LAB MEMBERS

Activities:	- Open Days at NICO - Organization of the 5th International Symposium on Peripheral Nerve Regeneration (ISPNR) (Porto 8-9 July 2019)

4. Research activity in 2019

a. Summary (500 characters)

The research activities of the group have been focused on the study of peripheral nerve repair and regeneration. Different aspects have been studied: i) biological processes occurring during peripheral nerve regeneration, ii) techniques of repair for nerve substance loss lesions, iii) strategies to improve the regeneration of peri-prostatic nerves, iv) evaluation of neurodynamic treatment on peripheral nerves.

b. Background and rationale (3000 characters)

Although peripheral nerve fibres retain a considerable regeneration potential also in the adulthood, recovery after injury is usually poor, especially in case of large nerve defects.

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

In case of severe traumas (especially at limb level) with substance loss, the direct repair is not possible, in this case, a graft is required to bridge the proximal and distal stumps of the injured nerves. Nerve

fibers can regenerate inside the graft and reach 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 harvesting of a healthy nerve that 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.
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 outcome can be affected by several factors, including i) the lesion site, ii) the time between the injury and the surgical repair, iii) the inability of denervated muscle to accept reinnervation and to recover from muscle atrophy, iv) the reduced ability of injured axons to regenerate after a long axotomy and v) the loss of the Schwann cell (SC) capability to support regeneration.
Such research brings together different disciplines which might contribute, not only to increase knowledge about the biological mechanisms that underlie the complex sequence of events which follows nerve damage, but also to define the best strategies for optimizing posttraumatic nerve regeneration and, eventually, the full recovery of the patient's motor and sensory function.

c. Objectives (1000 characters)

The objectives of the group activities were to better understand the biological process implicated in nerve regeneration and to study how improving functional recovery after peripheral nerve injuries.
These goals have been reached: i) studying biological events, such as cellular colonization of conduits and gene expression, during peripheral nerve regeneration after nerve repair; ii) evaluating different techniques to repair nerve lesion with substance loss (new conduits made of natural biopolymers and decellularized nerve allograft); iii) studying new strategies (chitosan membrane application) to improve the nerve regeneration of the neurovascular bundle after radical prostatectomy; iv) evaluating *in vitro* the effect of neurodynamic treatment on neuronal cells.

d. Results (4000 characters)

According to the different goals, all the results are summarized below.

<u>The role of fibroblasts during nerve regeneration by means of chitosan hollow tube</u>
Investigating the expression of different genes within a hollow tube used to repair a nerve lesion, we demonstrated that, in the early time points after repair, the expression of nerve fibroblast markers was observed, while Schwann cell (SC) marker expression was barely detectable.
In vitro analysis on primary culture of sciatic nerve fibroblasts show that fibroblasts express high levels of different NRG1 isoforms, while NRG1 receptors are not expressed, thus indicating that nerve fibroblasts signal in a paracrine manner. The presence of different soluble NRG1 isoforms inside the tube in the early steps after injury, suggests that NRG1 released by nerve fibroblasts might play a key role in the following SC migration inside the tube.

<u>Peripheral nerve repair with conduits made of natural biopolymers</u>
Results about techniques of nerve repair have been obtained mainly testing different biomaterials.
In collaboration with two different companies, the potential of an innovative homopolymer obtained from bacterial fermentation (PHA) and of a Silk fibroin conduits were tested.
Both were tested to repair a nerve lesion in a rat model. Results of these pre-clinical *in vivo* studies strongly suggest that both conduits are suitable medical devices for the reconstruction of peripheral nerve injuries with loss of substance.

<u>Study of decellularized nerve: research of the more reliable method</u>
A nerve allograft could be a good solution to repair large nerve lesion with substance loss but displays the important problem of immunogenicity. For this reason a decellularized nerve could represent an alternative strategy to the autograft, retaining the 3D structures and the extracellular matrix

components with the complete removal of immunogenic components. In this study different protocols have been tested in order to decellularize human (median and ulnar) and rats (sciatic) nerves. Preliminary morphological results of human decellularized nerves have been shown high degradation of myelin sheath and absence of cellular components. Rat nerves displayed the presence of cellular components and residual myelin sheath. Further studies are needed to find a more appropriate and standardized protocol suitable for both nerves.

Strategies to improve the functional recovery after radical prostatectomy
Radical prostatectomy for the removal of prostatic cancer often results in erectile dysfunction due to damage of the peri-prostatic nerve bundles.
The regenerative and anti-cancer properties of a biomedical device consisting of chitosan, a derivative of chitin, that is achieving resounding interest both in basic research and in clinical settings exerting pro-regenerative action on nerves was tested for the study of the autonomic regeneration. At the same time the anti-proliferative properties of chitosan were tested on different prostatic cancer cell lines in vitro. Results displayed that this biomaterial represents a suitable substrate to improve axonal regeneration in autonomic explants ganglia e to reduce the proliferation of cancer cells.

Assessment of the effects caused by mechanical stimulation on peripheral nervous system
Neurodynamic Treatment (NDT) is a type of intervention used by the physiotherapist to treat the diseases of the musculoskeletal system.
The aim of this work is to evaluate *in vitro* the effects of NDT on neuronal cells as regarding the cell morphology, neuroplasticity/cell growth phenomena, the biological and chemical behavior, comparing two different protocols for intensity and assess any adverse effects.
Preliminary results have shown that NDT seems to have no side effects and can affect neurites orientation, cell differentiation and avoids apoptosis. Interestingly, a protocol of NDT downregulates the expression of TLR2, a gene linked to mechanical allodynia.

e. Advancement in the field (1000 characters)

Results of our research, allowed to reach the FDA approval for the chitosan membrane tested to improve the regeneration of the peri-prostatic nerves. Moreover, results from Silk fibroin *in vivo* studies allowed to proceed towards the submission of a first-in-human clinical study aimed at evaluating the reconstruction of digital nerve defects in humans (ClinicalTrials.gov identifier: NCT03673449). In addition, the continuation of translational work for complex nerve conduits are ongoing activities.

f. Publications

1: Alessandrino A, Fregnan F, Biagiotti M, Muratori L, Bassani GA, Ronchi G, Vincoli V, Pierimarchi P, Geuna S, Freddi G. SilkBridge™: a novel biomimetic and biocompatible silk-based nerve conduit. Biomater Sci. 2019 Oct 1;7(10):4112-4130. doi: 10.1039/c9bm00783k. Epub 2019 Jul 30. PubMed PMID: 31359013.

2: Colonna MR, Fazio A, Costa AL, Galletti F, Lo Giudice R, Galletti B, Galletti C, Lo Giudice G, Dell'Aversana Orabona G, Papalia I, Ronchi G, Geuna S. The Use of a Hypoallergenic Dermal Matrix for Wrapping in Peripheral Nerve Lesions Regeneration: Functional and Quantitative Morphological Analysis in an Experimental Animal Model. Biomed Res Int. 2019 Jun 17;2019:4750624. doi: 10.1155/2019/4750624. eCollection 2019. PubMed PMID: 31317030; PubMed Central PMCID: PMC6601684.

3: Ronchi G, Morano M, Fregnan F, Pugliese P, Crosio A, Tos P, Geuna S, Haastert-Talini K, Gambarotta G. The Median Nerve Injury Model in Pre-clinical Research - A Critical Review on Benefits and Limitations. Front Cell Neurosci. 2019 Jun 28;13:288. doi: 10.3389/fncel.2019.00288. eCollection 2019. Review. PubMed PMID: 31316355; PubMed Central PMCID: PMC6609919.

4: Jaminet P, Schäufele M, Mager A, Fornaro M, Ronchi G, Geuna S, Schaller HE, Rosenberger P, Köhler D. Expression patterns and functional evaluation of RGMa during the early phase of peripheral

nerve regeneration using the mouse median nerve model. Restor Neurol Neurosci. 2019;37(3):265-272. doi: 10.3233/RNN-190913. PubMed PMID: 31177252.

5: Muratori L, Fregnan F, Ronchi G, Haastert-Talini K, Metzen J, Bertolo R, Porpiglia F, Geuna S. New basic insights on the potential of a chitosan-based medical device for improving functional recovery after radical prostatectomy. BJU Int. 2019 May 28. doi: 10.1111/bju.14834. [Epub ahead of print] PubMed PMID: 31134718.

6: Crosio A, Fornasari BE, Gambarotta G, Geuna S, Raimondo S, Battiston B, Tos P, Ronchi G. Chitosan tubes enriched with fresh skeletal muscle fibers for delayed repair of peripheral nerve defects. Neural Regen Res. 2019 Jun;14(6):1079-1084. doi: 10.4103/1673-5374.250628. PubMed PMID: 30762022; PubMed Central PMCID: PMC6404480.

7: Diogo CC, da Costa LM, Pereira JE, Filipe V, Couto PA, Geuna S, Armada-da-Silva PA, Maurício AC, Varejão ASP. Kinematic and kinetic gait analysis to evaluate functional recovery in thoracic spinal cord injured rats. Neurosci Biobehav Rev. 2019 Mar;98:18-28. doi: 10.1016/j.neubiorev.2018.12.027. Epub 2019 Jan 3. Review. PubMed PMID: 30611796.

8: Colonna MR, Pino D, Battiston B, d'Alcontres FS, Natsis K, Bassetto F, Papadopulos NA, Tiengo C, Geuna S. Distal nerve transfer from the median nerve lumbrical fibers to the distal ulnar nerve motor branches in the palm: An anatomical cadaveric study. Microsurgery. 2019 Jul;39(5):434-440. doi: 10.1002/micr.30402. Epub 2018 Dec 17. PubMed PMID: 30556926.

9: Mancini C, Hoxha E, Iommarini L, Brussino A, Richter U, Montarolo F, Cagnoli C, Parolisi R, Gondor Morosini DI, Nicolò V, Maltecca F, Muratori L, Ronchi G, Geuna S, Arnaboldi F, Donetti E, Giorgio E, Cavalieri S, Di Gregorio E, Pozzi E, Ferrero M, Riberi E, Casari G, Altruda F, Turco E, Gasparre G, Battersby BJ,Porcelli AM, Ferrero E, Brusco A, Tempia F. Mice harbouring a SCA28 patient mutation in AFG3L2 develop late-onset ataxia associated with enhanced mitochondrial proteotoxicity. Neurobiol Dis. 2019 Apr;124:14-28. doi: 10.1016/j.nbd.2018.10.018. Epub 2018 Oct 30. PubMed PMID: 30389403.

10: Gambarotta G, Raimondo S, Udina E, Phillips JB, Haastert-Talini K. Editorial: Peripheral Nerve Regeneration. Front Cell Neurosci. 2019 Oct 15;13:464. doi:10.3389/fncel.2019.00464. eCollection 2019. PubMed PMID: 31680873; PubMed Central PMCID: PMC6803521.

11: Gonçalves NP, Mohseni S, El Soury M, Ulrichsen M, Richner M, Xiao J, Wood RJ, Andersen OM, Coulson EJ, Raimondo S, Murray SS, Vægter CB. Peripheral Nerve Regeneration Is Independent From Schwann Cell p75(NTR) Expression. Front Cell Neurosci. 2019 May 29;13:235. doi: 10.3389/fncel.2019.00235. eCollection 2019. PubMed PMID: 31191256; PubMed Central PMCID: PMC6548843.

7. Future directions and objectives for next years

a. Summary (up to 2000 characters):

The first goal of the group will be to realize an innovative therapies to improve the patients' outcome after peripheral nerve damage. In collaboration with companies will be developed advanced prosthesis made of natural biopolymers for the repair of severe nerve lesions. In particular, silk conduits will be filled with silk fibroin fibers of different topography, while PHA conduits (homopolimer derived from bacterial fermentation) will be improved for the repair of more complex nerve injuries.
In addition, protocols for nerve decellularization will be standardized on human nerves harvested from donor cadaver in order to create the basis for the creation of a nerve tissue bank.
The second goal will be the study of a chitosan membrane (FDA approved) with a nanostrcutured grating for the improvement of axonal regeneration in patients undergoing radical prostatectomy in order to enhance the functional recovery. Experiments in this fields are in collaboration with Professor Porpiglia the head of the Department of Urology in San Luigi Gonzaga Hospital.
Moreover, the efficacy of neurodynamic treatments on peripheral nervous system will be studied in an *in vitro* and *ex vivo* model and on the vagus nerve of healthy subjects.

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

Improvement 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.

Strategies to improve the functional recovery after radical prostatectomy

Prostatic cancer is the most frequent cancer in males. Whereas the progress in early cancer detection and surgical removal has made significant improvement in patient survival, erectile dysfunction often results after radical prostatectomy due to damage of the peri-prostatic nerves. This condition is associated with impairment of quality of life. The application of new techniques and new materials such as chitosan membrane would result in minor inconvenience for patients and allow to extend the treatment also for applications in oncology.

Effects of neurodynamic treatments on peripheral nervous system

Reating the disorders of the PNS and CNS is a main part of clinical practice of the physiotherapist, the knowledge of the biomechanical properties of these systems is essential to manage their damage or alteration. Neurodynamic treatment is applied by the physiotherapist to treat the diseases of the musculoskeletal system. It is not known what biological mechanism can be induced on the PNS cells.
Considering the strong impact that low back pain and neck pain have on the health of the population, this project is expected to be relevant for clinical and economic relapse.

Validation and inter-rater reliability of the vagus nerve neurodynamic test among healthy subjects.

A growing body of evidence have shown that the Vagus Nerve (VN) is not only the main anatomical structure responsible for the brain and guts communication but is also a target for many interventions in which drugs or classic treatments have failed. The VN cervical tract stimulation have reported positive results for high social burden problems like acute and chronic pain, psychiatric diseases, disturbs of consciousness and epilepsy. Moreover it is well known that the selective tension of the Peripheral Nervous System, or neurodynamic test (NDT), is useful for diagnosis and treatment of neuropathic diseases and pain. Over the last 30 years NDTs were validated for upper and lower limbs nerves but nowadays a VN-NDT is lacking and could be a potential alternative in diagnosis and treatment for critical or neglected conditions.

Defining the central and peripheral sensitization mechanism in delayed onset muscle soreness among sport climbers in a randomized control study.

Muscular pain resulting from non-habitual physical activity (DOMS) is a very common phenomenon among those who start to practice a sport such as climbing. Normally the sore peaks in the 2-3 days

following physical activity causes a feeling of weakness and muscle uncoordination. This physiological phenomenon does not depend on mechanisms of inflammatory response or intra or extracellular muscle damage. Recently, transient irritation or sensitization processes of the Peripheral and or Central Nervous System have been hypothesized.

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

The general aim of the 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 psychosocial and relational problem. 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.

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

i) Improving axonal regeneration after traumatic lesion. This objective will be pursued by investigating innovative strategies of tissue engineering of the peripheral nerve. These include the construction of nanostructured scaffolds, cell transplantation, gene therapy, and physical stimulation of tissue repair. Moreover, based on the results obtained from decellularized protocols applied to human and rats nerves, this project related is an ongoing activity in order to study the best protocol and to perform translation study in *in vivo* model. Preliminary results have been shown that the decellularization could be influenced by several factors such as the segment of the nerve harvested, the species considered (human or rats) and the different reagents used. This objective will be pursued investigating the standardization of the protocol allowing to obtain a complete removal of immunogenic elements and maintaining an intact basal lamina to help axon regeneration. The second step will be the study of nerve regeneration *in vivo through* the implanted decellularized allograft.

ii) Developing a nanostructured chitosan medical device for its application in the urological clinical field. This objective will be pursued testing a grating nanostructured membrane for the repair of prostatic nerves in rats. Particularly, this project aims to develop functionalized nanostructured membrane to support and promote nerve regeneration and functional recovery after iatrogenic damage to the periprostatic autonomic neurovascular bundles to preserve erectile function.
The membrane will be made of chitosan, an FDA approved biodegradable biomaterial of natural origin, and it will provide mechanical cues and support during tissue regeneration. To promote nerve protection and regeneration the membrane will be nanopatterned and chemically functionalized. The controlled release of phosphodiesterase inhibitors will be used to chemically promote nerve regeneration and functional recovery.
In vitro and *ex vivo* experiments will be carried out to identify the best devices before their use for in vivo experiments in which they will be applied to stimulate nerve regeneration and erectile functional recovery after crush of cavernous nerves in adult male rats.

iii) Developing a protocol of neurodynamic treatments with impact on motor impairment and rehabilitation and also on acute and chronic pain. This objective will be pursued with different in vitro and in vivo analysis.

iv) Validation and inter-rater reliability of the vagus nerve among healthy subjects. This objective will be pursued performing different neurodynamic test on human.

v) Defining the central and peripheral sensitization mechanism in delayed onset muscle soreness among sport climbers in a randomized control study. The purpose of this innovative project is to investigate the relationship between the presence of muscular pain resulting from non-habitual physical activity and central or peripheral sensitization in a sample of healthy subjects. The study will evaluate the muscular strength and resistance of the upper limbs, the peripheral sensitivity of the upper limbs and central sensitization by the completion of a completed self-questionnaire before, after 48 hours and 96

hours from training in a sport climbing gym. The relationship between fatigue-induced muscle pain and central sensitization will be determined.

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

The unique features of our project research are the following.

1) The project research represents one of the most innovative approaches in Europe focused on the study of peripheral nerve repair and regeneration.
2) The research group brings together interdisciplinary competencies and skills.
3) The project research is carried out under good laboratory practice (GLP)-inspired procedures
4) the research group focus on the translational approach, i.e. on the applicability of the research results for developing new therapeutic strategies that could successfully 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 FDA approval of the chitosan membrane tested to repair peri-prostatic nerves.

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

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

FONDAZIONE
CAVALIERI OTTOLENGHI

NICO
Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: Neuroendocrinology

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

GianCarlo Panzica

Degree: PhD | Birthdate: 17/08/1949

Nationality: Italian | Gender: male

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Email: giancarlo.panzica@unito.it

Personnel

1 Stefano Gotti

Degree: PhD | Birthdate: 17/06/1971

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Position: Associate Professor

Role & expertise: Co-PI

2 Giovanna Ponti

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Position: Researcher RTD

Role & expertise: Researcher; Neurogenesis, phytoestrogens

3 Alice Farinetti

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Position: Post-Doc, assegno di ricerca

Role & expertise: Researcher; Neurogenesis, Gonadal hormones, eating disorders models

4 Marilena Marraudino

Degree: PhD | Birthdate: 08/06/1988

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Position: Post-Doc, assegno di ricerca

Role & expertise: Researcher; Control of reproduction, endocrine disruptors

5 Brigitta Bonaldo

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Role & expertise: Researcher; Neurodegenerative disorders models, endocrine disruptors

6 Godstime Stephen K. Morgan

Degree: Master Degree Birthdate: 27/06/1993

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Phone: 011 670 6632

Email: godstime.morgan272@edu.unito.it

Position: PhD-Student

Role & expertise: Researcher; eating disorders models

2. CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2013-2015	Effetti cerebrali degli obesogeni	Panzica, PI	UNITO-ex 60% 2012	Coordinator	3053	8%
2012-2014	Participación del óxido nítrico en el control neurohormonal de la ingesta	Panzica, PI	Ministerio de Ciencia e Innovación (Spain)	PI of research unit	60.000	8%
2014-2016	Effetti cerebrali degli obesogeni: azione della TBT sul circuito a NPY	Panzica, PI	UNITO-ex 60% 2013	Coordinator	2.127	8%
2015-2017	Distruttori endocrini e circuiti che regolano l'assunzione di cibo ed il metabolismo energetico	Panzica, PI	UNITO-ex 60% 2014	Coordinator	2343	8%
2016-2019	Involvement of estradiol on feeding neurohormonal circuit programming in the rat	Panzica, PI	Ministerio de Ciencia e Innovación (Spain)	PI of research unit	70.000	8%
2017 2019	Effetti dell'esposizio	Panzica, PI	UNITO-ex 60% 2016-17	Coordinator	7.095	8%

	ne a distruttori metabolici sui circuiti ipotalamici che controllano l'assunzione di cibo ed il metabolismo energetico					
2018 2020	Neuropeptidi e sviluppo dei disturbi del comportamento nel periodo evolutivo: modelli sperimentali e potenzialità terapeutiche'	Panzica, PI	UNITO-ex 60% 2018	Coordinator	2.800	8%
2019 2021	Bisfenolo A e sclerosi multipla: sviluppo di un modello traslazionale	Panzica, PI	UNITO-ex 60% 2019	Coordinator	2900	8%
2019 2021	Dipendenze dalle nuove tecnologie: sviluppo di un modello animale per future applicazioni sull'uomo	Panzica, PI	Fondazione CRT	Coordinator	30000	10%
pending	Ruolo degli ormoni steroidei nella patogenesi e nello sviluppo dei tumori gliali: nuove frontiere per approcci terapeutici	Panzica, PI	UNITO-ex 60% 2019, progetti traslazionali	Coordinator	15000	8%
2013 2018	**Neurosteroidi e modulazione della neurogenesi nell'ippocampo di ratto**	Gotti, Group member	UNITO-ex 60% 2012	Coordinator	2787	8%
2017 2019	Fattori ambientali nella sclerosi multipla: effetti in seguito a esposizione a	Gotti, Group member	UNITO-ex 60% 2018	Coordinator	1690	8%

	bisfenoli.					
2017 2019	Morphological and behavioral study of an Anorexia Nervosa rat model	Gotti, Group member	UNITO-ex 60% 2019	Coordinator	2545	8%
pending	Back to the future: updating *h*DHODH inhibitors for a new therapy in MS	Gotti, Group member	FISM 2019	Coordinator	167000	10%
2017 2018	Valutazione multifattoriale del benessere animale in avicunicoltura	Ponti G, Co-PI	UNITO-ex 60% 2017	Coordinator	10120	8%
2018 2019		Ponti G, Co-PI	UNITO-ex 60% 2018	Coordinator	7398.33	8%

3. SCIENTIFIC ACTIVITIES IN 2019

GianCarlo Panzica, PI

Supervised PhD students: 2: Brigitta Bonaldo, Godstime Stephen K. Morgan

Honors, prizes, awards: na

Outreach activities

- International collaborations: Cooperation with dr. J. Balthazart (University of Liege, Belgium)
  Cooperation with dr. M.A. Ottinger (University of Maryland, College Park, USA)
  Cooperation with dr. N. Harada (Kyoto University, Japan)
  Cooperation with dr. J. Bakker (University of Liege, Belgium)
  Cooperation with dr. P. Collado (UNED, Madrid, Spain)
  Cooperation with L.M. Garcia Segura (Cajal Institute, Madrid, Spain)
  Cooperation with M. Keller (INRA, Tours, France)
- Invited talks: 29° Congresso GISN – Bari – Special Lecture
  CervellaMente – Milano – Invited public lecture
  Update in Neuroscienze di base – Palermo – Plenary Lecture
  Circolo Be Curious – Torino – Invited public lecture
- Science communication: Scienza in Rete: Ossitocina e vasopressina: nuovi orizzonti per vecchi ormoni
- Editorial duties: ***Member of the Editorial board of:***
  Biology of Sex Differences (2013-today)
  Cell and Tissue Research (1996-today)
  Frontiers in Endocrinology (2015-today)
  Frontiers in Neuroscience (2018-today)
- others

Organizational activities and responsabilities at NICO:

Speakers invited:

Other organizational activities:

Workshops, Schools or Conferences organized:	International Meeting Steroids and Nervous System, Torino. February 2019 (10th edition). Satellite Symposium: Steroids and the Nervous System: Past and Future, Torino, February 2019
Technology transfer achievements (patents, etc.):	
Stefano Gotti, Co-PI xxx	
Supervised PhD students:	2: Brigitta Bonaldo, Godstime Stephen K. Morgan
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Cooperation with dr. P. Collado (UNED, Madrid, Spain)
• Invited talks:	
• Science communication:	
• Editorial duties:	**Journal Reviewer:** *Brain Research, Journal of Chemical Neuroanatomy, Cell and Tissue Research, Physiology and Behavior, Neurological Science, Histology and Histopathology, Neurobiology of Disease, Molecular and Cellular Neuroscience*
• others	
Organizational activities and responsabilities at NICO:	First aid and fire safety officer
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Coordinator of the Local Organizing Committee: - International Meeting Steroids and Nervous System, Torino. February 2019 (10th edition). - Satellite Symposium: Steroids and the Nervous System: Past and Future, Torino, February 2019 - School conference (April 2019) for Santorre di Santarosa Institute of Higher Education (Torino) with the title: "Hormones and Brain".
Technology transfer achievements (patents, etc.):	
ALL LAB MEMBERS	
Activities:	Members of the Local Organizing Committee: - International Meeting Steroids and Nervous System, Torino. February 2019 (10th edition). - Satellite Symposium: Steroids and the Nervous System: Past and Future, Torino, February 2019 NICO OPEN DAY 2019 NOTTE DEI RICERCATORI 2019

4. Research activity in 2019

a. Summary (500 characters)

Our research's lines have been focused to: the study of the interactions among steroids and nervous circuits, the effect of endocrine disrupting chemicals (EDCs) in the derangement of the circuit involved in the control of energetic metabolism, and the behavioral study of a rodent model of Anorexia Nervosa.

b. Background and rationale (3000 characters)

Gonadal hormones play a key role in the development of phenotypical characteristics in higher vertebrates, including several steroid dependent behaviors and neural circuits. After the demonstration that both nuclear estrogen receptors (ERα and ER β) and membrane receptor (GPER-1) are expressed in many brain areas during ontogeny, it was realized that estrogens may modulate neuronal differentiation, notably by influencing cell migration, survival and death, and synaptic plasticity.
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 behavioral alterations in many species.
Due to the fact that many EDCs are xenoestrogens (XEs) or xenoandrogens (XAs), they could, even in very low concentrations, deeply influence the development and the function of gonadal hormones-dependent neural circuits and related behaviors. Behavioral responses represent the culmination of several integrated systems, therefore, even small changes of neural or neuroendocrine components are likely to disrupt or modify behavior. Importantly, disturbances in normal behavior may influence the individual fitness and, therefore, assume a real biological significance in both animal and human ecosystems.
EDCs acting at low levels can exert subtle effects by interfering with gene expression and other cellular activities, which can cause transient responses (activational), or permanent impairment (organizational). Thus, the impact of EDCs will vary depending upon a variety of factors, including when exposure occurs, 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 or neonatal exposure may be drastically different from those of adult exposure. This occurs 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.
Moreover, considering our interest in gender differences, critical periods and alteration of food intake circuits we collaborate with clinicians in a project focused on Anorexia Nervosa, an eating disorder that typically affects women. In order to elucidate the neurobiological mechanisms that may play a role in this disorder, we studied an animals model for activity-based anorexia (ABA) and the relations with the maternal separation in both sexes.

c. Objectives (1000 characters)

Our main goal is the study of the interactions steroid hormones-neural circuits-behavior. This include the study of sexual differences at any level, in particular the sexually dimorphic action of EDCs on several behaviors including the food intake and the metabolism control, the involvement of neurosteroids in several diseases and the neurobiological mechanisms of anorexia nervosa, including the role of parental cure.

d. Results (4000 characters)

Gonadal hormones in physiological conditions and in diseases
a) Estradiol receptors and modulation of Vasopressin expression in the rat hypothalamus **(Lagunas et al., 2019).**
17β-estradiol is known to regulate the release, expression and immunoreactivity of arginine-vasopressin (AVP) in the supraoptic and paraventricular hypothalamic nuclei of rodents. We have examined the effects of an acute administration of estradiol or of specific agonists of the estrogen receptors α, β and GPER-1 on the hypothalamic AVP immunoreactivity in adult ovariectomized female rats. The treatment resulted, after 24 h, in a significant decrease in the number of AVP neurons. The use of specific estrogen receptors agonists

suggests that the action of estradiol on AVP neurons is mediated in the supraoptic nucleus by GPER-1 and in the paraventricular nucleus by both estrogen receptor β and GPER-1.

b) Gonadal hormones and retinal disorders **(Nuzzi et al., 2019)**
Estrogen, androgen, and progesterone receptors are present throughout the eye and these steroids are locally produced in ocular tissues. Sex hormones can have a neuroprotective action on the retina and modulate ocular blood flow. There are differences between male and female retina; moreover, sex hormones can influence the development (or not) of certain disorders. For example, exposure to endogenous estrogens, depending on age at menarche and menopause and number of pregnancies, and exposure to exogenous estrogens, as in hormone replacement therapy, appear to protect against age-related macular degeneration, whereas exogenous testosterone therapy is a risk factor for central serous chorioretinopathy. Progestin therapy appears to ameliorate the course of retinitis pigmentosa. Diabetic retinopathy may be more common among men than women. In conclusions, we observed a correlation between many retinopathies and sex, probably as a result of the protective effect some gonadal hormones may exert against the development of certain disorders. These observations may have implications for the use of hormone therapy in the treatment of eye disease, and of retinal disorders in particular.

Neuroendocrine disruption.
We have examined the action of two EDCs:
- bisphenol A (BPA), a chemical compound present in many consumer products (plastics, PVC, food packaging). It interacts with a variety of hormone receptors (ER α, ER β, GPER-1, androgen receptor). We focalized our attention to the perinatal period to understand the effects of a BPA exposition on the onset of puberty and related neural circuits (**Ruiz-Pino F et al., 2019**).
-genistein (GEN), a natural phytoestrogen that may interfere with the development of estrogen-sensitive neural circuits. Due to the large and increasing use of baby formulas characterized by a high content of phytoestrogens, there are some concerns that this could result in an impairment of some estrogen-sensitive neural circuits and behaviors. We published two research studies on GEN: one was describing the in vivo effects after postnatal administration of GEN (miming the baby formula) to alter dopaminergic hypothalamic circuits (**Ponti et al., 2019**). The other study was performed in vitro to analyses the mechanism of action of GEN in neuronal differentiation (**Marraudino et al., 2019**).
Additionally, we reviewed recent findings related to the role of some EDCs, referred as "metabolic disruptors", in the alteration of the neuroendocrine circuits controlling food intake (**Marraudino et al., 2019**).

Translational models of mental disease (Farinetti et al., 2019)
We continued our cooperation with our colleagues in Psychiatry. We analyzed male and female adolescent rats and tested the effect of emotional deprivation, induced by the maternal separation in a model of Anorexia nervosa. Our results indicate that the maternal separation induces a greater hyperactive behavior in females than in males.

e. Advancement in the field (1000 characters)

The health problems related to endocrine disruptors (in particular, those related to obesity) gained more attention in these years. Our studies, as well as those performed in other laboratories, established some new end-point 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.

f. Publications

Ruiz-Pino F, Miceli D, Franssen D, Vazquez MJ, **Farinetti A**, Castellano JM, **Panzica G**, Tena-Sempere M. Environmentally Relevant Perinatal Exposures to Bisphenol A Disrupt Postnatal Kiss1/NKB Neuronal Maturation and Puberty Onset in Female Mice. Environ Health Perspect. **2019** Oct;127(10):107011. doi: 10.1289/EHP5570. Epub 2019 Oct 25. PubMed PMID: 31652106.

Ponti G, Farinetti A, Marraudino M, Panzica G, Gotti S. Postnatal genistein administration selectively abolishes sexual dimorphism in specific hypothalamic dopaminergic system in mice. Brain Res. **2019** Dec 1;1724:146434. doi: 10.1016/j.brainres.2019.146434. Epub 2019 Sep 3. PubMed PMID: 31491419.

Marraudino M, Farinetti A, Arevalo MA, **Gotti S, Panzica G**, Garcia-Segura LM. Sexually Dimorphic Effect of Genistein on Hypothalamic Neuronal Differentiation in Vitro. Int J Mol Sci. **2019** May 18;20(10). pii: E2465. doi: 10.3390/ijms20102465. PubMed PMID: 31109056; PubMed Central PMCID: PMC6567056.

Nuzzi R, Scalabrin S, Becco A, **Panzica G**. Sex Hormones and Optic Nerve Disorders: A Review. Front Neurosci. **2019** Feb 11;13:57. doi: 10.3389/fnins.2019.00057. eCollection 2019. Review. PubMed PMID: 30804741; PubMed Central PMCID: PMC6378504.

Lagunas N, **Marraudino M**, de Amorim M, Pinos H, Collado P, **Panzica G**, Garcia-Segura LM, Grassi D. Estrogen receptor beta and G protein-coupled estrogen receptor 1 are involved in the acute estrogenic regulation of arginine-vasopressin immunoreactive levels in the supraoptic and paraventricular hypothalamic nuclei of female rats. Brain Res. **2019** Jun 1;1712:93-100. doi: 10.1016/j.brainres.2019.02.002. Epub 2019 Feb 4. PubMed PMID: 30731078.

Marraudino M, Bonaldo B, Farinetti A, Panzica G, Ponti G, Gotti S. Metabolism Disrupting Chemicals and Alteration of Neuroendocrine Circuits Controlling Food Intake and Energy Metabolism. Front Endocrinol (Lausanne). **2019** Jan 9;9:766. doi: 10.3389/fendo.2018.00766. eCollection 2018. Review. PubMed PMID: 30687229; PubMed Central PMCID: PMC6333703.

Montarolo F, Perga S, Martire S, Brescia F, Caldano M, Lo Re M, **Panzica G**, Bertolotto A. Study of the NR4A family gene expression in patients with multiple sclerosis treated with Fingolimod. Eur J Neurol. **2019** Apr;26(4):667-672. doi: 10.1111/ene.13875. Epub 2018 Dec 22. PubMed PMID: 30565812.

Farinetti A, Aspesi D, **Marraudino M**, Marzola E, Amianto F, Abbate-Daga G, **Gotti S**. Sexually dimorphic behavioral effects of maternal separation in anorexic rats. Dev Psychobiol. **2019** Sep 9. doi: 10.1002/dev.21909. [Epub ahead of print] PubMed PMID: 31502241.

7. Future directions and objectives for next years

Please describe the following information relevant to the research that you are planning to do – Character limit is mandatory. Please highlight the added value of collaborations within the NICO where applicable.

a. Summary (up to 2000 characters):

Our long-term cooperation with the laboratory of dr. Collado in Madrid, is dedicated to the study of the involvement of gonadal hormones (chiefly estradiol) on neuroendocrine circuits regulating feeding in rodents. In particular, we will investigate if estradiol during first stage of development participates in the programming/organization of these circuits as well as in the expression of the feeding behavior and the receptor pathways that are involved.
All these research lines will include groups of animals treated with several EDCs, in order to see how the exposure to these compounds will impact on these steroid hormone-dependent neuronal systems and behaviors.
We will continue our cooperation with our colleagues in Psychiatry. We started to analyze several circuits of male and female adolescent rat model of Anorexia Nervosa in consequences of the behavioral results that we recently published **(Farinetti et al., 2019)**.

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

Steroid hormones, which are synthesized in adrenal glands, gonads or placenta, exert a large array of biological effects on the nervous system. These hormones play important roles in the development, growth, maturation, differentiation and protection of the central (CNS) and peripheral nervous system (PNS). In addition, the nervous system itself is capable to metabolize or *de novo* synthesize active steroids (*neurosteroids*) which may control the activity and survival of nerve cells. In the living animal, there are mechanisms (i.e. the alpha-fetoprotein in rodents or the sex hormone binding globulin in primates) that protect the brain from the circulating gonadal hormones, in order to prevent "mistakes" in the differentiation of gonadal hormone-dependent circuits during specific windows of activity (critical period) that occurs in the pre- or/and postnatal development.
Endocrine disrupting chemicals (EDCs) are compounds that are biologically active and mimic endogenous hormones (often estrogens or androgens), thereby altering hormone-modulated responses. They are not blocked by protective mechanisms, as the alpha-fetoprotein, therefore they have been shown to disrupt embryonic development, sexual differentiation, reproduction, immune function, behavior, and responses mediated by hormones. The issue of EDCs has gained increasing attention as it has become clear that these environmental contaminants have endocrine activity in humans, as well as in wildlife and domestic animal species. Some of these chemicals, most notably the plant phytoestrogens largely present in the food, may play an important role in the reproductive cycles of small rodents as well as have positive (or negative) effects in other animals including humans.
A range of EDC effects has now been documented in a number of animal species, both in laboratory studies and in wild populations, demonstrating that xenoestrogenic or xenoandrogenic compounds may exert deleterious effects, even long time after exposure. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. Experimental data in galliforms and rodents showed that EDCs exposure, though nonlethal, left the individual impaired or even incapable of reproducing (Panzica et al., 2007). A recent acquisition is the concept of metabolic disruptors, i.e. substances that are able to induce profound alterations of the metabolism and inducing diseases like the diabetes (Heindel et al., 2015).
Many studies on EDCs have a toxicological approach and are performed in vitro. Among those done in vivo, only a few studies have considered the brain as a major target. In this field, our laboratory is very active and we will continue our researches covering several research lines dealing with different aspects of the interactions gonadal hormones-nervous system.

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

Our aim is to understand how the steroid hormones may interact and regulate the neural circuits that are involved in the control of several important physiological activities (i.e reproduction, food intake, metabolism), 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 and/or steroid-dependent. 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 steroid hormones receptors, are good candidates for this environmental action. The elucidation of how these compounds interact with nervous circuits will open a new frontier in our knowledge. For this reason we have planned to collaborate with clinician groups in proposing research project trying to correlate Parkinson Disease and Multiple Sclerosis with EDCs/environmental factors.

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

The specific objectives for next year are listed below:

- **Effect of EDCs on cerebral circuits and behavious**.

- *Genistein effects on circuits controlling reproduction and food intake*.

In our previous studies we demonstrated that early postnatal GEN administration, at doses similar to that of infant formulas, may interfere with the development of specific neuronal circuits (vasopressin, nitrergic system, dopaminergic system), now we want to test if genistein may interfere with reproductive activity (puberty, development of gonads) and of circuits implicated in the control of reproduction (kisspeptin). In addition, we want also to test the hypothesis that genistein is affecting food intake and the neuroendocrine

control of energetic metabolism. To do this we will measure some simple parameter (weight, food consumption) and some orexinergic (orexin) and anorexinergic (POMC) pathways.

- *Organizational effects of perinatal exposure to tributyltin*

In our previous studies we demonstrated an effect of TBT on NPY circuits after chronic adult exposure. Now we want to explore the possibility that perinatal exposure to TBT may interfere with the differentiation of the NPY system in adulthood. The study will be conducted with different doses of TBT orally administered during gestation and lactation.

- **Effect of steroids on neuroendocrine circuits controlling food intake.**

We will investigate if estradiol during first stage of development participates in the programming/organization of neuroendocrine circuits regulating feeding in rodents. In particular we will treat mice pups from PND 5 to PND 12 with injections of Estradiol and Estrogen Receptor antagonists. At PND50 animals will be subjected to several behavioral and food preference tests; the brain of these mice will be taken to analyze different circuits involved in food intake with immunohistochemical techniques. These systems consist of neurons and fibers that express some neuropeptides, including NPY (Neuropeptide Y) and POMC (pro-opium-melanocortin) and are located in the hypothalamus, particularly in the following nuclei: paraventricular nucleus, arcuate nucleus, dorsomedial nucleus, ventromedial nucleus. Neurons that express NPY regulate hunger, while neurons expressing POMC regulate the sense of satiety: it will be interesting, therefore, to study whether these two populations of neurons are modified by the experimental treatments.

- **Translational studies.**

ABA model. By using our model, we have demonstrated that the maternal separation induces in the females a greater hyperactive behavior than in males. We want to analyze this effect on the reward system: for this reason we will study putative changes in the dopamine neurons distribution in the ventral tegmental area and in the pars compacta of the substantia nigra and the serotonin neurons distribution in the dorsal raphe nucleus. Moreover, we want to analyze the effects of the maternal separation on brain areas involved in the control of the hyperactivity / anxiety-like behavior. We will focalize our attention on the hippocampal neurogenesis.

Mice model for Multiple Scleroses (MS) and EDCs. We have an ongoing PhD project related to a possible involvement of Endocrine Disruptors (EDCs) in the onset of the MS. In this study we will investigate the effects of perinatal exposure (from mating until weaning) to BPA (4μg/kg BW/day, according to the new European TDI) in one of the most widely used murine model of MS, the Experimental Autoimmune Encephalomyelitis (EAE). We will evaluate, by daily examination, the consequences of BPA exposure on the disease onset and progression (rotarod performance and clinical score) and on some physiological parameters (body weight, food intake, vaginal opening, ano-genital distance). Thereafter, we will focalize our attention to possible involvement of EDCs in the onset and progression of the disease.

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

Our research unit is devoted to the study of the interactions among steroid hormones and the nervous tissue, using as main physiological end-point the behavior. Due to the large distribution of steroid hormone receptors within the brain and the importance that steroid hormones have for neuronal and glial differentiation, survival and protection, a better understanding of the relationships steroids-nervous system seems to be of crucial importance.

In addition to this, the importance of gender medicine is increasing, and the interactions among gonadal hormones and the nervous system can partly explain gender differences in both physiological and pathological conditions (Panzica and Melcangi, 2016 for a review).

This is the general context, but, in last two decades the problem of how the environment can interact with human and animal physiology to induce pathologies became an important topic for the biomedical sciences. It is not surprising that a large number of synthetic substances may interact with hormone receptors and therefore induce endocrine unbalance and diseases. However, for many years the neuroendocrine effects were understimated 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 during the development.

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.

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: **Neurophysiology of Neurodegenerative Diseases**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Filippo TEMPIA

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Personnel

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Position: tenure track Assistant Professor

Role & expertise: Supervision, patch-clamp, molecular biology

2 Ilaria BALBO

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Email: ilaria.balbo@unito.it

Position: PhD student

Role & expertise: behavioral experiments, histology, molecular biology

2 CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
01/02/2019 10/06/2021	Identification of new markers and therapeutic targets for mood disorders	Prof. Filippo Tempia (PI) Prof. Giuseppe Maina (co-PI)	Fondazione Cassa di Risparmio di Torino	Coordinator	€ 17,500	€ 1,400
pending	The role of the Sonic Hedgehog signaling pathway in Cerebellar injury due to Obstructive Sleep Apnea	Prof. Roberto Pola (PI) Prof. Filippo Tempia (co-PI)	Riscrca Finalizzata 2019 / Italian Ministry of Health	PI of research unit	€ 88,500	€ 7,080

3 SCIENTIFIC ACTIVITIES IN 2019

Filippo TEMPIA, PI

Supervised PhD students: 1

Honors, prizes, awards: na

Outreach activities

- International collaborations: University of Texas Medical Branch (UTMB)
- Editorial duties: Associate Editor of Frontiers in Aginf Neuroscience, Frontiers in Synaptic Neuroscience, Frontiers in Dementia, Journal of Neuroscience and Rehabilitation, International Journal of Brain Science, The American Journal of Alzheimer's Disease
- Other: Guest Editor of 2 special issues of Frontiers in Cellular Neuroscience

Organizational activities and responsabilities at NICO:. Group Leader of Neurophysiology of Neurodegenerative Diseases; Director of the NICO Animal Facility

Speakers invited: Dr. Sathya Puthanveettil, Department of Neuroscience - The Scripps Research Institute, Florida, USA
Dr. Chiara Verpelli, CNR Institute, Milano

Other organizational activities:

Workshops, Schools or Conferences organized: na

Technology transfer achievements (patents, etc.): na

Eriola HOXHA, Supervisor and Researcher

Supervised PhD students: 1

Honors, prizes, awards: na

Outreach activities

- International collaborations: University of Texas Medical Branch (UTMB)
- Invited talks: na
- Science communication: na
- Editorial duties: Editor of Frontiers in Aging Neuroscience
- others na

Organizational activities and responsabilities at NICO: Responsible for the water ultrapurification systems

Speakers invited:

Other organizational activities: na

Workshops, Schools or Conferences organized: na

Technology transfer achievements (patents, etc.): na

ALL LAB MEMBERS

Activities: Open days at NICO

4. Research activity in 2019

a. Summary (500 characters)

The main project for the year 2019 was to complete the study of the Elovl5 knock-out mouse, model of the spino-cerebellar ataxia type 38 (SCA38). We found an impairment of the endocannabinoid-mediated suppression of excitation, a form of short-term synaptic plasticity. In addition, in the animal model of SCA38 the conduction velocity of peripheral and central axons was significantly reduced, in parallel with structural and molecular myelin damage. We started rescue experiments of the SCA38 model with a diet enriched of the main missing molecules. We acquired the animal model of mood disorder for the study funded by CRT and started the analysis of blood samples from patients.

b. Background and rationale (3000 characters)

Aim 1. Spinocerebellar ataxias (SCAs) are autosomal dominant neurological disorders characterized by gait ataxia, incoordination of eye movements, speech, and hand movements, and usually associated with cerebellar atrophy. We recently identified a novel form of spinocerebellar ataxia (SCA38) due to missense mutations in the gene *ELOngase of Very Long chain fatty acids 5*, *ELOVL5*. The molecular pathogenesis of SCA38 has not been studied yet. We have recently demonstrated that the deletion of *Elovl5* in mice causes symptoms that recapitulate SCA38, suggesting that human mutations found in patients act by a loss-of-function mechanism. The most abundant brain long chain PUFAs are the omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the omega-6 arachidonic acid. These molecules are substrates for the production of a huge variety of active substances, including prostaglandins, protectins and recoverins, involved in induction and resolution of inflammation. Since the actions of these lipidic mediators in the brain is largely unknown, it is likely that important roles in physiology and in reaction to pathology are yet to be discovered. The *Elovl5* knock mouse is an excellent model to discover new molecular mechanisms, in addition to allowing studies about the pathogenic mechanism of SCA38. In addition, although some redundancy is present among Elovl enzymes, the lack of Elovl5 causes a complex disruption of the lipidic pattern, as shown by preliminary lipidomics data of the laboratory of Milan. This fact suggests that functions dependent on long chain lipids might be affected. Moreover, proper function of myelin sheaths in allowing high velocity action potential conduction requires a correct lipid composition. Cerebellar function is based on precise timing of neuronal signals, so that a delay due to a myelin defect might disrupt the cerebellar contribution to motor control and cause ataxia. For this reason it is highly relevant to investigate the consequences of Elovl5 loss on myelin.

Aim 2. Currently the molecular mechanisms of depression are not understood and antidepressant drugs have a low rate of efficacy. GSK3 has been implied by preliminary studies on patients and animal models, but its role in mood disorders is still far from clear and the neural mechanisms are unknown.

If GSK3 can be confirmed as a central player in the control of susceptibility to depression, this finding would open a new avenue to the study of the molecular basis of this disease, which is the leading cause of lifelong disability due to its high prevalence in the population.

c. Objectives (1000 characters)

Aim 1: **Mechanisms of SCA38 ataxia**

In *Elovl5* knockout mice, our model of SCA38, neuronal excitability and synaptic transmission are intact in the cerebellum except for an impairment of a form of short-term plasticity mediated by endocannabinoids. For this reason we hypothesized that the unbalance of the lipidic profile might cause a disruption of myelin. The objective of year 2019 was to study central and peripheral myelin to uncover physiological, structural and molecular disfunctions due to the lack of *Elovl5*. Another objective was to verify whether motor symptoms can be attributed, at least in part, to the altered lipidic profile. To this aim we plan to administer a diet containing the main omega3 and omega6 fatty acids, which are deficient in *Elovl5*$^{-/-}$ mice.

Aim2. The project on the role of GSK3 in mood disorders started in the middle of 2019. The aims of this year were to obtain the permissions from the Bioethical Committee of the Hospital for the study on patients and from the Ministry of Health for the animal model of susceptibility to depression. Another aim was to obtain the Material Transfer Agreement to acquire the animal model and import 2 couples of breeders to start the colony.

d. Results (4000 characters)

Aim 1. The velocity of action potential conduction was measured in a peripheral nerve, finding a consistent and significant reduction. This suggests that Elovl5 is required for proper myelin function. In order to assess action potential conduction in a cerebellar axon, we evoked antidromic spikes by stimulation of the Purkinje cell axon while recording form the cell body. Also in this case the velocity was significantly slower. The correct conduction of action potentials in the nervous system is guaranteed by the lamellar structure of myelin that enwraps the axon and acts as an insulator to speed the transmission of electrical signals. To investigate whether the observed reduced action potential conduction was associated with myelin abnormalities, we performed high-resolution EM analysis of sciatic nerve myelin in both mutant and wild type mice. Elovl5$^{-/-}$ sciatic nerves displayed lower G ratio (internal /external diameter) compared to control littermates for fibres of any calibre while the axonal diameter exhibited no changes. These data indicate a thickening of myelin. Such thickening may result from alterations in myelin structure. In facts, Elovl5$^{-/-}$ sciatic nerves showed expanded myelin periodicity relative to Elovl5$^{+/+}$ nerves. This result is in agreement with the knowledge that myelin needs to be compact in order to speed up action potential conduction. Our results show a loss of compactness, in line with the physiological findings. These results suggest a role of Elovl5 and of its enzymatic products to achieve the correct myelin compactness. The layers of myelin sheaths are kept together by specific structural proteins. We quantified by western blot the amounts of the central myelin proteins MBP, CNPase, PLP; of peripheral proteins MPZ, MBP, PMP-22, CNPase. We found a significant reduction of MPB and CNPase in central myelin. These results suggest that myelin requires specific amounts of saturated vs. unsaturated and long-chain vs. shorter chain lipids. A loss of long-chain polyunsaturated fatty acids, as in Elovl5$^{-/-}$ mice, has deleterious consequences on the structure of myelin, with an impairment of compactness and a deficit in action potential velocity.

Aim 2. We obtained the permissions from the Bioethical Committee of the Hospital for the study on patients. We set up the technique to extract proteins from mononuclear blood cells and we tested the antibodies specific for the two phosphorylated isoforms of GSK3. A first set of blood samples from patients diagnosed with either major depression of bipolar disorder was processed. The permission from the Ministry of Health for the animal model of susceptibility to depression is still pending. A first submission was done in February and rejected in May with a request of integrations. The project was resubmitted in June with the requested amendments. The Material Transfer Agreement to acquire the animal model has been signed in May. We imported 2 couples of breeders and we will soon start the colony.

The publications of the year 2019 reflect the result obtained in 2018.

e. Advancement in the field (1000 characters)

The requirements for the structural determinants of myelin are not known. More specifically, the importance of polyunsaturated vs. saturated long-chain fatty acids present in phospholipids is far from clear. *Elovl5*$^{-/-}$ mice were exploited to find answers to this question. Our results showed that, with an improper ratio of polyunsaturated vs. saturated fatty acids, myelin losses its compactness and becomes unstable with a decrease of structural proteins. Such structural alteration causes a reduction of action potential velocity. In addition to the physiological relevance of this finding, our results suggest that a slow action potential conduction is the principal mechanism of motor symptoms of patients with SCA38.

f. Publications

1. Eriola Hoxha, Andrea Marcinnò, Francesca Montarolo, Linda Masante, Ilaria Balbo, Fernanda Laezza, Filippo Tempia (2019) Emerging roles of Fgf14 in behavioral control. Behavioral Brain Research 356: 257-265. https://doi.org/10.1016/j.bbr.2018.08.034.

2. Mancini C*, Hoxha E*, Iommarini L, Brussino A, Richter U, Montarolo F, Cagnoli C, Parolisi R, Gondor Morosini DI, Nicolò V, Maltecca F, Muratori L, Ronchi G, Geuna S, Arnaboldi F, Donetti E, Giorgio E, Cavalieri S, Di Gregorio E, Pozzi E, Ferrero M, Riberi E, Casari G, Altruda F, Turco E, Gasparre G, Battersby BJ, Porcelli AM, Ferrero E, Brusco A*, Tempia F*. (2019) Mice harbouring a SCA28 patient mutation in AFG3L2 develop late-onset ataxia associated with enhanced mitochondrial proteotoxicity. *contributed equally. Neurobiol Dis. 124: 14-28. https://doi.org/10.1016/j.nbd.2018.10.018.

3. Manes M, Alberici A, Di Gregorio E, Boccone L, Premi E, Mitro N, Pasolini MP, Pani C, Paghera B, Orsi L, Costanzi C, Ferrero M, Tempia F, Caruso D, Padovani A, Brusco A, Borroni B (2019) Long-term efficacy of Docosahexaenoic acid (DHA) for Spinocerebellar Ataxia 38 (SCA38) treatment: an open label extension study. Parkinsonism & Related Disorders. https://doi.org/10.1016/j.parkreldis.2019.02.040.

7. Future directions and objectives for next years

a. Summary (up to 2000 characters):

Mood disorders are an important health problem of the modern society and currently available therapies require long-term treatment and have limited efficacy, but the cellular and molecular mechanisms are largely unknown. We aim at studying the role GSK3 in mood disorders: 1. by analysis of its regulatory phosphorylation in blood sample from patients; 2. by single cell recordings in a mouse model of depression to uncover the responsible neuronal disfunction. A second project is to study the expression and physiological role of the potassium current mediated by Kv7 channels in cerebellar Purkinje cells, as a basis to understand the involvement of such current in neurologic disorders. A third project is to analyze the alterations of GABAergic signaling in the cerebellar cortex of a murine model of the Phelan McDermid syndrome, which is a genetic disease associated with motor deficits and autism.

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

Aim 1. Role of GSK3 in mood disorders. Mood disorders, including Major Depressive Disorder (MDD) and Bipolar Disorder (BD), are an important health problem of the modern society. Currently available therapies require long-term treatment and have limited efficacy. The discovery of the cellular and molecular mechanisms of depression is required for the development of therapies with higher efficacy. The involvement of GSK3 in mood disorders is supported by genetic studies [1-3] and investigations on the mechanisms of action of lithium, mood stabilizers and antidepressant drugs [4-5]. GSK3 controls neuronal excitability and synaptic transmission [6]. GSK3 is negatively regulated by phosphorylation at serine residues, while tyrosine phosphorylation promotes its activity. An aberrant GSK3 hyperactivity is present in patients with mood disorders [4,7.] but its activity and the specific role in MDD relative to BD are still unknown. Mutant mice with a constitutive GSK3 hyperactivity have

increased susceptibility to depression [8], but the molecular and electrophysiological mechanisms are not known.

Aim 2. Identification and physiological roles of Kv7 potassium channels in PCs.
Regulation of the resting membrane potential and the repolarization of neurons are important in regulating neuronal excitability. One ionic current that plays a key role in stabilizing neuronal activity is the M-current (I_M), a slowly deactivating, non-inactivating potassium current first identified nearly 40 years ago as the one underlying the excitatory effect of acetylcholine. The M-current is produced by the action of Kv7 channels encoded by members of the *KCNQ* gene family *KCNQ1–KCNQ5*, each of which encodes an individual potassium channel subunit Kv7.1–Kv7.5. Mutations in *KCNQ2* and *KCNQ3*, the genes encoding Kv7.2 and Kv7.3, cause a neonatal form of epilepsy, and activators of these channels have been identified as novel antiepileptics and analgesics. In hippocampal pyramidal neurons, Kv7 channels underlie several electrophysiological functions, including medium afterhyperpolarization, excitability control, spike frequency adaptation, and subthreshold resonance. In entorhinal cortex layer II pyramidal cells, Kv7 channels control membrane excitability. Despite the important physiological roles of Kv7 currents in the cell types where they have been studied, nothing is known about their properties or functional role in cerebellar Purkinje cells (PCs). PCs possess a unique repertoire of voltage gated channels with specific localization either in the dendrites or in the cell body or in the axon. As a consequence, electrical signaling and data processing in PCs is strikingly different relative to pyramidal cells. Current model simulations of PC function completely lack a Kv7 conductance, because of the gap of knowledge in this cell type. Finding the expression and the functional roles of Kv7 channels in PCs is highly relevant for a full understanding of the signal processing properties in the cell type and in cerebellar physiology.

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

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

The majority of our projects are aimed at finding the molecular and neural mechanisms of diseases caused by cerebellar dysfunction. The final aim of our project on SCA38 was to understand its pathogenic mechanism and to design a specific therapy. Regarding GSK3, a possible link with depression would open a new field of research on the molecular and neuronal mechanisms of this psychiatric disease, which is a main mission of our Institute. Knowledge of the role of Kv7 currents in PCs is necessary for the construction of biologically relevant simulations of this cell type and for the implications in specific brain disorders. Regarding autism, we want to provide mechanistic explanations of the role of the cerebellum in this disorder, so that therapeutic interventions can be envisaged. The mission of the Institute is exactly the same as ours, namely to advance scientific knowledge regarding brain disorders, including neurologic diseases like spino-cerebellar ataxias, and psychiatric diseases such as depression and autism and physiological functions implied in brain disorders.

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

AIM 1: Role of GSK3 in mood disorders.
Subaim 1.1: to discover GSK3 alterations in mood disorders. This part of the study concerns the expression and activity of GSK3 in patients with different mood disorders (DSM-5 Depressive and Bipolar Disorders). Specifically, we aim at studying patients with Major Depressive Disorder and Bipolar Disorder type I and type II. A first goal is to identify the GSK3 phosphorylation pattern specific for each type of mood disorder. We also plan to assess the effects of drugs, commonly used in mood

disorders therapy, on the GSK3 phosphorylation pattern. Alterations of the GSK3 phosphorylation pattern will be investigated in drug-free patients starting a treatment with lithium or other mood stabilizers like valproic acid or antipsychotics or antidepressants. The objective is to find which drugs interfere with GSK3, opening the way to studies on the role of GSK3 modulation in the therapeutic efficacy. We expect to also find correlations with dosage. Patients will be recruited both as inpatients and outpatients by Prof. Maina among subjects referring to the SCDU Psychiatry of the San Luigi Gonzaga Hospital. All patients with a DSM-5 diagnosis of MDD and BD will be enrolled; we will recruit patients in euthymia, during major depressive episodes and during (hypo)manic episodes. All patients will undergo a standardized clinical evaluation including clinical interview and rating scales (YMRS, HAM-D, MADRS). With patients' informed consent, the clinical evaluation will be accompanied by the collection of a blood sample, which will be analyzed by western blotting using antibodies for FGF14, total GSK3α and GSK3β, and for the phosphorylated forms of GSK3α, GSK3β. Patients will be divided in subgroups according to type and duration of therapy. A subsample of drug-free patients (both MDD and BD, at least 4 weeks) will also be recruited and followed since the beginning of therapy administration. GSK3α and GSK3β phosphorylated forms and total GSK3 and FGF14 protein in blood samples will be assessed, in order to find the changes induced by each type of therapy. For drug-free patients at least a second assessment of FGF14 and GSK3 activity will be performed after 8 weeks of therapy

Subaim 1.2: to discover the neuronal mechanisms of mood disorder in Gsk3 knock-in mice. The neuronal mechanisms controlled by GSK3 will be investigated in Gsk3 knock-in mice, which have a high sensitivity to mood disturbances. The first goal is to detect and study neuronal activity alterations in the prefrontal cortex of Gsk3 knock-in mice. We recently acquired Gsk3 knock-in mice, with constitutively active Gsk3. It has been reported that such mice have a high sensitivity to mood disturbances. Depressed behavior will be induced by chronic social defeat stress in mutant and wild-type mice, in which Gsk3 expression and phosphorylation will be studied. An electrophysiological analysis will be conducted in wild type and Gsk3 knock-in mice with induced depressive behavior. We plan to conclude this part of the study near the end of the first year of research. To study neuronal dysfunction related to mood disorders we'll record action potential firing in slices of prefrontal cortex of the murine models. Depression in patients and mice is associated with decreased neuronal activity in this brain region. Our goal is to detect action potential firing alterations caused by changes of Gsk3 activity in Gsk3 knock-in mice both naive and following induction of depressive-like behavior (compared with wild-type controls).

AIM 2. Identification and physiological roles of Kv7 potassium channels in PCs. Our project about Kv7 channels is aimed at identifying the subunits expressed in PCs, to ascertain whether a significant I_M current is present and which physiological roles are played in this type of neuron with unique functional features. In fact, PCs display large dendritic calcium spikes regulated by several potassium conductances, generate complex spikes in response to climbing fiber activity, and produce peculiar action potential firing patterns, which are a crucial signal of cerebellar motor control. The expression profile of Kv7 subunits will be assessed by RT-PCR and refined by immunohistochemistry. The Kv7 current and its role in PC firing will be studied by patch-clamp recordings in slices of cerebellum. A computer simulation of the PC will be constructed to better understand the role of Kv7 channels.

AIM 3: Role of cerebellum in autism spectrum disorders. Our latest results showed that Shank3 mutant mice have intact excitatory synaptic transmission in the cerebellar cortex, including the mGlu1 receptor mediated postsynaptic current. Preliminary results show that GABAergic signaling is altered in these mice. In the next year we plan to search for the alterations of GABAergic synaptic transmission in the cerebellum of Shank3 mutant mice and to relate them to the symptoms.

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

By the experiments of Aim1 we expect to identify the type/types of mood disorder associated with GSK3 alterations. A possible clinical impact is the possibility to utilize a GSK3 phosphorylation assay to guide and refine the diagnosis. We expect to characterize the GSK3-modulation profile of each type of therapy. A new assay to estimate the efficacy of therapy in each individual patient might derive from

this result. By the experiments of Gsk3 knock-in mice we expect to find a correlation between Gsk3 alterations in the animal model with those of patients. The study of action potential firing in the prefrontal cortex will be a first result in a new line of research aimed at discovering the neuronal mechanisms of mood disorders. We expect to find alterations in action potential firing caused by dysregulation of the Gsk3 pathway. This would open the way to the development of new drugs with a better efficacy relative to current therapies.

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

We plan to perform some of the measures of neuronal activity by *in vivo* two photon imaging, to confirm results derived from experiments in tissue slices. We plan to acquire mice with a genetically encoded calcium sensitive fluorescent protein or with a voltage-sensitive one (in collaboration with Dr. Knopfel of the Imperial College of London). In the next year we don't plan to perform stimulation experiments, but, following identification of the brain areas or nuclei where GSK3 modulates depression, we'll use *in vivo* optogenetic stimulation to assess the effects of activation or inhibition of specific neuronal populations in the relevant structures. This will allow us to identify the neurons and the pathways involved in the control of depression.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2019

Laboratory name: **Brain development and disease**

1 LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

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Personnel

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Role & expertise: Molecular Biology

2 ELENA TAMAGNO

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Role & expertise: Alzheimer Disease

3 MARINA BOIDO

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Role & expertise: Spinal cord injury, motor neuron diseases, Huntington disease, stem cells

4 MICHELA GUGLIELMOTTO

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Role & expertise: Alzheimer Disease

5 SERENA STANGA

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Role & expertise: Alzheimer Disease, Spinal muscular atrophy

6 ROBERTA SCHELLINO

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Role & expertise: Neurogenesis, Spinal muscular atrophy, Huntington disease, behavior

7 DANIELA RASA'

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Position: Scholarship holder

Role & expertise: Cell culture, Spinal muscular atrophy

8 ELENA SIGNORINO *

Degree: PhD Birthdate: 06/10/1976

Nationality: Italian Gender: Female

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Email: elena.signorino@gmail.com

Position: Technician

Role & expertise: Cell culture, molecular biology

* Since November 2019, ES has been hired by the Dept. Neuroscience as full time, permanent technician at NICO

2 CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Directly Available to NICO
2016-20	My-AHA	Vercelli A.	Horizon 2020	Coordinator	395000 €	8%
2017-20	Generation of functional striatal neurons for brain repair in Huntington Disease; ID 2015AY9AYB_002	Vercelli A.	PRIN (MIUR)	PI of research unit	75530 €	8%
2019		Vercelli A.	Smarathon	PI of research unit	14000 €	10%
2019		Vercelli A.	Atrofia spinale onlus	PI of research unit	25000 €	10%
2019	Spinal cord injury	Vercelli A.	FORB	PI	20000 €	10%
2019	Identification of new druggable targets andpotential therapeutic compounds for Spinal Muscular Atrophy, using a C. elegans model ofneurodegeneration	Vercelli A.	Telethon	PI of research unit	28000 €	10%
2017-2020	I mitocondri nell'Atrofia Muscolare Spinale: disfunzioni e mitofagia; ID 2017.2052	Boido M.	CRT	PI of research unit	28000 €	Not allowed
2019-2020	Development of combinatorial therapies for SMA; ID 22346	Boido M.	AFM Telethon	PI of research unit	12815 € (1st year)	Not allowed
2019-2022	The role of SMN protein in translation: implications for Spinal Muscular	Boido M.	Fondazione Telethon	PI of research unit	83600 €	10%

	Atrophy; ID GGP19115A					
2020-2021	Development of combinatorial therapies for SMA; ID 22346	Boido M.	AFM Telethon	PI of research unit	56000 € (2nd year)	Not allowed
pending	LAB-ON-CHIP E BIOSENSORI FUNZIONALI PER LO STUDIO DI BIOMARCATORI E MODELLI CELLULARI DI NEUROPATOLOGIA PER UNA MEDICINA PREDITTIVA E DI PRECISIONE (LAMARC)	Vercelli A	FISR	Coordinator	2155638.94	8% of the DNS part
Pending (first stage)	myFRAIL	Vercelli A	Horizon 2020	Coordinator	4,300,000	8% of the DNS part
pending	Targeting mitochondria in SMA with systems biology and drug repositioning	Vercelli A	CureSMA	PI	200000 USD	8%
pending	Sex hormones trigger different effect on protein Tau. A translational study to investigate the role of estrogens and oxidative stress to develop new gender antioxidant	Tamagno E.	Univ. Turin	coPI	10000 €	8%

	therapeutic approaches.					
pending	Ubiquitin C-terminal hydrolase (UCh) -L1 regulation a crucial link between obstructive sleep apnea syndrome (OSAS) and Alzheimer disease	Guglielmotto M.	Univ. Turin	coPI	10000 €	8%
pending	An innovative approach to contrast muscular atrophy in SMA: aids from octopus	Boido M.	MDA	Coordinator	200000 €	10%
pending	Scientific Meeting Grant "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies"	Boido M.	Company of Biologists	PI	2000 £	Not allowed

3 SCIENTIFIC ACTIVITIES IN 2019

Alessandro Vercelli, PI

Supervised PhD students: M. Lorenzati (Co-tutorship with A.Buffo), A. Naldi (co-tutorship with M. Bergui)

Honors, prizes, awards: Innovation Prize 4.0 A&T Torino February 2019
President Italian Society for Neuroscience (2020-2025)

Outreach activities

- International collaborations: 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); we have collaborations with Switzerland (University Lausanne, mithophagy in the CNS), and UK (miRNAs in spinal cord injury).
- Invited talks: 6 May 2019 Le scoperte di Giuseppe Levi a Torino. Accademia delle Scienze di Torino.
  12 July 2019 Invecchiare in modo attivo e in salute per prevenire la fragilità senza farmaci. University of Cagliari

18 July 2019 Development, MORPHOLOGY and CONNECTIVITY OF PYRAMIDAL NEURONS, University of Gdansk, Poland
2 October 2019 Molecular mechanisms of neuronal degeneration, University of Pisa

- Science communication: March 2019: Organiser of the Brain Awareness Week, Circolo dei Lettori, Torino
21 March 2019 La neurobiologia della fragilità. Convegno La malattia di Alzheimer, Ospedale San Luigi di Orbassano
3 October 2019 App e robot per combattere l'invecchiamento. Ciclo di conferenze della mostra l'Uomo Virtuale, INFN, Torino
- Editorial duties: Member of the Board of Editors of the Journal Digitcult
Associate Editor of Frontiers in Ageing Neuroscience
- others

Organizational activities and responsabilities at NICO: Scientific Director of the Foundation Cavalieri Ottolenghi
Speakers invited: E. Cherubini, EBRI (F.Rossi Lecture, 24 January)
Other organizational activities: Brain Awareness Week
Workshops, Schools or Conferences organized:
Technology transfer achievements (patents, etc.):

Marina Boido, Associate Professor

Supervised PhD students: na
Honors, prizes, awards: na
Outreach activities

- International collaborations: Prof. Artero, Univ. Valencia; Prof. Puyal, University of Lausanne, Switzerland; Prof. Soler, University of Lleida, Spain; Pharmafox company, Switzerland; Dr. Martinat, I-STEM, Corbeil-Essonnes
- Invited talks: Boido M. Mechanisms of cell death in Spinal Muscular Atrophy. Stazione Zoologica Anton Dohrn, 04.11.2019
- Science communication: - Boido M. Apoptosi e autofagia: due facce della stessa medaglia nella patogenesi della SMA? Update in Neuroscienze di Base: Morfologia e dintorni, Palermo, 21-22.01.2019.
- Boido M., Schellino R., Vercelli A. Autophagy and apoptosis: alternative or cooperating pathways in SMA? INN: Prospettive di Neuroscienze, Ferrara, 8-9 luglio 2019
- Boido M., Schellino R., Butenko O., Vrijbloed J.W., Fariello R.G., Vercelli A. ActR-Fc-nLG3: a new protein which improves motor performance with a limited hypertrophic effect in young and old mice. 18th National Congress of the Italian Society for Neuroscience, SINS 2019, Perugia 26-29.09.2019.
- Boido M., Schellino R., Butenko O., Vrijbloed J.W., Fariello R.G., Vercelli A. A new protein to improve motor performance with a limited hypertrophic effect in young and old mice. Neuroscience 2019, Chicago, 18-23.10.2019.
- Boido M., Ghibaudi M., Gentile P., Favaro E., Fusaro R., Tonda-Turo C. Chitosan-based hydrogel as a promising tool to support the paracrine activity of mesenchymal stem cells in spinal cord injury. 2nd BraYn - Brainstorming Research Assembly for Young Neuroscientists, Milano, 14-16.11.2019.
- Editorial duties: Guest Associate Editors for Biomaterials, proponent of the Research Topic "Advances in the Development and Application of

	Natural-Based Polymers for Nervous- and Musculoskeletal-Associated Disease Treatment".
• others	Involvement in the conception of the "Brain Game" installation for the Exhibition "Uomo virtuale. Corpo, mente, cyborg", at Mastio della Cittadella, Torino, 04.01.2019 – 13.10.2019).
Organizational activities and responsabilities at NICO:	Responsible for the infrastructure in open access "In vivo and behavioral studies"; responsible for "Leica SP5 confocal microscope", "E800 Nikon fluorescence microscope and Neurolucida software (Neurolucida Vercelli)"; responsible for cryostat room
Speakers invited:	na
Other organizational activities:	CEO of S&P BRAIN SRL spinoff
Workshops, Schools or Conferences organized:	Regional coordinator (Piedmont) of Olympics in Neuroscience; Regional stage, Torino, 16.03.2019 Giornate del Dipartimento di Neuroscienze, Torino 12-14.12.19
Technology transfer achievements (patents, etc.):	na

Serena Stanga, Assistant Professor in tenure track (RTDa)

Supervised PhD students:	na
Honors, prizes, awards:	Winner of a Travel Grant for the participation at the National Congress of the Italian Society for Neuroscience (SINS) September 26/29-2019, Perugia (Italy).
Outreach activities	
• International collaborations:	Pr. Pascal Kienlen-Campard, UCLouvain, Bruxelles (Belgium); Pr. Giulio Muccioli, Louvain Drug Research Institute - LDRI, UCLouvain, Bruxelles (Belgium); Pr. Donatienne Tyteca, Institut de Duve, UCLouvain, Bruxelles (Belgium).
• Invited talks:	"The crucial role of mitochondria in neurodegenerative diseases: focus on Alzheimer's disease and Spinal Muscular Atrophy" Metabolism meeting, Molecular and Biotechnology Center MBC, Torino, Italy, 12.11.2019
• Science communication:	- Stanga S, Pasini G, Pergolizzi B, Mezzanotte M, Roetto A, Boido M, Vercelli A; "Mitochondrial dysfunction: a new biomarker candidate for Spinal Muscular Atrophy?". 2nd BraYn - Brainstorming Research Assembly for Young Neuroscientists, Milano 14-16/11/2019. - Stanga S, Pasini G, Pergolizzi B, Boido M, Vercelli A; "Mitochondrial dysfunction in Spinal Muscular Atrophy". 18th National Congress of the Italian Society for Neuroscience, SINS 2019, Perugia 26-29/09/2019. - Stanga S, Schellino R, Signorino E, Rasà D, Boido M & Vercelli A; "Meccanismi di morte neuronale nell'atrofia muscolare spinale", Giornate del Dipartimento di Neuroscienze, Torino 12-14.12.19.
• Editorial duties:	Review editor for the section Neurodegeneration of the Journal Frontiers in Neuroscience
• others	na
Organizational activities and responsabilities at NICO:	Responsible for the Cell Culture room and for the Molecular Biology room
Speakers invited:	Pr. Pascal Kienlen-Campard, UCLouvain, Bruxelles Belgium), 30.05.2019
Other organizational activities:	na

Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Roberta Schellino, Postdoc fellow

Supervised PhD students:	na
Honors, prizes, awards:	- Fellowship as selected participant at the ISN-JNC Flagship School. International Society of Neurochemistry. Alpbach, Austria. September 9-16. -Selected speaker at the 2nd Brainstorming Research Assembly for Young Neuroscientists (BRAYN). November 14-16, 2019. Milan, Italy.
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	- "Impact of long-term transplantation of hMSN progenitors and enriched environment on striatal circuits reconstruction and recovery in a rat model of Huntington's disease" Schellino R, Boido M, Besusso D, Parolisi R, Vercelli A, Cattaneo E, Buffo A. Conferenza NECTAR. Cardiff, Galles (UK). 28-29 Novembre 2019. - "Human medium spiny neuron progenitors grafted into an HD rat model early integrate into the host circuits, express striatal markers and support functional recovery." Schellino R, Boido M, Besusso D, Parolisi R, Belloli S, Murtaj V, Moresco RM, Buffo A, Cattaneo E, Vercelli A. 2nd Brainstorming Research Assembly for Young Neuroscientists (BRAYN). Milano, Italia. 14-16 Novembre, 2019.
• Editorial duties:	na
• others	na
Organizational activities and responsabilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:

- Olympics in Neuroscience; Regional stage, Torino, 16.03.2019
- Alternanza Scuola-lavoro: 10-days-long stages (11-21.06.2019) for high school students (tutoring and laboratory activities, formulation and validation of scientific hypothesis, data collection, interpretation and discussion of results).
- NICO porte aperte: Open day at the Neuroscience Institute Cavalieri Ottolenghi

4. Research activity in 2019

a. Summary (500 characters)

We study CNS development (from the embryo to the aged) and the common neurobiological mechanisms and molecular pathways leading to normal development and to neurodegeneration. We are interested in neuronal cell death pathways, which we study in development and in experimental models of SMA and AD. Finally, we are studying cell therapy in preclinical experimental models of ALS, SCI and HD.

b. Background and rationale (3000 characters)

The study of the CNS represents a great challenge to the scientist of the 21st century, and neurodevelopmental and neurodegenerative disorders provide major insights in the understanding of its anatomy, physiology and pathology and the design of new therapies. Many cellular events and mechanisms occurring during development may have profound influences on the adult nervous system, and healthy aging may be considered as the last phase of neural development.

Recently, Europe with the Human Brain Project and USA with the Connectome project, together with similar projects launched by other countries such as Japan and China, targeted the micro-, meso- and macro-connectome from a normal and pathological point of view. In the meanwhile, collaborative projects such as the JPND and ERA-NET Neuron in Europe aim to investigate the basic mechanisms underlying neurodegenerative diseases, with a translational aim to design new diagnostic/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 ("hubs") may be more liable to disease. Therefore, only a multidisciplinary and holistic approach, from molecules to brain areas, from development to disease, can provide new insights and concept on brain function, disease and repair. Understanding the CNS development 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. We have also 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, since 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.

c. Objectives (1000 characters)

We aim to understand the structural/functional building blocks of the cerebral cortex and their circuitry, as substrate for brain activities and entities which may be disrupted in several congenital and degenerative diseases. 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 disease models

In several neurodegenerative diseases, the pathology is not cell-autonomous: therefore, we study neuroinflammation in ALS and SMA, and how to prevent it.

Stem cells are still a growing field of research: we study the integration of hES-derived striatal progenitors grafted into the striatum (in HD). Moreover, we use neural and/or mesenchymal stem cells to treat neurodegenerative/traumatic diseases (in ALS and SCI), to provide trophic and immunomodulatory substances to host neurons.

d Results (4000 characters)

Development of cerebral cortex
We study the development of corticofugal axons. With A. Buffo we study the axon/oligodendrocyte (OL) relationship, and the role of OL activity: we identified a MAP-kinase, JNK, as a key molecule in OL development and axon myelination, both *in vitro* and *in vivo*.

Mechanisms of neuronal death and neuroinflammation in ALS, SMA and AD
Re SMA, we are studying mitochondrial alterations, *in vitro* and *ex vivo*, by IF, proteomic analysis and 3D electron microscopy. We are also testing SMN-independent therapeutic approaches (including a GHRH agonist and MSC-derived exosomes) to delay disease progression and counteract muscular atrophy.
Re ALS, we evaluated the role of Nurr1, a nuclear receptor implicated both in neuroprotection and immunomodulation in PD and MS: in our ALS mouse model, Nurr1 activation can modulate neuroinflammation and protect motor neurons; with A. Bertolotto, we evaluated Nurr1 expression in ALS patients (*submitted to Disease Models and Mechanisms*). Moreover, with IZS Torino, we contributed to characterize a transgenic pig model of ALS (Crociara et al., Neurobiol Dis 2019).
Re AD, we study the role of transcription factor NF-κB as a player in this event. We found that the ischemic damage alone or in association with Aβ1-42 activates the NF-κB pathway, induces an increase of BACE1 and a parallel inhibition of Uch-L1 and TREM2, both *in vitro* and *in vivo*, in Tg 5XFAD and in human brains of sporadic AD. This mechanism creates a synergistic loop that fosters inflammation. We also demonstrated a significant protection exerted by the restoration of Uch-L1 activity. The rescue of the enzyme is able to abolish the decrease of TREM2 and the parameters of neuroinflammation.

Stem cell therapy in HD and ALS
With E. Cattaneo and A. Buffo, we are exploring the potential of human ESCs in an experimental model of HD (animals were housed in standard cages or enriched environment conditions, to further boost the cell integration), observing good results in terms of cell replacement, establishment of new connections and behavioral performance, both at short- *(submitted to Stem Cell Reports)* and long-terms *(in preparation)*.
With A. Vescovi, we contributed to assess the therapeutic potential of clinical-grade human neural stem cells in a rat model of ALS (Zalfa et al., Cell Death Dis. 2019).

Spinal cord injury
We previously demonstrated the therapeutic effects of stem cells (NPs and MSCs) in SCI. To further improve the graft success, with Dr. Tonda-Turo (Polito), we developed and tested biomimetic injectable hydrogels (chitosan) in which stem cells can be encapsulated. The *in vitro* and *in vivo* results revealed the capability of chitosan to support survival and paracrine activity of MSCs (Boido et al., Sci Rep. 2019).
Finally, with Prof. Dalmay, we performed a profiling of miRNA expression in a mouse model of SCI, in order to identify key-miRNAs involved in the regulation of axon growth (*in preparation*).

Active and Healthy Ageing
AV coordinates the Horizon 2020 project entitled My-AHA (Active and Healthy Ageing). Stemming from a holistic view of interrelated frailties, cognitive decline, physical frailty, depression and anxiety, social isolation and poor sleep quality, My-AHA proposes an ICT platform for early detection of pre-frailty and intervention to sustain active and healthy ageing and slowing or reversing further decline. The main aim of My-AHA is to reduce frailty risk by improving physical activity and cognitive function, psychological state, social resources, nutrition, sleep and overall well-being in older adults. After a pilot study on a limited number of subjects, a randomized controlled study will end in December 2019 and the data will be analysed in the first trimester 2020. Preliminary results are very positive. In a limited number of subjects (control vs pre-frail vs pre-frail + intervention) a fMRI study is undergoing at the Brain Imaging Center in Turin.

e Advancement in the field (1000 characters)

Our group is working in several hot topics in Neuroscience, such as axonal development/growth in the normal brain and disease, 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. Also, we are involved in the technical revolution about microscopy to investigate micro- and meso-connectome, with the use of confocal microscopy and 2P microscopy. We are collaborating with groups working on fMRI aiming to macroconnectome, and the PI is involved in the Brain Imaging center of the Univ. Torino. We are also involved in several studies to identify and test new drugs for neurodegenerative diseases, and new biomaterials to support CNS repair. Moreover, we are involved in studies using Internet of Things, Medical Devices and A.I. 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.

f Publications

Boido M, Ghibaudi M, Gentile P, Favaro E, Fusaro R, Tonda-Turo C. Chitosan-based hydrogel to support the paracrine activity of mesenchymal stem cells in spinal cord injury treatment. Sci Rep. 2019 Apr 25;9(1):6402.

Cofano F*, Boido M*, Monticelli M, Zenga F, Ducati A, Vercelli A, Garbossa D. Mesenchymal Stem Cells for Spinal Cord Injury: Current Options, Limitations, and Future of Cell Therapy. Int J Mol Sci. 2019 May 31;20(11). pii: E2698.

Crociara P, Chieppa MN, Vallino Costassa E, Berrone E, Gallo M, Lo Faro M, Pintore MD, Iulini B, D'Angelo A, Perona G, Botter A, Formicola D, Rainoldi A, Paulis M, Vezzoni P, Meli F, Peverali FA, Bendotti C, Trolese MC, Pasetto L, Bonetto V, Lazzari G, Duchi R, Perota A, Lagutina I, Quadalti C, Gennero MS, Dezzutto D, Desiato R, Boido M, Ghibaudi M, Valentini MC, Caramelli M, Galli C, Casalone C, Corona C. Motor neuron degeneration, severe myopathy and TDP-43 increase in a transgenic pig model of SOD1-linked familiar ALS. Neurobiol Dis 2019 Apr;124:263-275.

Guglielmotto M, Repetto IE, Monteleone D, Vasciaveo V, Franchino C, Rinaldi S, Tabaton M, Tamagno E. (2019) Stroke and Amyloid-β Downregulate TREM-2 and Uch-L1 Expression that Synergistically Promote the Inflammatory Response. J Alzheimers Dis.71:907-920.

Naldi A, Provero P, Vercelli A, et al. Optic nerve sheath diameter asymmetry in healthy subjects and patients with intracranial hypertension [published online ahead of print, 2019 Oct 5]. Neurol Sci. 2019;10.1007/s10072-019-04076-y.

Roig-Puiggros S, Vigouroux RJ, Beckman D, Bocai NI, Chiou B, Davimes J, Gomez G, Grassi S, Hoque A, Karikari TK, Kiffer F, Lopez M, Lunghi G, Mazengenya P, Meier S, Olguín-Albuerne M, Oliveira MM, Paraíso-Luna J, Pradhan J, Radiske A, Ramos-Hryb AB, Ribeiro MC, Schellino R, Selles MC, Singh S, Theotokis P, Chédotal A. Construction and reconstruction of brain circuits: normal and pathological axon guidance. J Neurochem. 2019 Oct 20. doi: 10.1111/jnc.14900. Review.

Schellino R, Boido M, Vercelli A. JNK signaling pathway involvement in spinal cord neuron development and death. Cells, Vol. 8, Pag 1576.

Zalfa C, Rota Nodari L, Vacchi E, Gelati M, Profico D, Boido M, Binda E, De Filippis L, Copetti M, Garlatti V, Daniele P, Rosati J, De Luca A, Pinos F, Cajola L, Visioli A, Mazzini L, Vercelli A, Svelto M, Vescovi AL, Ferrari D. Transplantation of clinical-grade human neural stem cells reduces neuroinflammation, prolongs survival and delays disease progression in the SOD1 rats. Cell Death Dis. 2019 Apr 25;10(5):345.

7. Future directions and objectives for next years

Please describe the following information relevant to the research that you are planning to do – Character limit is mandatory. Please highlight the added value of collaborations within the NICO where applicable.

a. Summary (up to 2000 characters):

We intend to exploit our previous research on i) axonal growth in the CNS, ii) molecular and cellular mechanisms of neuronal death in neurodegenerative diseases, iii) network analysis at multiscale level, iv) stem cell therapy. We want to identify some new therapeutic targets (such as autophagy for neurodegenerative diseases, JNK-related molecules for neuronal death, miRNAs for neural development, neuronal cell death and axonal growth). Recently, there is a growing interest on mitochondria in neurodegenerative diseases: we have therefore started a new line of research. We also intend, in collaboration with internal and external groups, to import in the institute new techniques, such as the organoids for in vitro analysis of brain development and disease modeling, 2 photon microscopy for in vivo analysis and 3D electron microscopy. We will continue to collaborate with the Brain Imaging center to study human brain morphology (voxel-based morphometry and tractography) and functional networks (fMRI).

Moreover, the PI is the coordinator of a 4-year Horizon 2020 grant, my-AHA (my Active and Healthy Aging), from January 2016. His task is mostly related to the overall organization of the activities, to be performed on human subjects and not involving directly the structures of NICO. Nevertheless, being the scientific coordination acted by A. Vercelli, this will allow the NICO to receive overheads. In particular we are preparing applications for the next EC calls (deadline April 2020) on Ageing to improve early detection of age-related frailty in the individual by use of Artificial Intelligence (machine and deep learning). This will be the follow up of the my-AHA project. On the same subjects, in collaboration with F. Cauda (Dept. Psychology) and I. Rainero (Dept. Neurosci) we are performing morphometric (voxel-based morphometry and tractography) and functional (fMRI) analysis of the brain and networks.

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

There is a growing interest in studying the development and disease of the CNS in terms of networks: genes, miRNAs and molecular networks at a ultramicroscopic level of magnitude, synaptic networks at the microscale, and anatomical and functional networks at the meso- and macroscale. Perturbances in the networks at the different scale levels may result in developmental or neurodegenerative disorders. To this extent, one may refer to “damage networks”: is it possible that some brain areas are more vulnerable than others to damage, or maybe more relevant than others for the onset of disease and of functional disorders? Such perturbances may be responsible for developmental disorders, such as schizophrenia, autism, epilepsy where there is an altered connectivity in terms of synapses and axonal connections, and of excitability. Also, neural networks may underlie the spread of neurodegenerative diseases in the CNS, such as for the Braak hypothesis of the molecular and cellular damage. Understanding the mechanisms of the onset and establishment of neural disorders at different scale levels is dramatically relevant to design neuroprotective and repair strategies to prevent and modify disease progression. These strategies therefore may be at a genetic, molecular, cellular and behavioral level, and must be considered in a holistic strategy. To this aim we will collaborate with F. Di Cunto (molecular biologist at NICO) and F. Cauda (fMRI) to investigate the existence of damage networks in some neurodegenerative and psychiatric diseases. We are strongly connected to clinicians working within the field of neurodegenerative diseases, such as A. Chiò (Turin, ALS), I. Rainero (Turin, AD), Tabaton (Genoa, AD), P. Rocca (Turin, Schizophrenia) and T. Mongini (Turin, SMA): we intend to continue and implement this kind of collaborations in order to have a continuous exchange of ideas, data and therapeutic strategies to favor a back and forth flow of information and bidirectional translation to find innovative therapeutic solutions.

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

Our research aims to understand some basic mechanisms of neural development, whose alterations may be involved in the onset of neuropsychiatric diseases, and of neuronal cell death in neurodegenerative diseases. We are also interested in investigating the micro-, meso- and macro-scale of the CNS as the fundamental principles of brain function and disease.

Our findings are finally aimed to develop new therapeutic strategies to prevent neurodegenerative diseases and to brain repair. Therefore, we believe that our research is perfectly fitted to study "the interdependence between physico-chemical state of the human body and the expression of the psyche", and fully integrated with the mission of the Neuroscience Institute.

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

Axonal growth

A fundamental issue in the building of neural connections and their conductive properties consists in axon formation and maintenance. We will study with A. Buffo the relationship between cortical axons and OLs and their precursors, and some molecules which may affect this interaction in the normal brain and in experimental models of disease (as schizophrenia).

SCI: we intend to employ nanocarriers, trackable by MRI, attracted by inflammatory sites, and able to deliver in situ molecules/drugs inducing axonal growth/sprouting: in collaboration with E. Terreno (MBC Turin) we are currently developing similar nanocarriers to label lymphocytes (B and T) and evaluate their trafficking in MS (FISM pilot grant, Dr. Boido co-applicant). Moreover, with G. Fiorito (SZN), we intend to exploit the outstanding regenerative abilities of the cephalopod mollusk Octopus vulgaris, to identify key genes responsible of axonal sprouting in octopus and then verify their functionality in murine models of SCI or SMA.

Stem cell therapy

HD: with A. Buffo and E. Cattaneo, we intend assess the long-term survival, maturation, integration and function of optimized hPSCs-derived MSNs; we will assess the impact of mutant HTT associated pathology on grafted MSNs survival and function; we will also improve the graft functional efficacy through physical training and enriched environment.

Molecular mechanisms of cell death and neuroinflammation, and therapeutic approaches

We will investigate the role of specific genes and molecules involved in mechanisms of neuronal cell death and neuroinflammation in neurodegenerative diseases.

SMA: we are evaluating the activation of glial cells and the apoptotic processes at central and peripheral level. To fully understand the functions of SMN, with G. Viero (CNR, Trento), we will verify the presence of translation defects in our murine model of SMA: by analyzing both transcriptome and translatome in SMA mice, we will evaluate the translation efficiency in different stages of disease. Finally, we will test both SMN-dependent and -independent approaches for SMA: i) with R. Artero (Univ. Valencia), we will test some FDA-approved drugs with the ability to increase SMN protein levels; ii) with R. Mariotti (Univ. Verona), the ICV injection of MSC-derived exosomes in SMA mice, to counteract neuroinflammation and apoptosis, and to delay the disease progression; iii) with R. Granata (Torino) MR-409 (a GHRH agonist) to improve muscular functionality.

AD: our overall aim is to pursue the hypothesis that gender influences the effect of Aβ42 monomers on pathological Tau conformational change. A series of clues support the amyloid hypothesis: accumulation of Aβ peptides is the primary and early event that induces neuronal degeneration, characterized by accumulation of conformational altered and aggregated Tau protein. To investigate this hypothesis, we developed a powerful system based on male mice expressing the WT human Tau which were subjected to ICV injections of Aβ peptides. We discovered that Aβ42 monomers, but not oligomers: i) produce PHF-like conformation of Tau protein, ii) induce two phosphorylated epitopes absent in normal Tau (Ser396 and Ser422) through the activation of GSK3β, JNK and ERK 1/2 kinases. Our results have practical implication, because currently the major efforts of AD therapy are focused on removal of Aβ oligomers, and not monomers. Surprisingly, our preliminary data revealed that Aβ42 monomers are not able to produce the conformational changes of Tau protein in female mice suggesting that the abnormal conformation of Tau induced by monomers could be affected by gender. Recent

epidemiological studies showed that two-thirds of AD patients are women, and this cannot be attributed only to their higher life expectancy. The connection among these events is probably due to the loss of protective actions exerted by estrogens during the fertile life and this relationship will be investigated.

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

Some of our i) research topics, ii) methodologies employed and iii) external collaborations with top institutes, scientists and biotech companies, allow us to be involved in hot topics of research. Our studies on axonal thickness and its plasticity depending on the neuronal pattern of origin/projection, and on activity represent a new field which may have very important significance not only for normal development but also for disease.

Our experience on some molecular pathways related to neuronal death, such as JNK and those related to autophagy is a specific competence which allowed us to design new therapeutic drugs.

The emergence of new concepts in brain function and disease in terms of networks and damage networks may be fundamental for investigating the onset of disease and eventually prevent its full development. Also, our group is one of the major groups working with stem cell therapy at a preclinical level in Italy and Europe.

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

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. The collaboration with other groups at the Polytechnic and INRIM (Istituto Nazionale di Ricerca Metrologica) will allow to design biosensors and lab-on-chip to the detection of biomarkers.

We plan to use of human brain organoids derived from iPSCs, to study the CNS development and to "mimic" model of neurodegenerative diseases.

We also intend to exploit the already existing clearing protocols and, in association with light sheet microscope use (coming soon at NICO), we will perform detailed anatomical studies (e.g. to deeply analyze the integration of human embryonic stem cells in an experimental model of HD). The forthcoming entry of Dr. Calì in our group, and the recent installation at the Polytechnic of Turin of a 3D EM, will provide access to a new technique in our studies.

The collaboration with the group of prof. Cauda (Department of Psychology), which already allowed to obtain significant results on the functional connectivity of the human insula by fMRI, will allow to use voxel-based morphometry, fMRI and tractography to study human anatomical and functional connectivity, and structure of the brain in ageing subjects.

Recently, we established a collaboration with G. Boella (Head of the Department of Informatics) to apply Artificial Intelligence (machine and deep learning) to our studies on neurodegenerative diseases in patients and human subjects. A collaboration is also under discussion to develop a neuroinformatic approach in studies of neurodegenerative diseases with G. Boella, F. Di Cunto and P. Provero (Scientific Director, Genomics and Bioinformatics Torino University Service, soon joining the Department of Neuroscience).

27/9
Alan Perotti, Researcher at the Institute for Scientific Interchange (ISI Foundation), Turin
DEEP LEARNING. Solutions and a problem

19/9
Ludovic Telley, Faculty of Biology and Medicine - University of Lausanne, Switzerland
Genetic aspects of the temporal patterning of cortical neurogenesis

14/6
Giovanni Petri, ISI Foundation, Torino
Brain functional shapes: how topology characterizes healthy and altered brain function

31/5
Pascal Kienlen-Campard, Université catholique de Louvain
How protein folding drives the onset and the progression of Alzheimer's disease

30/5
Prabahan Chakraborty, National Centre for Biological Sciences, Tata Institute of Fundamental Research, India
ALL IN THE TIMING: EFFECT OF STRESS ON MORPHOLOGY AND BEHAVIOUR

3/5
Samuele Chiaramello, PhD
Cultivating Emotional Balance

19/4
Alessia Caramello, Francis Crick Institute, London
Early SOX9 expression in the archicortex is required for gliogenesis, granule neuron progenitor migration and dentate gyrus development

10/4
Mehrnaz JAFARIAN-TEHRANI, Université de Paris Descartes
Alpha-secretase ADAM10: a therapeutic target for remyelination after CNS demyelination

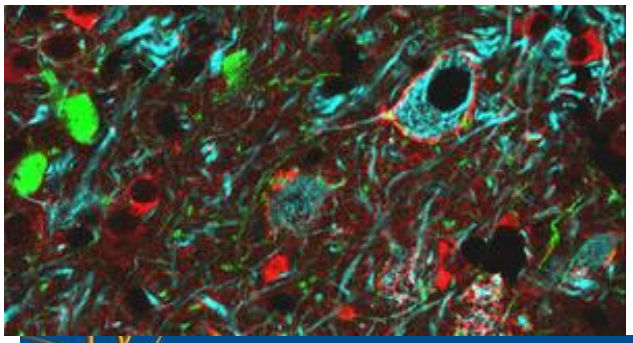
14/3
Francesco Bifari, BIOMETRA - Università di Milano
Meninges are a reservoir for new functional cortical neurons.

1/2
Giovanni Ferrara, Università di Genova
Does Nerve-glial antigen 2 (NG2) play a role in dendritic cell activation?

12/12
Seminars in Neuroscience (DBIOS - Dottorato in Neuroscienze - NICO)
Dipartimento di Scienze della Vita e Biologia dei Sistemi

Prof. Stefano Vicini, Department of Pharmacology and Physiology - Georgetown University, Washington DC, USA - Visiting Professor in Neurophysiology
Electroconvulsive Shock Enhances Responsive Motility and Purinergic Currents in hippocampal Microglia

Dr. Gabriele Losi, Istituto di Neuroscienze CNR - Dip. Scienze Biomediche, Università di Padova
The role of astrocyte calcium microdomains in prolonged LTP maintenance in the perirhinal cortex
7/6
Live Cells Analysis Workshop
9/4
Polo didattico dell'AOU San Luigi Gonzaga (Aula blu)
Monitoring Health and Disease Progression with Ultrasensitive Biomarker Analysis



Istituto Nazionale di Neuroscienze
Consorzio Interuniversitario di Neuroscienze

NICO PROGRESS REPORT 2019

INN Open Neuroscience Forum

Ogni due venerdì i nostri giovani ricercatori aggiornano i colleghi sulle loro ricerche.
http://www.nico.ottolenghi.unito.it/Agenda/NICO-Progress-Report

20/12 - Ilaria Bertocchi (gruppo Eva)

11/12 - Marina Boido (gruppo Vercelli)
Nanoparticles and biomaterials as tools for the study and the treatment of spinal cord injury

29/11 - **Serena Stanga** (gruppo Vercelli)
The crucial role of mitochondria in neurodegenerative diseases: focus on Alzheimer's disease and Spinal Muscular Atrophy

4/10 - **Enrica Boda** (gruppo Buffo)
Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model.

20/9 - **Marco Fogli** (gruppo Peretto)
Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion

6/9 - **Gianmarco Pallavicini** (gruppo Di Cunto)
Characterization of Citron kinase dead mice.

26/7 - **Roberta Parolisi** (gruppo Buffo)
Detrimental and protective action of microglial extracellular vesicles on myelin lesions

21/6 - **Giulia Nato** (gruppo Buffo)
Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion

17/5 - **Valentina Cerrato** (gruppo Buffo)

26/5 - **Ilaria Balbo** (gruppo Tempia)
Diet-based effects of PUFAs on a murine model of Spinocerebellar Ataxia 38 (SCA38)

5/4 - **Martina Lorenzati** (gruppo Buffo e Vercelli)
Allele specific silencing in ADLD: a proof-of-principle in OPCs and iPSCs-reprogrammed neurons

21/4 - **Brigitta Bonaldo** (gruppo Panzica)
CHRONIC PERINATAL EXPOSURE TO BISPHENOL A: ANALYSIS OF THE EFFECTS IN EAE MODEL OF MULTIPLE SCLEROSIS

8/3 - **Francesca Montarolo** (gruppo Bertolotto)
ADHD-like phenotypes in NURR1 knock-out mice

22/2 - **Isabella crisci** (gruppo Peretto)
Fate mapping of adult hippocampal neural stem/progenitor cells in a model of neuroinflammation

8/2 - **Marilena Marraudino** (gruppo Panzica)
GPER neuronal and glial cells expression in the hypothalamus of adult rats: sexual dimorphic distribution and differences during the estrous cycle

25/1 - **Roberta Schellino** (gruppo Vercelli)
STEM CELL THERAPY FOR HD: WHEN THE ENVIRONMENT MATTER