FONDAZIONE
CAVALIERI OTTOLENGHI

NICO
Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2020

INDEX

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

NICO
Neuroscience Institute Cavalieri Ottolenghi

2010-2020 10 years Anniversary

NICO 2020 by the numbers

9
Research Groups
57 Scientists
55 Graduating Students

72
Peer-Reviewed
Publications

64
Collaborative Initiatives
with
International
Research Groups

61
On-going/Granted
Research Projects

3
Scientific
Conferences/workshops
organized
by NICO members

20
Invited speakers

1
Spin-off Company

1
Biobank

54
Outreach Activities
17 Invited Talks
37 Science Dissemination Initiatives

14
trained
PhD students

3900
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, Lisbon, 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.

“On January 13-14, 2020 the Evaluation Committee made by members of the scientific advisory board conducted an onsite visit and met with PIs and lab members of each research group presently active at NICO to evaluate the scientific productivity and business organization of the institute and to suggest future directions. Over these two days of discussion the committee has come up with the following conclusions and suggestions concerning the institute as a whole and the individual research groups it hosts. Overall the committee had a very positive impression of the institute, its scientific and administrative accomplishments and of the many investigators that foster its scientific productivity. All committee members were impressed by the spirit of collaboration existing between the different research groups, and by the wide spectrum of techniques and projects. It was also noted that through a well-managed shared instrumentation plan the institute maximizes the return on investment for advanced instrumentation. A few shortcomings were also noted, mostly, but not entirely, related with the overall ecosystem of scientific research in Italy. The specific points that were discussed are summarized here, starting with the general considerations and then followed by evaluations of the individual research groups. “Overall, the Institute has been very successful in establishing an excellent, collaborative research environment at the University of Turin. Outreach activities are outstanding and far exceed what is typical of italian institutions, showing notable commitment to engage with the public and to search for non-conventional funding sources.” (file:///C:/Users/Alessandro/Downloads/NICO%20Report%20Jan%202020%20site%20visit%20(8).pdf).

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 and in neurologic and psychiatric diseases.

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 14 PhD students. Moreover, as lecturers at the schools of Biology, Biotechnology, Pharmacy, Medicine, Psychology and Veterinary Medicine, they are involved in the preparation of many theses for Bachelor and Master degrees.

Currently, NICO laboratories hosts students who are developing their Bachelor or Master thesis projects and stage students, 55 in total. 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 (Microscopia avanzata | Università di Torino (unito.it)).

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 and 2019 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. In 2020, the third "virtual" edition was held in live streaming, with about 600 registered participants and 195 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 (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). 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)
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 (renewed in January 2021) and with San Luigi Hospital of Orbassano (renewed in 2019), the NICO hosts:
- **University staff**: 1 honorary professor, 5 full professors, 9 associate professors, 11 university research assistants, 1 technician, 19 post-docs and 14 PhD students;
- **Hospital staff**: 1 Head physician, 1 manager biologist, 3 specialists in Clinical Biochemistry, 3 laboratory technicians.

About 55 graduating students of Biology, Biotechnology, Medicine and Psychology perform experiments for their thesis at the NICO. This is a reduced number compared to the previous years, due to the restrictions to the access to the structure due to the COVID-19 pandemics.

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. A Zeiss Axioscan high throughput image acquisition station was bought and installed in 2019 and is currently used by researchers of the Institute. Also, in 2020 the light sheet UltraMicroscope II (Miltenyi Biotec) was installed. 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 devoted to the experimental animals include rooms dedicated to animal housing and husbundry, 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 for the use in animals of 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. A cell culture room exclusively devoted to the development of human

in vitro models (derivatives of human pluripotent stem cells) for neurodevelopmental and neurodegenerative diseases has been established in 2020.
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 and action potential conduction. There is one imaging system for real time fluorimetric measurement of free ions (calcium, sodium and others) in single cells, associated with path-clamp recording of membrane voltage.

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 2020

In 2020, all instruments of the microscopy facility (see above) were put together to create PICO. PICO (Platform for Imaging Cavalieri Ottolenghi; https://www.nico.ottolenghi.unito.it/eng/PICO-

Platform-for-Imaging-Cavalieri-Ottolenghi) is a research centre that offers services for both sample preparation and state of the art in situ, in vivo and in vitro imaging.
Since imaging and neuroanatomy are a cultural heritage for the University of Torino, NICO invested consistent funding to potentiate the PICO imaging platform, as it was identified as a strategic target for the local, national and international growth of both the institute and the university. In this frame, as highlighted above, substantial funding (1.7 million euros) from the MIUR grant for the Departments of Excellence awarded to the Department of Neuroscience was used to empower the facility with the Axioscan, the light sheet microscope and the implementation of additional features to the two-photon microscope Nikon (A1MP).
By doing so, NICO further potentiated the technical specialties offered by the facility, one of the few in Italy. As a reflection of the growing quality of research of the institute, the University of Torino has also decided to contribute to the development of the facility with several initiatives. There will be one opening for a tenured position for a full time technician that the Department of Neuroscience will dedicate to the imaging, working side by side with the imaging scientist at PICO.

Personnel
New personnel were recruited by the University Departments collaborating at NICO: in particular, Dr. Calì that joined the group of A. Vercelli. In the meanwhile, Dr. Boda was promoted associate professor.

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 he joined 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 organized a series of international 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".
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 has remained stable in 2020 (3,224,000 €), compared to 2019 (approximately 3,170,000 €). The number of funded projects was 61 in 2020, compared to 53 in 2019 reflecting the increased number of funding requests in the previous year.

Effects of COVID-19 pandemics on NICO

The general lockdown occurring in Italy from beginning of March due to the diffusion of COVID-19 affected also the lab activities at NICO. Even though research institutes were allowed to continue to work, in agreement with the University of Torino the NICO entered the lockdown in March 2. During this period only essential activities were allowed, i.e. work related to the Neurologic Clinic of the Clinical Neurobiology lab and maintenance of animals and cell cultures.

Nevertheless, we started an intense activity of online seminars (either from internal or external speakers) and progress reports, which were increased to three times/a week. In parallel, each group increased their online lab meetings, to discuss research project and prepare publications. This was a very profitable and productive period, which allowed our community to huddle together and react from the psychological point of view. This was particularly important for our students and young researchers. We also took advantage of the situation to implement home working by allowing remote access to some of our scientific instruments (microscopes and servers for data storage).

On May 11, some weeks in advance compared to the University Departments, the Institute resumed the lab activity, and the webinars were limited again to once a week, in order to allow lab work. The work was organised in two rounds, 7-13 and 14-1930, with very strict safety rules to prevent the diffusion of the virus. Insite activities were restricted to the lab, whereas all office activities were performed in smart working. The rules never relapsed, even during the summer, so that when the second wave of COVID-19 diffusion occurred in Italy we were prepared. From May 11 the NICO never stopped lab activities.

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.

NICO is responsible of the 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.

In occasion of the celebrations for the first ten years of NICO, a series of movies were filmed concerning the work of each lab (see at the link Decennale NICO (unito.it)). In the same period, a famous rock group, the Subsonica (Subsonica) interested in Neuroscience, visited the Institute and played a promotion video.

SCIENTIFIC SEMINARS AT NICO
Since 2020, prompted by the pandemics, Marina Boido, Corrado Calì and Enrica Boda have taken the lead of the 'NICO neurowebinar series' that is currently providing a very efficient platform to discuss science with external speakers and share advancements of research generated at NICO.

For invited speakers, see the attached list.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: Clinical Neurobiology

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator
ANTONIO BERTOLOTTO

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

Personnel

1) SERENA MARTIRE

Degree: MSc
Birthdate: 01/08/1987
Nationality: Italian
Gender: F
Phone: +39 011 670 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: +39 011 670 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

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

Phone: +39 011 670 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: +39 011 670 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: +39 011 670 6635

Email: paolaval81@hotmail.com

Position: Medical biotechnologist

Role & expertise: biomarkers studies, management of biological material in CRESM Biobank

6) ARIANNA SALA

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

Nationality: Italian — Gender: F

Phone: +39 011 670 6601

Email: sala.arianna72@gmail.com

Position: resident biologist

Role & expertise: CSF analysis, diagnostic/prognostic tests for MS and NMO, drug immunogenicity

7) **FEDERICA BRESCIA**

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

Nationality: Italian — Gender: F

Phone: +39 011 670 6635

Email: fedeb2684@hotmail.it

Position: Technician

Role & expertise: CRESM Biobank technician.

8) SILVIA FEDELE

Degree: 3-years level degree in Laboratory Techniques

Birthdate: 03/12/1996

Gender: F

Nationality: Italian

Phone: +39 011 670 6635

Email: silviafedele7@gmail.com

Position: Technician

Role & expertise: sample processing and storage in CRESM Biobank, diagnostic tests for MS patients

9) SERENA CRISAVOLA

Degree: 3-years level degree in Laboratory Techniques

Birthdate: 11/01/1990

Gender: F

Nationality: Italian

Phone: +39 011 670 6635

Email:serecrisavola@gmail.com

Position: Technician

Role & expertise: diagnostic tests for MS patients, sample processing and storage in CRESM Biobank.

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
2019-20	Comunicazione fra paziente e centro Sclerosi Multipla: implementazione di strumenti e sistemi informatici	Dr. Bertolotto	Roche	PI of research unit	25000
September 2016-March 2021	"Improving therapeutic appropriateness of Multiple Sclerosis treatments using Biological approaches to personalize therapy and save pharmaceutical spending" RF-2013-02357497	Dr.Bertolotto	Ministero della Salute, Ricerca Finalizzata 2013	PI of research unit	381880
Pending	Searching for a link between EBV infection status, genetically determined BAFF overexpression and MS risk in Sardinians and mainland Italians	Dr. Bertolotto	Fondazione Italiana Sclerosi Multipla (FISM)	Unit of a Multicentric study (PI Francesca Aloisi, ISS Rome)	140645
Pending	"Exploring the link between EBV infection, genetically determined BAFF overexpression and MS risk"	Prof. Vercelli, Dr. Bertolotto PI: Francesca Aloisi ISS Rome	Program Announcement for the Department of Defense Defense Health Program Congressionally Directed Medical Research Programs Multiple Sclerosis Research Program Exploration – Hypothesis Development Award	Unit of a Multicentric study	125000
Pending	Placenta-derived extracellular vesicles as a potential modulator of multiple	Dr. Perga	Fondazione Italiana Sclerosi Multipla (FISM)	PI of research unit	112900

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.
[2] Coordinator/PI of research unit/team component.

	sclerosis disease activity				
Pending	Investigation on the potential Gd retention in biological fluids of MS patients upon multiple administrations of Gadolinium Based Contrast Agents	Dr. Montarolo	Fondazione Italiana Sclerosi Multipla (FISM)	PI of second research unit (PI of the project Dr. Eliana Gianolio, UNITO)	34600
Pending	Development and neuropharmacological characterization of new NLRP3 inflammasome inhibitors for the treatment of Multiple Sclerosis	Dr. Montarolo	Fondazione Italiana Sclerosi Multipla (FISM)	PI of second research unit (PI of the project Prof. Massimo Bertinaria, UNITO)	15000

3. SCIENTIFIC ACTIVITIES IN 2020

Antonio Bertolotto, PI

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:[1]	FAD "SM e gravidanza: la gestione attuale tra nuove evidenze ed esperienza clinica 1/06/2020 – 31/12/2020; "Symphony: confronto fra esperti in neurologia sull'impatto del Covid-19 sulle strategie terapeutiche e sui bisogni emergenti per i pazienti con sclerosi multipla", Webinar 15/10/2020; Riunione via Web Gruppo Multidisciplianre di Esperti sugli Interferoni, 6/11/2020; "SM Regional Experience", webinar 25/09/2020 – 13/11/2020; FAD "IX Convegno su cognitività e malattie neurologiche", 13/11/2020; FAD "Itinerari SNO in FAD – Focus on Sclerosi Multipla – Update sulla gestione del paziente con SM: terapia, vaccinazioni e gravidanza", 11/12/2020; "Convegno Regionale SIN Piemonte, Liguria e Val d'Aosta", 15/12/2020

[1]Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

• Science communication:[1]	Back to the Future: dalla neuropatologia di frontiera al trattamento avanzato della Sclerosi Multipla, Torino 14/02/2020: "I neurofilamenti"; Advisory Board "Back to Lem" 21/02/2020: Webinar "SMRR: nuove soluzioni terapeutiche tra le terapie di seconda linea" 1/02/2020 – 27/02/2020; Advisory Board – Webinar "Simphony in MS", 29/06/2020; Advisory Board – Webinar "Spettro dei disordini da neuromielite ottica: il percorso diagnostico-terapeutico del paziente con NMOSD in Italia", 16/07/2020; Stand Alone – Webinar "AUROOM è tempo di Teriflunomide", 30/09/2020; Advisory Board – Webinar "I dati di efficacia e sicurezza di Eculizumab a lungo termine nei pazienti NMOSD AQP4+", 4/12/2020
• Editorial duties:	Membro della commissione giudicatrice Premio Merck Serono 2020 "Innovazione Digitale in Sclerosi Multipla"
• others[2]	**Posters** 8th joint ACTRIMS-ECTRIMS meeting, 2020 Sep 11-13: P0286 - Alemtuzumab following natalizumab: a multicentric Italian real-world experience, P0913 - Risks associated with wash-out duration when switching from fingolimod to cell-depleting agents, P1115 - A first characterization of placenta-derived extracellular vesicles in patients with multiple sclerosis, LB1219 - Normal serum NFL levels: a proposal of cut-off strategy definition for the clinical practice, LB1220 - Real-life experience with sNFL in Multiple Sclerosis patients, as monitoring and treatment decision biomarker, LB1221 - Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation, LB1245 - BB-CRESM: a structured institutional biobank for quality research in Multiple Sclerosis. 3rd Brainstorming research assembly for young neuroscientists (BRAYN), 2020 November 25-26: NNP36 - NURR1 deficiency in mice is associated with sex-dependent altered behavioral phenotypes. 51°Congresso SIN virtual edition:

[1] Public engagement
[2] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

	- "Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation"; -"BB-CRESM: An institutional biobank to support quality research in Multiple Sclerosis" **Oral communications** 51°Congresso SIN virtual edition: - "Normal serum NFL levels: a proposal of cut-off strategy definition for the clinical practice" -"Real-life experience with sNFL in MS patients, as monitoring and treatment decision biomarker"
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[1]	na
Workshops, Schools or Conferences organized:	Webinar for the European Biotech Week: "La Biobanca del CRESM: un servizio per potenziare la ricerca sulla Sclerosi Multipla"
Technology transfer achievements (patents, etc.):	na

Serena Martire, Biostatistician

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Back to the Future: dalla neuropatologia di frontiera al trattamento avanzato della Sclerosi Multipla, Torino 14/02/2020: "Trapianto di cellule staminali emolinfopoietiche come opzione terapeutica"
• Editorial duties:	na
• others	**Posters** 8th joint ACTRIMS-ECTRIMS meeting, 2020 September 11-13: P1115 - A first characterization of placenta-derived extracellular vesicles in patients with multiple sclerosis, LB1219 - Normal serum NFL levels: a proposal of cut-off strategy definition for the clinical practice, LB1220 - Real-life experience with sNFL in Multiple Sclerosis patients, as monitoring and treatment decision biomarker, LB1221 - Applicability of sNFL in Multiple Sclerosis as additional

[1] No university appointments.

	measure in clinical practice and implications in NEDA-3 evaluation. 51°Congresso SIN virtual edition: "Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation"; 3rd Brainstorming research assembly for young neuroscientists (BRAYN), 2020 November 25-26: NNP36 - NURR1 deficiency in mice is associated with sex-dependent altered behavioral phenotypes
Organizational activities and responsabilities at NICO:	Speaker at the NICO NeuroWebinar Statistic course I) "Biostatistics: Back to the Basics", 30/03/2020 II) "Introduction to hypothesis testing", 20/04/2020 III) "Correlation and linear regression", 04/05/2020
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Francesca Montarolo, PhD Biologist

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	United Kingdom (UK) MS Tissue Bank of the Imperial College of London
• Invited talks:	
• Science communication:	na
• Editorial duties:	na
• others	NICO NeuroWebinar 20/03/2020 **Posters** 8th joint ACTRIMS-ECTRIMS meeting, 2020 September 11-13: P1115 - A first characterization of placenta-derived extracellular vesicles in patients with multiple sclerosis; 3rd Brainstorming research assembly for young neuroscientists (BRAYN), 2020 November 25-26: NNP36 - NURR1 deficiency in mice is associated with sex-dependent altered behavioral phenotypes **Referee for grant agencies** FISM (Federazione Italiana Sclerosi Multipla), since 2018; National Science Center Poland NCN, 2020.
Organizational activities and responsabilities at NICO:	na
Speakers invited:	Dr. Roberta Magliozzi NICO NeuroWebinar 30/10/2020

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

Simona Perga, PhD Biotechnologist

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	United Kingdom (UK) MS Tissue Bank of the Imperial College of London
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	**Posters** 8th joint ACTRIMS-ECTRIMS meeting, 2020 September 11-13: P1115 - A first characterization of placenta-derived extracellular vesicles in patients with multiple sclerosis. 3rd Brainstorming research assembly for young neuroscientists (BRAYN), 2020 November 25-26: NNP36 - NURR1 deficiency in mice is associated with sex-dependent altered behavioral phenotypes
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, MSc Biotechnologist

Supervised PhD students:	na
Honors, prizes, awards:	1st place in the e-poster presentation award at the European Biobank Week Congress 2020
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	**Posters:**

	8th joint ACTRIMS-ECTRIMS meeting, 2020: LB1245 "BB-CRESM: a structured institutional biobank for quality research in Multiple Sclerosis"; LB1219 - Normal serum NFL levels: a proposal of cut-off strategy definition for the clinical practice; LB1220 - Real-life experience with sNFL in Multiple Sclerosis patients, as monitoring and treatment decision biomarker; LB1221 - Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation; Europe Biobank Week 2020: "sNFL as a biomarker for routine management of MS patients: the role of disease-based biobank." 51°Congresso SIN virtual edition: - "Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation"; -"BB-CRESM: An institutional biobank to support quality research in Multiple Sclerosis" BBMRI Italia Working groups "Accesso e sharing" and "Biobancaggio COVID19". Co-author in the following documents: "*Accesso e processo di condivisione: per una buona pratica della condivisione (dai dati ai risultati) nel biobanking di ricerca*". "*Modello di consenso informato semplificato" e "Strumenti ELSI informativi" nell'ambito del biobancaggio COVID-19"* (https://www.bbmri.it/nodo-nazionale/elsi-covid-19/).
Organizational activities and responsabilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Webinar for the European Biotech Week: "La Biobanca del CRESM: un servizio per potenziare la ricerca sulla Sclerosi Multipla"
Technology transfer achievements (patents, etc.):	na

Paola Valentino, Msc Biotechnologist

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na

• Invited talks:	Simoa Success Webinars 2020: "Real-life experience with sNFL in Multiple Sclerosis at CRESM: from cut-off values definition to application in the monitoring of MS patients"
• Science communication:	na
• Editorial duties:	na
• others	**Posters** 8th joint ACTRIMS-ECTRIMS meeting: - LB1219 "Normal serum NFL levels: a proposal of cut-off strategy definition for the clinical practice"; - LB1220 "Real-life experience with sNFL in Multiple Sclerosis patients, as monitoring and treatment decision biomarker"; - LB1221 – "Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation"; - LB1245-"BB-CRESM: a structured institutional biobank for quality research in Multiple Sclerosis"; - P1115 - A first characterization of placenta-derived extracellular vesicles in patients with multiple sclerosis Europe Biobank Week 2020: "sNFL as a biomarker for routine management of MS patients: the role of disease-based biobank." 51°Congresso SIN virtual edition: - "Applicability of sNFL in Multiple Sclerosis as additional measure in clinical practice and implications in NEDA-3 evaluation"; -"BB-CRESM: An institutional biobank to support quality research in Multiple Sclerosis"
Organizational activities and responsabilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Webinar for the European Biotech Week: "La Biobanca del CRESM: un servizio per potenziare la ricerca sulla Sclerosi Multipla"
Technology transfer achievements (patents, etc.):	na

Arianna Sala, Biologist

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

Federica Brescia, Lab technician

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Europe Biobank Week 2020: "sNFL as a biomarker for routine management of MS patients: the role of disease-based biobank."
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

Silvia Fedele, Lab 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
Technology transfer achievements (patents, etc.):	na

Serena Crisavola, Lab 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
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[1]	

[1] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.

4. Research activity in 2020[1]

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, Neurofilament light chain (NFL) are the most promising biomarkers to investigate clinical activity and treatment efficacy in MS. NFL are released upon axonal damage in the cerebrospinal fluid and, in low concentration, in serum (sNFL). Whilst correlation between NFL and clinical outcomes is established at group level, their implementation in clinical practice is still to be addressed. Instead 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 in 2018 we purchased the SR-X instrument (Quanterix) for the quantification of serum Neurofilaments (sNFL).
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.
An interesting tool useful to elucidate the MS pathological mechanisms is the animal model of the disease. Specifically, one of the most well-studied is the immune-mediated experimental autoimmune encephalomyelitis (EAE) model. Although EAE model cannot fully replicate the MS disease course, it has been developed to recapitulate the neuro-inflammatory mechanisms, to improve the monitoring of the disease and to address future therapeutic intervention.
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

[1] Use times new roman 11 for the text.

distribute data and samples obtained from MS patients to other researchers, following rigorous ethical and technical guidelines.

c. Objectives (1000 characters)

I) To monitor disease activity and treatment efficacy through the implementation in the clinical practice of biological tools, such as the quantification of drugs level, ADA, biological activity and new biomarkers such as sNFL. In particular, we aimed to define if sNFL could be informative in real-life clinical practice as additional biological measure in the routine monitoring of individual patients
II) To investigate the pregnancy-related mechanisms of immunomodulation though the phenotypic and functional characterization of placenta-derived extracellular vesicles (EV).
III) To improve Magnetic Resonance Imaging (MRI) diagnostic modality and to propose new therapeutic intervention at pre-clinical level using the EAE model.
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 and NAT-treated patients to evaluate ADA and biological activity.
To implement sNFL in clinical practice, the definition of widely accepted normative values represents a crucial step. We analysed sNFL in 79 Healthy individuals, confirming a strong positive correlation between sNFL levels and age; different cut-off values specific for each age-decade were calculated, later used to interpret sNFL levels in individual MS patients. We performed a real-life cross-sectional study to describe sNFL in a large cohort of MS patients (n=1130 samples from 961 MS patients). 1) Progressive MS patients showed higher sNF-L levels and a greater prevalence of high pathological sNFL levels (32% in PPMS and 26% in SPMS) relative to RRMS patients (16%), with respect to the previously determined cut-off values. 2) All DMTs notably lower sNF-L in RRMS patients relative to untreated patients; though, 12% of treated patients still demonstrated high NFL levels. 3) Patients experiencing disease activity showed higher levels than stable patients. High NFL levels were observed in a substantial percentage (72-75%) of patients showing radiological or clinical activity; also, 10% of patients without evidence of disease activity still demonstrate high NFL levels. Different categories of stable patients with high NFL levels were identified showing borderline values, comorbidities, chronic ongoing fatigability, a history of very active disease, and conceivable inflammation not detectable by RMN or clinics.
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 enriched the collection of placental tissues from both MS and healthy women. We characterized EV at the transmission electron microscope and we identified for the first time surface markers differentially expressed on EV derived from MS and healthy women, which reflects their function and cell of origin..
III) Thanks to the collaboration with Prof Silvio Aime, Department of Molecular Biotechnology and Health Sciences, University of Torino (Italy), we quantitatively investigated the extent of gadolinium

retention in the central nervous system (CNS), and peripheral organs of EAE mice. Gadolinium, the contrast agent often used to enhanced MRI during the monitoring of the disease, accumulates preferentially in the most affected CNS regions of EAE mice.
Within the collaboration with Prof. Tiziana Crepaldi, Istituto di Candiolo - Fondazione del Piemonte per l'Oncologia (FPO), IRCCS, we are exploring the therapeutic value of anti-Met antibodies in the EAE mouse model by preventing demyelination and neuron damage. During 2020, we defined the timing and doses of the anti-Met antibody to be administered.
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. The CRESM Biobank was recognized as a structured biobank by the AOU San Luigi in January 2020. Specific protocols regulate the timing and modalities of biological sample collection, ensuring privacy of
subjects. Scientists can apply to the director of BB-CRESM specifying number, types and quantity of required samples and data. More than 1000 participants (healthy controls and MS patients) have enrolled in the BB-CRESM; over 20000 tubes of biological material (cerebrospinal fluid, serum, plasma, PBMCs, RNA and DNA) have been collected and stored.

e. Advancement in the field (1000 characters)

Results obtained may lead to the setup of biological assays that can improve the monitoring of disease activity and treatment efficacy, to 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.
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) which collects, stores and distributes samples and associated data to researchers all over the world, and is funded by Fondazione Italiana Sclerosi Multipla (FISM) since 2015.

f. Publications[1]

Masuccio FG, Lo Re M, **Bertolotto A**, Capobianco M, Solaro C. (2020). *Benign SARS-CoV-2 infection in MOG-antibodies associated disorder during tocilizumab treatment*. Mult Scler Relat Disord. Nov;46:102592. doi: 10.1016/j.msard.2020.102592. Epub 2020 Oct 21.

Perga S, Montarolo F, Martire S, Bonaldo B, Bono G, Bertolo J, Magliozzi R, **Bertolotto A**. (2020). *Overexpression of the ubiquitin-editing enzyme A20 in the brain lesions of Multiple Sclerosis patients: moving from systemic to central nervous system inflammation*. Brain Pathol. 2020 Oct 14:e12906. doi: 10.1111/bpa.12906. Online ahead of print.

Bandettini di Poggio M, Toni D, Gandolfo C, Paolicelli D, Zini A, Agostoni E, Bandini F, Ragno M, Altavista MC, **Bertolotto A**, Siciliano G, Vecchio M, Tambasco N, Gambardella A, Manganotti P, Melis M, Onofrj M, De Michele G, Reale N, Tedeschi G, Mancardi G. (2020) *Coverage of the requirements of first and second level stroke unit in Italy*. Neurol Sci. Jul 31. doi: 10.1007/s10072-020-04616-x. Online ahead of print.

[1] Plese DO NOT include papers in press or submitted.

Bertolotto A, **Martire S**, Mirabile L, Capobianco M, De Gobbi M, Cilloni D. (2020). *Autologous Hematopoietic Stem Cell Transplantation (AHSCT): Standard of Care for Relapsing-Remitting Multiple Sclerosis Patients.* Neurol Ther. Dec;9(2):197-203. doi: 10.1007/s40120-020-00200-9. Epub 2020 Jun 16.

Bertolotto A, Arroyo R, Celius EG, Comi G, Havrdova EK, Honeycutt WD, Hunter SF, Izquierdo G, Kornek B, Miller T, Mitsikostas DD, Singer BA, Ziemssen T, Chung L, Daizadeh N, Afsar S, Hashemi L, Senior P. (2020) *Quality of Life Improves with Alemtuzumab Over 6 Years in Relapsing-Remitting Multiple Sclerosis Patients with or without Autoimmune Thyroid Adverse Events: Post Hoc Analysis of the CARE-MS Studies*. Neurol Ther. Dec;9(2):443-457. doi: 10.1007/s40120-020-00191-7. Epub 2020 May 14.

Montarolo F, **Perga S**, Tessarolo C, Spadaro M, **Martire S**, **Bertolotto A**. (2020) *TNFAIP3 Deficiency Affects Monocytes, Monocytes-Derived Cells and Microglia in Mice*. Int J Mol Sci. Apr 18;21(8):2830. doi: 10.3390/ijms21082830.

Comi G, Pozzilli C, Morra VB, **Bertolotto A**, Sangalli F, Prosperini L, Carotenuto A, Iaffaldano P, Capobianco M, Colombo D, Nica M, Rizzoli S, Trojano M. (2020) *Effectiveness of fingolimod in real-world relapsing-remitting multiple sclerosis Italian patients: the GENIUS study*. Neurol Sci. Oct;41(10):2843-2851. doi: 10.1007/s10072-020-04380-y. Epub 2020 Apr 21.

Valsecchi V, Boido M, **Montarolo F**, Guglielmotto M, Perga S, Martire S, Cutrupi S, Iannello A, Gionchiglia N, Signorino E, Calvo A, Fuda G, Chiò A, **Bertolotto A**, Vercelli A. (2020). *The transcription factor Nurr1 is upregulated in amyotrophic lateral sclerosis patients and SOD1-G93A mice*. Dis Model Mech. May 15;13(5):dmm043513. doi: 10.1242/dmm.043513. PMID: 32188741 Free PMC article.

Marangon D, Boda E, Parolisi R, Negri C, Giorgi C, **Montarolo F**, **Perga S**, **Bertolotto A**, Buffo A, Abbracchio MP, Lecca D. (2020) *In vivo silencing of miR-125a-3p promotes myelin repair in models of white matter demyelination.* Glia. Oct;68(10):2001-2014. doi: 10.1002/glia.23819. Epub 2020 Mar 12.

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. (2020) *Assessing measurement invariance of MSQOL-54 across Italian and English versions*. Qual Life Res. Mar;29(3):783-791. doi: 10.1007/s11136-019-02352-0. Epub 2019 Nov 9.

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 monitor disease activity and treatment efficacy, we evaluated sNFL in a large real-life cross-sectional study: specific decade-related cut-off values were identified, to discriminate high pathological from normal values in MS patients. The evaluation of sNFL in MS patients during their routine follow-up demonstrated that the implementation of NFL in routine blood test could be informative: indeed 10-12% of treated patient, despite being clinically stable and clinically responsive to treatments, still showed pathological sNFL levels, suggesting inflammation or disease progression not yet detectable by clinics and/or MRI. The clinical monitoring of patients showing high NFL levels despite disease stability is ongoing
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 unveil the contribution of placental EV, through their preliminary phenotypic and functional characterization.
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.
In particular we plan 1) to implement sNFL as additional biological measure in the routine monitoring of individual MS patients to monitor disease activity and treatment efficacy; 2) to assess sNFL in different clinical contexts, as during the switch of NAT-treated patients to the extend-dose or to other second-line therapies, during pregnancy, for the evaluation of non-responder patients; 3) to assess sNFL along patients follow-up to evaluate the possible future implementation of individual cut-off values. 4) to assess sNFL in a growing number of healthy individuals and patients with other neurological disorders.
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 the immunomodulatory potential of placental EVs and their contribution to the pregnancy-induced disease amelioration observed in patients with MS. In particular, we will treat human blood cells with placental EVs from MS and healthy women, in the absence/presence of lymphocytes stimulation and we will evaluate the proliferation of lymphocytes, the expression of phenotypic and activation markers by leucocytes and the release of cytokines in the supernatant. In addition, we will explore the therapeutic effects of the EVs in EAE mice by evaluating the neuropathology signs (leukocyte infiltration, demyelination and gliosis), the inflammatory phenotype, the proliferation of splenocytes and the amount of regulatory T cells.
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 placental EVs will contribute to clarify their immunomodulatory role in pregnancy and in pregnancy-induced MS disease amelioration. They also have a therapeutic potential, since EVs can be produced in large scale and used as vectors for nanoparticles and drug delivery.
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): <u>please fill-out this section only in the case of innovative technologies</u>

“SR-X Ultra-Sensitive Biomarker Detection System” instrument (Quanterix) is a new instrument recently purchased by Clinical Neurobiology Laboratory and CRESM. The SR-X System is a benchtop instrument based on the innovative Simoa bead technology. This is based upon the isolation of individual immunocomplexes on paramagnetic beads using standard ELISA reagents. The main difference between Simoa and conventional immunoassays lies in the ability to trap single molecules in femtoliter-sized wells, allowing for a “digital” readout of each individual bead to determine if it is bound to the target analyte or not. The digital nature of the technique allows an average of 1000 times sensitivity increase over conventional assays with CVs less than 10 percent. This technology enables the ultra-sensitive detection of biomarkers in the range of subfemtomolar concentrations (below 1 pg/ml), in a variety of biological samples, including serum, plasma, cerebrospinal fluid (CSF), cell lysates.

The technology is currently being used for applications in a majority of therapeutic areas, including oncology, neurology, cardiology, inflammation and infectious disease. The SR-X is designed to support multiplexed detection of up to four biomarkers per sample, with low volume requirements to increase throughput and productivity while conserving precious samples.
In neurological field, this technology is widely used in different neurological disorders to measure NFL, proteins released following axonal damage in CSF, and also in blood, at very low concentrations. Thanks to its ultra-sensitivity, Simoa technology enables quantification of NFL also in blood, down to concentrations occurring in healthy persons. Several other neurological biomarkers can be assessedby Simoa technology on SR-X instrument including GFAP, TAU, Ab42, Ab40, alpha-sinuclein. In addition, the technology enables to set-up custom assays, when specific antibodies are available for the analyte of interest.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: Adult neurogenesis

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator 1

PAOLO PERETTO

Degree: PhD Birthdate: 18/09/1963

Nationality: Italian Gender: M

Phone: 00 39 011 6706605

Email: paolo.peretto@unito.it

Principal Investigator 2

LUCA BONFANTI

Degree: DVM, PhD Birthdate: 19/05/1962

Nationality: Italian Gender: M

Phone: 00 39 011 6706606

Email: luca.bonfanti@unito.it

Personnel

1. SILVIA DE MARCHIS

Degree: PhD Birthdate: 14/09/1966

Nationality: Italian Gender: F

Phone: 00 39 011 6706605

Email: silvia.demarchis@unito.it

Position: Associate professor

Role & Expertise: *in vivo* and *in vitro* molecular and cellular analyses

2. FEDERICO LUZZATI

Degree: PhD Birthdate: 20/10/1974

Nationality: Italian Gender: M

Phone: 00 39 011 6706615

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

Email: federico.luzzati@unito.it

Position: Assistant professor

Role & Expertise: morphological analyses and 3D reconstructions

3. SERENA BOVETTI

Degree: PhD Birthdate: 13/09/1977

Nationality: Italian Gender: F

Phone: 00 39 011 6706613

Email: serena.bovetti@unito.it

Position: Assistant professor (RTD-B)

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

4. CHIARA LA ROSA

Degree: PhD Birthdate: 01/07/1988

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: chiara.larosa@unito.it

Position: PostDoc

Role & Expertise: two-photon and lightsheet microscopy, comparative analyses of immature neurons

5. ISABELLA CRISCI

Degree: Biological Sciences Birthdate: 17/12/1989

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: isabella.crisci@unito.it

Position: PhD

Role & Expertise: cellular and molecular analyses of AN in the hippocampus

6. SARA BONZANO

Degree: PhD Birthdate: 22/03/1987

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: sara.bonzano@unito.it

Position: PostDoc

Role & Expertise: cellular and molecular analyses of AN in the hippocampus; morphometric assessment on mitochondria *in vivo*

7. 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: cellular and molecular analyses of lesion-induced neurogenesis

8. MARCO FOGLI

Degree: Biological Sciences Birthdate: 23/09/1993

Nationality: Italian Gender: M

Phone: 00 39 011 6706632

Email: marco.fogli@unito.it

Position: PhD

Role & Expertise: cellular and molecular analyses of lesion-induced neurogenesis

9. MARCO GHIBAUDI

Degree: Biological Sciences Birthdate: 29/05/1992

Nationality: Italian Gender: M

Phone: 00 39 011 6706632

Email: marco.ghibaudi@unito.it

Position: PhD

Role & Expertise: cellular and molecular analyses of immature neurons in mammals

10. YIFEI LIU

Degree: Veterinary Medicine

Birthdate: 22/01/1995

Nationality: Chinese

Gender: F

Phone: 00 39 011 6706632

Email: yifei.liu@unito.it

Position: PhD

Role & Expertise: cellular and molecular analyses of immature neurons in mammals

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
November 2020	Sounds and pheromones: neural networks merging olfactory and acoustic cues in sexual imprinting.	Serena Bovetti	Human Frontier Science Program HFSP	Coordinator	117.000 USD per year for a period of three years
December 2019	"Potenziare le cellule staminali per combattere l'invecchiamento e il declino cognitivo"	Serena Bovetti	Fondazione CRT	Coordinator	15.000 Euro (second year)
Pending 2021	imprinted SCENTs: odour control of mate preference	Applicant: Stefano Zucca Supervisor: Serena Bovetti	Marie Skłodowska-Curie Individual Fellowships 2020	Coordinator -Referent	171473,28 Euro
July 2020	Understanding the role of NR2F1 on mitochondrial functions to gain new insight into the Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome	Silvia De Marchis	Fondazione Jerome Lejeune	Coordinator	20000,00 per year for a period of two years)
July 2020	Studio dei fattori molecolari di controllo della funzione	Silvia De Marchis	Università Italo-Francese-Programma Vinci –	Coordinator	71.082,92 (euro) (for a three year scholarship)

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.

[2] Coordinator/PI of research unit/team component.

	mitocondriale nelle cellule staminali neurali adulte: implicazioni nei disordini cognitivi		Finanziamento borse dottorato		
March 2020	Local research funding	Bonfanti, Peretto, De Marchis, Luzzati, Bovetti	University of Turin	Single projects for each researcher	15,000
Year 2020	Neuroni alternativi	Bonfanti	External donations	Scientific supervisor	8,000
01/2019-08/2021	Imaging cellular dynamics in the central nervous system	Luzzati	Compagnia di San Paolo	Coordinator	25,000 (second year)

3. SCIENTIFIC ACTIVITIES IN 2020

Luca Bonfanti (PI)

Supervised PhD students:	Marco Ghibaudi (second year); Yifei Liu (second year)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Irmgard Amrein: ETH Zurich and Institute of Anatomy, University of Zurich (Switzerland) - Chet C Sherwood: Department of Anthropology, George Washington University (USA) - Frederic Lévy and Elodie Chaillou: University of Tour and INRA, Nouzilly (France) - Sebastien Couillard Despres: Paracelsus Medical University, Salzburg, Austria - Bruno Cozzi: University of Padova and Mediterranean Marine Mammal Tissue Bank (Italy)
• Invited talks:	"*Is there adult neurogenesis in the mammalian neocortex?*" Adult neurogenesis digital talks, (virtual meeting from Germany organized by Gerd Kempermann), October 6, 2020 "*Neuronal plasticity in large-brained mammals: Adult neurogenesis or immature neurons?*" (virtual meeting from USA) J. B. Johnston Club meeting, October 27, 2020
• Science communication:	- Organization of "*10 piccoli neuroni per 10 grandi libri*" a series of meetings in a book shop between researchers and the general public (Libreria il Bardotto, Torino) - "*Neuroni giovani nel cervello adulto*" a series of conferences for the general public and secondary school students, in Festival dell'innovazione (Settimo torinese), Genitorinsieme (Asti), and different secondary schools

	- "*La neurogenesis nell'uomo esiste?*" partecipazione a UNISTEM DAY 2020 (organized by NICO) - "*La plasticità cerebrale: l'unica salvezza per gestire il cambiamento?*" Incontro interdisciplinare organizzato dal Politecnico di Torino - "*Cervello: è capace di rigenerarsi? E se si, come?*" partecipazione a Scienza Show, il talk show online di Renato Sartini
• Editorial duties:	- Editor in Chief *Frontiers in Neurogenesis* - Editor of Special issue in IJMS: *Neuron and Brain Maturation* (with Sebastien Couillard-Despres)
• others	na
Organizational activities and responsabilities at NICO:	na
Speakers invited:	na
Other organizational activities:[1]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Paolo Peretto (PI)

Supervised PhD students:	Marco Fogli (co-tutored, second year)
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:	"Amor scientifico e Amor Poetico"; Festival dell'innovazione e della scienza, Settimo Torinese 16 Ottobre 2020.
• Editorial duties:	Associate Editor Frontiers in Neuroscience
• others	Referee for Scientific Journals
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

[1] No university appointments.

Technology transfer achievements (patents, etc.):	na

Silvia De Marchis

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:[1]	na
Science communication:[2]	na
Editorial duties:	Reviewing Editor Frontiers
others[3]	Referee for Scientific Journals - Member of the Committee ANR CES 16 (2020)
Organizational activities and responsibilities at NICO:	Comitato di gestione Piattaforma Imaging e microscopia
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Federico Luzzati (Senior Researcher)

Supervised PhD students:	Marco Fogli (co-tutor)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Matteo Bergami, University Hospital Cologne; Benedikt Berniger, King's College London; Philip Greulich, Univ of Southampton
• Invited talks:[5]	na

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[2] Public engagement

[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[4] No university appointments.

[5] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

• Science communication:[1]	"C'era una volta un neurone": Festival dell'innovazione e della scienza, Settimo Torinese 16 Ottobre 2020 and Giornata nazionale dell'afasia (https://www.youtube.com/watch?v=wZ_se2ramNE)
• Editorial duties:	Referee for Scientific Journals
• others[2]	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[3]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Serena Bovetti (Senior Researcher)

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); Dr. Tommaso Fellin: Istituto Italiano di Tecnologia, Genova (Italy).
• Invited talks:[4]	na
• Science communication:[5]	"Amor scientifico e Amor Poetico"; Festival dell'innovazione e della scienza, Settimo Torinese 16 Ottobre 2020.
• Editorial duties:	Referee for Scientific Journals (e.g. Scientific Reports; Molecular Neurobiology; Brain structure and Function)
• others[6]	na
Organizational activities and responsibilities at NICO:	Person in charge of the BSL2 surgical room and of two-photon microscope.
Speakers invited:	Dr. Dania Vecchia, 17/7/20 Dr. Stefano Zucca, 4/12/20

[1] Public engagement

[2] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[3] No university appointments.

[4] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[5] Public engagement

[6] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

Other organizational activities:[1]	na
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:	- Prof. Dustin Penn: Konrad Lorenz Institute of Ethology, Veterinary Medicine University, Vienna (Austria); - Prof Sylvain Gigan: Laboratoire Kastler-Brossel Sorbonne Université, Paris (France); - Dr. Tommaso Fellin: Istituto Italiano di Tecnologia, Genova (Italy); - Dr. Irmgard Amrein: ETH Zurich and Institute of Anatomy, University of Zurich (Switzerland); - Chet C Sherwood: Department of Anthropology, George Washington University (USA).
• Invited talks:[2]	na
• Science communication:[3]	Organizing member of the "Beautiful mind" sessions for International Festival "Pint of Science" in Turin (postponed for COVID19 emergency)
• Editorial duties:	Referee for Scientific Reports
• others[4]	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[5]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Giulia Nato (Postdoc)

Supervised PhD students:	
Honors, prizes, awards:	na

[1] No university appointments.

[2] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[3] Public engagement

[4] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[5] No university appointments.

Outreach activities	
• International collaborations:	Matteo Bergami, University Hospital Cologne; Benedikt Berniger, King's College London; Philip Greulich, Univ of Southampton
• Invited talks:[1]	BRAYN, 3rd Brainstorming Research Assembly for Young Neuroscientists, Online Congress, 25-27 November 2020
• Science communication:[2]	na
• Editorial duties:	na
• others[3]	Chairperson at the Workshop "Neuro-glia crosstalk in health and disease" Poster presenter at the Online Workshop "Neuro-glia crosstalk in health and disease" (October 1st-3rd, 2020)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Sara Bonzano (Postdoc)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- R. Berckervordersandforth, D.C. Lie (FAU - Erlangen, Germany) - M. Studer (iBV, UCA, CNRS, INSERM - Nice, France)
• Invited talks:[5]	na
• Science communication:[6]	na
• Editorial duties:	Referee for Metabolic Brain Disease and Journal of Experimental Neuroscience
• others[7]	Chairperson at the Workshop "Neuro-glia crosstalk in health and disease" (session: Glial cells in CNS aging and pathology)

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[2] Public engagement
[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[4] No university appointments.
[5] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[6] Public engagement
[7] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

	Poster presenter at the Online Workshop "Neuro-glia crosstalk in health and disease" (October 1st-3rd, 2020) "COUP-TFI/Nr2f1 overexpression in the GLAST-lineage perturbs migration and morphology of an adult-born hippocampal neuron subpopulation" Bonzano S, Michelon F, Crisci I, Griego F, Studer M, De Marchis S
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[1]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Isabella Crisci (PhD)

Supervised PhD students:	na
Honors, prizes, awards:	*March 2020*: awarded by the University of Turin with a research scholarship of 7 months for a project entitled "Potenziare le cellule staminali neurali per combattere l'invecchiamento e il declino cognitive" at the Dept. of Life Sciences and Systems Biology (March – October 2020).
Outreach activities	
• International collaborations:	na
• Invited talks:[2]	na
• Science communication:[3]	na
• Editorial duties:	na
• others[4]	Poster presenter at the Online Workshop "Neuro-glia crosstalk in health and disease" (October 1st-3rd, 2020)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[5]	na
Workshops, Schools or Conferences organized:	na

[1] No university appointments.
[2] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[3] Public engagement
[4] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[5] No university appointments.

Technology transfer achievements (patents, etc.):	na

Marco Fogli (PhD)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Benedikt Berninger (King's College, London); Philip Greulich (Mathematical Sciences, Univ of Southampton)
• Invited talks:[1]	na
• Science communication:[2]	na
• Editorial duties:	na
• others[3]	Invitation to chair the session "Unveiling CNS development and glial cell heterogeneity through multi-omic approaches" at the Glial cells-neuron crosstalk in CNS health and disease (Online Workshop). Poster presentation at: - Glial cells-neuron crosstalk in CNS health and disease (Online Workshop, 1-3 October, 2020) - BraYn - 3rd Brainstorming Research Assembly for Young Neuroscientists (Online Meeting, 25-27 November, 2020) Poster title "*Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion"*- Marco Fogli, Giulia Nato, Philip Greulich, Paolo Peretto, Annalisa Buffo & Federico Luzzati
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[2] Public engagement

[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[4] No university appointments.

Marco Ghibaudi (PhD Student)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Irmgard Amrein: ETH Zurich and Institute of Anatomy, University of Zurich (Switzerland) - Chet C Sherwood: Department of Anthropology, George Washington University (USA)
• Invited talks:[1]	na
• Science communication:[2]	na
• Editorial duties:	na
• others[3]	*Immature neurons in the amygdala of cat and marmoset*, talk at GISN
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Yifei Liu (PhD Student)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:[5]	na
• Science communication:[6]	na
• Editorial duties:	na
• others[7]	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[2] Public engagement

[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[4] No university appointments.

[5] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[6] Public engagement

[7] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

Other organizational activities:[1]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[2]	

4. Research activity in 2020[3]

a. Summary (500 characters)

Different aspects of adult brain structural plasticity were addressed.
i) *hippocampal neurogenesis:* cell-intrinsic and extrinsic control of adult neural stem cells and newborn neurons;
ii) *reproductive*: understanding GnRH system role in adult neurogenesis an in olfactory bulb 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)

A lot of unanswered questions remain, concerning both the mechanisms and functions of adult neurogenesis (AN) in different mammalian species/brain regions. 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 set-up 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 and when the external and internal cues integrate with AN to sustain behaviors essential for survival, such as reproduction?

[1] No university appointments.
[2] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.
[3] Use times new roman 11 for the text.

The rationale of the research carried out in 2020 can be summarized as follows:
i) The age-related decrease in hippocampal neurogenesis might contribute to brain pathologies, such as Alzheimer's disease and age-related memory loss. 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), nevertheless 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 heterogeneously distributed in the mammalian brain (Piumatti et al., 2018, J Neurosci; La Rosa et al., 2020, eLife) we established a method to quantify in a comparable manner the amount of immature neurons in the subcortical regions of 10 mammals, including small-brained and large-brained species.
iv) Previously, we showed modulation of AN during puberty in female mice (Oboti et al., 2017, 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 cellular/molecular mechanisms controlling/regulating the activity of adult neural stem cells and their progeny (i.e. proliferation and fate) in young adults with possible implications for intellectual disability.
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 GnRH secretion on peri-pubertal AN modulation, and the activity of olfactory bulb GnRH neuronal population.
iv) Establishing whether subcortical "immature" neurons are heterogeneous in mammals, and possibly more extended in large-brained species; starting the study of cortical immature neurons in human fetal brains.

d. Results (4000 characters)

Hippocampal neurogenesis.
We found that the disease gene COUP-TFI/NR2F1, whose mutations cause the rare ID (intellectual disability)-associated human disease BBSOAS, controls not only adult NSCs proliferation and fate, but also the correct development of newborn neurons through a direct regulation of nuclear-encoded mitochondrial genes. By exploiting mouse genetics and retroviral mediated labelling of mitochondria *in vivo* we showed an altered mitochondria morphology/distribution upon COUP-TFI conditional knock-out. Moreover, we demonstrated that Tamoxifen, a drug used to induce the Cre recombinase system, which is an essential and largely used tool to study gene function, exerts a direct pro-neurogenic effect in the adult hippocampal neurogenic niche in a LPS-induced mouse model of neuroinflammation.

Neurogenesis and reproduction. By exploiting a transgenic GnRH deficient mouse model, that progressively loses GnRH expression during postnatal development (GnRH::Cre;DicerloxP/loxP mice), we found that a postnatally acquired dysfunction in the GnRH system affects the process of adult neurogenesis in both the hippocampal dentate gyrus and the subventricular-zone neurogenic niches, in a sexually dimorphic way. Moreover, by examining adult subventricular-zone neurogenesis in adult females ovariectomized prior to puberty, we found that among the HPG-axis secreting factors, the circulating level of gonadal hormones during pre-/peri-pubertal life is critical to set-up the process of adult neurogenesis.
During 2019 we also identified and characterized a new population of GnRH neurons located around the olfactory bulb (OB-GnRH neurons) , whose role is still unknown. In 2020, by using two photon imaging we started to examine their functional activity after exposure to male and female pheromones.

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. We showed that in large brain mammals, in spite of well-preserved morphological and molecular features, the distribution of cortical immature neurons was highly heterogeneous, particularly abundant 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 hippocampal neurogenic niche, we unraveled a yet unknown function for COUP-TFI/NR2F1 in modulating mitochondrial dynamics in newborn hippocampal neurons, moreover, we added relevant information for the use of Tamoxifen in inducible Cre-LoxP transgenic mice.
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 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. In addition, we found that the OB-GnRH neuron population is functionally activated by pheromones. 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[1]

La Rosa C, Cavallo F, Pecora A, Chincarini M, Ala U, Faulkes CG, Nacher J, Cozzi B, Sherwood CC, Amrein I, **Bonfanti L** (2020). *Phylogenetic variation in cortical layer II immature neuron reservoir of mammals*. eLife 9:e55456.

La Rosa C, Parolisi R, **Bonfanti L** (2020). *Brain structural plasticity: From adult neurogenesis to immature neurons.* Front Neurosci 14:75.

Cozzi B, **Bonfanti L**, Canali E, Minero M (2020). *Brain waste: The neglect of animal brains.* Front. Neuroanat. 14:573934.

Trova S, **Bovetti S**, Pellegrino G, **Bonzano S**, Giacobini P, **Peretto P** (2020). *HPG-Dependent Peri-Pubertal Regulation of Adult Neurogenesis in Mice*. Front Neuroanat. 27;14:584493.

Antonini A, Sattin A, Moroni M, **Bovetti S**, Moretti C, Succol F, Forli A, Vecchia D, Rajamanickam VP, Bertoncini A, Panzeri S, Liberale C, Fellin (2020). *Extended field-of-view ultrathin microendoscopes for high-resolution two-photon imaging with minimal invasiveness*. eLife. 9:e58882.

Fornasari BE, El Soury M, **Nato G**, Fucini A, Carta G, Ronchi G, Crosio A, Perroteau I, Geuna S, Raimondo S, Gambarotta G. (2020). *Fibroblasts Colonizing Nerve Conduits Express High Levels of Soluble Neuregulin1, a Factor Promoting Schwann Cell Dedifferentiation*. Cells. 1;9(6):1366.

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 to some aspects of reproductive behavior and the comparative approach to phylogenetic variation of 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 and immature neuron function in both physiological and pathological conditions, the identification of brain circuits/neuronal populations integrating salient sensory cues underlying

[1] Plese DO NOT include papers in press or submitted.

reproductive behavior, and a further characterization of the immature neuron "reservoirs" in widely different mammals, including humans. In addition, we will focus on the identification of neural circuits responsible for sexual imprinting in female mice (according to HFSP founded research project).

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, so called "neurogenesis without division"), 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. During the next years the analyses will be extended to several brain sub-cortical regions and to human fetal brains.

C) how, when and where salient sensory cues are integrated in the brain to sustain behaviors essential for survival (e.g., reproduction)? To this aim we will focus on: i) the role of OB-GnRH neurons in mediating pheromonal perception, and ii) the identification of neural circuits responsible for sexual imprinting in female mice (according to the founded HFSP research project). As to point ii, it is known that female mice use olfactory and acoustic cues from parents to learn and form memories of conspecifics and close kin, which enables them to avoid heterospecific matings as adults.
This process, called sexual imprinting, has been largely studied in different animal species but little is known about the sensory processing underlying representation of imprinted cues and how they shape brain circuits to drive mate selection.

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/related forms of plasticity 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/young 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* and possible implication in the causative mechanisms and pathogenesis of intellectual disability. Stemming from our recent data on COUP-TFI/NR2F1 function we propose to pursue three main objectives: i) to further characterize mitochondrial architecture and dynamic in newborn neurons of COUP-TFI mutant mouse models exploiting functional imaging of mitochondria in COUP-TFI-icKO/HET hippocampi by *in vivo*/*ex vivo* approaches; ii) to identify COUP-TFI targets by combining genome-wide and *in silico* analyses; iii) to provide an experimental validation of the most promising targets.
- *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.
As concerns the project on the mice sexual imprinting, it is important to note that this new research line relies on complementary disciplines/groups: team 1 (our team at NICO) with expertise in neuroscience, which will lead the neuroanatomical and neurophysiological part; team 2 (located in Paris) with expertise in light propagation in highly scattering media, which will develop the micro-endoscopic system for in vivo photons detection and signal processing analysis; team 3 (located in Vienna) with expertise in animal behavior and communication, which will direct the behavioral aspects on laboratory and wild-mice.
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.

- *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 (from mice to humans). 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 vision (intended as a constitutive, continuous genesis of new neurons in rodents), 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:
- To address the functional role of olfactory GnRH-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 (GCaMP) in head

restrained anesthetized and awake mice. Two-photon imaging is also used to study *in vivo* mitochondrial dynamics in neurogenic regions.

- As concern the HFSP research project, 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.

- 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 already 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.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: Physiopathology of neural stem cells

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator

ANNALISA BUFFO

Degree: PhD | Birthdate: 25-12-1967

Nationality: Italian | Gender: F

Phone: 00 39 011 6706614

Email: annalisa.buffo@unito.it

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: Associate Professor of Anatomy

Role & expertise: Lead responsible of research on oligodendroglial physiopathology

3. ROBERTA PAROLISI

Degree: PhD | Birthdate: 23-01-1985

Nationality: Italian | Gender: F

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

Phone: 00 39 011 6706632

Email: Roberta.parolisi@unito.it

Position: Senior PostDoc

Role & expertise: Expert in myelin ultrastructure and responsible of EM investigations

4. 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 and single cell RNA sequencing analyses

5. 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 and reactivity

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

Role & expertise: Expert in oligodendroglia biology, *in vitro* assays and biochemical analyses

7. MARTA RIBODINO

Degree: Bachelor in Medical Biotechnology Birthdate: 1-06-1996

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: marta.ribodino@edu.unito.it

Position: Junior Researcher

Role & expertise: Developer of protocols to derive glia from hPSCs.

8. MARYAM KHASTKHODAEI ARDAKANI

Degree: Master in Anatomical Sciences Birthdate: 30-06-1991

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: maryam.khastkhodaeiardakani@unito.it

Position: PhD student

Role & expertise: Addressing oligodendrocyte heterogeneity and response to DNA damage

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded
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 €
2020-2024	NSC-Reconstruct Novel Strategies for Cell-based Neural Reconstruction #874758	Annalisa Buffo	H2020-SC1-BHC-2018-2020	PI of research unit, WP coordinator	680,000 €
2020-2022	Allele-specific siRNAs as therapeutic option for ADLD: in vitro pre-clinical validation on unique human experimental models	Annalisa Buffo	ELA Foundation	PI	200,000 €

2020-2022	Studio e cura dei disturbi dello spettro autistico: sviluppo di un laboratorio speciale per la ricerca su neuroni e mini-cervelli umani	Annalisa Buffo	CRT Fondazione	PI	20,000 €
2020-2022	Developmental trajectories of cerebellar astrocytes and neurons	Annalisa Buffo	RiLo	PI	2,200 €
2019-2021	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	20,000 €
2020	European Society of Neurochemistry (ESN) Initiative Funding	Enrica Boda	European Society of Neurochemistry (ESN)	Proponent	500 €
2020-2023	Role of particulate matter (PM) exposure in neuroinflammation and demyelination.	Enrica Boda	Local Funds of the Department of Neuroscience (University of Turin)	PI	2,205 €
2019-2022	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 €
2020	Uncovering the unfolding of mouse and human astrocyte lineages through high throughput RNA sequencing in the cerebellum	Valentina Cerrato	IBRO - PERC InEurope Short Stay Grant	Proponent	3.000 €

2020	IBRO-PERC Workshops, Conferences and Meetings Grant	Valentina Cerrato	IBRO-PERC	Proponent	4.000 €
2021	Approcci d'avanguardia per lo studio traslazionale in uomo e topo dello sviluppo dei circuiti e delle patologie cerebellari	Valentina Cerrato	Banca d'Italia	Proponent	25.000 € (pending)
2020	Qki-mediated mRNA cleavage in astrocytes to counteract MS pathology	Annalisa Buffo	FISM	Co-proponent	5.723 € (pending)

3. SCIENTIFIC ACTIVITIES IN 2020

Annalisa Buffo, PI

Supervised PhD students:	Martina Lorenzati (co-supervised with A Vercelli); Maryam Khastkhodaei (co-supervised with E Boda).
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Neural stem cell (NSC) reconstruct network (E Cattaneo, University of Milano, M Parmar, University of Lund; A Bosio, Miltenyi Biotec, Koln; M Gotz, University of Muenchen); L López-Mascaraque and F de Castro (Inst Cajal, Madrid); S Goldman (University of Rochester); see also lab members below.
• Invited talks:	na
• Science communication:	*Staminali, medicina rigenerativa ed etica*, in the frame of the "Festival 2020 dell'Innovazione e della Scienza", cycle of lectures addressed to the public (15 Oct 2020, Biblioteca Civica Paola e Nicolò Francone, Chieri, TO). https://www.nico.ottolenghi.unito.it/News/STRANA-MENTE-dal-10-al-17-10-torna-il-Festival-dell-Innovazione-e-della-Scienza)

• Editorial duties:	- Topic Editor: Pharmacology of neurogenesis, Current Opinion in Pharmacology; - Editorial board member of Scientific reports; - Ad hoc reviewer for the following journals: The Journal of Neuroscience, Frontiers in Neuroscience, Scientific Reports, Advanced Science, Biochemical Pharmacology, Science Advances, The Neuroscientist.
• others	- Member of the task force for Ataxia (Society for Research on the Cerebellum and Ataxias) - Reviewer for the following Agencies/Charities: FISM (Federazione Italiana Sclerosi Multipla), Spain Ministry for Science, Innovation & University. Nectar 2020, 19th – 20th November 2020 ISSCR 2020 meeting 23-27 June, 2020
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:	Luigia Pace Armenise-Harvard Immune Regulation Lab. - IRCCS Candiolo)
Other organizational activities:	UNISTEM Day Torino (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), October 2020, Torino Organizer of the first workshop of the EU consortium Neural Stem cell Reconstruct 'How new methodologies can drive the development of next generation stem cell products' November 24-25 2020
Technology transfer achievements (patents, etc.):	na

Enrica Boda, Lead responsible of research on oligodendroglial physiopathology

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with A Buffo)
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:	*Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model.* 28 July 2020, invited seminar at Institute of Neuroscience, National Research Council (CNR), Pisa, Italy (webinar). Host: Dr. Eleonora Vannini *Oligodendroglia heterogeneity in physiology and pathology: a confocal study in vivo and in vitro.* 26 May 2020, invited seminar in the frame of the 9th workshop "Advanced microscopy techniques for biomedical applications", Dept. of Clinical and Biological Sciences, Orbassano (Turin), Italy (webinar). Host: Prof. Saverio F. Retta
• Science communication:	*Inquinamento, infiammazione e sclerosi multipla. Polveri sottili nell'aria: un fattore di rischio per le patologie del sistema nervoso*, in the frame of the "Festival 2020 dell'Innovazione e della Scienza", cycle of lectures addressed to the public (15 Oct 2020, Biblioteca Civica Paola e Nicolò Francone, Chieri, TO). https://www.nico.ottolenghi.unito.it/News/STRANA-MENTE-dal-10-al-17-10-torna-il-Festival-dell-Innovazione-e-della-Scienza)
• Editorial duties:	Guest Editor for the Research Topic **"**The Role of Astroglia and Oligodendroglia in CNS Development, Plasticity, and Disease – Novel Tools and Investigative Approaches", Frontiers in Cellular Neuroscience (https://www.frontiersin.org/research-topics/13033/the-role-of-astroglia-and-oligodendroglia-in-cns-development-plasticity-and-disease---novel-tools-an) Review Editor for Frontiers in Neuroanatomy Review Editor for Frontiers in Cellular Neuroscience – Section Non-neuronal cells Review Editor for Frontiers in Neurogenesis Ad-hoc reviewer for Frontiers in Neuroscience, Scientific Reports, Mechanisms of Ageing and Development, Journal of Alzheimer's Disease, 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	Other oral communications: *Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model.* 1 October 2020, oral communication in the frame of the Workshop: "Glial cell-neuron crosstalk in health and disease" 1-3 October 2020, Turin, Italy (webinar) Posters at meetings: Parolisi R, Montarolo F, Pini A, Rovelli S, Cattaneo A, Bertolotto A, Buffo A, Bollati V, Boda E (2020). *Exposure to particulate matter hampers myelin repair in a mouse model of demyelination.* European Journal of Histochemistry 2020; vol. 64; supplement 3; p.12 (Proceedings of the 30th National Conference of the Italian Group for the Study of Neuromorphology) Parolisi R, Montarolo F, Pini A, Rovelli S, Cattaneo A, Bertolotto A, Buffo A, Bollati V, Boda E (2020). *Exposure to particulate matter hampers myelin repair in a mouse model of demyelination.* 3rd BraYn (Brainstorming Research Assembly for Young Neuroscientists) Conference, 25-26 November 2020 Attended meetings: - 2nd National Meeting "Morfologia e dintorni" (Amici dell'Anatomia), 22-23 February 2020, Turin, Italy - 3rd National Meeting "Morfologia e dintorni" (Amici dell'Anatomia), 26 September 2020, Turin, Italy - XXXI Meeting of the Italian Group for the Study of Neuromorphology (GISN), 13-14 November 2020, Turin, Italy (via webinar) - Neural Stem Cell Reconstruct workshop "How new methodologies can drive the development of next generation stem cell products", 24 November 2020 (via webinar)

	- 3rd BraYn (Brainstorming Research Assembly for Young Neuroscientists) Conference, 25-26 November 2020 (via webinar) - Annual Scientific Congress of the Italian MS Foundation (FISM), 27 November 2020 (via webinar) Grant Reviewer for: Italian Foundation Multiple Sclerosis (FISM) Membership of Scientific Societies: Federation of the European Neuroscience Societies (FENS) International Society of Neurochemistry (ISN) European Society of Neurochemistry (ESN) Italian Society of Neuroscience (SINS) Italian Group for the Study of Neuromorphology (GISN) Italian Society of Anatomy and Histology (SIAI)
Organizational activities and responsibilities at NICO:	- Responsible for the Histology Lab at NICO; - Organization of the Progress Report seminar series at NICO.
Speakers invited:	-Dr. Giovanni Ferrara, Dipartimento di Neuroscienze, riabilitazione, oftalmologia, genetica e scienze materno-infantili, Università di Genova, Italy; -Dr. Eleonora Vannini, Institute of Neuroscience, National Research Council (CNR), Pisa, Italy -Dr. Stefano Angiari, Trinity College, Dublin, Ireland -Dr. Alice Staffa, Instituto de Neurociencias de Alicante, Spain
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, 25-26 November 2020, https://www.braynconference.com/ - Workshop: “Glial cell-neuron crosstalk in health and disease” 1-3 October 2020, Turin, Italy (via webinar) https://www.nico.ottolenghi.unito.it/Agenda/GLIAL-CELLS-NEURON-CROSSTALK-IN-CNS-HEALTH-AND-DISEASE (with V Cerrato and A Buffo).

	- Secretariat of the Organizing Committee of the XXXI Meeting of the Italian Group for the Study of Neuromorphology (GISN), 13-14 November 2020, Turin, Italy (via webinar) - Member of the Organizing Committee of the 2nd National Meeting "Morfologia e dintorni", 22-23 February 2020, Turin, Italy
Technology transfer achievements (patents, etc.):	na

Roberta Parolisi, PostDoc, expert in myelin ultrastructure and responsible of EM investigations

Supervised PhD students:	na
Honors, prizes, awards	na
Outreach activities	
• International collaborations:	Neuro stem cell repair (NSCR) network (coordinated by Prof. Elena Cattaneo), related to NSC transplantation in a model of Huntington's disease.
• Invited talks:	Parolisi R, Lombardi M, Fumagalli M, Bonfanti E, Verderio C, Buffo A. Microglia-derived extracellular vesicles regulate proliferation and differentiation of oligodendrocyte precursor cells. Online International Workshop On: Glial Cells-Neurons Cross Talk In Health And Disease, 3 october 2020. Oral Presentation.
• Science communication:	na
• Editorial duties:	Ad hoc reviewer for Neurochemical Research, Journal of the Neurological Sciences and Frontiers Aging Neuroscience.
• others	-Parolisi R, Montarolo F, Pini A, Rovelli S, Cattaneo A, Bertolotto A, Buffo A, Bollati V, Boda E. Exposure to fine particulate matter (PM2.5) hampers myelin repair in a mouse model of white matter demyelination. Online XXX Convegno Nazionale Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.), 13 november 2020. Oral Presentation. -Parolisi R, Montarolo F, Pini A, Rovelli S, Cattaneo A, Bertolotto A, Buffo A, Bollati V, Boda E. Exposure to fine particulate matter (PM2.5) hampers myelin repair in a mouse model of white matter demyelination. Online III

	incontro Nazionale Morfologia e Dintorni, 26 october 2020. Oral Presentation.
Organizational activities and responsibilities at NICO:	In charge of the maintenance of Nikon CS1 confocal microscope one and of the light sheet microscope, hosted at NICO microscopy facility.
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Valentina Cerrato, PostDoc, lead 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 a 6-month stay in the Laboratory of Prof. Ludovic Telley, University of Lausanne. Title of the project: "Uncovering the unfolding of mouse and human astrocyte lineages through high throughput RNA sequencing in the cerebellum". - IBRO-PERC in Europe Short Stay Grant, for a 3-month stay in the Laboratory of Prof. Ludovic Telley, University of Lausanne. Title of the project: "Uncovering the unfolding of mouse and human astrocyte lineages through high throughput RNA sequencing in the cerebellum".
Outreach activities	
• International collaborations:	Prof. Ludovic Telley (University of Lausanne, Switzerland); Dr. Andreas Bosio (Miltenyi Biotec B.V. &Co, Bergisch Gladbach, Germany)

• Invited talks:	- "Single-cell RNA Sequencing unveils an unprecedented molecular and functional heterogeneity of cerebellar astrocytes". 4th Cerebellum day, 21-22 January, 2021 (Online). - "*in vivo* clonal analyses to study the ontogenesis of cerebellar astrocytes: from confocal microscopy, to automatic segmentation and 3D reconstruction tools", 9th Workshop of Advanced Microscopy Techniques for Biomedical Applications, University of Turin, Orbassano (TO), May 26th, 2020.
• Science communication:	- "Ricercatori in classe: la scienza e la ricerca incontrano i giovani", online event organized by Fondazione Umberto Veronesi with High School Students of Santorre di Santarosa, May 27, 2020
• Editorial duties:	Ad hoc reviewer for International Journal of Developmental Neuroscience and Neurochemical Research.
• others	Talk:"The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning". Online Workshop on Glial cells-neuron crosstalk in CNS health and disease, October 1-3, 2020. - Poster: "The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning". CSHL virtual meeting "Glia in Health and Disease", July 16-19, 2020 - Poster: "Single-cell RNA Sequencing unveils an inedited molecular and functional heterogeneity of cerebellar astrocytes".Virtual Meeting "From Stem cells to human development", September 8-11, 2020.
Organizational activities and responsibilities at NICO:	- Responsible of the ZEISS Axio Scan.Z1 use at NICO - Responsible of the Neurolucida system II
Speakers invited:	Dr. Erica Staurenghi, Dept of Clinical and Biological Sciences (DSCB), University of Turin.
Other organizational activities:	na
Workshops, Schools or Conferences organized:	- Workshop GLIAL CELLS-NEURON CROSSTALK IN CNS HEALTH AND DISEASE (with E. Boda and A. Buffo), October 1-3, 2020 (Online event)

	https://www.nico.ottolenghi.unito.it/Agenda/GLIAL-CELLS-NEURON-CROSSTALK-IN-CNS-HEALTH-AND-DISEASE
Technology transfer achievements (patents, etc.):	na

Giulia Nato, PostDoc, lead responsible of research on astrocyte neurogenic activation and reactivity

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	-Matteo Bergami, University Hospital Cologne; -Benedikt Berniger, King's College London; -Philip Greulich, Univ of Southampton
• Invited talks:	BRAYN, 3rd Brainstorming Research Assembly for Young Neuroscientists, Online Congress, 25-27 November 2020
• Science communication:	na
• Editorial duties:	na
• others	-Chairperson at the Workshop "Neuro-glia crosstalk in health and disease" -Poster presenter at the Online Workshop "Neuro-glia crosstalk in health and disease" (October 1st-3rd, 2020)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Martina Lorenzati, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Prof. Fernando De Castro (Cajal Institute, Madrid, Spain)
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Talks: (i) 2° incontro nazionale MORFOLOGIA E DINTORNI Turin (Italy), Title: "c-Jun N-terminal Kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination"; (ii) SINS (Italian Society for the Neurosciences) Congress – Online, Title: "c-Jun N-terminal Kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination". Attended meetings: Workshop GLIAL CELLS-NEURON CROSSTALK IN CNS HEALTH AND DISEASE, October 1-3, 2020 – Online.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	PhD representative (PhD in Neuroscience, Department of Neuroscience "Rita Levi Montalcini" – Turin); co-organizer of the COVID-19 fundraising for Amedeo di Savoia Hospital and ASL "Città di Torino"
Workshops, Schools or Conferences organized:	Co-organizer of the workshop "Success and Science" held by Allegra Via (Sapienza University, Rome) for the PhD program in Neuroscience.
Technology transfer achievements (patents, etc.):	na

Maryam Khastkhodaei , PhD student

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

Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Attended: -SINS (Italian Society for the Neurosciences) PhD Webinar,September 29-30,2020 -Workshop of GLIAL CELLS-NEURON CROSSTALK IN CNS HEALTH AND DISEASE, October 1-3, 2020 (Online event) -BraYn - 3rd Brainstorming Research Assembly for Young Neuroscientists,November 25 - 26, 2020, - Webconference
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Marta Ribodino, Junior Researcher

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Attended meetings:

	Reactive features and neurogenic potential of striatal astrocytes upon excitotoxic lesion: role of the transcription factor SOX2. Online BraYn Conference, 26 November 2020. Poster Presentation. NSC-Reconstruct workshop (1-3 October 2020), online.
Organizational activities and responsibilities 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:[1]	na

4. Research activity in 2020[2]

a. Summary (500 characters)

In 4 collaborative studies published in prestigious journals, our group demonstrated that: 1) hESC-derived progenitors grafted in the striatum of a rat model of Huntington's disease integrate in neural circuitries and improve functional recovery; 2) miR-125a-3p negatively modulates remyelination; 3) oxysterols enriched in Alzheimer's Disease brain induce astrocyte reactivity and, in turn, synaptotoxicity. Also, we participated in a drug screening approach to identify compounds modulating LMNB1 levels.

b. Background and rationale (3000 characters)

In 2020 we have put our main focus on the investigation of the contribution of glia to CNS physiopathology. The pandemics significantly impacted on some areas of our research (studies on therapeutic approached in ADLD that required a training period abroad and acquisition of cell lines from distinct labs). However, overall, we maintained a good productivity level.

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

[1] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.

[2] Use times new roman 11 for the text.

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, as well as to define strategies to rescue OL dysfunctions. To meet these needs, we worked to disclose both intrinsic and extrinsic/environmental factors affecting OPC maturation and myelin deposition. We also approached the study of OL maturation and myelin deposition during human fetal cerebellar development, with the aim of disclosing any existing spatiotemporal pattern in relation with neuronal development and maturation.

In regard to astrocyte (AS) lineages, AS heterogeneity remains poorly understood. The disclosure that an ontogenetic program, tightly regulated in space and time, determines AS heterogeneity in the cerebellum (Cerrato et al., PLoS Biol 2018) and that astrocytes are involved in the onset and progression of several neurodegenerative diseases such as cerebellar ataxia (Cerrato, Journal of Clinical Medicine 2020) prompted new questions on how mechanistically such diversity is achieved, and about lineage relationships between distinct AS types and cerebellar neurons. To these aims, we approached cutting edge analytic tools such as single nuclei RNA sequencing (snRNAseq) alone or combined with ATAC-seq analyses (multi-omic analyses), that allow to clarify with an unprecedented resolution AS transcriptional profiles and their regulatory mechanisms. This approach will allow to disclose the mechanisms that regulate astrocyte development and mature states in mice with an unprecedented spatial and temporal resolution. Single cell RNAseq analyses have also been transferred to neuroblasts derived from the activation of striatal AS (Nato et al., Development 2015) to delineate relation to other neuronal populations and ontogenic profile. Moreover, we investigated mechanisms of AS toxicity in neurodegeneration.

In parallel, we have continued the work to develop cell therapies for Huntington's disease (HD) as a promising complementary option for disease modifying therapies that do not solve the problem of lost neurons. We investigated the therapeutic effects of transplantation of human striatal cells 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 OL phenotypes;
(c) disclose how astrocytes heterogeneity is achieved, with temporal and spatial resolution;
(d) investigate astrocytes pathological and therapeutic roles in neurological diseases;
(e) explore cell replacement strategies based on hESC derivatives and promotion of circuit plasticity.

d. Results (4000 characters)

(a) We demonstrated 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. (Coll.

with Dr. D. Lecca and Prof. MP Abbracchio, Univ of Milano; Dr. C Giorgi, EBRI and CNR; Dr. A Bertolotto, NICO). doi: 10.1002/glia.23819

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)

We demonstrated that the post-injury exposure to airborne PM2.5 hampers remyelination, disturbs oligodendroglia differentiation dynamics and survival, and promotes astroglia and microglia reactivity. (**Parolisi R**, *Montarolo F.., Bertolotto A*, **Buffo A**,.., **Boda E.**, under rev, Neurochem Int).

We found that the germinal ablation of the MAPK c-Jun N-Terminal Kinase isoform 1 (JNK1) results in persistent myelin abnormalities *in vivo* and cell-autonomously determines alterations of OPC proliferation and OPC/OL branching architecture. (**Lorenzati M**, **Boda E**, **Parolisi R**,…, **Buffo A**, *Vercelli A.*, under rev Sci Rep)

(b) We contributed to validate a novel drug screening strategy for identifying compounds that modulate the levels of lamin B1 (LMNB1), whose overexpression causes ADLD. This approach identified alvespimycin, an HSP90 inhibitor, as capable of significantly reducing LMNB1 levels in different cell lines and rat primary glial cells. (Coll with Dr. E. Giorgio, University of Pavia; Prof. A Brusco, Dept Medical Sciences, Univ of Turin; Dr. N Pedemonte, IRCCS Gaslini, Genova; Prof. P Cortelli, University of Bologna). doi: 10.1002/humu.24147.

(c) Toward a better understanding of the emergence of cerebellar AS heterogeneity, we identified ACSA-2 as a new marker of glial-restricted precursors of the prospective white matter in the postnatal cerebellum. This marker, in combination with GLAST, allows to distinguish and isolate astrocyte-committed progenitors from multipotent progenitors of the postnatal cerebellum (Kantzer C, Parmigiani E, **Cerrato V**, …, **Buffo A**, Bosio A, under rev, J of Neurosci Research).

(d) We found that oxysterols typically found in the AD brain induce AS reactivity and the release of several mediators that, in turn, affect neuronal health and cause synaptotoxicity. In this frame, a major role is played by lipocalin-2 (Lcn2) released by reactive AS. (Coll. with Prof. G. Leonarduzzi, DSCB, UniTO. Staurenghi E, **Cerrato V**, …, **Buffo A**, Noble W, Gomez Perez-Nievas B, Leonarduzzi G.) doi: 10.1016/j.redox.2020.101837

We contributed to show that mesenchymal stem cells attenuate detrimental features of reactive astroglia and that AS may be empowered in their protective and reparative actions by MSC. (Vigo T, Voulgari-Kokota A.. , **Buffo A**, Kerlero de Rosbo N, Uccelli A., University of Genova) doi: 10.1002/glia.23958.

(e) In replacement studies implanted human cells showed high rate of survival in the absence of uncontrolled overgrowth and partly progressed towards mature striatal neurons. Human neurons also displayed integration into the host tissue by reaching striatal target regions and by establishing synaptic connections. Behavioral analysis demonstrated functional recovery in lesion-dependent

sensorimotor responses. (Besusso D*, *Schellino R*, *Boido M*., **Parolisi R**.., *Vercelli A*, **Buffo A**# and Cattaneo E#). Italic, NICO collaborators; * cofirst,# colast authors doi: 10.1016/j.stemcr.2020.03.018

e. Advancement in the field (1000 characters)

Among published papers, we foresee that the following findings will have the major impact:
(i) Evidence that miR125a-3p manipulation could be exploited to foster oligodendroglia maturation and white matter repair in vivo
(ii) Evidence that oxysterols found in AD brain can induce a synaptotoxic astrocyte reactivity mediated by Lcn2 release
(iii) The development and validation of a novel drug screening strategy for identifying potential drugs for treating genetic diseases associated with deletions/duplications and paving the way toward Phase II clinical trials
(iv) Advancements towards the validation of cell replacement strategies in HD

f. Publications[1]

Vigo T, Voulgari-Kokota A, Errede M, Girolamo F, Ortolan J, Mariani MC, Ferrara G, Virgintino D, **Buffo A**, Kerlero de Rosbo N, Uccelli A. (2020) *Mesenchymal stem cells instruct a beneficial phenotype in reactive astrocytes*. Glia. Dec 31. doi: 10.1002/glia.23958.

Staurenghi E, **Cerrato V**, Gamba P, Testa G, Giannelli S, Leoni V, Caccia C, **Buffo A**, Noble W, Perez-Nievas BG, Leonarduzzi G. (2020) *Oxysterols present in Alzheimer's disease brain induce synaptotoxicity by activating astrocytes: A major role for lipocalin-2.* Redox Biol. 39:101837. doi: 10.1016/j.redox.2020.101837.

Giorgio E, Pesce E, Pozzi E, Sondo E, Ferrero M, Morerio C, Borrelli G, Della Sala E, **Lorenzati M**, Cortelli P, **Buffo A**, Pedemonte N, Brusco A. (2020) *A high-content drug screening strategy to identify protein level modulators for genetic diseases: a proof-of-principle in Autosomal Dominant LeukoDystrophy (ADLD).* Hum Mutat. Nov 30. doi: 10.1002/humu.24147.

Besusso D, Schellino R, Boido M, Belloli S, **Parolisi R**, Conforti P, Faedo A, Cernigoj M, Campus I, Laporta A, Bocchi VD, Murtaj V, Parmar M, Spaiardi P, Talpo F, Maniezzi C, Toselli MG, Biella G, Moresco RM, Vercelli A, **Buffo A***, Cattaneo E. (2020) *Stem Cell-Derived Human Striatal Progenitors Innervate Striatal Targets and Alleviate Sensorimotor Deficit in a Rat Model of Huntington Disease.* Stem Cell Reports. 14(5):876-891. * co-corresponding author

Buffo A, Ceruti S. (2020) *Editorial overview: Modulation of neurogenesis*. Curr Opin Pharmacol. 50:96-99. doi: 10.1016/j.coph.2020.03.001.

Marangon D, **Boda E**, **Parolisi R**, Negri C, Giorgi C, Montarolo F, Perga S, Bertolotto A, **Buffo A**, Abbracchio MP, Lecca D. (2020) *In vivo silencing of miR-125a-3p promotes myelin repair in models of white matter demyelination.* Glia. 68(10):2001-2014. doi: 10.1002/glia.23819.

[1] Please DO NOT include papers in press or submitted.

Mitoma H, **Buffo A**, Gelfo F, Guell X, Fucà E, Kakei S, Lee J, Manto M, Petrosini L, Shaikh AG, Schmahmann JD. (2020) *Consensus Paper. Cerebellar Reserve: From Cerebellar Physiology to Cerebellar Disorders*. Cerebellum. 19(1):131-153. doi: 10.1007/s12311-019-01091-9.

Becerra-González M, Varman Durairaj R, Ostos Valverde A, Gualda EJ, Loza-Alvarez P, Portillo Martínez W, Gómez-González GB, **Buffo A**, Martínez-Torres A. (2020) *Response to Hypoxic Preconditioning of Glial Cells from the Roof of the Fourth Ventricle*. Neuroscience. 439:211-229. doi: 10.1016/j.neuroscience.2019.09.015.

Finetti F, Schiavo I, Ercoli J, Zotta A, **Boda E**, Retta SF, Trabalzini L. (2020) *KRIT1 loss-mediated upregulation of NOX1 in stromal cells promotes paracrine pro-angiogenic responses*. Cell Signal. 68:109527. doi: 10.1016/j.cellsig.2020.109527.

Boda E, Rigamonti AE, Bollati V. (2020) *Understanding the effects of air pollution on neurogenesis and gliogenesis in the growing and adult brain*. Curr Opin Pharmacol. 50:61-66. doi: 10.1016/j.coph.2019.12.003.

Cerrato V. (2020) *Cerebellar Astrocytes: Much More Than Passive Bystanders In Ataxia Pathophysiology*. J Clin Med. 9(3):757. doi: 10.3390/jcm9030757.

Fornasari BE, El Soury M, **Nato G**, Fucini A, Carta G, Ronchi G, Crosio A, Perroteau I, Geuna S, Raimondo S, Gambarotta G. (2020).*"Fibroblasts Colonizing Nerve Conduits Express High Levels of Soluble Neuregulin1, a Factor Promoting Schwann Cell Dedifferentiation*." Cells. 1;9(6):1366. doi: 10.3390/cells9061366.

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

In OL studies, we will extend the investigation of mechanisms underlying distinct vulnerability of dorsal and ventral cells in response to DNA damage 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. Further, we will assess the effect of the exposure of environmental pollutants, such as airborne PM, on the pathological course and plasmatic extracellular vesicles (EVs) abundance/source/content in murine models of MS. With this, we aim to identify intrinsic and extrinsic factors regulating the OPC maturation potential and possibly targetable for preclinical intervention. In addition, we will generate glial cells from pluripotent stem cells of ADLD patients to fully validate the therapeutic value of an allele silencing strategies with already proved effective in other models.

The investigation of AS heterogeneity will proceed through multi-omic analyses of cerebellar cells in both mouse and both mouse and human cerebella, to describe the unfolding of AS lineages, disclose the mechanistic milestones driving distinct trajectories, and highlight features of each AS type specific to its interplay with defined neuronal circuits. These topics will be addressed with a particular attention to the spatial topography of the cerebellum, highly relevant for both its development and functioning.

In replacement studies, we will specifically address the impact of environmental enrichment on graft therapeutic efficacy and investigate how long differentiation times of human cells act in tolerization protocols. In parallel, the study of the spontaneous neurogenic activation and reactivity of adult mouse striatal AS will define new neurons identities and propose 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; (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 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 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 rev). This is related to a differential ability to counteract oxidative stress and distinct levels of NRF2, a master regulator of cell detoxifying functions. Whether this or other mechanisms explain 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) could “spread” aging-related dysfunctions in a paracrine manner. Active components in OPC SASP remain to be dissected.
- Epidemiological studies show a strong association between exposure to airborne PM, increased prevalence of MS and higher rates of hospital admissions for MS and MS relapses. We also showed that PM exposure hampers myelin repair and oligodendroglia survival/differentiation (Parolisi et al., under rev). It remains to be clarified whether PM could affect the clinical course of MS experimental models and which mechanisms mediate the lung-to-CNS communication upon PM exposure.
- 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. Yet, how such extrinsic cues are translated within the cells in a permanent phenotype remains to be understood. 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 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 rescue (Besusso, Schellino, 2020). However, there is need to improve graft composition and circuit reconstruction with consequent functional impact. Moreover, devising immunotolerization protocols will help advancing the clinical applications of these strategies.

An alternative approach 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 2020 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;
- optimising 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, by a NRF2-directed pharmacological strategy, we will first assess the role of NRF2 as a possible cell-intrinsic mechanism at the basis of OPC divergent responses to DNA damage. By genetic/pharmacological approaches, we will also investigate the mechanisms upstream to NRF2 divergent regulation dorsal and ventral OPCs and expand our investigation to assess whether the postnatal and/or prenatal administration of NAC, an FDA-approved glutathione precursor, is able to correct Cit-K KO dependent pathology. Coll. with Prof. F Di Cunto and Dr. A Bertolotto (NICO).

Understanding the role of PM exposure as a possible trigger/risk factor for MS We will investigate whether PM exposure may operate as a trigger/risk factor for MS/MS relapses in predisposed subjects by combining the induction of experimental autoimmune encephalomyelitis (EAE) in mice and PM exposure. Clinical score and neuropathological evaluations will be performed to evaluate the onset and progression of the disease. Cellular and molecular mechanisms of PM effects and interactions will autoimmunity in MS pathogenesis will be studied. (Coll. with Prof. V Bollati, Dept Clinical Sciences and Community Health, University of Milan)

- **Validation of a therapeutic strategy in ADLD unique human disease models** We will develop human model of disease by generating human glia from induced pluripotent stem cells of ADLD patients and healthy subject, (ii) examine the transcriptome of ADLD cells to better understand ADLD ethiology and pathology (iii) prospectively, provide full validation on these models of therapeutic silencing strategies already proved effective in fibroblasts, mouse cells and human reprogrammed neurons (Giorgio, 2019). (Prof. A Brusco, Dept Medical Sciences, Univ of Turin; Prof. P Cortelli, University of Bologna).

- **Identification of molecular determinants of astrocyte diversity and ontogenesis.** We will examine embryonic and postnatal AS isolated from hGFAPGFP mice with single nuclei RNAseq. This approach will be complemented with labelling of distinct cohorts of progenitors and multi-omic analyses (ATAC-seq combined with snRNAseq). Moreover, we will analyze published datasets to reveal new level of molecular and functional diversity in adult cerebellar AS. Expanding to human cerebella we aim to delineate in both rodents and humans the unfolding of AS lineages, disclose the mechanistic milestones driving distinct trajectories, and highlight features of each AS type specific to its interplay with defined neuronal circuits. These topics will be addressed with a particular attention to the spatial topography of the cerebellum, highly relevant for both its development and functioning. (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**. Our efforts will be aimed at: (i) finalizing the study on the effects of enriched environment on graft maturation, connectivity and therapeutic efficacy; (ii) asking whether in utero or early postnatal graft of pluripotent stem cells derivative in rats or mice induces tolerization of the host immune system despite very slow maturation time of human cells; (iii) test in vivo human striatal cells derived from a second-generation protocols to examine whether they display increased in vivo clinical relevance.
In view to reprogram striatal AS toward striatal neuronal fates we will continue studying mechanisms driving the neurogenic activation of striatal AS by applying genome-wide surveys of gene regulation and by disclosing the role of SOX2. Moreover, we will qualify the produced neuroblasts according to morphology and transcriptional profile.
(Coll: Proff A Vercelli, M Boido, NICO; Prof. E Cattaneo, Univ of Milano; Prof F Luzzati, NICO; Prof. S Nicolis, Univ M Bicocca; Prof M Parmar, 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 2020

Laboratory name: **Embryonic neurogenesis**

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

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

2. GIORGIA IEGIANI

Degree: MoS in Molecular Biotechnology
Birthdate: 17/04/1996
Nationality: Italian
Gender: F
Phone: 011 6706616
Email: giorgia.iegiani@edu.unito.it
Position: PhD 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

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

Email: giadaonorato93@gmail.com

Position: PhD Student

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

CURRENT AND PENDING GRANTS

Starting-end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
01/01/2020 30/06/2025	Development of Citron Kinase as a therapeutic target for brain tumors. IG 23341	PI	AIRC	PI	855.000
04/12/2019 03/06/2022	Implementazione di modelli cellulari e genetici per la validazione di varianti genomiche associate a patologie neurodegenerative RF= 2019.2276	PI	CRT foundation	PI	35.000
01/01/2020 31/12/2020	Validation of primary microcephaly genes as new therapeutic targets for glioblastoma multiforme	Gianmarco Pallavicini	FIRC fellowship	PI	25000

2. SCIENTIFIC ACTIVITIES IN 2020

Ferdinando Di Cunto (PI)

Supervised PhD students:	Giorgia Iegiani Giada Onorato
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	- Prof. Stephanie Bielas, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA. - Prof. Wieland B. Huttner, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.

[2] Coordinator/PI of research unit/team component.

	- Prof. Pierre Gressens, Inserm, U1141, Paris, France; Univ Paris Diderot, Sorbonne Paris Cité, UMRS, 1141, Paris, France. - Dr. Yohann Couté, Laboratoire Biologie à Grande Echelle, Biosciences and Biotechnology Institute of Grenoble, France
• Invited talks:[1]	na
• Science communication:[2]	na
• Editorial duties:	- Associated Editor of PLoS ONE - Associated Editor of Frontiers in Neurogenesis
• others[3]	Board member of SINS (Società Italiana di Neuroscienze) Member of: American Society of Cell Biology ; Società Italiana di Biofisica e Biologia Molecolare (SIBBM); Associazione di Genetica Italiana (AGI)
Organizational activities and responsibilities at NICO:	Data management
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	17/03/2020 – Present: Scientific blog for general public on COVID-19 pandemics web personale: https://standandfightthevirus.blogspot.com/ 21/05/2020: Invited web seminar for general public: Fake news: diagnosi e terapia 04/06/2020: Invited web seminar for general public: Homo Deus: fantascienza o scienza del futuro? 25/05/2020: Web interview with #culturavirus association 27/05/2020: Invited seminar for the Brain Awareness Week
Technology transfer achievements (patents, etc.):	na

4. Research activity in 2020[5]

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.

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[2] Public engagement
[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[4] No university appointments.
[5] Use times new roman 11 for the text.

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 which are known to contribute to genetic microcephaly. Finally, considering the strong involvement of CITK in proliferation, we are addressing the hypothesis that it may be required also by brain tumor cells, in particular those that characterize the pediatric tumor medulloblastoma. If this possibility should be confirmed, CITK could be an excellent target for the development of new drugs for these devastating tumors.

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 arborizations induced by trisomy are not neuron-intrinsic, because they are not present in cultures. In contrast, the characteristic defects in dendritic spines are visible both in cultures and in vivo. Moreover, trisomic neurons may be characterized by delay of cell migration. We are now focusing our attention on the role played in these phenotype TTC3, a gene located in the region of Chr. 21 believed to play the strongest role in determining intellectual disability.
4. 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]

Pallavicini G, **Iegiani G**, Berto GE, Calamia E, Trevisiol E, Veltri A, Allis S, **Di Cunto F**. (2020) *CITK Loss Inhibits Growth of Group 3 and Group 4 Medulloblastoma Cells and Sensitizes Them to DNA-Damaging Agents*. Cancers (Basel). Feb 26;12(3):542. doi: 10.3390/cancers12030542. PMID: 32111106; PMCID: PMC7139701.

Frasca A, Spiombi E, Palmieri M, Albizzati E, Valente MM, Bergo A, Leva B, Kilstrup-Nielsen C, Bianchi F, Di Carlo V, **Di Cunto F**, Landsberger N. (2020). *MECP2 mutations affect ciliogenesis: a novel perspective for Rett syndrome and related disorders*. EMBO Mol Med. Jun 8;12(6):e10270. doi: 10.15252/emmm.201910270. Epub 2020 May 8. PMID: 32383329; PMCID: PMC7278541.

Onorato G, Di Schiavi E, **Di Cunto F**. (2020) *Understanding the Effects of Deep Space Radiation on Nervous System: The Role of Genetically Tractable Experimental Models*. Frontiers in Physics 8, 362 DOI=10.3389/fphy.2020.00362

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.

g. 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

[1] Plese DO NOT include papers in press or submitted.

biological significance of mutations identified in a clinical setting, in patients affected by neurodevelopmental and neurodegenerative disorders.

h. 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.

i. 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.

j. 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

k. 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.

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

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: Neuropsychopharmacology

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

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

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Position: Research associate

Role & expertise: Biotechnology, behavioral analysis, immunohistochemistry

2 ILARIA BERTOCCHI

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Nationality: Italian

Gender: Female

Phone: +390116706632

Email: ilaria.bertocchi@unito.it

Position: Research contract

Role & expertise: Behavioral analysis, immunohistochemistry, biotechnology

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
2020-2022	Nuove prospettive terapeutiche nel trattamento della sindrome dell'X-fragile	Carola Eva	Fondazione CRT	PI	**35000**

3. SCIENTIFIC ACTIVITIES IN 2020

Carola Eugenia Eva (PI)

Supervised PhD students:	Supervised PhD students: Mattia Ghigo
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	● 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' -Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherlands -Christoph Thiemermann, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK. NLRP3 inflammasome
● Invited talks:[3]	na
● Science communication:[4]	na
● Editorial duties:	Neuropharmacology European Journal of Pharmacology

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.

[2] Coordinator/PI of research unit/team component.

[3] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

[4] Public engagement

	Biomolecules
• others[1]	**Poster:** 'Metabolic and behavioral sex-related differences induced by conditional inactivation of Npy1r gene in mice' 12° FENS, 11-15 July 2020
Organizational activities and responsibilities at NICO:	In charge for hygiene anti-smoke rules
Speakers invited:	na
Other organizational activities:[2]	Founding member and President of the spinoff S&P BRAIN
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Alessandra Oberto, Research contract (RU)

Supervised PhD students:	Mattia Ghigo
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	-Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) -Mazahir T. Hasan, PhD Ikerbasque Professor Laboratory of Memory Circuits Achucarro Basque Center for Neuroscience Meibergdreef 47 - 1105 BA Amsterdam - The Netherlands -Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University -Mark Shlomchik, MD, PhD, UPMC Endowed and Distinguished Professor of Immunology Chair, Department of Immunology, University of Pittsburgh School of Medicine. -William Wisden, Imperial College London Dept Life Sciences Laboratory of Molecular Neuroscience
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	**Poster:** 'Metabolic and behavioral sex-related differences induced by conditional inactivation of Npy1r gene in mice' 12° FENS, 11-15 July 2020
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

[1] Posters at meetings, participation in the board of scientific societies, referee for grant agencies

[2] No university appointments.

Technology transfer achievements (patents, etc.):	na

Ilaria Bertocchi, Research contract (RTDA)

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 -José María Delgado García, Division de Neurociencias Universidad Pablo de Olavide Sevilla-41013, España (Spain) -Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University -David M. Bannerman, Department of Experimental Psychology, University of Oxford, Radcliffe Observatory, Anna Watts Building, Woodstock Rd, Oxford, OX2 6GG, UK -Christoph Thiemermann, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK
• Invited talks:	'The hidden role of NLRP3 inflammasome in obesity-related COVID-19 exacerbations: lessons for drug repurposing'. 21 Ottobre 2020, ore 15:00 GMT. 'The pharmacology of drugs for COVID-19' International Webinar, sponsored by the British Journal of Pharmacology and the British Journal of Clinical Pharmacology
• Science communication:	na
• Editorial duties:	Behavioural Brain Research
• others	NICO NeuroWebinar 9/10/2020 **Poster:** 'Metabolic and behavioral sex-related differences induced by conditional inactivation of Npy1r gene in mice' 12° FENS, 11-15 July 2020
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:	na
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[1]	na

4. Research activity in 2020[2]

a. Summary (500 characters)

We used conditional mutant mice to investigate the role of NPY-Y1R in: 1) susceptibility to metabolic syndrome in female mice; 2) vulnerability to diet-induced obesity in male mice 3) changes in plasticity and perineuronal nets (PNN) and cognitive functions. We also investigated the effects of the Grin2A(N596S) mutation in mice.

b. Background and rationale (3000 characters)

Neuropeptide Y (NPY) is one of the most abundant neuropeptides within the CNS that regulates several important physiological functions. Our previously generated conditional knockout mouse model ($Npy1r^{rfb}$ mice) (Bertocchi et al., 2011) was used to investigate the following hypotheses:

1) Sex differences are important in epidemiology, pathophysiology, treatment, and outcomes in many diseases. Low estrogens levels, in conditions such as menopause or ovariectomy (OVX), are associated with higher risk of hypertension, obesity, diabetes, and severe cardiovascular disease. It's therefore crucial to understand the role of estrogens in energy balance and metabolism, besides its principal regulation of the reproductive axis. Limbic Y1R may represent a key target through which estrogens modulate energy metabolism in relation to reproductive functions.
2) Dysfunctions of the NPY system have been implicated in human diseases such as obesity, type II diabetes and metabolic syndrome, raising the possibility that intervention on this system may provide therapeutic benefits for these diseases. Low limbic Y1R in male mice might affect susceptibility to metabolic challenges in adulthood and vulnerability to obesity and associated disorders.
3) NPY is expressed in GABAergic neurons and acts as a homeostatic regulator of cortical and hippocampal excitatory neurotransmission in particular via Y1R. NPY-Y1R signaling has a role in modulating perineuronal nets (PNNs), important plasticity regulators. NPY-PNN interaction in different brain areas may contribute to cognitive and emotional functions.

We carried on a comprehensive and thorough study about a mouse model carrying the point mutation Grin2a(N615S), analogous to a *de novo* mutation found in a patient affected by early onset epileptic encephalopathy. GluN2A(N615S) mice may be a valuable murine model to elucidate different neuropathological mechanisms.

[1] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.
[2] Use times new roman 11 for the text.

c. Objectives (1000 characters)

Objective 1: to uncover the role of Y1R in sex related differences in behavioural and metabolic functions using the Npy1r^{rfb} conditional knockout mouse model. In particular we investigated:

1) sex related difference in cognitive and emotional function between control and Npy1r^{rfb} mice;
2) sex dependent differences in the susceptibility to metabolic challanges of control and Npy1r^{rfb} mice;
3) role of estrogen/Y1R interaction in resiliency of females to metabolic syndrome

Objective 2: to investigate whether the selective ablation of Npy1r in excitatory neurons of the limbic system can modulate perineuronal nets (PNNs) expression and, in turn, cognitive and emotional functions

Objective 3: to provide experimental evidence on the role of the NMDAR voltage-dependent signaling in GRIN2-human disorders.

Results (4000 characters)

We demonstrated that:

1) female mice are resilient to hormonal and metabolic effects of limbic *Npy1r* gene inactivation, suggesting the existence of an estrogen-dependent relay necessary to ensure the maintenance of the homeostasis, that can be mediated by hypothalamic Y1R (Bertocchi et al., 2020). In line with this study, we reviewed data in the literature concerning metabolic and behavioral phenotype of rodents of both sexes, transgenic and knockout for NPY and Y receptor genes, with a particular focus on energy balance, bone homeostasis and emotional and cognitive behavior (Eva et al., 2020).
2) despite Npy1r^{rfb} male mice showed slower body weight growth from weaning to adulthood, following HFD they showed increased body weight, abdominal adipose tissue and blood glucose level compared to their control littermates (Npy1r^{2lox}). These results suggest that a low expression of *Npy1r* in limbic areas induced high susceptibility to diet-induced obesity and glucose intolerance in male mice uncovering a specific contribution of the limbic *Npy1r* gene in the pathogenesis of obesity and type-2 diabetes (Paterlini et al., in preparation).
3) unlike males, both control and Npy1r^{rfb} female mice are resilient to metabolic changes induced by a high fat diet (HFD) for three months since they show no significant changes in body weight growth, adipose tissue weight, glucose intolerance and leptin levels. The resilience to obesity and associated disorders in response to metabolic challenges observed in females is reverted by ovariectomy, an effect that is more pronounced in Npy1r^{rfb} female mice suggesting that estrogens play a role in maintaining energy homeostasis in case of Y1R malfunctioning (Oberto et al., in preparation).
4) low expression levels of Npy1r in the limbic system in adult mice, induced by poor maternal care or by postnatal depletion of *Npy1r* gene (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. This behavioural dysfunction is associated with a different distribution of PNN around PV+ neurons in the PFC. Moreover, Npy1r^{rfb} mice showed impaired spatial learning that is associated with a significant increase in the number of pyramidal cells expressing c-Fos, an established marker for neural activity, and in 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., 2020).
5) in collaboration with Prof. Sprengel (University of Heidelberg, Germany) we provided (i) experimental evidence that the inherent voltage-dependent Ca^{2+} signaling of NMDA receptors is essential for maintaining appropriate responses to sensory stimuli and (ii) a mechanistic explanation for the neurological manifestations seen in the NMDAR-related human disorders with GRIN2-mediated intellectual disability and focal epilepsy.

d. Advancement in the field (1000 characters)

We convincingly demonstrated the sexual dimorphic nature of the NPY-Y1R system, which may contribute to the sex-related differences in several diseases associated to metabolic, emotional and cognitive dyfunctions. Our results suggest that a low expression of *Y1R* in limbic areas induced high susceptibility to diet-induced obesity and glucose intolerance in male mice, uncovering a specific contribution of the limbic *Npy1r* gene in the pathogenesis of obesity and type-2 diabetes.
We also highlighted a previously unknown functional link between NPY-Y1R transmission and PNNs, which may play a role in the control of dorsal hippocampal excitability and related cognitive functions.
Moreover, we studied the role of the tightly regulated Mg^{2+} block of the NMDAR, and thus the voltage-controlled Ca^{2+}-influx, in different regions of the nervous system. This has rarely been tested directly at the behavioral level.

e. Publications[1]

-**Bertocchi I**, Mele P, Ferrero G, **Oberto A**, Carulli D, **Eva C**. (2020) *NPY-Y1 receptor signaling controls spatial learning and perineuronal net expression.* Neuropharmacology 184:108425. Online ahead of print.

-**Eva C**, **Oberto A**, Longo A, Palanza P, **Bertocchi I**. (2020) *Sex differences in behavioral and metabolic effects of gene inactivation: The neuropeptide Y and Y receptors in the brain*. Neurosci Biobehav Rev. 119:333-347.

-**Bertocchi I**, **Oberto A**, Longo A, Palanza P, **Eva C**. (2020) *Conditional inactivation of Npy1r gene in mice induces sex-related differences of metabolic and behavioral functions*. Horm Behav. 125:104824. Epub 2020

-**Bertocchi I**, Foglietta F, Collotta D, **Eva C**, Brancaleone V, Thiemermann C, Collino M. (2020) *The hidden role of NLRP3 inflammasome in obesity-related COVID-19 exacerbations: Lessons for drug repurposing*. Br J Pharmacol. 177(21):4921-4930.

- Mastrocola R, Collotta D, Gaudioso G, Le Berre M, Cento AS, Ferreira Alves G, Chiazza F, Verta R, **Bertocchi I**, Manig F, Hellwig M, Fava F, Cifani C, Aragno M, Henle T, Joshi L, Tuohy K, Collino M. (2020) *Effects of Exogenous Dietary Advanced Glycation End Products on the Cross-Talk Mechanisms Linking Microbiota to Metabolic Inflammation*. Nutrients. 12(9):2497.

[1] Plese DO NOT include papers in press or submitted.

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 future directions are oriented towards a deeper characterization of the functional link existing between NPY and PNNs in regulating plasticity and behavior. Our results indeed suggest that limbic Y1Rs are key targets of maternal care-induced programming of anxiety and cognitive functions. To better correlate the emotional and behavioral deficits found in mice with low expression levels of limbic Npy1r with neuronal plasticity, we will extend PNN analysis to the brain regions crucial for emotional and cognitive functions, such as the prefrontal cortex and the amygdala.
Moreover, we will also investigate: i) how the impairment of PNN formation, expression and composition in different brain areas of Fmr1KO mice affects cognitive and behavioral functions and neuronal excitability and the critical developmental stages at which PNN and correlated behavioral and electrophysiological alterations can be more efficaciously targeted by therapeutic interventions; ii) if mice expressing the GluN2A(N615S) mutation can represent a valuable mouse model of GRIN-related EOEE and for studying the mechanisms underlying epilepsy and its most common related death: sudden unexpected death in epilepsy (SUDEP).

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

1) Role of perinatal environment on neuronal plasticity inhibitors modulation
The lower expression levels of Npy1r in the adult limbic system can be induced not only via gene targeted inactivation in mice but also by using poor perinatal environmental conditions such as low levels of maternal care (Bertocchi et al, 2011). Control mice who received low levels of maternal care during the postnatal period showed behavioral deficits such as higher anxiety and cognitive dysfunction, that may be possibly coupled with PNN alterations in different limbic areas. Indeed, our preliminary findings suggest that mice raised by low caring dams show anxiety and cognitive deficits that are associated with alterations of PNN expression in PFC and hippocampus. It can be therefore of interest to further characterize maternal care induced behavioural deficits and alteration of PNN development and expression.
2) New therapeutic perspectives in the treatment of fragile X syndrome.
Converging evidence from ours and others' laboratories shows that PNNs are critically involved in cognitive and emotional processes and are affected in several neurodisorders. Fragile X syndrome (FXS) is the main monogenic cause of inherited intellectual disability. FXS is caused by the silencing of the fragile X mental retardation 1 (Fmr1) gene, encoding the FMRP protein, or, much more rarely, by point mutations or deletions of this gene. Alterations in dendritic spines and synaptic transmission in the brains of FXS patients and of Fmr1KO mice indicate a disturbance of the development, maintenance and plasticity of neuronal network connections. Matrix metalloproteinases-9 (MMP-9) regulates the formation and organization of PNNs through cleavage of ECM. Recent studies suggested a link between PNNs, MMP-9 activity and PV activity in FXS. FMRP negatively regulates MMP-9 translation and levels of MMP-9 are elevated in FXS. Fmr1 KO mice display delayed PNN development and increased MMP-9 activity levels in the developing auditory cortex (AUC), leading

to impaired PV+ cell development and reduced inhibition. Genetic or pharmacological reduction of MMP-9 levels in Fmr1KO mice normalize PV/PNN development and reverse the hyperresponsiveness of auditory cortical neurons. Although no approved curative therapies exist for FXS, there are several potential medications targeting different pathways involved in FXS pathophysiology that can be beneficial in patients with FXS depending on the individual constellation of comorbid symptoms. As PNNs may play a major role in FXS pathology, the development of therapeutic interventions targeting them may be crucial in reversing several FXS phenotypes, including behavioral deficits and neuronal hyperexcitability.

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

Recently, several mutations have been identified in GRIN2A, encoding the GluN2A subunit of the NMDA receptor, in children suffering from early onset epileptic encephalopathies (EOEE). EOEE comprise a large, heterogeneous group of devastating epileptic disorders mainly characterized by pharmaco-resistant polymorphous epilepsy, severe EEG abnormalities, and developmental regression. Gene-targeted mice expressing the GluN2A(N615S) mutation, analogous to a *de novo* mutation found in a patient affected by EOEE, are strongly prone to audiogenic seizures leading to respiratory arrest and exhibit cognitive deficits and behavioral endophenotypes associated with ADHD, representing the first viable and valuable mouse model of GRIN-related EOEE. The model can be used as a preclinical tool to test the therapeutic potential of new pharmacological approaches to rescue the functional and cognitive disabilities associated with the disease. Moreover, they can be used to get more knowledge about the mechanisms underlying SUDEP.

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

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

Our projects well integrate with the mission since:

-project 1 and 2 will be focused on the understanding of neurobiological, biochemical and neurophysiological mechanisms underlying structural neuronal plasticity and, in turn, a wide range of neuropsychiatric disorders characterized by unbalanced excitatory and inhibitory systems, including FXS;

-project 3 will be focused on better understanding the neurological manifestations seen in the NMDAR-related human disorders with GRIN2-mediated intellectual disability and focal epilepsy;

-all projects aim to reveal new targets for therapeutic interventions in neuropsychiatric disorders.

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

1) *Role of perinatal environment on neuronal plasticity inhibitors modulation.*

Our data suggest that mice expressing lower levels of Npy1r (Npy1r^{rfb} mice and mice experiencing poor levels of maternal care) show alterations in PNN expression in the hippocampus (Bertocchi et al 2020) and in the PFC (preliminary). Such imbalance may alter brain function and plasticity, having important repercussions on behavior. Accordingly, these mice show deficits in tests known to be sensitive to prefrontal and hippocampal integrity (e.g. cognitive and executive functions), lower sociability and high anxiety levels. To further demonstrate the presence of a functional link between NPY-Y1R system and PNN in regulating plasticity and, consequently, behavior, we will deepen the characterization of our murine models behaviorally and molecularly, with particular emphasis on the

number and intensity of PNNs especially in brain areas important for emotional and cognitive functions, such as the PFC, hippocampus, amygdala and hypothalamus. This goal will be achieved also using pharmacological and genetic manipulation aimed at modulating PNNs and the NPYergic system, respectively.

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

We have recently started a collaboration with Prof. Chiurazzi and Dr Tabolacci (Chatholic University of Rome) and with Prof. Tempia and Dr. Hoxha (Nico) to investigate the role of PNN in FXS using a) fibroblast and iPS cell derived neurons from FXS patients and b) Fmr1KO mouse model. The main goal is to identify drugs, available clinically or on clinical trials, targeting PNNs and characterize their therapeutic efficacy in reversing molecular, physiological and behavioral phenotypes in both cellular and mouse models of FXS.

Thanks to the concomitant analysis of behavior and PNNs (studies performed by our laboratory) and of neuronal excitability and inhibition/excitation balance (studies performed by Tempia and Hoxha) along three strategic phases of postnatal development (P20, P40 and P60), we expect to find changes in PNN formation, expression and compositions in brain regions other than the AUC and that these alterations will correlate with specific behavioral and physiological dysfunctions in Fmr1KO mice. Treating mice at different developmental stages with drugs clinically available or in clinical trials (previously screened on ECM in human cells by Chiurazzi and Tabolacci), we then expect to observe a rescue effect on behavioral, molecular and physiological deficits in Fmr1KO mice. We also expect to observe differences in their efficacy, depending on the time of initiation of the treatment, that could correlate with the developmental window of PNN formation in the different brain regions analyzed.

3) *Treating GRIN2A-related epileptic encephalopathies: a preclinical study*. The main objectives are: i) validation of the GluN2A(N615S) mice as animal models of GRIN2A-associated EOEE; ii) individuation of specific brain areas and new molecular targets; iii) assessment of the potentiality of therapeutic approaches. These objectives will be developed thanks to the use of behavioral, electrophysiological, molecular and pharmacological tools. The phenotypic effects of the mutation are clearly visible and can be easily scored, moreover the model allows for preclinical assessment of different treatments to study epilepsy and epilepsy-related disorders at different developmental stages. These features are difficult to find in other animal models and they open experimental strategies impossible to achieve with clinical studies. Moreover, in collaboration with Prof. Palanza (University of Parma), we will record ECG signals (i) during resting conditions, (ii) under selective pharmacological manipulation of cardiac vagal activity, and (iii) during audiogenic seizures. Moreover, to investigate the possible mechanisms underlying SUDEP, we will analyze via time- and frequency-domain analysis the heart rate variability.

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

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

1. Role of perinatal stress on neuronal plasticity inhibitors modulation. Epidemiologic evidence suggests a strong association between poor postnatal environment and the development of psychiatric disorders in adult life. The neuronal plasticity associated with brain development during early infancy might be considered a possible risk factor for psychopathology but also a potent mechanism for compensation. In this project we will investigate the role of NPY induced modulation of plasticity-regulatory molecules in early-life stress-induced anxiety and cognitive deficits. Moreover, we expect to elucidate whether pathological behaviours can be reverted to normal by genetic and pharmacological manipulation in adulthood. The knowledge that will originate from this application

may help to find novel therapeutic approaches and early intervention strategies for the cure and possibly prevention of mental disorders related to early life adversities.

2. *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. 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.

3. *Treating GRIN2A-related epileptic encephalopathies: a preclinical study.* GRIN-associated EOEE syndromes still lack comprehensive genetic models; indeed none of the animal models generated so far, carrying mutation/deletion of Grin genes showed epileptic activity. Thus, in addition to next generation genetic sequencing and *in vitro* studies, there is a great need to consider the functional impact of mutations within a more systems-wide context. GluN2A(N615S) mice represent the first valuable murine model for GRIN-associated EOEE. Also, they represent one of the best animal models for elucidating the mechanisms underlying SUDEP, which are still largely unknown

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 2020

Laboratory name: Neuroendocrinology

3. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator
GIANCARLO PANZICA

Degree: PhD
Birthdate: 17/08/1949
Nationality: Italian
Phone: 011 670 6607
Gender: M
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Personnel

1 STEFANO GOTTI (CoPI)

Degree: PhD
Birthdate: 17/06/1971
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Phone: 011 670 6610
Gender: M
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Position: Associate Professor
Role & expertise:Co-PI

2. ALICE FARINETTI

Degree: PhD
Birthdate: 23/12/1981
Nationality: Italian
Phone: 011 670 6632
Gender: F
Email: alice.farinetti@unito.it
Position: Post-Doc, assegno di ricerca
Role & expertise: Researcher; Neurogenesis, Gonadal hormones, eating disorders models

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

3. MARILENA MARRAUDINO

Degree: PhD Birthdate:

Nationality: Italian 08/06/1988

Phone: 011 670 6632 Gender: F

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marilena.marraudino@unito.it

Position: Post-Doc, assegno di

ricerca

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

4. BRIGITTA BONALDO

Degree: Master Degree Birthdate: 30/01/1992

Nationality: Italian Gender: F

Phone: 011 670 6632

Email: brigitta.bonaldo@unito.it

Position: PhD-student

Role & expertise: : Researcher; Neurodegenerative disorders mo endocrine disruptors

5 GODSTIME STEPHEN K. MORGAN

Degree: Master Degree Birthdate:27/06/1993

Nationality: Ghana Gender: M

Phone: 011 670 6632

Email: godstime.morgan272@edu.unito.it

Position: PhD-Student

Role & expertise: Researcher; eating disorders models

4. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
2019-2022	Dipendenze dalle nuove tecnologie: sviluppo di un modello animale per future applicazioni sull'uomo	Panzica, PI	Fondazione CRT	Coordinator	30000
2020-2022	Ruolo degli ormoni steroidei nella patogenesi e nello sviluppo dei tumori gliali: nuove frontiere per approcci terapeutici	Panzica, PI	UNITO-Dipartimento Progetti traslazionali	Co-PI (other Co-PI Prof. Garbossa)	15000
2019-2022		Panzica-Gotti, PI	UNITO-autofinanziata	Coordinator	13648
Pending	Exposure to bisphenols as new environmental risk factor for Multiple Sclerosis	Panzica, PI	FISM 2020	Coordinator	25882
2019-2021	Neuropeptidi e sviluppo dei disturbi del comportamento nel periodo evolutivo	Panzica-Gotti, PI	UNITO-erogazioni privati	Coordinator	1465
2020-2022	La deprivazione affettiva nell'Anoressia Nervosa: possibile ruolo dell'Ossitocina; studio sul modello animale ABA.	Gotti, PI	Fondazione CRT	Coordinator	35000
2020-2022		Gotti, PI	Unito-Autofinanziata	Coordinator	1609
2020-2023	The role of gonadal hormones receptors in sex-related tracts of multiple sclerosis	Gotti, PI	Unito-ex 60% 2020	Coordinator	2228
Pending	Back to the future: updating hDHODH inhibitors for a new therapy in MS (a Pilot Project)	Gotti, PI	FISM 2020	Coordinator	29969

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.
[2] Coordinator/PI of research unit/team component.

5. SCIENTIFIC ACTIVITIES IN 2020

Giancarlo Panzica (PI)

Supervised PhD students:	Brigitta Bonaldo (co-supervisor with Stefano Gotti)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	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) Cooperation with M. Tena Sempere (Cordoba, Spain) Cooperation with D. Grassi (Universidad Autonoma, Madrid, Spain)
• Invited talks:[1]	na
• Science communication:[2]	na
• 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) • Journal of Neuroendocrinology (2019-today) ***Guest editor:*** • Journal of Neuroendocrinology - *10th International Meeting Steroids and Nervous System* 32: Wiley ISSN:1365-2826. • Frontiers in Neuroendocrinology - *Steroids and Nervous System: Past and Future* Elsevier ISSN: 0091-3022.
• others[3]	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	President of the XXX Convegno Nazionale Virtuale del Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.)
Technology transfer achievements (patents, etc.):	na

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[2] Public engagement
[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[4] No university appointments.

Stefano Gotti, Co-PI[1]

Supervised PhD students:	Brigitta Bonaldo (co-supervisor with GianCarlo Panzica), Godstime Stephen K. Morgan
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Cooperation with dr. P. Collado (UNED, Madrid, Spain)
• Invited talks:	na
• Science communication:	na
• 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	na
Organizational activities and responsibilities at NICO:	First aid and fire safety officer
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Scientific Committee of the XXX Convegno Nazionale Virtuale del Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.)
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[2]	Members of the Local Organizing Committee: International Meeting Steroids and Nervous System, Torino (all lab) 9th edition of 'Premio Nazionale Giovedìscienza', Turin, Italy. (Marraudino 4th Place Prize) Into the brain - Festival 2020 dell'innovazione e della scienza. Chieri (TO), Italy (Marraudino) **FRIDA Unito. Non è sempre sano quel che germoglia! Gli effetti della soia sul nostro sistema endocrino (Marraudino)**

[1] Please duplicate the module for the various lab members
[2] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.

4. Research activity in 2020[1]

a. Summary (500 characters)

Our research's lines have been focused to study the interactions among steroids and nervous circuits, and the behavioral and morphological 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.
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 animal 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 among steroid hormones-neural circuits-behavior. This includes the study of sexual differences at any level, in particular the sexually dimorphic expression of estrogen receptors, the involvement of neurosteroids in several diseases, the effects of the exposure to some endocrine disruptors, and the neurobiological mechanisms of anorexia nervosa, including the role of parental cure.

d. Results (4000 characters)

Estradiol receptors expression in the rat brain
The membrane-associated G protein-coupled estrogen receptor 1 (GPER) immunoreactivity was detected in hypothalamic neurons, astrocytes and oligodendrocytes. Sex and regional differences and changes during the estrous cycle were detected in the total number of immunoreactive cells and in the proportion of neurons, astrocytes and oligodendrocytes that were GPER immunoreactive (**1**). Moreover, GPER mediates actions of estradiol on anxiety, social recognition and spatial memory, but little was known about the regional distribution of GPER in the amygdala and dorsal hippocampus and whether this distribution is affected by sex or by the estrous cycle. In our morphometric analysis

[1] Use times new roman 11 for the text.

of GPER immunoreactivity, we discovered the presence of sex dimorphism and fluctuations of the receptor during the estrous cycle in the amygdala and in dorsal hippocampal formation (**2)**.

Neuroactive steroids (3)
In a commentary about neuroactive steroids we illustrated the collection of papers including many of the invited lectures presented during the last edition of the "Steroids and Nervous System" meeting (2019). The papers cover classical themes (gonadal steroids and glucocorticoids) and very new topics such as the involvement of neuroactive steroids in the control of energy homeostasis and the development of translational models for a variety of neural diseases in which neuroactive steroids are implicated.

Historical overview of neuromorphological studies in Italy (4)
Celebrating the 30° anniversary of the foundation of the Italian Group for Neuromorphological Studies, we wrote a commentary about the origins and the development of the studies of the nervous system in Italy, starting from the XVI century to nowadays.

Diet and psychiatric disorders
Neuropsychiatric disorders stem from gene-environment interaction and their development can be, at least in some cases, prevented by the adoption of healthy and protective lifestyles.
We participate in a review **(5)** about the current evidence in relation to Healthy Eating education, Physical Activity programs, and Sleep hygiene promotion components in the prevention and management of neuropsychiatric disorders and we provide suggestions for clinical practice.

Translational models of mental disease
Eating disorders affect females about 10 times more often than males, but the mechanisms are poorly understood. The ABA animal model for Anorexia Nervosa provide a tool to better understanding these mechanisms. In our study (**6**) we confirmed the impact of the ABA protocol on physical activity, eating behavior and weight loss in a sex-dependent way. Furthermore, when maternal separation (MS) is associated with the ABA protocol there is a further strengthening of the behavioral differences between the sexes.
Due to these results, we investigated the effects of MS on brain areas involved in controlling anxious behavior. We focused our attention on the neurogenesis of the adult hippocampus (**7**), a process involved in the response to environmental stimuli and stressful conditions. The highest number of Ki67 cells (marker of neurogenesis) was found in ABA females who underwent early separation from their mother, while the other female groups showed significantly fewer proliferative cells. It is, therefore, possible that MS is a vulnerability factor that changes, in a sexually dimorphic way, the response to a chronic stressful condition that intervenes later such as the ABA protocol. Due to the different effect of MS in the two sexes, it is plausible to think that sex hormones strongly mediate this process.
To confirm the idea that the hippocampus is a focal point for AN we participated to a multicenter study (**8**) and confirmed the alteration in the volume of the hippocampus in anorexic patients (women) that showed a reduced hippocampal volume compared to healthy women, with no substantial differences between patients with recent onset and those with a longer duration of the disease.

e. Advancement in the field (1000 characters)

Our findings suggest an important estrogenic regulation of hypothalamic function through the membrane receptor GPER: the action may be different in males and in females and may fluctuate during the estrous cycle in females.
Moreover, our behavioral and morphological studies of a rodent model for Anorexia Nervosa led us to think that sex hormones are strongly involved in the different effects observed among the two sexes.

f. Publications[1]

Llorente R, **Marraudino M**, Carrillo B, **Bonaldo B**, Simon-Areces J, Abellanas-Pérez P, Rivero-Aguilar M, Fernandez-Garcia JM, Pinos H, Garcia-Segura LM, Collado P, Grassi D. **2020** *G Protein-Coupled Estrogen Receptor Immunoreactivity Fluctuates During the Estrous Cycle and Show Sex Differences in the Amygdala and Dorsal Hippocampus.* Front Endocrinol (Lausanne). Aug 7;11:537. doi: 10.3389/fendo.2020.00537.

Marraudino M, Carrillo B, **Bonaldo B**, Llorente R, Campioli E, Garate I, Pinos H, Garcia-Segura LM, Collado P, Grassi D. **2020** *G protein-coupled estrogen receptor immunoreactivity in the rat hypothalamus is widely distributed in neurons, astrocytes and oligodendrocytes, fluctuates during the estrous cycle and is sexually dimorphic.* Neuroendocrinology. Jun 22. doi: 10.1159/000509583.

Panzica GC, Melcangi RC. **2020** *Neuroactive steroids and the new decade*. J Neuroendocrinol. Jan;32(1):e12832. doi: 10.1111/jne.12832.

Panzica GC, Michetti F, Tayebati SK, De Giorgio R, Mariotti R, Pacini A, Quartu M **2020** *A bridge among history and new multidisciplinary approaches: the role of G.I.S.N. in the field of neuromorphology*. European Journal of Histochemistry 64, suppl.3: 1 https://doi.org/10.4081/ejh.2020.3200

Briguglio M, Vitale JA, Galentino R, Banfi G, Zanaboni Dina C, Bona A, **Panzica GC**, Porta M, Dell'Osso B, Glick ID. **2020** *Healthy Eating, Physical Activity, and Sleep Hygiene (HEPAS) as the Winning Triad for Sustaining Physical and Mental Health in Patients at Risk for or with Neuropsychiatric Disorders: Considerations for Clinical Practice.* Neuropsychiatr Dis Treat. Jan 8;16:55-70. doi: 10.2147/NDT.S229206.

Farinetti A, Aspesi D, **Marraudino M**, Marzola E, Amianto F, Abbate-Daga G, **Gotti S. 2020a** *Sexually dimorphic behavioral effects of maternal separation in anorexic rats*. Dev Psychobiol. Apr;62(3):297-309. doi: 10.1002/dev.21909.

Farinetti A, Aspesi D, **Marraudino M**, Marzola E, Abbate-Daga G, **Gotti S. 2020b** *Maternal Separation in ABA Rats Promotes Cell Proliferation in the Dentate Gyrus of the Hippocampus*. Neuroscience. Oct 15;446:238-248. doi: 10.1016/j.neuroscience. 2020.08.005.

Collantoni E, Tenconi E, Solmi M, Meneguzzo P, Marzola E, D'Agata F, **Gotti S**, Daga GA, Manara R, Favaro A. **2020** *Hippocampal volumes in anorexia nervosa at different stages of the disorder.* Eur Eat Disord Rev. Nov 13. doi: 10.1002/erv.2806.

[1] Plese DO NOT include papers in press or submitted.

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 will develop several different lines of research:
1 - Collaboration with the laboratory of Prof. Collado in Madrid to investigate the role of gonadal hormones (chiefly estrogens) on neuroendocrine circuits regulating feeding behavior in rats (Madrid) and mice (Torino)
2 - Collaboration with the laboratory of Prof. D. Grassi in Madrid to investigate the action of different estrogen receptors in the regulation of hypothalamic neuroendocrine circuits, chiefly located in the paraventricular nucleus.
3 - Effects of some EDCs on hypothalamic circuits (NPY, POMC, orexin) controlling feeding behavior. The studies will be performed to observe both effects of exposure on adult animals as well as the effects of prenatal exposure to EDCs.
4 - Effects of prenatal exposure to BPA or BPS in EAE model of multiple sclerosis.
5 - Effects of genistein on neural circuits controlling reproduction, metabolism control and other physiological parameters.
6 - 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 consequence of the behavioral results that we have recently published.
7 - With the aid of a research grant from the CRT, we will start the development of an animal model to study the Internet Gaming Disorder (IGD) in order to understand the mechanisms that are underlying the sex differences observed in humans.
8 - In cooperation with the division of neurosurgery, we will investigate the role of estrogens in the development of glioblastoma, and its putative role to generate the observed sex differences in humans.

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 and peripheral nervous system. 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 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. In particular, gonadal hormones are implicated in the development of sexually dimorphic circuits and functions, in the control of important physiological activities as reproduction, metabolism, parental behavior, social behaviors, aggressive behavior and others. Therefore, it is extremely important to elucidate the mechanisms involved in their function, in particular what type of estrogen receptor is implicated in the control of these different circuits and activities.

A large part of our previous and future works is dealing with the study of sex differences at any level, with the effect of the stimulation of different estrogen receptors, as well as the effects of the environment on the nervous system and behaviors.
The environment may, in fact, have an impact on neural circuits due to different factors.
On one side many substances may alter the functions of the endocrine system which is intimately connected to the development and functioning of the nervous system. These compounds are collectively named Endocrine Disrupting Chemicals (EDCs), many of them are xenoestrogens or xenoandrogens, and they could, even in very low concentrations, deeply influence the development and the function of gonadal hormones-dependent neural circuits and related behaviors. EDCs can exert subtle effects by interfering with gene expression and other cellular activities, which can cause transient responses, or permanent impairment. Thus, the impact of EDCs will vary depending by a variety of factors, including way of exposure, duration, and amount of the exposure. The 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. 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. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. 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.
Another important environmental effect is linked to the parental behavior. It has been, in fact, demonstrated that lack of maternal cure may induce permanent alterations of some behaviors in the pups when adult, as well as induce permanent changes in neuroendocrine circuits. Therefore, the maternal separation became an important method to understand, in different situations, how this could impact in behavior and neural circuits. In many cases the effects are different in males and females and this is probably due to an involvement of the gonadal hormones in this mechanism.

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. For this reason, we have planned to collaborate with clinician groups in proposing research project trying to correlate Multiple Sclerosis and Anorexia Nervosa with EDCs/environmental factors.

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

We will focus towards three main topics:

- **Effect of steroids**

One of our aim is to understand how the steroid hormones may interact with the nervous system; we will continue our cooperation with Prof. Collado to study the role of the estrogen receptors in the regulation of the neural circuits involved in the control of food intake.
An important center controlling neuroendocrine circuits linked to feeding behavior is the Hypothalamic Paraventricular Nucleus; recently our cooperation with Prof. Grassi resulted in a series of papers covering different aspects of the role played by estrogens in regulating the neural circuits of PVN. Now we want to explore better the functional significance of recent findings.

- **Effect of EDCs**

EDCs effects in adult mice. Neuroendocrine circuits controlling feeding behavior are targets for the action of EDCs. We will continue our studies exploring the effects on NPY and POMC circuits in adult mice chronically exposed to different EDCs (BPA, TBT, DES). We will also investigate the effects of different bisphenols (BPA and BPS) on maternal behavior as well as on related neuroendocrine circuits (vasopressin and oxytocin).
Genistein effects on circuits controlling reproduction and food intake. We demonstrated that early postnatal GEN administration, at doses similar to that of infant formulas, may interfere with the development of vasopressinergic, nitrergic, and dopaminergic circuits; now we want to test if GEN may interfere with reproductive activity and with circuits implicated in the control of reproduction. In addition, we also want to test if GEN affect food intake and the neuroendocrine control of energy metabolism.

- **Translational studies**

Multiple Scleroses (MS) and EDCs. We have an ongoing PhD project related to a possible involvement of EDCs in the onset of the MS. We will investigate the effects of perinatal exposure to BPA in a murine model of MS, the Experimental Autoimmune Encephalomyelitis. We will evaluate the consequences of BPA exposure on the disease onset and progression and on some physiological parameters.
Anorexia Nervosa and ABA model. Using our ABA model, we want to analyze the effect on the reward system: dopamine neurons (in the Ventral Tegmental and in the Substantia Nigra) and the Serotonin neurons (in the Dorsal Nucleus of the Rafe). Both these systems are altered in the Anorexia Nervosa and this could explain the hyperactivity we observed in anorexic female rats subjected to maternal separation.
Internet Gaming Disorder (IGD). IGD has been recently recognized as a psychiatric condition affecting the younger population. In cooperation with the Child Neuropsychiatric team, we are preparing a review considering chiefly the sex and age differences that are reported in the literature. For IGD is, however, lacking an affordable animal model to investigate the mechanisms that are at the basis of this disease. With the aid of a specific grant from CRT we will try to develop an animal model. We will train young rats to interact with a screen showing some simple play and to have a reward when they touch it. We want to test if we will have a sex difference in the conditioning, and, later, if there are changes in neural circuits (for example in the reward system).
Steroid hormones and glioblastoma. The aim of this project (financed through the translational grant of the department) is to study the role of steroid hormones in the genesis and development of primitive brain tumors of the glial series. Numerous evidencies recognize the importance of the interaction between the glial tumor cell and its microenvironment for the active growth of the neoplasm. Among these, steroid hormones seem to play a crucial role. Therefore, we will study

the role of neuroactive steroids and their agonists/antagonists in the development and growth of gliomas with evaluations performed in animal models and related in vitro concentration, spectrum of action, regulatory capacity, any risk factors and exposure.

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 endpoint 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 (see Panzica and Melcangi, 2016 for a review).
This is the general context, but, in last two decades the problem of how the environment can interact with human and animal physiology to induce pathologies became an important topic for the biomedical sciences. It is not surprising that a large number of synthetic substances may interact with hormone receptors and therefore induce endocrine unbalance and diseases. However, for many years the neuroendocrine effects were underestimated and the nervous tissue was not the main target of studies as well as, more importantly, it was not considered as an important endpoint to be included to develop toxicological tests for the regulations of the EDCs use. Our research, 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 research 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 2020

Laboratory name: Peripheral Nerve Regeneration Unit

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator

1 STEFANIA RAIMONDO

Degree: PhD in Physiology — Birthdate: 25/02/1977

Master's degree in Biological Sciences, University of Turin — Gender: F

Nationality: Italian

Phone: +39 011/6705433

Email: stefania.raimondo@unito.it

Position: Associate Professor

Personnel

1 GIULIA RONCHI

Degree: PhD in Neuroscience — Birthdate: 27/11/1982

Master's degree in Neurobiology, University of Turin — Gender: F

Nationality: Italian

Phone: +39 011/6705433

Email: giulia.ronchi@unito.it

Position: RTDB

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

2 GIOVANNA GAMABAROTTA

Degree: PhD in Cellular Sciences and Technologies, — Birthdate: 22/08/1967

Master's degree in Biological Sciences, University of Turin — Gender: F

Nationality: Italian

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Email: giovanna.gambarotta@unito.it

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

Position: Assistant Professor

Role & expertise: *In vitro* models and biomolecular analysis for peripheral nerve regeneration study

3 FEDERICA FREGNAN

Degree: PhD in Neuroscience
Master's degree in Biological Sciences, University of Turin
Nationality: Italian
Phone: +39 011/6705433
Email: federica.fregnan@unito.it
Position: Research Technician
Role & expertise: *In vitro* model for peripheral nerve regeneration study

Birthdate: 02/07/1976
Gender: F

4 LUISA MURATORI

Degree: PhD in Experimental Medicine and Therapy
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Nationality: Italian
Phone: +39 011/6705433
Email: luisa.muratori@unito.it
Position: Post-doctoral fellowship recipient
Role & expertise: *In vitro* and *in vivo* models for autonomic nervous system regeneration

Birthdate: 02/05/1984
Gender: F

5 BENEDETTA FORNASARI

Degree: PhD in Neuroscience
Master degree in Molecular and Cellular Biology, University of Turin
Nationality: Italian

Birthdate: 11/07/1989
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Position: Post-doctoral fellowship recipient

Role & expertise: In vivo models and biomolecular analysis of peripheral nerve regeneration study

6 MARWA EL SOURY

Degree: Master's Degree in Molecular Biology and Biotechnology, Alexandria University

Birthdate:22/04/1991

Gender: F

Nationality: Egyptian

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Email: marwa.elsoury@unito.it

Position: PhD Student, PhD Program in Neuroscience

Role & expertise: Biomolecular analysis of peripheral nerve regeneration

7 GIACOMO CARTA

Degree: Master's degree in Rehabilitation Science

Birthdate: 24/06/1986

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Gender: M

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

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

8 ALESSANDRIO CROSIO

Degree: Master degree Medicine and surgery, Birthdate: 04/08/1987

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

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

9 FEDERICA ZEN

Degree: Master degree in Industrial Biotechnologies, Birthdate: 06/04/1995

University of Padova Gender: F

Nationality: Italian

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

Role & expertise: Biomolecular analysis of peripheral nerve regeneration

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded
2019-2021	Ricerca Finalizzata 2018	Prof. Stefano Geuna	Regione Piemonte	PI of Research Unit	56500
2019- 2021	Fondo per la Ricerca Locale	Prof. Stefano Geuna	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2.859,70
2019- 2021	Fondo per la Ricerca Locale	Prof.ssa Stefania Raimondo	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2874,70

2019- 2021	Fondo per la Ricerca Locale	Dr.ssa Giulia Ronchi	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2.721,39
2019- 2021	Fondo per la Ricerca Locale	Dr.ssa Giovanna Gambarotta	Ricerca Scientifica finanziata dall'Università di Torino	Coordinator	2703,47

3. SCIENTIFIC ACTIVITIES IN 2020

Stefania Raimondo, PI

Supervised PhD students:	Giacomo Carta (PhD) Alessandro Crosio (PhD) Federica Zen (PhD)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Prof. Victor Sebastian Carriel, Tissue Engineering Group, Dept. Histology, University of Granada, Granada, Spain. - Dott. Ana Colette Maurício, Porto University, Porto, Portugal - Dott. Artur Varejão, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
• Invited talks:	na
• Science communication:	na
• Editorial duties:	- Member of the Editorial Board of *Frontiers in Neuroanatomy* - Member of the Editorial Board of *Biomedicines* - Guest associate editor of *Frontiers in Cellular Neuroscience*
• others	-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

Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	- Scientific director of ECM course "Ridefinire le neuropatie da trauma meccanico", Torino, 19-20 November 2020.
Workshops, Schools or Conferences organized:	Member of the local organizing committee of the GISN 2020 congress
Technology transfer achievements (patents, etc.):	na

Giulia Ronchi, Assistant Professor

Supervised PhD students:	Giacomo Carta (PhD) Alessandro Crosio (PhD)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Prof. Kirsten Haastert-Talini, Institute of Neuroanatomy and Cell Biology, Hannover Medical School (MHH) - Kerimedical, private company, Germany
• Invited talks:	na
• Science communication:	na
• Editorial duties:	- Member of Editorial Board of *Neural Regeneration Research* - Guest associate editor of *International Journal of molecular Sciences*
• others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na

Technology transfer achievements (patents, etc.):	na

Giovanna Gambarotta, Assistant Professor

Supervised PhD students:	Marwa El Soury Federica Zen
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Member of the European Society for the Study of Peripheral Nerve Repair and Regeneration (ESPNR) - Prof. Victor Sebastian Carriel, Tissue Engineering Group, Dept. Histology, University of Granada, Granada, Spain. - Prof. Mohammed Bennis, Laboratory of Pharmacology, Neurobiology and Behavior, Cadi Ayyad University, Marrakech, Morocco
• Invited talks:	na
• Science communication:	na
• Editorial duties:	- Member of Editorial Board of *Brain Sciences* - Guest Associate Editor for *Frontiers in Cellular Neuroscience*
• Others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	Luca Bartesaghi (co-host with Corrado Calì)
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Federica Fregnan, Technician

Supervised PhD students:	Giacomo Carta Federica Zen
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Member of the European Society for the Study of Peripheral Nerve Repair and Regeneration (ESPNR)
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• Others	na
Organizational activities and responsibilities 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:	

4. Research activity in 2020[1]

a. Summary (500 characters)

The research activities of the group have been focused on the study of peripheral nerve repair and regeneration. In 2020 different research topics have been studied: i) understanding the biomolecular and biological processes occurring during peripheral nerve regeneration, ii) methodology and

[1] Use times new roman 11 for the text.

techniques to repair severe injury with nerve substance loss, iii) strategies to improve the regeneration of autonomic peri-prostatic nerves, iv) evaluation of neurodynamic treatment on peripheral nerves.

b. Background and rationale (3000 characters)

Although peripheral nervous system retains 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 represents an enormous stimulus for more research in peripheral nerve regeneration field 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 biomolecular and biological processes involved 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 the cellular colonization of conduits and gene expression, during peripheral nerve regeneration after nerve gap repair with a chitosan conduit; 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.

The role of nerve fibroblasts during nerve regeneration by means of chitosan hollow conduit

Investigating the expression of different genes within a hollow chitosan conduit 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. Biomolecular results were confirmed by immunofluorescence analysis.

In vitro analysis shows that fibroblasts express high levels of different soluble 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 conduit in the early steps after injury, suggests that NRG1 released by nerve fibroblasts might play a key role in the following SC migration and de-differentiation to a "repair" phenotype, contributing to the nerve regeneration inside the conduit.

Peripheral nerve repair with conduits made of natural biopolymers

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.

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.

Decellularized nerve: research of the more reliable decellularization method

A decellularized nerve could represent an alternative strategy to the autograft for repairing injured nerves. Indeed, decellularized nerve retains the 3D structure useful to sustain axons during the regeneration with the complete removal of immunogenic components. Different protocols have been tested in order to obtain the complete decellularization of human, rat, pig and horse nerves and a comparative analysis between the different species was performed. Morphological analysis demonstrated that human decellularized nerves have shown the best degradation of myelin sheath and absence of cellular components than other species analyzed. Further in vivo studies are needed to prove the efficacy of the protocol on human nerves in allowing nerve regeneration.

Strategies to improve the functional recovery after radical prostatectomy

Radical prostatectomy for the removal of prostatic cancer 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 was tested for the study of the autonomic regeneration. *In vitro* results displayed that this biomaterial represents a suitable substrate to improve axonal regeneration in autonomic explant ganglia and to reduce the proliferation of cancer cells. In order to improve the pro-regenerative effect of the chitosan membrane, grating nanostructured membranes have been preliminary tested on primary sensory neurons cultures. Morphological results showed neurites orientation along grating structure.

Assessment of the effects caused by mechanical stimulation on peripheral nervous system

Back pain is the main cause of disability worldwide and clinical and preclinical studies have shown that neurodynamic treatment (NDT), is a successful intervention for this lumbar radiculopathy. Unfortunately, no standard protocol is available, and even if NDT reduces disability and pain, the biological processes involved in the target tissues are still unknown.

In vitro results obtained on neuronal cells models, demonstrated that NDT induces significant dose-response changes promoting cell differentiation, neurite outgrowth, and neuron survival, especially

for sensory and nociceptive neurons without any side effects. Notably, NDT significantly upregulates gene expression of PIEZO1, able to suppress mechanic pain, but not of other genes involved in mechanical allodynia related to neuroinflammation.

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]

Fregnan F, **Muratori L**, Bassani GA, **Crosio A**, Biagiotti M, Vincoli V, **Carta G**, Pierimarchi P, **Geuna S**, Alessandrino A, Freddi G, **Ronchi G**. 2020. *Preclinical Validation of SilkBridge™ for Peripheral Nerve Regeneration*. Front Bioeng Biotechnol.; 8, 835. doi: 10.3389/fbioe.2020.00835. eCollection 2020. PubMed PMID: 32850714.

Pinho AC, Vieira Branquinho M, Alvites RD, Fonseca AC, Caseiro AR, Santos Pedrosa S, Luís AL, Pires I, Prada J, **Muratori L**, **Ronchi G**, **Geuna S**, Santos JD, Maurício AC, Serra AC, Coelho JFJ. 2020. *Dextran-based tube-guides for the regeneration of the rat sciatic nerve after neurotmesis injury.*Biomater Sci.; 8,798-811. doi: 10.1039/c9bm00901a. PubMed PMID: 31904045.

Ronchi G, Gambarotta G, Morano M, **Fregnan F**, Pugliese P, Tos P, **Geuna S**, Haastert-Talini K. 2020 *Critical analysis of the value of the rabbit median nerve model for biomedical research on peripheral nerve grafts*. J Tissue Eng Regen Med.; 14,736-740. doi: 10.1002/term.3036. Pubmed PMID: 2203643.

Fornasari BE, El Soury M, Nato G, Fucini A, **Carta G**, **Ronchi G**, **Crosio A**, Perroteau I, **Geuna S**, **Raimondo S**, **Gambarotta G**. 2020. *Fibroblasts Colonizing Nerve Conduits Express High Levels of Soluble Neuregulin1, a Factor Promoting Schwann Cell Dedifferentiation*. Cells.; 9,1366. doi: 10.3390/cells9061366. Pubmed PMID: 32492853.

Fornasari BE, **Carta G**, **Gambarotta G**, **Raimondo S**. 2020 *Natural-Based Biomaterials for Peripheral Nerve Injury Repair*. Front Bioeng Biotechnol. 2020; 8,554257. doi:10.3389/ fbioe.2020.554257. eCollection. Pubmed PMID: 33178670.

Fornaro M, Giovannelli A, Foggetti A, **Muratori L**, **Geuna S**, Novajra G, Perroteau I. 2020 *Role of neurotrophic factors in enhancing linear axonal growth of ganglionic sensory neurons in vitro*. Neural Regen Res.; 15,1732-1739. doi: 10.4103/1673-5374.276338. Pubmed PMID: 32209780.

Ait-Bali Y, Ba-M'hamed S, **Gambarotta G**, Sassoè-Pognetto M, Giustetto M, Bennis M. 2020 *Pre and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments*

[1] Plese DO NOT include papers in press or submitted.

in adult mice: evidence of cortical and hippocampal dysfunction. Arch Toxicol; 94,1703-1723. doi: 10.1007/s00204-020-02677-7. Pubmed PMID: 32067069.

7. Future directions and objectives for next years

a. Summary (up to 2000 characters):

The first goal of the group will be to realize innovative therapies to improve the patients' outcome after peripheral nerve damage both somatic and autonomic. In collaboration with companies, advanced devices made of natural biopolymers for the repair of severe nerve lesions will be developed. In particular, silk and chitosan conduits will be further tested *in vivo* to evaluate nerve regeneration and functional recovery at longer time points post-surgery (12/18 months).
The nerve regeneration within empty conduits used to repair a nerve gap will be further studied, to assess the role of the different cell populations and factors involved in the regeneration process, with the aim of improving the outcome when larger nerve gaps are repaired.
In addition, protocols for nerve decellularization will be standardized on human nerves harvested from donor cadaver in order to create the basis for the organization of a nerve tissue bank and *in vivo* tests will be performed in order to study the ability of the decellularized nerve to sustain nerve regeneration.
As regard the study on the chitosan membrane (FDA approved), two different nanostructured gratings will be tested *in vitro* and *in vivo* on rat animal model to study the improvement of axonal regeneration in patients undergoing radical prostatectomy and to enhance the functional recovery.
Moreover, the efficacy of neurodynamic treatments on the peripheral nervous system will be studied in an *in vivo* experimental model and on the vagus nerve of healthy human subjects.
Finally, the group is going to approach an innovative research topic focused on the microbiota alteration and its involvement in several peripheral nerve disorders.

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

<u>Improvement of axonal regeneration</u>
New strategies for promoting the outcome after nerve trauma are needed, especially in cases of severe nerve lesions. The stages of nerve bridge formation when the nerve gap is small were deeply studied, showing the important role played by endothelial cells in the migration of Schwann cells from the proximal and distal stumps. On the contrary, the role played by newly formed blood vessels as a substrate for guiding Schwann cell migration and cord formation within an empty conduit needs further investigation and could contribute to understand a further role, in addition to oxygenation, played by vascularization in nerve regeneration.

<u>Strategies to improve the functional recovery after radical prostatectomy</u>
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 would result in minor inconvenience for patients and allow to extend the treatment also for applications in oncology.

<u>Effects of neurodynamic treatments on PNS</u>

Treating 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 gut 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 PNS, or neurodynamic treatment (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.

Microbiota and PNS
In the last decades significant progress has been made in supporting the role of bidirectional and constant communication between the CNS and the gastrointestinal tract (brain-gut axis); recent advances in research have described the importance of gut microbiota in influencing these interactions (leading to broadening the term into “brain-gut-microbiota”, BGM axis) though neural, immune, endocrine, and metabolic signaling. Perturbation in the communication between brain and gut is implicated not only in the pathogenesis and pathophysiology of classic brain-gut disorders such as irritable bowel syndrome, inflammatory bowel disease and other functional gastrointestinal disorders, but also a growing list of neurodegenerative diseases, neurologic pathologies and psychiatric disorders has been demonstrated to be influenced by intestinal microbiota. Dysbiosis has been associated also with liver diseases, cardiovascular diseases, kidney diseases and others. No data are available on the relationship between microbiota alteration and peripheral nerve structure, function and regeneration.

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

The general aim of the group is to study innovative solutions for improving functional recovery after traumatic nerve lesion and iatrogenic nerve injuries. Nerve damage represents one of the major causes of neuronal disability with significant influences on the patient’s 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 investigating innovative strategies of tissue engineering on 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, rat, horse and pig nerves, this project related is an ongoing activity in order to study the best protocol and to perform translational study in *in vivo* models. Preliminary results showed that the decellularization could be influenced by several factors such as the segment of the nerve harvested, the species considered (human, rat, pig, horse) and the different reagents used. This objective will be pursued through the standardization of the protocol allowing to obtain a complete removal of immunogenic elements and maintaining an intact basal lamina to assist axon regeneration. After protocol standardization the further step will be the *in vivo* implantation of decellularized allograft in rat animal model.

ii) Developing a nanostructured chitosan medical device for its application in the urological clinical field. This objective will be pursued testing grating nanostructured membrane with two different topographies for the repair of prostatic nerves in rats. Particularly, this project aims to develop functionalized nanostructured membranes 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 device with the best performance for the following *in vivo* implantation. At this purpose, chitosan membrane will be applied after injury of cavernous nerve in the rat animal model in order to study the ability of the device to sustain nerve regeneration and to achieve the functional recovery.

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 *ex vivo* organotipic culture, behavioral test and *in vivo* analysis on rat animal model.

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) Studying the relationship between microbiota alterations and peripheral nerve structure, function and regeneration. This objective will be pursued by first analyzing the nerve structure, morphology and gene expression in Germ-Free mice compared to conventional mice (in collaboration with Hannover Medical School). The second step will be to study the response to nerve injury when the microbiota is altered.

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 competences and skills.

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

4) the research group focuses 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).
In collaboration with Giulia Nato and Marco Fogli, we are obtaining a confocal microscopy 3D reconstruction of the regenerating nerve within a chitosan conduit at different time points after injury, showing the interaction between Schwann cells and newly formed blood vessels.
A new collaboration with Serena Bovetti and Chiara La Rosa is starting, with the aim of using the iDISCO clarification technique and the light sheet microscopy to obtain whole nerve visualization during the different phases of nerve regeneration after injury and repair.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: Neurophysiology of neurodegenerative diseases

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator

FILIPPO TEMPIA

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Position: tenure track Assistant Professor

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

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Role & expertise: behavioral experiments, histology, molecular biology

[1] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
01/02/2019 10/06/2022	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
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)	Ricerca Finalizzata 2019 / Italian Ministry of Health	PI of research unit	€ 88,500
01/09/2020 31/08/2021	Are early cerebellar circuitry deficits preceding neurodegeneration in A-T disease?	Dr. Eriola Hoxha (PI)	Associazione Nazionale Atassia Telangiectasia	Coordinator	€ 15,000

3. SCIENTIFIC ACTIVITIES IN 2020

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 Aging 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
• others	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

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.
[2] Coordinator/PI of research unit/team component.

Eriola Hoxha, Supervisor and Researcher[1]

Supervised PhD students:	1
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	University of Texas Medical Branch (UTMB), Columbia University
• Editorial duties:	Editor of Frontiers in Aging Neuroscience
Organizational activities and responsabilities at NICO:	Responsible for the water ultrapurification systems
Other organizational activities:	
Workshops, Schools or Conferences organized:	

ALL LAB MEMBERS

Activities:[2]	Open day at NICO

4. Research activity in 2020[3]

a. Summary (500 characters)

In the year 2020 the main effort was to complete the lines of research about the Elovl5 knock-out mouse, model of the spino-cerebellar ataxia type 38 (SCA38). Four manuscripts are submitted or in preparation: 1) the role of Elovl5 in myelin to enable fast action potential conduction along the axon; 2) expression pattern of Elovl5 in the central nervous system; 3) dietary rescue the SCA38 phenotype; 4) the central deficits due to lack of Elovl5. Mood disorder study funded by CRT: we prepared the breeders of the animal model and analyzed a first set 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

[1] Please duplicate the module for the various lab members
[2] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.
[3] Use times new roman 11 for the text.

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 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.** We hypothesized that, in *Elovl5* knockout mice - model of SCA38, the unbalance of the lipidic profile might cause a disruption of myelin. First objective of 2020 was to conclude the study of the defects of myelin due to *Elovl5* deletion. Second objective was to determine the expression pattern of Elovl5 in the central nervous system. Third objective was to verify whether the motor deficits can be rescued by a diet containing the main lipids that are deficient in *Elovl5*$^{-/-}$ mice. Fourth objective was to search for functional deficits in central neurons involved in SCA38.
Aim2: **Role of GSK3 in mood disorders**. Objectives were to obtain the authorizations for the patients and for the animal parts of the study, to collect and analyze a first set of patients and to prepare the animal model to start experiments in 2021.

d. Results (4000 characters)

Aim 1, Objective 1. In the year 2020 we finished the study of the role of Elovl5 in peripheral nerve function. We found a consistent and significant reduction of the velocity of action potentials. 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 fact, 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; and of peripheral proteins MPZ, MBP, PMP-22, CNPase. We found a significant reduction of MBP 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. We also started a study of the nodes of Ranvier and paranodes, which are involved in the modulation of the speed of action potentials. The manuscript about peripheral nerve myelin has been submitted to the journal Glia, and it was judged as acceptable upon revision.
Aim 1, Objective 2. The localization of Elovl5 in the central nervous system has been finished and the figures for the manuscript have been prepared. We found a widespread distribution of Elovl5 in all brain and spinal regions, with specific patterns of expression.
Aim 1, Objective 3. Numerous sets of Elovl5$^{-/-}$ mice were studied, as they reached the weaning age. We found a significant improvement in motor performance in the group that received long chain polyunsaturated fatty acids from weaning or form birth, while administration in the adult was not associated with any change in motor parameters. The study is now completed and the manuscript is in preparation.
Aim 1, Objective 4. We extended the experiments about the functional role of Elovl5 in the central nervous system by analyzing long-term synaptic plasticity. The results show that, in contrast to the deficit in the short-term plasticity mediated by endocannabinoids, long-term mechanisms are not affected.
Aim 2. In addition to the permissions from the Bioethical Committee of the Hospital for the study on patients, we also obtained the authorization of the Ministry of Health for the study on the animal model. Taking advantage from the fact that in 2019 we set up the technique for the analysis of blood samples from patients, in year 2020 we collected a large population of cases, extracted mononucleated cells and stored them at -80°C. We conducted the western blot analysis of a first cohort of patients with major depressive disorder and with different subtypes of bipolar disorder. Our preliminary results showed a reduction of Gsk3 beta levels in patients compared to control subjects. The animal model, consisting of double GSK3 knock-in mice, was backcrossed with a wild-type strain to obtain double heterozygotes, that were further crossed to obtain double homozygotes and double wild-types (expected rate 1/16 for each genotype).

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 loses its compactness and becomes unstable with a decrease of structural proteins, causing a reduction of action potential velocity. Our results suggest that a slow action potential conduction is the principal mechanism of motor symptoms of patients with SCA38.
The expression pattern of Elovl5 in the nervous system was unknown. We showed a strong expression by the principal neurons of cerebellum and olfactory bulb, in line with the symptoms of patients. We showed for the first time that Elovl5 is necessary for proper function of brain neurons.

The finding of a reduced level of Gsk3 protein in patients suggest that the Gsk3 pathway might be a good starting point to identify biochemical markers for psychiatric diseases.

f. Publications[1]

Lippiello P, **Hoxha E**, **Tempia F**, Miniaci MC. (2020) *GIRK1-mediated inwardly rectifying potassium current is a candidate mechanism behind Purkinje cell excitability, plasticity and neuromodulation.* Cerebellum, Online First 02 July 2020. https://doi.org/10.1007/s12311-020-01158-y.

Lippiello, Pellegrino; **Hoxha, Eriola**; Cristiano, Claudia; Malvicini, Emilia; Stanley, Adrien; Russo, Roberto; **Tempia, Filippo**; Miniaci, Maria. (2020) *Role of β3-adrenergic receptor in the modulation of synaptic transmission and plasticity in mouse cerebellar cortex*. J Neuroscie Res 98: 2263-2274. https://doi.org/10.1002/jnr.24712.

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.

g. 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. A fourth project is to study the link between the deficiency of the ataxia-telangiectasia-mutated kinase (ATM) and the selective vulnerability of Purkinje cells (PCs) in ataxia telangiectasia (A-T) disease. The last project is to study the role of the sonic-hedgehog pathway in cerebellar neurodegeneration induced by chronic hypoxia and to try a pharmacological rescue.

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

1. Currently available therapies for mood disorders, including Major Depressive Disorder (MDD) and Bipolar Disorder (BD), 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] Plese DO NOT include papers in press or submitted.

and investigations on the mechanisms of action of lithium, mood stabilizers and antidepressant drugs. GSK3 controls neuronal excitability and synaptic transmission. It 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 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, but the molecular and electrophysiological mechanisms are not known.

2. Regulation of the resting membrane potential and the repolarization of neurons are important in regulating neuronal excitability. The M-current (I_M), a slowly deactivating, non-inactivating potassium current due to Kv7 channels encoded by members of the *KCNQ* gene family *KCNQ1–KCNQ5*. Mutations in *KCNQ2* and *KCNQ3* cause a neonatal form of epilepsy, and activators of these channels have been identified as novel antiepileptics and analgesics. Despite the important roles of Kv7 currents in the cell types where they have been studied, nothing is known about their properties or functional role in cerebellar 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 other 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

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. Mice with a deletion of exon 11 of Shank3, 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.

4. A-T is an autosomal recessive disorder caused by loss of function of the ATM kinase and is characterized by an early onset, progressive, cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, pulmonary disease and increased risk of developing cancer. Histological studies from autoptic brain material revealed an important degeneration of PCs with an unavoidable compromised cerebellar structure. Atm protein modulates the correct presynaptic vesicle release at glutamatergic synapses, controls GABAergic tone during development and maintains a proper mitochondria and peroxisome homeostasis. Transcriptomics on A-T cerebellum in early asymptomatic phase have showed a possible compromised cerebellar glutamatergic signaling paralleled by a deranged calcium homeostasis which might anticipate PCs death and ataxia.

5. Obstructive sleep apnea is a sleep disorder of breathing that affects 2-9% of the Italian population. Clinical and neuroimaging studies demonstrate that obstructive sleep apnea creates regional alterations in brain morphology and structure and the cerebellum is among the areas most severely affected. Sleep disorders of breathing are frequent in patients affected by sporadic degenerative ataxias. A striking upregulation of the Shh pathway was found in the adult cerebellum upon exposure to chronic hypoxia, using an established model of obstructive sleep apnea. In mammals, the Shh gene encodes a morphogen that is implicated in a wide range of signaling activities during embryonic development, by interacting with the receptor Ptch1 and the transmembrane protein Smoothened. In

embryonic and neonatal cerebellum, Shh is expressed by PCs and acts as a mitogen for the cells of the external granular layer.

i. 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 projects on SCA38 and sonic-hedgehog are aimed at understanding the pathogenic mechanisms and to design specific therapies. 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

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

AIM 1: Role of GSK3 in mood disorders.
Subaim 1.1: 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). 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. 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. 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 total GSK3α and GSK3β, and for the phosphorylated forms of GSK3α, GSK3β.

Subaim 1.2: 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 either acutely by tail suspension or 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.

AIM 4: Are early cerebellar circuitry deficits preceding neurodegeneration in A-T disease? The project will start with the study of a new murine model of the disease. We will perform motor behavioral tests to validate the murine model. Our electrophysiological study will be performed in the early phase of the disease. We will study excitatory and inhibitory transmission in the cerebellar cortex together with the action potential conduction along myelinated PC axon in Atm model mice.

AIM 5: Role of Sonic Hedgehog (Shh) signaling in cerebellar neurodegeneration caused by obstructive sleep apnea. A first part of the project will determine the spatial and temporal changes in expression of Shh following intermittent hypoxia, which is an experimental model of obstructive sleep apnea. The second part of the project is based on the hypothesis that an upregulation of Shh protects cerebellar Purkinje cells from degeneration. Genetic and pharmacological upregulation of Shh will be obtained and the effects of neurodegeneration will be assessed.

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

1. 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.
2. The study on Kv7 channels will fill a gap of knowledge about the intrinsic membrane properties of PCs and allow the construction of more complete simulation models.

3. Results about the involvement of the cerebellum in the Phelan McDermid syndrome will broaden the knowledge on the role of this brain center in autism spectrum disorders and disclose a new specific mechanism in this disease.
4. In ataxia-teleangiectasia, an alteration of synaptic signaling might be responsible for PC degeneration. A discovery about this topic would open the way to design new treatments to rescue PCs and prevent ataxia in patients with AT.
5. The results of the study about Shh might be exploited to prevent neurodegeneration in patients with obstructive sleep disorders.

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

Neuronal activity can be measured *in vivo* in awake and behaving animals by an optic method bases on optic fiber laser illumination of a fluorescent activity reporter expressed by the cells under investigation. With an associated optic fiber the activity-dependent fluorescence signals can be acquired. This technique is now available at NICO. We plan to record neuronal activity during induction of depression to detects the neuronal signals involved in mood disorders.
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). Following identification of the brain areas or nuclei where GSK3 modulates depression, we plan to 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. For these techniques, the collaboration with Dr. Serena Bovetti of the NICO group led by Bonfanti/Peretto will allow us to rapidly acquire the skills and knowledge to successfully perform the experiments.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2020

Laboratory name: **Brain development and disease**

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Role & expertise: mitochondrial dysfunctions, biomarkers, Alzheimer's disease, motor neuron diseases

7 ROBERTA SCHELLINO

Degree: PhD Birthdate: 11/02/1985

Nationality: Italian Gender: F

Phone: +390116706632

Email: roberta.schellino@gmail.com

Position: Post-doc fellow

Role & expertise: Neurogenesis, spinal muscular atrophy, Huntington's disease, confocal imaging, behavior

8 GIOVANNA MENDUTI

Degree: PhD Birthdate: 14/04/1991

Nationality: Italian Gender: F

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Email: giovanna.menduti@unito.it

Position: Post-doc fellow

Role & expertise: Cellular and molecular neurobiology, spinal muscular atrophy

9 VALERIA VESCIAVEO

Degree: PhD student Birthdate: 03/11/1993

Nationality: Italian Gender: Female

Phone: +390116707764

Email: valeria.vesciaveo@edu.unito.it

Position: PhD student

Role & expertise: Pathogenesis of Alzheimer's disease

10 ANNA CARETTO

Degree: PhD student Birthdate: 08/07/1995

Nationality: Italian Gender: F

Phone: +390116706632

Email: anna.caretto@edu.unito.it

Position: PhD student

Role & expertise: Spinal muscular atrophy, neuromuscular diseases

11 DANIELA MARIA RASA'

Degree: Master degree in Biology | Birthdate: 11/09/1990

Nationality: Italian | Gender: F

Phone: +390116706632

Email: danielamaria.rasa@unito.it

Position: Scholarship holder

Role & expertise: Cell culture, spinal muscular atrophy

12 NOEMI MARINO

Degree: MSc | Birthdate: 16/03/1993

Nationality: Italian | Gender: F

Phone: +390116706632

Email: noemi.marino@unito.it

Position: Scholarship holder

Role & expertise: Spinal muscular atrophy, neuroinflammation

2. CURRENT AND PENDING GRANTS

Starting- end date	Project Title and ID	Beneficiary[1]	Funding Program/Agency	Role of the unit[2]	Overall Amount Funded
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 €
2020	SMA	Vercelli A.	Smarathon	PI of research unit	14000 €
2020	SMA	Vercelli A.	Atrofia spinale onlus	PI of research unit	25000 €
2020	Spinal cord injury	Vercelli A.	FORB	PI	20000 €
2020	Identification of new druggable targets and	Vercelli A.	Telethon	PI of research unit	28000 €

[1] Include names of the lead beneficiary: PI or group members. Please avoid duplications and list first all the PI grants, then those of the other lab members.
[2] Coordinator/PI of research unit/team component.

	potential therapeutic compounds for Spinal Muscular Atrophy, using a C. elegans model of neurodegeneration				
2021-2023	The involvement of the small heat shock protein HSPB8 in amyotrophic lateral sclerosis	Vercelli A.	AFM Telethon	PI of research unit	36000
2021	My-AHA	Vercelli A.	EC	coordinator	173400
2021-22	Mental health and urban green/blue	Vercelli A.	UNITO-DNS	coordinator	65000
2021-2022	Approcci innovativi per comprendere la diffusione di nuove sostanze stupefacenti nella popolazione 2020.AI1481.U1610	Salomone A. Tamagno E. Prina F. Guglielmotto M.	CRT	coPI	28000
2020-2022	Caloric restriction in the prevention of Alzheimer's disease	Tamagno E.	Ricerca Locale 2020	PI	670
2021-2024	Carrier mediated nose-to-brain delivery of 24-hydroxycholesterol: a new strategy for Alzheimer's disease?	Gamba P. Tamagno E.	Bright Focus Foundation	coPI	296000
2017-2021	I mitocondri nell'Atrofia Muscolare Spinale: disfunzioni e mitofagia; ID 2017.2052	Boido M.	CRT	PI	28000 €
2020-2021	Development of combinatorial therapies for SMA; ID 22346	Boido M.	AFM Telethon	PI of research unit	34700 € (2nd year)
2019-2022	The role of SMN protein in translation: implications for Spinal Muscular Atrophy; ID GGP19115A	Boido M.	Fondazione Telethon	PI of research unit	83600 €
2021-2023	La bio-stampa 3D: neurobiologia e ingegneria unite per studiare e curare le lesioni al midollo spinale. ID 2020.1801	Boido M.	CRT	PI	30000 €
2020	Virtual meeting "Motor neuron diseases: understanding the pathogenetic	Boido M. (co-organizer Stanga S.)	The Company of Biologists	Proponent	1.773,45 €

	mechanisms to develop therapies"				
2020	Virtual meeting "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies"	Boido M. Stanga S.	Zeiss company Media System Lab	Meeting organizers	1.250 €
01/01.2020 – 31/12/2021	Starting Grant	Calì C.	UNITO	PI	2857,14 €
14/07/2021 – 31/05/2023	RILO 2020: Analisi Ultrastrutturale della Neuroanatomia cellulare della Corteccia con tecniche di Realta Virtuale	Calì C.	UNITO - RILO	PI	2261,67 €
01/04/2021 - 31/03/2023	SHED: A new class of visual analytics tools for comparison on 3D neuronal structures from segmented human EM Data	Calì C.	CRG KAUST Program	PI	140000 € Euro
01/01/2020 – 31/12/2021	"Assegnazione Fondo Dipartimentale destinato ai nuovi ricercatori assunti nel 2019"	Stanga S.	Dip. Neurosci. UNITO; Fondo Dipartimentale	PI	2.857,14€
14/07/2020 – 31/05/2023	"Role of mitochondrial dysfunctions in the aetiology and progression of neurodegenerative diseases"	Stanga S.	Dip. Neurosci. UNITO; Finanziamento di progetti di ricerca dall'Università degli studi di Torino (ex 60%)	PI	1.854,57€
pending	ActR-Fc-nLG3: an effective therapy to counteract muscle deficits due to sedentary behavior, in old, convalescent and disabled people. ID FISR2020IP_04005	Boido M.	Bando FISR 2020	PI	25000 €
pending	C2T - Sviluppo di un Capnografo per la valutazione ed il monitoraggio remoto di dispnea cronica da coronavirus	Calì C.	FISR	PI	80000 €
pending	VR Playground	Calì C.	TIM	PI	11000 €

3. SCIENTIFIC ACTIVITIES IN 2020

Alessandro Vercelli, PI

Supervised PhD students:	M. Lorenzati (co-tutorship with A.Buffo), A. Naldi (co-tutorship with M. Bergui), A. Caretto (co-tutorship with M. Boido)
Honors, prizes, awards:	Elected member of the Academy of Medicine, Torino, president-elect Italian Society for Neuroscience
Outreach activities	
• International collaborations:	G. Aumayr (Austria); M. Summers (Australia); C. Rouaux (France)
• Invited talks:[1]	
• Science communication:[2]	Organiser of the 2020 Brain awareness week
• Editorial duties:	Editor Frontiers in Ageing Neuroscience
• others[3]	
Organizational activities and responsibilities at NICO:	Scientific director
Speakers invited:	Angelo Bifone (UNITO, IIT); Francesca Cirulli (ISS, Rome); Ludovico Minati (Univ. Trento)
Other organizational activities:[4]	Vice-Rector for Biomedical Research, UNITO; vice-director for research, Department of Neuroscience; president of the spinoff committee, UNITO
Workshops, Schools or Conferences organized:	Two Meetings of the Amici dell'Anatomia (February and September)
Technology transfer achievements (patents, etc.):	

Marina Boido, Associate Professor

Supervised PhD students:	A. Caretto (co-tutorship with A. Vercelli), F. Virla (co-tutorship with R. Mariotti, UNIVR)
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:	"Experimental approaches for the study of the spinal cord: from development to pathology", virtual lecture at Milan University, 18/12/20

[1] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[2] Public engagement
[3] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[4] No university appointments.

• Science communication:	Conference "Il neurone suicida", in the seminar cycle "10 piccoli neuroni per 10 grandi libri", libreria Bardotto, Torino, 14/01/2020 Dissemination article on Pharmacon magazine (the magazine of council-run pharmacies), February 2020: https://www.pharmacom.news/nazionali/ricerca-e-salute/conoscere-e-capire-la-sma) UniStem Day, virtual seminar "Lesioni spinali: presente e futuro delle cellule staminali mesenchimali", 29/05/2020
• 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". Review Editor in Frontiers in Aging Neuroscience
• others	Poster presenter "Mitochondrial dysfunction in Spinal Muscular Atrophy" (M. Boido, S. Stanga, G. Pasini, B. Pergolizzi, A. Vercelli), SMA Europe, Evry 05-07/02/20 Member of the local organizer committee of 2nd National meeting "Morfologia e dintorni". Torino, 22-23/02/2020 Member of the organizer committee of the 3rd National meeting "Morfologia e dintorni". Torino, Italy, 26/09/2020, online meeting Member of the local organizer committee of XXX National virtual meeting of "Gruppo Italiano per lo Studio della Neuromorfologia (GISN)", 12-14/11/2020 Vice-President of "Amici dell'Anatomia" group (https://www.amicidellanatomia.it)
Organizational activities and responsibilities 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 system I)", lightsheet microscope From March 2020, organization of the NICO NeuroWebinars
Speakers invited:	Pamela Imperadore (Stazione Zoologica Napoli)
Other organizational activities:	CEO of S&P BRAIN SRL spinoff
Workshops, Schools or Conferences organized:	Organization of the virtual meeting "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" (co-organized with S. Stanga), 06-07/11/20

Technology transfer achievements (patents, etc.):	Patent request number 2020/19 (together with G. Menduti), in collaboration with Univ. Valencia and INSERM

Corrado Calì, Assistant Professor RTD-B

Supervised PhD students:	Maria Fernanda Veloz-Castillo
Honors, prizes, awards:	COVID prize "Intravides" startup
Outreach activities	
• International collaborations:	Pierre Magistretti (Brain Energy Metabolism: KAUST, Saudi Arabia); John Morrison (3D Reconstruction of human neurons: UC Davis, USA); Markus Hadwiger and Hanspeter Pfister (Visual Analytics of 3D models: KAUST, Saudi Arabia and Harvard, USA)
• Invited talks:	"The use of 3D models and Augmented Reality for Neurosurgery", 2nd National meeting "Morfologia e dintorni". Torino, Italy, 22-23/02/2020 "Investigating Neuroanatomical basis of Brain-Energy Metabolism using 3D models and VR tools", Glial cells-neurons crosstalk Workshop, Online, 03.10.2020 "The Neuroanatomical basis of Brain Energy Metabolism", KAUST Student Led Seminar, Online, 11.11.2020 "Neuroanatomical basis of brain energy metabolism in the mammalian brain" GISN, Online, 13.11.2020
• Science communication:	na
• Editorial duties:	na
• others	Member of the reviewing board panel CE45 (Mathématiques et sciences du numérique pour la biologie et la santé) for the Agence Nationale Francaise de la Recherche (ANR) Member of the reviewing board panel for the BraYn starting grant 2020
Organizational activities and responsabilities at NICO:	na
Speakers invited:	Pierre Magistretti (KAUST, Saudi Arabia); Luca Bartesaghi (Karolinska Institute, Stockholm); Joao Filipe Oliveira (Univ. Miño, Braga, Portugal); Renaud Jolivet (Cern, Ginevra)
Other organizational activities:	President and founder of "Intravides" startup
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	AR Surgical Goggles (Intravides)

Serena Stanga, Assistant Professor RTD-A

Supervised PhD students:	Sabrina Contino, UCLouvain, PhD thesis entitled "Rôle des présénilines dans la morphologie et la fonctionnalité mitochondriale" discussed in September 30th 2020, Supervisor: Pr. Pascal Kienlen-Campard, Co-Supervisor: Serena Stanga.
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Pr. Pascal Kienlen-Campard, Institute of Neuroscience, UCLouvain, Belgium; Pr. Giulio Muccioli, Louvain Drug Research Institute - LDRI, UCLouvain, Bruxelles (Belgium); Pr. Donatienne Tyteca, Institut de Duve, UCLouvain, Bruxelles (Belgium); Dr. Emilie Auoduard, Institut National de la Santé et de la Recherche Médicale (INSERM).
• Invited talks:	na
• Science communication:	na
• Editorial duties:	Review editor for the MDPI Journal International Journal of Molecular Sciences (IJMS) section Biology Review editor for the Journal Frontiers in Neuroscience section Neurodegeneration
• others	Poster at meeting: Stanga S. "Mitochondrial dysfunctions and mitophagy in Spinal Muscular Atrophy", 2nd National meeting "Morfologia e dintorni", Torino, Italy, 22-23/02/2020 Oral presentations at meetings: Stanga S, Pavarino G, Monteleone F, Pergolizzi B, Boido M, Vercelli A. "Mitochondrial Alterations In Spinal Muscular Atrophy" XXX GISN 2020 (Gruppo Italiano per lo Studio della Neuromorfologia) online meeting (Torino), 12-14.11.2020; Proceedings of the 30th National Conference of the Italian GISN published in EHJ, 2020; V(64)/supplement 3; ISSN 1121-760X Stanga S, Caretto A, Boido M, Vercelli A. "Mitochondrial dysfunctions are the early event eliciting the shift towards pathological neurobiological processes", 3rd National meeting "Morfologia e dintorni", Torino, Italy, 26/09/2020, online meeting Stanga S. "Mitochondrial dysfunctions and mitophagy in Spinal Muscular Atrophy", 2nd National meeting "Morfologia e dintorni", Torino, Italy, 22-23/02/2020 Member of the Organizational Committee "2nd National meeting "Morfologia e dintorni", Torino, Italy, 22-23/02/2020

Organizational activities and responsabilities at NICO:	Responsible for the Cell Culture room at NICO (floor 0); Responsible for the Dissection room at NICO (floor -1) Organization of the data meetings 2020 of the Group "Brain development and disease"
Speakers invited:	Paolo Porporato (MBC – Torino)
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Organization of the virtual meeting "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" (co-organized with M. Boido), 06-07/11/20
Technology transfer achievements (patents, etc.):	na

SCHELLINO ROBERTA, Postdoc

Supervised PhD students:	na
Honors, prizes, awards:	Individual Research Fellowship (Assegno di Ricerca), Department of Neuroscience "Rita Levi Montalcini", University of Torino. Project title: "Impact of pathological environmental signals on survival, differentiation and integration of human neuron transplants into preclinical models of Huntington's disease"
Outreach activities	
• International collaborations:	Prof. Malin Parmar, Lund University (Swe)
• Invited talks:	na
• Science communication:	na
• Editorial duties:	Review editor for the journals Brain Sciences (MDPI journal) and Frontiers in Neuroscience
• others	Oral presentations: "Anatomical and functional integration of human MSN precursors grafted into a rat model of Huntington's Disease". Schellino R, Boido M, Besusso D, Parolisi R, Buffo, A, Cattaneo E, Vercelli A. 2nd National meeting "Morfologia e dintorni". Torino, Italy, 22-23/02/2020 "ActR-Fc-nLG3: a novel protein to sustain neuromuscular junction innervation and improve motor endurance in old sarcopenic mice." Schellino R, Wrijbloed JW, Fariello R, Boido M. 3rd National meeting "Morfologia e dintorni". Torino, Italy. 26/09/2020, online meeting "A novel biological (Actr-Fc-nLG3) to sustain neuromuscular junction innervation in Sarcopenia." Schellino R, Boido M, Vrijbloed JW, Fariello R, Vercelli A. XXX G.I.S.N. meeting (Gruppo Italiano per lo studio della Neuromorfologia). 12-14/11/2020, online meeting

Organizational activities and responsibilities at NICO:	Responsible for "E800 Nikon Eclypse fluorescence microscope"
Speakers invited:	na
Other organizational activities:[1]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Giovanna Menduti, Postdoc

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Poster presenter: "Drug Screening and Drug Repositioning; Modern Horizons in Spinal Muscular Atrophy Therapy", Menduti G*., Rasà D.M.*, Stanga S., Boido M.; Virtual meeting "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies", 06-07/11/2020
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	Patent request number 2020/19 (together with M. Boido), in collaboration with Univ. Valencia and INSERM

Anna Caretto, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na

[1] No university appointments.

• others	Oral presentation: "Efficacy evaluation of the GHRH agonist MR409 in a SMA murine model", Caretto A., Gesmundo I., Schellino R., Schally A.V., Boido M., Granata R. and Vercelli A. XXX Convegno Nazionale del Gruppo Italiano per lo Studio della Neuromorfologia (GISN). Torino, Italia. 12-14/11/2020 Poster presenter: "A new potential supportive role of MR409, a GHRH agonist, in an experimental mouse model of Spinal Muscular Atrophy", Caretto A., Gesmundo I., Schellino R., Schally A.V., Boido M., Granata R. and Vercelli A. Motor Neuron Diseases understanding the pathogenetic mechanisms to develop therapies. Torino, Italia. 6-7/11/2020 Poster presenter: "Valutazione dell'efficacia terapeutica di MR409, un agonista del GHRH, in un modello sperimentale di Atrofia Muscolare Spinale", Caretto, A., Boido, M. 2nd National meeting "Morfologia e dintorni". Torino, 22-23/02/202
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Daniela Maria Rasà, Scholarship holder

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• Others	Poster presenter: "Drug Screening and Drug Repositioning; Modern Horizons in Spinal Muscular Atrophy Therapy", Menduti G*., Rasà D.M.*, Stanga S., Boido M.; Virtual meeting "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies", 06-07/11/2020
Organizational activities and responsibilities 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:	Video-shooting and interviews on the occasion of 10th year NICO Anniversary

4. Research activity in 2020[1]

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 neurodegeneration. We are interested in neuronal cell death pathways (in development and in experimental models of SMA and AD) and in the fine-tuning of brain-energy metabolism, a complex paradigm involving a strong astrocyte-neuron interplay. We are also studying cell therapy in preclinical experimental models of SCI, ALS and HD.

b. Background and rationale (3000 characters)

The study of the CNS represents a great challenge 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.
Europe with the Human Brain Project and USA with the Connectome project, together with similar projects launched by Japan and China, targeted the micro-, meso- and macro-connectome from a normal and pathological point of view. 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. Moreover, astrocytes interface at various level with neurons in brain parenchyma, although their support to the neuronal energetic needs remains to be fully understood. To explore these aspects, we take advantage of the study of normal brains and of the brains of TG

[1] Use times new roman 11 for the text.

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, to study the molecular mechanisms involved and treat 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 to foster a translation from bench to bedside, but also to get 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. Indeed, 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 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 (networks and connectivity), as substrate for brain activities and entities which may be disrupted in several congenital and degenerative diseases. We also aim to clarify the astrocyte-neuron metabolic interplay, by analyzing the overall morphology of individual astrocytes, through machine learning, high-throughput 3D imaging, 3D models, VR tools.
We study the neuronal death mechanisms (excitotoxicity, apoptosis, autophagy and oxidative stress) during development and disease. We also study neuroinflammation and how to prevent it.
Stem cells (SCs) are still a growing field of research: we study the integration of hES-derived striatal progenitors grafted into the striatum (in HD). We also use neural and/or mesenchymal SCs to treat neurodegenerative/traumatic diseases (in ALS and SCI), to provide trophic and immunomodulatory factors 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*.

Astrocytes-neurons structural crosstalk
So far, we managed to design a semi-automated tool for skeletonizing cells in VR, to quantify features of cell structures like branching order, total length, and overall cell complexity. VR eases in particular the skeletonization of astrocytes, a still unexplored field. We have also obtained high-resolution EM stacks from a human sample that will be used for further processing and reconstruction. We are starting a set of IHC analysis of visual cortex of rodent samples to identify sites of interactions between dendritic bundles and astrocytes, since literature suggests a potential metabolic hotspot in layer 2/3 among the two, under control of Noradrenaline (NA) and Vaso-Intestinal Peptide (VIP).

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 and proteomic analysis. We are also testing both SMN-dependent (repurposed drugs, in collaboration with Univ. Valencia and CNR Naples) and -independent therapeutic approaches (a GHRH agonist and MSC-derived

exosomes) to delay disease progression and counteract muscular atrophy. Moreover, with G. Viero (CNR, Trento) we are deepening the SMN functions, analyzing the presence of translation defects in our SMA mice.
Re AD, we found that biological sex influences the effect of Aβ42 monomers on pathological Tau conformational change. In this study we used TG mice expressing the WT human Tau (hTau) which were subjected to ICV injections of Aβ peptides. We found that Aβ42 produces pathological conformational changes and hyperphosphorylation of Tau protein in male or ovariectomized female mice, but not in control females. The treatment of ovariectomized females with estradiol replacement protects against the pathological conformation of Tau and seems to be mediated by antioxidant activity as well as the ability to modulate the expression of miRNA 218 linked to Tau phosphorylation.

Stem cell therapy in HD
With E. Cattaneo and A. Buffo, the graft of hESCs in an experimental model of HD assured good results in terms of cell replacement, establishment of new connections and behavioral performance *(Stem Cell Reports, 2020)*: we are now evaluating longer time points and the effect of enriched environment conditions *(in preparation)*.

Spinal cord injury
With E. Terreno (MBC, Turin), we have developed a method to label macrophages with perfluorocarbon-based compounds: we can image inflammatory processes *in vivo* at the injury site by MRI and identify the best therapeutic window (*in preparation*).
With Prof. Dalmay (Univ. East Anglia), 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 (*submitted to Front. Mol. Biosci.*).

Active and Healthy Ageing
AV coordinated 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 ended in 2019, and the manuscript accepted in 2020. In a limited number of subjects (control vs pre-frail vs pre-frail + intervention) a fMRI study is ongoing 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, study of cell complexity and interplay through 3D skeletonization, cell death and 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. We are involved in the technical revolution about microscopy to investigate micro- and meso-connectome, with the use of confocal 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 empower the elderly in their everyday life and to improve early detection and personalized prevention of disease.

f. Publications[1]

Agus M, Al-Thelaya K, Cali C, **Boido MM**, Yang Y, Pintore G, Gobbetti E, Schneider J. 2020 *InShaDe: Invariant Shape Descriptors for Visual Analysis of Histology 2D Cellular and Nuclear Shapes*. Eurographics Workshop on Visual Computing for Biology and Medicine (VCBM), 10.2312/vcbm.20201173

Besusso D, **Schellino R**, **Boido M**, Belloli S, **Parolisi R**, Conforti P, Faedo A, Cernigoj M, Campus I, Laporta A, Bocchi VD, Murtaj V, Parmar M, Spaiardi P, Talpo F, Maniezzi C, Toselli MG, Biella G, Moresco RM, **Vercelli A**, Buffo A, Cattaneo E. 2020 *Stem Cell-Derived Human Striatal Progenitors Innervate Striatal Targets and Alleviate Sensorimotor Deficit in a Rat Model of Huntington Disease*. Stem Cell Reports. May 12;14(5):876-891.

Boges DJ, M Agus M, Magistretti PJ, **Calì C**. 2020 *Forget About Electron Micrographs: A Novel Guide for Using 3D Models for Quantitative Analysis of Dense Reconstructions*. Neuromethods, Volume Microscopy, 263-604.

Boges D, Agus M, Sicat R, Magistretti PJ, Hadwiger M, **Calì C**. 2020. *Virtual reality framework for editing and exploring medial axis representations of nanometric scale neural structures.* Computers & Graphics 91, 12-24

Boido M, Butenko O, Filippo C, **Schellino R**, Vrijbloed JW, Fariello RG, **Vercelli A**. 2020 *A new protein curbs the hypertrophic effect of myostatin inhibition, adding remarkable endurance to motor performance in mice*. PLoS One. Mar 11;15(3):e0228653.

Boido M, **Schellino R**, **Vercelli A**. *Neuromuscular correlations: development, pathology and aging*. Pages 169-189. Giornale della Accademia di Medicina di Torino 2019. Anno CLXXXII.

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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 and expand our previous research on i) axonal growth in the CNS, ii) the astrocytic morphology and the complex astrocyte/neuron interplay, iii) molecular and cellular mechanisms of neuronal death in neurodegenerative diseases, iv) network analysis at multiscale level, v) stem cell therapy. We aim at investigating how astrocytic morphology relates in particular to dendritic bundles, whose activity might be coordinated by surrounding astroglial cells. There is also a growing interest on mitochondria in neurodegenerative diseases: that we are extending to several pathologies (SMA and PD). We aim at identifying some new therapeutic targets for traumatic and neurodegenerative diseases (as SCI, SMA, ALS and HD). With internal and external groups, we are importing new techniques, such as the organoids for *in vitro* analysis of brain development and disease modeling, light sheet microscopy and innovative clearing protocols, semi-automated tool for skeletonizing cells in VR, and 3D EM. 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, as a followup of the Horizon 2020 grant, my-AHA, is preparing applications for the next EC calls on Ageing to improve early detection of age-related frailty in the individual by use of Artificial Intelligence (machine and deep learning). On the same subjects, in collaboration with F. Cauda (Dept. Psychology) and I. Rainero (Dept. Neurosci) we 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. Recently, we have submitted a project together with Prof. Rocca (Psychiatry) to study the effects of living in the green (close to city parks) or near the blue (close to the rivers) on schizophrenia and psychiatric diseases, from a clinical, behavioral and biochemical marker point of view. This will be a preliminary study in order to prepare the group to the new Green deal program of Horizon Europe.
Moreover, the fine-tuning of brain-energy metabolism is another key process and hot topic in the CNS study. The fact that neurons express the machinery allowing them to provide for their energetic needs is counterintuitive with respect to the assumption that astrocytes support their metabolism. Also, recent evidence points to the fact that metabolism is just one face of the coin, the other one being gliotransmission, a property that makes astrocytes competent to directly fine-tune synaptic activity. The high spatial compartmentalization of astrocytes might be the key to solve such complex interplay between the two. Recent evidence has shown that glycogen, a mechanism of energy storage in astrocytes, is specifically located around strategic neuronal compartments, such as synapses and large dendrites containing long mitochondrial bundles. Also, we know that glycogen stores can be mobilized and used on request by activating astrocytic receptors to NA or VIP. Such arrangement has been suggested already in the late 80s, where scientists have described the presence of VIP and NA fibers targeting L2/3 dendrites in visual cortex. More recently, it has been shown how in the same region, dendrites from pyramidal cells located in deep cortical layers arrange in bundles corresponding to cell bodies of neurons projecting to common targets. We aim at exploring whether the activity of bundles could be coordinated by astrocytes, as suggested in previous works in the hippocampus.

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. We aim to explore the astrocyte-neuron crosstalk, to decipher whether this activity could be mediated by gliotransmission or metabolic support, and whether these two are spatially co-localized or not: understanding physiological processes can help to treat pathological states of the brain. Our findings are finally aimed to develop new therapeutic strategies to prevent neurodegenerative diseases and to support 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 Institute.

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

Axonal growth
SCI: in collaboration with Dr Tonda-Turo (Polytechnic of Turin), we will develop a 3D cellularized construct by bioprinting technique for preliminarily screen treatments for SCI: 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. 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.

Astrocyte-neuron interplay
In order to unveil the complex astrocytes-neurons interplay, we will deepen the knowledge regarding the compartmentalization of astrocytes at different level (parenchyma, neuropil). As a model, we consider using rodent visual cortex, because of its stereotyped organization and the previous knowledge available regarding the spatial arrangement of glycogenolytic fibers (VIP, NA) compared to dendritic bundles in L2/3. In particular, we intend to set in place the expansion microscopy (ExM) protocol (a modified version of the tissue clearing, already sat up in the Institute by Dr Bovetti) to increase imaging resolution by physical expanding the tissue size.

Stem cell therapy
Our group has collaborated with A. Buffo and E. Cattaneo, in stem cell transplantation in HD, to assess the long-term survival, maturation, integration and function of optimized hPSCs-derived MSNs, thanks to new *in vitro* protocols and cutting edge tracing techniques *in vivo*. This part of the project, as far we are concerned, is at the last experiments.
Regarding ALS, In collaboration with A. Vescovi, we will further assess the therapeutic potential of clinical-grade human neural stem cells in a mouse model of ALS.

Molecular mechanisms of cell death and neuroinflammation, and therapeutic approaches
SMA: we will extend our studies on mitochondria alterations, by also investigating GABA signalling and interneuron functionality, and their possible correlation in mitochondrial dynamics in SMA. In collaboration with G. Viero (CNR, Trento), we will also verify how SMN translational defects can affect mitochondrial protein synthesis. 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 (alone or in combination) 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: nutrition (particularly fasting-mimicking diets and re-feeding periods) may be effective in limiting AD progression in mouse models. Dietary restriction (DR) regimen reduces excitotoxic damage to CA1 and CA3 neurons compared to mice fed ad libitum. We will test the role for periodic DR cycles in reducing the pathologic conformation of Tau protein in 5XFAD TG mice expressing hTau. In 2020, we found that Aβ42 produces pathological conformational changes and hyperphosphorylation of Tau protein in male or ovariectomized female mice but not in control females. The treatment of ovariectomized females with estradiol replacement protects against the pathological conformation of Tau and seems to be mediated by antioxidant activity as well as the ability to modulate the expression of miRNA218 linked to Tau phosphorylation. Emerging evidence suggests that estrogens can regulate miRNAs in many pathological conditions. We focused our attention on miRNA218, since implicated in the phosphorylation of Tau upon estrogen receptor α and β activation. We will measure miRNA218 levels in the CSF and blood of MCI and AD women to understand if it can be used as a disease biomarker.

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. Moreover, the current collaborations (with Pharmafox and Univ, Valencia) will give us the opportunity to patent some of the tested treatments.
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. Moreover, our group is one of the major groups working with stem cell therapy at a preclinical level in Italy and Europe.
Finally, our unique approach combining 3D models and VR has previously put our research in evidence, and we currently collaborate with a network of top-ranked scientists in the Visual Computing community, including Harvard (USA; Hanspeter Pfister), KAUST (Saudi Arabia; Markus Hadwiger) and Hamad Bin Khalifa University (Qatar; Marco Agus).

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

The collaboration with 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.
The recent installation of the light sheet microscope is pushing many teams to explore the tissue clearing protocol: we intend to perform detailed anatomical studies (e.g. to deeply analyze the integration of human embryonic stem cells in an experimental model of HD). Moreover, a step further we intend to set up is the ExM protocol, to further improve resolution, as intermediate step before 3D EM. All these techniques will require development of novel visualization and analysis techniques that will be developed using the aid of VR. Moreover, together with IIT and CNR, we plan to exploit STED microscopy, to evaluate localization and interactions of SMN protein.
The collaboration with the group of prof. Cauda (Department of Psychology), which already allowed obtaining significant results on the functional connectivity of the human insula by fMRI, will allow using 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).

NICO Neuroscience Institute Cavalieri Ottolenghi 2010-2020 10 years Anniversary

07/01/20 - Lecture
Alice Staffa, Cellular and System Neurobiology Unit, Development and refinement of neuronal circuits group - Instituto de Neurociencias de Alicante, Spain
NMDA receptors containing GluN3A subunits influence myelination during development and after injury

24/01/20 - Lecture
***Francesca Cirulli**, Ph.D., Center for Behavioral Sciences and Mental Health*
Istituto Superiore di Sanità, Roma
Healthspan vs longevity: translating research from animal models to promote healthy ageing in humans

07/02/20
Stefano Espinoza, PostDoc, Non-coding RNAs and RNA-based therapeutics, IIT Genova
SINEUPs: a functional class of lncRNAs that activates translation as a novel strategy for gene therapy of neurological disorders

10/04/20 - Lecture
Livio Oboti (PhD - Institut für Biologie Humboldt Universität zu Berlin)
Corticofugal feedback circuits in the mouse vomeronasal system
Host: Silvia De Marchis

15/04/20 - Lecture
Pierre Magistretti (KAUST - King Abdullah University, Saudi Arabia)
Neuron-glia metabolic coupling: relevance for plasticity and neuroprotection
Host: Corrado Calì

24/04/20 - Lecture
Erica Staurenghi (Dept. of Clinical and Biological Sciences, Univ. Torino)

Brain oxysterols in Alzheimer's disease: could they contribute to neuronal damage by inducing astrocyte reactivity?
Host: Annalisa Buffo

27/04/20 - Lecture
Ludovico Minati (CIMeC, University of Trento, Italy)
Across neurons and silicon: new bridges between biology and electronics

06/05/20 - Lecture
Angelo Bifone (University of Torino)
Alcoholism and the insular cortex: insights from neuroimaging
Host: Alessandro Vercelli

15/05/20 - Lecture
Pamela Imperadore (SZN)
Neural regeneration: tales from the octopus
Host: Marina Boido

5/06/20 - Lecture
Paolo Porporato (MBC - Torino)
Altered iron metabolism controls muscle wasting and cachexia
Host: Serena Stanga

19/06/20 - Lecture
Eleonora Vannini (CNR, Pisa)
Synaptic vesicles dynamics in focal epilepsy
Host: Enrica Boda

3/07/20 - Lecture
Giovanni Ferrara (San Martino Hospital, Genova)
Characterization of the possible role of specialized pro-resolving mediators (SPMs) in the generation of immature/tolerogenic dendritic cells (DCs).
Host: Enrica Boda

17/07/20 - Lecture
Dania Vecchia (IIT, Genova)
Temporal Sharpening of Sensory Responses by Layer V in the Mouse Primary Somatosensory Cortex
Host: Serena Bovetti

31/07/20 - Lecture
Stefano Angiari (Trinity College, Dublin)
Metabolic regulation of the immune response
Host: Enrica Boda

25/09/20 - Lecture
Luca Bartesaghi (Karolinska Institute, Stockholm)
Characterisation of the onset and progression of nervous system myelination in mice
Host: Corrado Calì and Giovanna Gambarotta

30/10/20 - Lecture
Roberta Magliozzi (Univ. Verona)
Meningeal inflammation and grey matter pathology in multiple sclerosis
Host: Francesca Montarolo

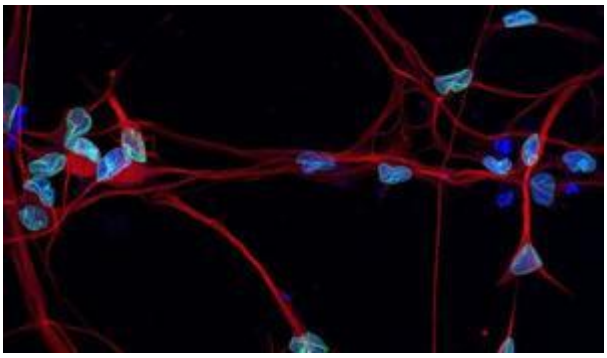
6/11/20 - Lecture
Joao Filipe Oliveira (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal)
The involvement of astrocytes in cognitive processing
Host: Corrado Calì

27/11/20 - Lecture
Luigia Pace (Armenise-Harvard Immune Regulation Lab. - IRCCS Candiolo)
Epigenetic and transcriptional control during CD8+ T cell fate commitment
A single cell approach to understand cell heterogeneity
Host: Annalisa Buffo

4/12/20 - Lecture
Stefano Zucca (IIT, Genova)
An inhibitory gate for State Transition in the Cortex
Host: Serena Bovetti

11/12/20 - Lecture
Renaud Blaise Jolivet (University of Geneva, Department of Nuclear and Corpuscular Physics and Geneva Neuroscience Center)
A New type of Plasticity within Neuroglial Networks
Host: Corrado Calì

NICO Neuroscience Institute Cavalieri Ottolenghi 2010-2020 10 years Anniversary



NICO Progress Report

Our young researchers present their work to collegues.

31/1/20 - **Progress Report**

Roberta Schellino (Group Vercelli)
Anatomical and functional integration of human MSN precursors grafted into a rat model of Huntington's Disease

14/2/20 - Progress Report

Marilena Marraudino (Group Panzica)
Postnatal treatment with estrogen receptor antagonists alters the sexual and feeding behaviors in male and female mice

13/03/20 - Progress Report

Martina Lorenzati (Group Vercelli)
c-Jun N-terminal Kinase 1 (JNK1) modulates OPC architecture, proliferation and myelination

16/03/20 - Journal club

Federico Luzzati
Glia Accumulate Evidence that Actions Are Futile and Suppress Unsuccessful Behavior

18/03/20 - Senior Lecture

Alessandro Vercelli
Development, morphology and connectivity of pyramidal neurons

20/03/20 - Progress Report

Francesca Montarolo (Group Bertolotto)
Effects of sphingosine-1-phosphate receptors modulators on nuclear receptor NR4As: an in vitro study using human blood and brain-resident cells

23/03/20 - Senior Lecture
Carola Eva
Conditional inactivation of Npy1r gene in mice induces sex-related differences of metabolic and behavioral functions

25/03/20 - Progress Report
Corrado Calì (Group Vercelli)
3D-EM and Virtual Reality tools in Brain Metabolism

27/03/20 - Progress Report
Chiara la Rosa (Group Bonfanti)
"Immature" neurons in mammals: a "reservoir" of young neurons in large-sized brains?

30/03/20 - Lecture
Serena Martire
Biostatistics: Back to the Basics

01/04/20
Barbara Magnani (Press Agent)
Communication@NICO: istruzioni per l'uso

03/04/20 - Progress Report
Brigitta Bonaldo (Group Panzica)
Something unexpected: the effects of chronic treatment with BPA on pregnant adult female mice?

06/04/20 - Progress Report
Giacomo Carta
Neurodynamics from bedside to in-vitro to refine a basic diagnostic and therapeutic intervention

17/04/20 - Progress Report
Isabella Crisci (Group Bonfanti – Peretto)
A new perspective on Tamoxifen effect within the adult hippocampal neurogenic niche

20/04/20 - Lecture
Serena Martire (II)
Introduction to hypothesis testing

22/04/20 - Senior Lecture
Alessandro Vercelli
Supporting healthy ageing with information and communication technology and artificial intelligence

29/04/20 - Progress Report
Ilaria Balbo (Group Tempia)
Spinocerebellar ataxia 38: role of Elovl5 in central and peripheral nervous system

04/05/20 - Lecture (III)
Serena Martire
Correlation and linear regression

08/05/20 - Senior Lecture
Luca Bonfanti
From adult neurogenesis to immature neurons: a 10.000 papers' Journal Club

22/05/20 - Senior Lecture
Giovanna Gambarotta
Cells & factors involved in peripheral nerve regeneration

29/05/20 - Progress Report
Sara Bonzano (Group Bonfanti – Peretto)
Shedding light on mitochondria in adult neurogenesis: a role for the transcription factor COUP-TFI/Nr2f1

12/06/20 - Progress report
Marco Fogli (Group Bonfanti – Peretto)
Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic injury

26/06/20 - Progress report
Daniela Rasà (Group Vercelli)
Drug screening and drug repositioning as promising approaches for SMA treatments

10/07/20 - Progress report
Roberta Parolisi (Group Buffo)
Air pollution and Multiple Sclerosis. Role of particulate matter (PM) exposure in de- and remyelination

24/07/20 - Progress report
Gianmarco Pallavicini (Group Di Cunto)
CIT kinase activity loss generates DNA damage in neural precursor

04/09/20 - Progress report
Giulia Nato (Group Buffo - Peretto)
Multistep transition of parenchymal astrocytes toward neurogenesis

18/09/20 - Progress report
Enrica Boda (Group Buffo)
Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model

9/10/20 - Progress report
Ilaria Bertocchi (Group Eva)
Voltage-independent GluN2A-type NMDA receptor Ca2+ signaling promotes audiogenic seizures, attentional and cognitive deficits

23/10/20 - Progress report
Serena Bovetti (Group Peretto)
Imaging the developing auditory cortex with two-photon and light-sheet microscopy

20/11/20 - Progress report
Serena Stanga (Group Vercelli)
Mitochondrial morpho-functional dysfunctions in Spinal Muscular Atrophy: focus on Aconitase2

18/12/20 - Progress report
Giorgia Iegiani (Group Di Cunto)
Citron Kinase modulates homologous recombination through microtubule dynamics